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Addition of milk to tea infusions: Helpful or harmful? Evidence from *in vitro* and *in vivo* studies on antioxidant properties

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ABSTRACT
Tea consumption is practised as a tradition, and has shown potential to improve human health. Maximal uptake of tea antioxidants and milk proteins without a negative impact on tea flavor is highly desired by consumers. There is a conflicting evidence of the effect of milk addition to tea on antioxidant activity. Differences in the type of tea, the composition, type and amount of milk, preparation method of tea–milk infusions, the assays used to measure antioxidant activity, and sampling size likely account for different findings. Interactions between tea polyphenols and milk proteins, especially between catechins and caseins, could account for a decrease in antioxidant activity, although other mechanisms are also possible, given the similar effects between soy and bovine milk. The role of milk fat globules and the milk fat globule membrane surface is also important when considering interactions and loss of polyphenolic antioxidant activity, which has not been addressed in the literature.

KEYWORDS
Polyphenols; milk proteins; antioxidant activity; bioavailability; milk fat globules; chemical interactions

Introduction

Tea is the second most popularly consumed beverage worldwide after water. The top 10 tea-producing countries are China, India, Kenya, Sri Lanka, Turkey, Vietnam, Iran, Indonesia, Argentina, and Japan (FAO 2004). Different varieties of tea, including black, green, oolong, white, and yellow tea, are derived from *Camellia sinensis*, but climatic differences and processing methods account for differences in the composition and degree of anti-oxidative behavior (Hayat et al. 2015). For example, black and green tea varieties, the two most common tea varieties, mainly differ in the presence or absence of a fermentation step, respectively. Polyphenol oxidase in tea leaves can aerobically oxidize tea phenolics after the enzyme comes into contact with the substrate upon the disruption of the intact leaf cell structure (Graham 1992). Black tea is fully fermented, thus possesses stronger flavor compared to less oxidized teas. Green tea accounts for only 20% out of 2.5 million metric tons (on dried basis) of manufactured tea leaves, with less than 2% for oolong tea. The chemical composition of tea determines the various putative health benefits such as anti-carcinogenic effects (Dufresne and Farnworth 2000; Gupta et al. 2002; Sun et al. 2006; Butt and Sultan 2009; Butt et al. 2015), anti-oxidative potential (Benzie and Szeto 1999), and mitigation of cardiovascular diseases (Sano et al. 2004; Kuriyama et al. 2006; Wang et al. 2011), strokes (Keli et al. 1996), atherosclerosis (Tijburg et al. 1997; Vinson et al. 2004), and other heart diseases (Hertog et al. 1997; Arts et al. 2001; Vinson 2000; Hayat et al. 2015).

The major constituents responsible for these health benefits are polyphenols that account for 30% of the dry leaf weight and constitute the largest group of chemical components in green tea, followed by carbohydrates and proteins. The polyphenols consist of catechins, theaflavins, tannins, and flavonoids (Leenen et al. 2000). Catechins, as the predominant polyphenolic substances in tea, can be partially converted into other components, such as theaflavins and thearubigins by oxidation in black tea (Hollman et al. 1997). The major catechins in green tea are epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC), gallocatechin (GC), and catechin (C), with EGCG being the most abundant and potent compound (Goto et al. 1996; Huang et al. 2011). These antioxidants are well-known effective electron donors and scavengers of physiologically relevant reactive oxygen species in vitro, including superoxide anions, peroxyl radicals, and singlet oxygen, and have been shown to account for some of the health benefits of tea consumption, especially antioxidant activity of human plasma (Michalak 2006; Yashin et al. 2011). Tea is a good source of methyl xanthines, which are primarily found in the form of caffeine, but the caffeine content in tea is only around one-third of coffee (known as the best source of caffeine among all foodstuffs). Small quantities of methyl xanthines, theobromine, and theophylline are also present in tea. Theanine, a specific and unusual non-protein amino acid derived from L-glutamic acid (glutamate), is thought to be largely responsible for the unique flavor of tea. This amino acid constitutes approximately one-half of the total amino acid composition of tea. Some other products, such as carotenoids, important as precursors in black tea, are also well known in tea, although these are present at low levels. β-Carotene, violaxanthin, lutein, and neoxanthin have been also identified (Engelhardt 2010).

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Milk is recognized as a natural and nutrient-rich beverage that provides good nutrition for new-born mammals, including humans. Although many kinds of milk are consumed by humans, including milk from sheep, goat, buffalo, camel, and horse, 85% of all milk produced by the dairy industry is from bovine cows (Gerosa and Skoet 2012). The standard bovine milk in the market is an opaque white liquid containing water (87%), proteins (3.0–3.5%, with ~80% associated into casein micelles), fat (3.4–4.0%), lactose (4.8–4.9%), minerals (mainly calcium phosphate, 0.2%), and vitamins (~0.03%) (Fennema 1996; Walstra et al. 2010). The casein micelles contain most of the calcium phosphate mineral. Fat is found in an emulsified form coated by the native milk fat globule membrane.

Although tea is consumed without any additional ingredients in many Asian countries, addition of milk to tea is a ubiquitous practice. Many studies have been carried out on the effects of milk components, especially proteins, on the antioxidant properties of tea catechins. These include: (1) a totally inhibitory (negative) or masking effect (Arts et al. 2002; Sharma et al. 2008; Dubeau et al. 2010; Ryan and Petit 2010; Ryan and Sutherland 2011), (2) a neutral effect (non-masking, with neither inhibition nor enhancement) (Leenen et al. 2000; Kyle et al. 2007), (3) a dual effect (both positive and negative for different attributes) (Dubeau et al. 2010), and (4) a positive effect (enhancement) of milk on the catechin absorption of tea (Xie et al. 2013). The effect of milk addition on tea polyphenolic activity depends upon the ratio of milk to tea, milk composition, tea type, temperature of brewing, and infusion method. There are no in vivo or in vitro reports published on the effect of milk addition on the activity, availability, and accessibility of tea caffeine. This review focuses on the effect of adding different types of milk to tea, and the resultant phenolic content, antioxidant activity, bioaccessibility, and bioavailability of tea polyphenols.

Tea production and composition

Understanding the manufacturing methods and leaf composition of various types of tea, and most importantly, the fundamental chemical changes that occur during the production of commercial tea products, is essential to interpret the outcome of adding milk to tea. Two major species of tea, *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica*, are steeped for consumption. The sinensis variety has small leaves of 5–12 cm, whereas the leaves of the assamica variety can be as long as 20 cm (Graham 1992). The exact classification of tea is mostly based upon the degree of fermentation, i.e. white, green, oolong, black, pu-erh, and flavored tea (Fig. 1). The properties, appearance, flavor, and the methods of preparing infusions of the main tea categories are presented in Table 1. Tea blends, through the combined use of these main categories of tea, also exist on the market. It is worth noting that many herbal infusions are also called "tea," although they are entirely unrelated to the tea plant (*Camellia sinensis*). The composition of two main types of tea (black and green), which vary with fermentation processing, climatic conditions, handling practices, season, leaf age, and tea variety, is shown in Table 2.

The development of more effective extraction, separation, and characterization methods should enable the appreciation of the full spectrum of bioactive phytochemicals, including catechindigallates, methylated catechins, and chalcan-flavans in fresh tea leaves. Tea contains flavor compounds that are also found in food spices (Dufresne and Farnworth 2001). Approximately 60 volatile components, including esters, acids, carbonyls, alcohols, and cyclic compounds, have also been identified in fresh tea leaves that contribute to the specific flavor of tea (Graham 1992). Dimericproanthocyanidins, phenolic compounds found in fresh tea leaves, contribute to tea flavor and astringency, and are derived products from tea catechins with a pyran ring and an "A" ring linked via C-C bonds (Quideau et al. 2011). Some important phenolic acids, such as gallic acid, are also present in a free form in tea leaves where they enter into oxidation reactions during fermentation. Quinic acid and caffeoylquinic acid are also found in the fresh leaf (Engelhardt 2010). The protein fraction of tea mainly includes the enzymes that are normally associated with the metabolism of plant cells. These enzymes are also responsible for synthesis of catechin. Polyphenol oxidase facilitates aerobic oxidation of tea catechins, especially in black tea. The presence of other enzymes, such as glycosidase (which catalyzes the hydrolysis of several aroma precursors), lipoxidase (which generates volatile aldehydes), and the enzymes responsible for the synthesis of methyl xanthine, have also been reported (Komes et al. 2010).

Milk composition

The composition of bovine milk is required to understand the mechanisms of interaction between proteins and tea polyphenols. Bovine milk contains casein proteins (α_{s1}-, α_{s2}-, β-, and

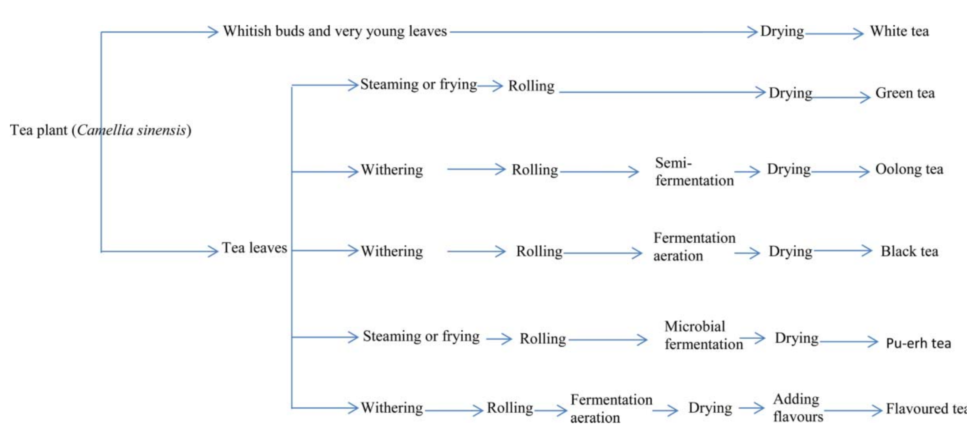

Figure 1. Processes for manufacturing different types of tea.

Table 1. The characteristics, appearance, flavor, and methods of infusion preparation for the main tea categories based on the degree of fermentation and processing.

Tea type	Properties	Appearance of the tea plant	Production method	Appearance and flavor of the beverage	Applicable methods for preparing the beverage
White	Almost the rarest tea category, and mainly produced in China.	Silvery white hairs found on the undeveloped parts of the plant (whitish buds)	Naturally sun- or steam-dried.	Relatively pale yellow with slightly sweet flavor and a sort of mellow nutty or creamy quality.	Brewing in hot water
Green	Nearly 20% of all of the tea produced globally.	Green or dark green leaves	Withering, followed by steaming or frying (usually pan-frying) Either artisanal or modern ways of firing might be used.	Greenish yellow with having an astringent grassy taste of the fresh tea leaves.	Brewing or steeping in hot water
Oolong	Semi-fermented traditional Chinese tea leaves.	Red-brown leaves	Short period of fermentation.	Pale yellow with a specific unique floral and fruity taste.	Brewing or steeping in hot water
Black	Most common tea type. People in China and neighboring countries may call it "red tea."	Black leaves	First withered (by blowing air) after harvesting and then fully fermented for several hours under controlled temperature and humidity and finally treated by heating or drying processes.	Reddish brown color contains a wide range of flavors with a hearty and more assertive quality than other types of tea.	Brewing or steeping in hot water
Pu-erh	A thin layer of mould on the tea leaves used for medicinal purposes.	Dark red Leaves	Fully anaerobically fermented with microbes through the processing phase. It is usually fermented twice instead of once (double oxidation process along with a period of maturation).	Dark red with a distinctive soil-like flavor recognized as off-putting by some people.	Brewing in hot water
Flavored	Not regarded as a different type of tea but refers to the tea leaves have been flavored with the addition of various flavors	Mainly black leaves	Mainly fully fermented	Depends on the added flavor.	Brewing or steeping in hot water

κ-caseins) and soluble serum proteins ($\sim$18%, collectively called whey proteins, including α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, and enzymes). Caseins form spherical aggregates (sized $\sim$0.1 μm), commonly called casein micelles, consisting of protein molecules bound together via nanoparticles of calcium phosphate (Horne 2006; Dalgleish and Corredig 2012). The outermost layer of the casein aggregate consists of negatively charged κ-casein that maintains colloidal stability under physiological conditions (Goff 2010).

Milk fat naturally occurs in the form of milk fat globules with a triacylglycerol content of up to 96–98%, surrounded by a milk fat globule membrane (MFGM) containing phospholipids and proteins. Milk fat composition and the size of fat globules vary according to breed and speciesof cows, and factors related to season, lactation stage, feed composition, feeding time, and nutritional status (Butler et al. 2008). Milk fat contains fat-soluble carotenoids and vitamins such as vitamins A, D, E, and K, along with more than 400 short-, medium- and long-chain saturated and unsaturated fatty acids, including linoleic and linolenic acids and some medium-chain fatty acids with reported health benefits. Homogenization can reduce the diameter of milk fat globules from 2–4to $\sim$ 0.4 μm (MacGibbon and Taylor 2006).

Some of the compounds in milk may also be counted as sources of extractable phenolic compounds and endogenous antioxidants. These include vitamin A, hydroxycinnamic acids, and flavonoids (Hilario et al., 2010; Rashidinejad et al., 2013), which are mostly transferred from the diet (O'Connell & Fox, 2001).

Addition of milk to tea infusions

It is debatable whether complexation between tea catechins and milk proteins can decrease or increase the antioxidant capacity of these bioactive compounds, and subsequently decrease the nutritional value of milk proteins. The nutritional impact of the addition of milk to tea is a controversial debate that has been ongoing for many years. Some of the contradictory findings of milk addition to tea are reviewed in the following sections.

Table 2. Major constituents of green and black tea leaves (modified from Dufresne and Farnworth 2001).

	Occurrence (% dry weight)		
	Green tea	Black tea	Structure
Catechins	30–42	10–12	
epigallocatechingallate	11		**B**(−)2,3-cis R1 = OH R2 = A
epicatechingallate	2		**B**(−)2,3-cis R1 = H R2 = A
gallocatechingallate	2		**B**(+)2,3-trans R1 = OH R2 = A
epicatechin	10		**B**(−)2,3-cis R1 = R2 = H
epigallocatechin	ND*		**B**(−)2,3-cis R1 = OH R2 = H
gallocatechin	ND		**B**(−)2,3-trans R1 = OH R2 = H
catechin	ND		**B**(−)2,3-trans R1 = R2 = H
Teaflavin		3–6	
theaflavin-3-gallate	ND		**C** R1 = OH R2 = OH
theaflavin-3`-gallate	ND		**C** R1 = A R2 = OH
theaflavin-3,3`-gallate	ND		**C** R1 = OH R2 = A
Thearubigens	2–3		**C** R1 = A R2 = A
Theogallin	ND	12–18	
Proanthocyanidin	ND		
Flavonols	5–10	6–8	
quercetin	ND		**D** R1 = OH R2 = H R3 = OH
kaempferol	ND		**D** R1 = R2 = H R3 = OH
rutin	ND		**D** R1 = OH R2 = H R3 = O-rutinose
Methylxanthines	7–9	8–11	
caffien	3–5		**E** R1 = R2 = CH_3
theobromine	0.1		**E** R1 = H R2 = CH_3
theophylline	0.02		**E** R1 = CH_3 R2 = H
Amino acids			
theanine	4–6		**F**

*Not determined

The method of making a perfect cup of tea (considering water temperature and the type of tea), as well as the suitability and method of adding milk to tea, has been debated for many years and contradictory findings have been reported. Although milk is usually added to black tea; milk can also be added to green, oolong, or even herbal infusions. It is generally accepted that adding milk to tea is either a dietary habit or an approach to reduce the astringency of plain tea and/or decrease the temperature of hot tea for immediate consumption (Hertog et al. 1997). It is thought that people more likely drink tea due to their interest in the benefits of tea rather than that of milk. Moreover, in the past, adding milk to tea before pouring tea could temper the tea and prevent porcelain from breaking (Dubrin 2010). A major barrier to adding milk to tea is a concern about the potential reduction of the health benefits of tea (Graham 1992; Katiyar and Mukhtar 1996; Gupta et al. 2002; Liu et al. 2005; Song et al. 2005; Kuriyama et al. 2006; Sun et al. 2006; Nath et al. 2012; Payton 2012; Xie et al. 2013).

Effect of milk addition on the health beneficial properties of tea

Results from previous studies on the controversial topic of adding milk to tea is summarized in Table 3 As stated before, the effects of adding milk to tea can be classified into negative, neutral, dual, and positive effect categories.

Negative (masking) effects of milk addition to tea: Possible interactions between tea and milk

Reports on the negative effects of adding milk to either green tea or black tea outnumber reports on the neutral and positive effects. Interestingly, Hertog et al. (1997) reported that consumption of flavonols after adding milk to tea was not inversely associated with an increased ischemic heart disease (IHD) risk for Welsh men aged 45–49 years due to suppression of plasma antioxidant raising capacity. In contrast, some epidemiologic studies report that consumption of polyphenols (flavonols, such as catechins) derived from tea is inversely associated with the occurrence of coronary problems in elderly Dutch men (Hertog et al. 1993) and stroke incidences in middle-aged Dutch men (Keli et al. 1996).

A high proportion of publications report that negative effects of milk addition to tea could be related to interactions between tea polyphenols (catechins) and milk proteins (Serafini et al. 1996; Langley-Evans 2000; Tewari et al. 2000; Arts et al. 2002; Bourassa et al. 2010; Livney 2010; Hemar et al. 2011; Kanakis et al. 2011; Xiao et al. 2011; Korir et al. 2014; Moser et al. 2014; Sabouri et al., 2015). Recently, Moser et al. (2014), Haratifar and Corredig (2014), and Ye et al. (2013) demonstrated that not only casein micelles but also whey proteins in milk can bind to tea catechins. These authors also pointed out that casein micelles exhibit greater affinity to highly polymerized tea polyphenols, whereas whey proteins showed a greater degree of binding to smaller polyphenolic molecules. The high affinity of casein micelles to phenolic compounds is well-recognized in many studies, brought about by the high level of proline amino acids in casein, resulting in binding to polyphenols via hydrogen bonds between the peptide carbonyl and the phenolic hydroxyl along with other interactions between phenolic rings and hydrophobic amino acid residues (Hofmann et al. 2006; Pascal et al. 2008). The cyclic structure of proline, despite decreasing the formation of hydrogen bonds inside the peptide backbone, can facilitate a more open and flexible protein conformation, enabling interactions between large-sized and proline-rich proteins with polyphenols (Williamson 1994). In comparison, proteins that are tightly coiled and globular-shaped likely have much lower affinities for polyphenols. Proline is also unique in its capability to form cis peptide bonds that are very rigid when forming complexes (Luck et al. 1994). A good example of this phenomenon is the salivary proteins that are rich in proline. Phenolic compounds, such as those found in tea, can also cross-link with proteins in food (Tantoush et al., 2012). Jöbstl et al. (2006) reported that β-casein molecules from bovine milk can wrap around green tea catechins, such as EGCG, leading to a stiffer and more tightly packed structure. These researchers (Jöbstl et al. 2006) concluded that this kind of binding can reduce the bioavailability of tea catechins while stabilizing the structure of caseins in milk. Generally, non-covalent binding between phenolic compounds and proteins correlates with the physicochemical parameters of proteins, such as the sequence of amino acids, the isoelectric point, and the secondary structure (Nagy et al., 2012). For example, some polyphenols, such as phloretin and EGCG, have a stronger affinity to proteins containing a high number of positive and negative charges, whereas others, such as procyanidin, can bind strongly to proteins containing a relatively higher number of proline residues (Nagy et al., 2012).

When food containing a high polyphenol content is consumed, astringency is mostly perceived because of the binding of salivary proteins to polyphenols (Hofmann et al. 2006). Milk caseins, as do salivary proteins, contain a high content of proline residues in the amino acid sequences, thus milk caseins have a relatively open structure. For instance, β-casein (the second most abundant milk protein) contains 35 prolines out of 209 amino acid residues. In β-casein, proline residues are evenly distributed throughout the peptide sequences with phosphorylated serine amino acids at five locations close to the N-terminus (by which β-casein peptide exerts an amphiphilic property to form casein micelles) (Eskin and Shahidi 2012). Thus, it is understandable how milk addition could induce polyphenol–protein interactions similar to those described herein. Although bovine milk contains a considerable amount of protein, it also contains a similar amount of fat in the form of globules (MacGibbon and Taylor 2006) stabilized by the milk fat globule membrane (Keenan and Mather 2006). Therefore, when adding high-fat milk to tea infusions, interactions between tea polyphenols and milk fat globules should be considered.

Differences among tea phenolic compounds, such as chemical structure and molecular size, may impact the affinity for casein micelles (Ye et al. 2013). Ryan and Petit (2010) compared the antioxidant capacity of five brands of tea with and without the addition of different amounts of whole, semi-skimmed, and skimmed milk. These authors concluded that the three types of milk significantly ($P \leq 0.05$) decreased the total antioxidant capacity of all brands of tea measured by the ferric reducing antioxidant power (FRAP) assay, although the effect of whole milk was less than for semi-skimmed or skimmed milk addition. Whole milk, compared with skimmed milk, contains several fat-soluble antioxidants, including

Table 3. Summary of the previous studies carried out on the effect of milk addition to tea.

Author	Tea type tested	Milk type added	Milk ratio (v/v %) added	The effect reported
Korir et al. (2014)	Black and green tea	Fresh cow's milk (full-fat)	2	Milk addition had a significant effect on decreasing the antioxidant activity (DPPH) of tea infusions in Swiss albino mice in a concentration-dependent manner.
Ye et al. (2013)	Black and green tea	Full-fat milk (3.3% fat)	40	There are strong interactions between milk proteins and polyphenols when added to both black and green teas. These bindings could alter the secondary structures of proteins in milk.
Egert et al. (2013)	Green tea	Skimmed milk (0.1% fat)	20	The bioavailability of total catechins in tea was significantly reduced by addition of skimmed milk.
Xie et al. (2013)	Green tea	Skimmed milk (fat content not reported)	10 and 25	Gallatedcatechins, including ECG and EGCG, were bound to milk proteins more than non-gallatedcatechins (EC and EGC), but, in general, milk addition at both concentrations increased bioavailability of catechins via enhancing their transepithelial absorption and their uptake from green tea.
Ryan and Sutherland (2011)	Black tea	Semi-skimmed milk (1.6% fat). Different brands of soy milk (1.2–2.2% fat content)	10	The addition of either bovine or soy milk decreased the total antioxidant activity (FRAP), while the effect of milk was greater than soy milk.
Dubeau et al. (2010)	Green and black tea	Semi-skimmed milk (2% fat)	5	Milk decreased the antioxidant activity (measured by the $ABTS^+$ method[a]) of both types of tea. In contrast, it improved the chain-breaking antioxidant capacity measured by a lipid peroxidation method.
Ryan and Petit (2010)	Black tea	Whole milk (3.5–3.7% fat), semi-skimmed milk (1.7–2% fat), and skimmed milk (<0.5% fat)	5, 7.5, 10	Addition of all three concentrations of all three types of milk decreased the total antioxidant activity (FRAP) of tea but the masking effect of skimmed milk was greater than other two.
Lorenz et al. (2009)	Black tea	Skimmed milk and soy milk	10	Both skimmed and soy milk hampered beneficial tea-induced vascular effects.
Kartsova and Alekseeva (2008)	Black and green tea	Milk (fat content not reported)	5–10	The milk addition can decrease the concentration of free polyphenols in tea due to their binding with milk caseins.
Sharma et al. (2008)	Black tea	Milk (fat content not reported)	40	Milk reduced the total phenolic content and radical scavenging activity (DPPH) of tea but stabilized the antioxidant activity (β-carotene–linoleic acid model).
Lorenz et al. (2007)	Black tea	Skimmed milk (fat content not reported)	10	The vascular protective effects of tea were completely blunted by the addition of milk.
Kyle et al. (2007)	Black tea	Skimmed milk (fat content not reported)	33.3	Total phenolic content, antioxidant capacity (FRAP), and catechin contents of tea were not affected by milk addition.
Reddy et al. (2005)	Black tea	Whole milk (3.5% fat)	20	The plasma antioxidant capacity of black tea may not be adversely affected by the addition of milk, although the absorption/ bioavailability of tea catechins might be affected.
Hollman et al. (2001)	Black tea	Milk (fat content not reported)	10	Addition of milk did not change the plasma concentration of antioxidants (ORAC) in tea.
Richelle et al. (2001)	Black tea	Milk (fat content not reported	10	Antioxidant activity of tea infusions (LDL oxidation assay) was not impacted by milk addition.
Leenen et al. (2000)	Black and green tea	Full-fat milk (fat content not reported)	20	Addition of milk did not remove the plasma antioxidant activity (FRAP) of either tea.
Tewari et al. (2000)	Black tea	Milk (fat content not reported)	Not reported (taking 300 mL of tea with milk)	Tea without milk showed better in vivo antioxidant potentials than tea with milk. Addition of milk can form a complex of polyphenols–milk protein, which results in increasing ionization of polyphenols and thus lowering their absorption through gastric mucosa.
Langley-Evans (2000)	Black tea	Semi-skimmed milk (fat content not reported)	10	The effect of tea on plasma antioxidant potential (FRAP) appeared to be totally negated by the addition of milk.
van het Hof et al. (1998)	Black tea	Semi-skimmed milk (fat content not reported)	16.7	The bioavailability of tea catechins was not impaired by milk addition.
Weisburger et al. (1997)	Black tea	Full-fat milk (4.5% fat)	32	Milk potentiated the inhibiting effects of tea on mammary and colon tumor induction in rats.
Hertog et al. (1997)	Black tea	Milk (fat content not reported)	Not reported	Adding milk removed the in vivo plasma antioxidant-raising capacity of tea.
Serafini et al. (1996)	Black and green tea	Whole milk (fat content not reported)	25	Simultaneous consumption of tea and milk resulted in a totally inhibition of in vivo activity of tea polyphenols by milk.

[a]2,2′-azino-bis.

carotenoids, tocopherols, and retinols, that can protect milk against lipid peroxidation and peroxyl/superoxide radical generation, thereby maintaining milk quality (Lindmark-Månsson and Åkesson 2000). Thus, a decreased fat content of milk lowers the amount of fat-soluble antioxidants in milk and consequently lowers the total antioxidant property of milk (Ryan and Petit 2010).

Some studies report no negative effect on the plasma antioxidant activity of black tea after adding whole milk (Leenen et al. 2000; Hollman et al. 2001; Reddy et al. 2005), whereas others report a negative effect (Hertog et al. 1997; Tewari et al. 2000; Kartsova and Alekseeva 2008). In comparison, most of the studies on the effect of low-fat milk on tea show a decrease (although to a different extent) in both the antioxidant activity of tea-infused milk and the bioavailability of tea antioxidants in vitro or in vivo (Lorenz et al. 2007; Lorenz et al. 2009; Dubeau et al. 2010; Ryan and Petit 2010; Ryan and Sutherland 2011; Egert et al. 2013; Xie et al. 2013; Moser et al. 2014). It should be noted that all types of milk contain enzymatic antioxidants, such as superoxide dismutase, catalase, glutathione, and peroxidase, along with non-enzymatic antioxidants such as lactoferrin and vitamin C (Lindmark-Månsson and Åkesson 2000).

Milk caseins, especially β-casein, can actively interact with most tea catechins, particularly EGCG, forming an association (complex) with a very compact core (Guinee et al. 2004; Jöbstl et al. 2006; Haratifar and Corredig 2014). Precipitation of both β-casein and bovine serum albumin is possible at different concentrations of EGCG (Jöbstl et al. 2006; Pascal et al. 2008). Heratifar and Corredig (2013) reported that EGCG in tea forms complexes with caseins, especially κ-casein, and decreasing EGCG concentration causes rennet gel formation time to increase. Based on these observations, it was proposed that EGCG associates with four proline residues near the hydrolysis point of κ-casein (in the 98–111 sequence of His-Pro-His-Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys). Finding an alternative to bovine milk to minimize the impact of milk on the health benefits of tea has generated further investigations. Soy-based emulsion beverages were proposed as a substitute since soy does not contain any casein (Ryan and Sutherland 2011). All the five brands of soy beverage used in this study had much higher antioxidant activities than semi-skimmed milk in the absence of tea. Three studies have recently showed that soy beverage can suppress vascular effects (Lorenz et al. 2009), bioavailability (Egert et al. 2013), and total antioxidant activity of tea (Ryan & Sutherland, 2011).

Many in vivo studies have been carried out with different results (van het Hof et al. 1998; Henning et al. 2004; Roura et al. 2007; Egert et al. 2013). Egert et al. (2013) reported on the inhibitory effects of skimmed bovine milk, caseinate, and soy beverage on the bioavailability of galloylated catechins from green tea based on the plasma concentrations of tea catechins in 24 non-smoking women (aged 23–32 years) with normal body weight. A negative effect of soy beverage on the antioxidant activity of tea polyphenols in vivo (Lorenz et al. 2009) and in vitro (Ryan & Sutherland, 2011) have been reported. Lorenz et al. (2009) found that 10% plain soy beverage can suppress the vascular antioxidant property of tea, possibly through interactions between proteins, such as β-conglycinin and glycinin, with tea antioxidants. Thus, soy proteins might exert effects similar to bovine milk caseins on the bioavailability and beneficial effects of tea antioxidants (Rawel et al. 2002; Ryan and Sutherland 2011; Egert et al. 2013). As Tewari et al. (2000) explained, the negative effect of milk on antioxidant activity of tea phenolic compounds is associated with the interference of increasing gastric pH (caused by milk) with the absorption of simple phenolics. Gastric acids are normally weak acids and easily absorbed in their non-ionized form, but are increasingly ionized after milk addition, causing a reduced passage rate through the gastric mucosa.

Neutral (non-masking) effects of milk addition to tea

Reports have shown weak to non-existent effects of milk addition to tea (van het Hof et al. 1998; Leenen et al. 2000; Hollman et al. 2001; Reddy et al. 2005; Kyle et al. 2007) but less in number than reports of negative effects. As mentioned above, milk is an inherently good source of antioxidants, thus addition of milk should increase the total antioxidant potential of a tea–milk beverage in the absence of any synergistic of confounding effects. A non-masking effect occurs when the inhibitory effects of added milk on the antioxidant potential in tea infusions are very weak and insignificant. For example, the addition of milk only weakly affected ($P < 0.07$, too weak to be measured as a negative effect) kaempferol activity (one of the tea flavonols) after tea intake (Kyle et al. 2007). Langley-Evans (2000) reported that tea flavonoids appear to be taken up across the gastrointestinal tract membrane, and van het Hof et al. (1998) showed that simultaneous consumption of black tea and milk did not impair catechin bioavailability based on the presence of tea catechins in plasma that decrease the concentration of circulating low-density lipoproteins.

It is worth noting that the absorption of other flavonoids, such as theaflavins and thearubigins in tea, has not yet been completely studied and more investigations are needed to confirm whether there is a neutral effect of milk addition on flavonoid activity. Variations in the methods used for measuring plasma antioxidant potential of polyphenols may lead to inconsistent results. For example, Maxwell and Thorpe (1996) reported no effect of tea consumption on plasma antioxidant status in the absence of added milk or derived milk products, even though catechins from tea were found to be absorbed rapidly into blood after ingestion (Langley-Evans 2000). A good demonstration of the antioxidant properties of tea flavonoids needs to be clearly shown. Thus, a lack of consistency in the findings may reflect the variety in methodologies used to measure plasma antioxidant potential of catechins, and this might be one of the reasons why researchers such as Maxwell and Thorpe (1996) reported no effect of tea consumption without any additives on plasma antioxidant status. Similarly, Benzie and Szeto (1999) claimed a small and transient effect of green tea on the plasma antioxidant activity (by the FRAP assay) after ingestion. However, this transient effect may be spurious as green tea flavonoids, especially catechins, have a short half-life (4.8 h) in human plasma (van het Hof et al. 1998).

Dual effects of milk addition to tea

Among all the studies reviewed, only one study describes the dual effect of milk addition on the antioxidant capacity of green and black teas (Dubeau et al. 2010). These authors examined the in vitro antioxidant activity of tea infusions, and infusions containing 5% semi-skimmed milk, using three complementary methods, ABTS+ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging, lipid oxidation, and voltammetry assays. Dubeau et al. (2010) reported that milk decreased the antioxidant activities, as measured by the ABTS+ and voltammetry assays, in all tea infusions, but increased the chain-breaking antioxidant property as determined by the lipid peroxidation assay. This study also showed that Darjeeling tea (usually available in the form of black tea) possessed the largest polyphenolic content and antioxidant capacity amongst the tested green and black teas, a result in disagreement with the findings of other studies where green tea has been shown to have a greater antioxidant content than black tea (van het Hof et al. 1998; Leenen et al. 2000; Anesini et al. 2008;

Ye et al. 2013). The antioxidant property of milk, due to intrinsic antioxidants (Lindmark-Månsson and Åkesson 2000), could have been masked by the negative effect of milk on antioxidant capacity of teas. Although Dubeau et al. (2010) found that the antioxidant capacity of tea–milk infusions by voltammetry and ABTS+ assays was significantly lower than for tea alone, these authors reported that milk addition positively affected ($P = 0.004$) the inhibition of lipid peroxidation by tea samples, suggesting a dual effect between tea polyphenols and milk components in the emulsion, such as linoleic acid. There are limitations to the study of Dubeau et al. (2010), given that only one milk concentration was examined (5%) and no in vivo bioavailability study was carried out. Apart from this, the data obtained from only one antioxidant activity assay (ABTS+) may not be enough to draw conclusions about the negative or positive effects of milk on the antioxidant activity of tea polyphenols. Previously, it was reported that oxygen radical absorbance capacity was the most suitable assay for measuring antioxidant activity of dairy products due to the suitability of the method in both hydrophilic and lipophilic systems (Rashidinejad et al. 2013).

Positive effects of milk addition to tea

Some studies report on the ability of milk proteins to facilitate intestinal transport of catechins originating from green tea by enhancing the absorption of these catechins. Xie et al. (2013) observed that the content of catechins increased significantly in digested green tea containing 10% skimmed milk compared with the corresponding undigested tea–milk samples, based on a Caco-2 cell in vitro model (a human colon epithelial cancer cell line). These authors also found that adding 10% milk significantly improved the recovery of all catechins in Caco-2 cells after 2-h incubation, whereas milk addition at 25% resulted in a slight decline in the recovery due to binding of green tea catechins to milk proteins (in agreement with the discussion in Section "Negative (Masking) Effects of Milk Addition to Tea: Possible Interactions Between Tea and Milk"). Xie et al. (2013) pointed out that all types of green tea catechins, with EGCG as the predominant compound, are still present at substantial concentrations in the digestive fluids, except for some degradation of catechins during digestion. Enzyme treatment can considerably increase the recovery of catechins, i.e. ~59% of EGCG, possibly due to disruption of the interactions between milk proteins and tea catechins after hydrolysis of proteins into peptides (Xie et al. 2013). The positive effect of milk addition to black tea was confirmed by Weisburger et al. (1997) in trials using rats with either mammary gland cancer or colon cancer. These researchers found that black tea alone decreased the mammary gland cancer, and black tea with milk exerted a greater protection against colon and breast tumors. Full-fat milk (4.5% fat) led to an even greater additive effect on the antioxidant property. In summary, these two studies show the positive effects of milk addition, although the mechanisms and the quantitative analysis of absolute values (rather than relative results) are currently not known.

Differences in the type of tea tested, type, composition, and concentration of milk products, method of preparation for tea and milk–tea infusions, the assay used to measure in vivo or in vitro antioxidant activity, and sampling size for analysis can account for variations among the findings reported by different authors. Moreover, bioaccessibility as a prerequisite for bioavailability of any substance, such as polyphenols in the gut, is an important index. The effectiveness of releasing bioactives from a food matrix, in addition to gastrointestinal stability, determines bioavailability (Xie et al. 2013). Chemical alteration of catechins by enzymatic glucuronidation, methylation, and sulfation is possible during absorption through intestinal epithelial cells (Lambert et al. 2007), which also affects the bioavailability assessment.

Conclusions

Highlights

- Milk addition to tea can decrease or completely inhibit tea antioxidant properties.
- Milk caseins interact with polyphenolic catechins from tea.
- Skimmed milk has a more negative effect on tea health benefits than whole milk.
- Proteins from soy and milk similarly affect the bioavailability of tea antioxidants.

Although it is a logical step to translate various and controversial scientific findings into a public health message, it will be a challenge, at least not straightforward, to do so for the impact of milk addition to tea on health. Based on the results of previously published studies with consideration of the variations among individuals, tea type, milk type, and methods of assessing antioxidant properties, the evidence appears to point toward a negative (masking) effect for adding milk, especially skimmed milk, to tea beverages because of the putative association between tea polyphenols (such as catechins) and milk proteins (specifically caseins). This effect may vary depending upon the concentration of milk proteins and tea phenolics. Despite of the various traditional habits for drinking tea, consumers are increasingly aware of the health benefits of both tea polyphenols and milk proteins, but there is insufficient evidence to support any recommendation for the appropriate ratio of milk to tea. Furthermore, it appears that the role of milk fat has been neglected, despite milk containing fat globules, which may interact with tea catechins as well. Another important aspect is the purpose of consuming tea by different people. Some may drink tea for pleasure whereas others may do so for the health benefits of either caffeine or antioxidants. Although the negative effects of the addition of milk on the activity of tea antioxidant have been broadly reported, there is lack of information about the effect of milk addition on the activity or bioavailability of caffeine in tea infusions. Further studies are required on the effect of milk addition to tea on the bioaccessibility and bioavailability of tea polyphenols and milk proteins. Even though the habits of drinking tea and the use of milk addition in different societies, or even between individuals, might be different, tea antioxidants can play an important role in the prevention of many chronic diseases and disorders in individuals who drink it regularly.

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