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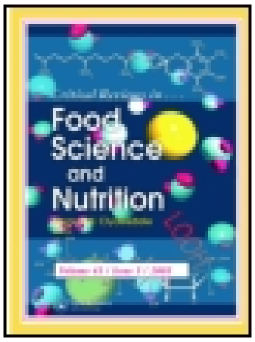

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REVIEW

Addition of milk to coffee beverages; the effect on functional, nutritional, and sensorial properties

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ABSTRACT
To date, there exists a debate on the effect of milk added to coffee infusions/beverages concerning the nutritional quality of coffee and the functional properties of its phenolic compounds. Yet, the full nutritional quality and functional properties of a coffee beverage without a significant negative impact on its sensorial profile are highly desired by the consumers. Negative/masking, positive, and neutral effects of milk on the antioxidant activity and bioavailability of coffee phenolics (particularly, chlorogenic acids) have been reported. Some potential factors including the type and amount of milk added, type of coffee beverage, the composition of both milk (protein and fat) and coffee (phenolic compounds), preparation method, assays used to measure antioxidant properties, and sampling size may account for the various reported findings. Interactions between phenolic compounds in coffee and milk proteins could account as the main responsible aspect for the reported masking/negative impact of milk on the antioxidant activity and bioaccessibility/bioavailability of coffee bioactives. However, considering the interactions between milk components and coffee phenolics, which result in the loss of their functionality, the role of milk fat globules and the milk fat globule membrane can also be crucial, but this has not been addressed in the literature so far.

HIGHLIGHTS

- In most cases, milk is added to the coffee beverages in several various ways.
- Effect of milk on the nutritional/functional properties of coffee is controversial.
- Enough evidence suggests negative effects of milk addition on properties of coffee.
- Interactions of coffee phenolics and milk proteins could account as the main aspect.
- The role of milk fat globules and milk fat globule membrane may also be crucial.

KEYWORDS
Antioxidant activity; coffee infusions/beverages; milk components; bioactive bioavailability; chemical interactions; chlorogenic acids

Introduction

Coffee, one of the most popular drinks (after water and tea) around the globe (Trevisan et al. 2016), is prepared by hot water extraction (85–93 °C) of the soluble material from the roasted grounds of coffee beans. Even though the earliest validated evidence of coffee drinking dates back to the early 15th century (Weinberg and Bealer 2001), only over the last few years, coffee has been considered as a functional food, owing to its high phenolic contents, mainly the chlorogenic acids (CGAs; accounting for about 1-4% of the total dried composition of roasted coffee beans) (Farah 2009). The phenolic compounds present in coffee are mostly the esters of hydroxycinnamic acids and quinic acid, the major compounds being caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and feruloylquinic acids (FQA), each of which contains at least three isomers (Clifford et al. 2003). Numerous studies have reported that the phenolic compounds in coffee possess several health-promoting effects in humans; e.g., antioxidant, antimicrobial, immune-stimulating, hepatoprotective, and hypoglycemic properties (Basnet et al. 1996; Nicolopoulos et al. 2020; Poole et al. 2019; Poole et al. 2017). Coffee is also known for the activation of the nervous system, enhancement of perception, and fatigue reduction, most of which being associated with caffeine (Sánchez-González, Jiménez-Escrig, and Saura-Calixto 2005). However, some studies have suggested that these types of activities may be linked with other compounds that

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result in the rise of blood pressure and the activation of the sympathetic nervous system (Corti et al. 2002; Klag et al. 2002). Consequently, numerous new coffee-based products are being developed globally and a substantial number of investigations is being carried out in the field of coffee, various coffee formulations, and delivery of its health benefits to humans.

Regardless of how much coffee is being consumed around the globe every day, once brewed, there are several various methods for preparing a cup of coffee and each is based on a specific formulation that has become part of the consuming habits in different countries around the world. While coffee is consumed without the addition of milk or cream in many countries, adding milk or cream is an essential part of the coffee preparation in other parts of the world. This includes the addition of whole, semi-skimmed or skimmed milk, and light or heavy cream, while the use of steamed milk, condensed milk, plant-based milk, or foamed milk is also popular. Although coffee is served as a hot drink, today, some types of coffee beverages are also served cold (e.g., iced coffee) (Hoffmann 2018; Thurston, Morris, and Steiman 2013).

With the invention of the Gaggia machine (1884, Italy), various formulations of espresso with milk (e.g., cappuccino and latte) spread in popularity to the UK (the 1950s), and then to America and the rest of the world (from 1980s) via coffeehouses and coffeehouse chains (Pendergrast 2010). According to the amount and type of the milk/cream added to a cup of coffee, espresso-based coffee comes with various possible formulations/presentations, but in the most basic form, it is served alone (i.e., a shot or short black, with or without hot water added; Caffè Americano, long black) (Castle and Nielsen 1999). Milk can be added to a cup of espresso in various forms; steamed milk in caffè latte (Fried 1993), equal parts of milk and milk froth steamed in cappuccino (Castle and Nielsen 1999), a dollop of hot foamed milk in caffè macchiato (Miller 2003), steamed hot milk (microfoam) in flat white (for bringing out the flavor and creating a velvety texture) (Kenneally 2014), and many other recipes. Therefore, the method of making a perfect cup of coffee and the amount and type of milk required, as well as the suitability and the method of adding milk, have been debated among people of different nations for many years.

It is mostly accepted that the addition of milk to a cup of coffee is an approach to decrease the astringency of black coffee and/or to decrease the temperature of the hot infusion for immediate consumption, or just as a dietary habit. Nevertheless, it is common sense that coffee is drunk more likely for gaining the benefits of coffee itself rather than for milk benefits. Furthermore, in the past, when hot drinks such as tea and coffee were served in the original porcelain, milk was added to the hot infusion before pouring it in the porcelain to temper the infusion and prevent the porcelain from breaking (Dubrin 2012).

Presumably, most people drink coffee for its immediate effect related to the action of caffeine, the best-known pharmacologically-active component of the infusion, or just as a habitual dietary practice (Lorist and Tops 2003). While the reasons for people to consume caffeine are manifold, it is commonly believed that drinking coffee can affect the energetic state of the drinker. Saying so, there is a robust body of literature confirming the effect of caffeine on increasing subjective energy and alertness (Bruce et al. 1986; Gevins, Smith, and McEvoy 2002; Lieberman 2001; Yu et al. 1991). Nevertheless, the consumption of coffee for its health-promoting effects associated with phenolic compounds is becoming more popular. For a long time, there have been endeavors made to develop a coffee plant that contains no or minor amount of caffeine in the beans (Smith 1985).

While the effect of milk addition on the bioefficacy of caffeine is currently unknown, a major barrier to adding milk to coffee is a concern about the potential decrease in the antioxidant properties of its phenolic compounds, especially CGAs. The previous studies have shown that the bioavailability and antioxidant properties of coffee polyphenols may be affected by the possible interactions with milk components, especially proteins (Al-Doghaither et al. 2017; Jeon et al. 2019; Quan et al. 2020). Both in vitro and in vivo assays have confirmed that proteins such as whey proteins and caseins can bind to CGAs by both covalent and noncovalent interactions, suggesting that the simultaneous consumption of coffee and milk may suppress the effective absorption of CGAs in humans (Duarte and Farah 2011; Muralidhara and Prakash 1995).

Although most of these studies have reported an inhibitory (negative or masking) effect of milk on the antioxidant properties of CGAs (Al-Doghaither et al. 2017; Jeon et al. 2019; Quan et al. 2020), others have reported a neutral effect (non-masking, with neither inhibition nor enhancement) (Otemuyiwa, Williams, and Adewusi 2017; Renouf et al. 2010) or even a positive effect (enhancement) (Alongi, Calligaris, and Anese 2019) of milk on the antioxidant activity and bioavailability of CGAs. According to the data available in the literature so far, the effect of milk addition on the functionality of coffee depends on some factors such as coffee type, milk composition, the ratio of milk to coffee, temperature, and method of preparation. We previously published the findings on the various aspects of the addition of milk to tea infusions (Rashidinejad et al. 2017). Thus, in the present study we aimed to extend that work by reviewing the available information on the addition of milk to coffee infusions and the possible effects in terms of nutritional, functional, and sensorial properties of coffee, with a focus on its phenolic compounds and their possible interactions with milk components.

Coffee; production, composition, processing, and drink preparation

Production

The term "coffee" is originated from "cafeto", which is a tropical bush. The wild coffee plant (*Coffea Arabica*) is an indigenous plant (discovered around AD 850) that belongs to Ethiopia in the Horn of Africa, but it is believed that the word "coffee" is derived from the Arabic word "quahweh" (Smith 1985). This word through its equivalent in Turkish (kahweh) was then pronounced "caffe" in Italian, "café" in

French, "Kaffee" in German, "koffie" in Dutch, and the well-known term of "coffee" in English. Also, it appears that the Latin term for the botanical genus of coffee (Coffea) is originated from the original Arabic term (Smith 1985).

The history of coffee growing and consumption (in the form of berry flesh) dates back around 1000 years ago in Arabia (Mussatto et al. 2011). By the 16th century, the boiling of the coffee beans and serving as a beverage to people spread around the world, starting in the Kaffa province of Ethiopia (Ghosh and Venkatachalapathy 2014). The coffee tree (shrub) belongs to the botanical family *Rubiaceae*, genus *Coffea*. There are around 100 varieties of *Coffea* genus available, among them, *Coffea Arabica* (Arabica) and *Coffea canephora* (Robusta) are the most commercial varieties of coffee beans (Laovachirasuwan et al. 2019).

Coffee is a tropical plant that needs special environmental conditions for the commercial cultivation; e.g., optimum temperatures of 15–24 and 24–30°C for Arabica and Robusta, respectively, with an annual rainfall of 1500–3000 mm for both varieties. Coffee cherries, the raw fruit of the coffee tree, consist of an inner kernel of two coffee beans (Ghosh and Venkatachalapathy 2014). Each of the beans is covered by a thin parchment-like skin and further surrounded by the pulp. These cherries reach their maturity after four to five years of the plantation of the tree and they bear fruit in clusters along with the stem and branches. Regarding the type of coffee plant, the 'berry' ripens about eight to nine months after the flowering stage. Coffee is harvested when the fruit turns red (berry-like) (Ghosh and Venkatachalapathy 2014; Mussatto et al. 2011).

Figure 1 presents the annual production and consumption of coffee beans around the world, based on the data from the International Coffee Organization (ICO 2019). The world's coffee production in the year 2018/19 was around 169 million bags (each bag is 60 kgs), which showed an increase of greater than 5% as compared with that of 2017/2018 (ICO 2019). However, ICO reported a 0.9% decline in coffee production in the year 2019/2020 (167.4 million bags). Brazil's smaller off-year of the biennial Arabica crop cycle and bad weather in parts of Central America and Asia were reported to be the most important reasons for this decline. Arabica and Robusta contribute to 57.2% and 42.8% of the world's coffee production, respectively. South America alone produces almost half of the world's production (around 46.6%), followed by Asia and Oceania (29.6%), Mexico and Central America (12.9%), and Africa (10.9%). In this regard, Brazil (78.08 million bags), Vietnam (31.2 million bags), and Colombia (14 million bags) are the three first largest coffee producers. Nonetheless, in line with the slower growth of the global economy, only a slight increase (1.5%) in coffee demand is projected (ICO 2019).

The chemical composition of coffee beans

The chemical composition of green coffee is related to factors such as its variety, stage of maturity, climate conditions, and processing method (Farah 2012). The coffee bean is constituted of water, carbohydrates/fibers (including cellulose; reducing sugars: arabinose, sucrose, galactose, glucose, fructose, and mannose; pectin, etc.), nitrogenous compounds (proteins; free amino-acids: arginine, alanine, asparagine, glutamic acid, cysteine, histidine, glycine, isoleucine, leucine, lysine, proline, methionine, threonine, phenylalanine, tyrosine, serine, and valine; caffeine; cafestol and kahweol diterpenes), lipids (triglycerides, esters, arachidic acid, stearic acid, oleic acids, linoleic acids, palmitic acid, amides, and diterpenes), minerals (zinc, sodium, potassium, magnesium, manganese, calcium, iron, rubidium, copper, strontium, chromium, vanadium, barium, nickel, cobalt, lead, molybdenum, titanium, and cadmium), B vitamins, organic acids, chlorogenic acids (CGAs), trigonelline, and caffeine (Akash, Rehman, and Chen 2014; Belitz, Grosch, and Schieberle 2009; Farah 2012).

Along the lines of the chemical composition of coffee beans, the recent evidence from the epidemiological and experimental research confirms the positive effect of coffee consumption on the prevention and decrease of the risk of several types of diseases, conditions, and disorders such as cardiovascular disease (Gebeyehu et al. 2020; Rodríguez-Artalejo and López-García 2018), liver disease (Hayat et al. 2021; Wijarnpreecha, Thongprayoon, and Ungprasert 2017), neurodegenerative diseases (Colombo and Papetti 2020; Herden and Weissert 2018), Parkinson's (Cho et al. 2018), and Type-2 diabetes (Alperet et al. 2020; Carlström and Larsson 2018). Therefore, this beverage can be part of a healthy diet (Grosso et al. 2017). Many of the health benefits attributed to coffee are originated from its phenolic compounds. Coffee contains various polyphenolic compounds, which contribute to about 10% of dry weight. CGAs and caffeine are the most important polyphenol compounds of coffee beans. CGAs are responsible for the astringent, bitter, and acidic taste of coffee infusions. Caffeoylquinic, dicaffeoylquinic, feruloylquinic, and *p*-coumaroylquinic acids and mixed diesters of ferulic acids and caffeic with quinic acid are the main groups of CGAs of green coffee beans (Clifford and Ramirez-Martinez 1991; Farah and Donangelo 2006). In particular, CGAs have shown great antioxidant properties as well as hepatoprotective, hypoglycemic, and antiviral activities (Farah 2012). The quantity of CGAs can vary in commercial coffee products, based on the origin of coffee beans, roasting degrees, and brewing procedures (Tfouni et al. 2013). Intensive roasting at high temperatures and during a long-time cause a decrease in the amount of CGAs, while it may not have a significant effect on the amount of caffeine, which is regarded to be resistant to roasting (Jeon et al. 2019). Thus, brewing methods that affect polyphenol content can significantly alter the antioxidant capacity of different types of coffee.

Table 1 presents the chemical composition of green and roasted coffee beans from both Arabica and Robusta varieties. The chemical composition of coffee beans significantly changes during the roasting process, due to the Maillard reaction, carbohydrate caramelization, and the pyrolysis of the organic compounds (Belitz, Grosch, and Schieberle 2009). Carbohydrates, proteins, lipids, minerals, and free amino acids in the coffee beans may be degraded and transformed into reactive compounds during this process (Table 1) (Farah 2012; Ginz et al. 2000; Rawel and Kulling 2007). Carbohydrates and nonvolatile nitrogenous compounds show

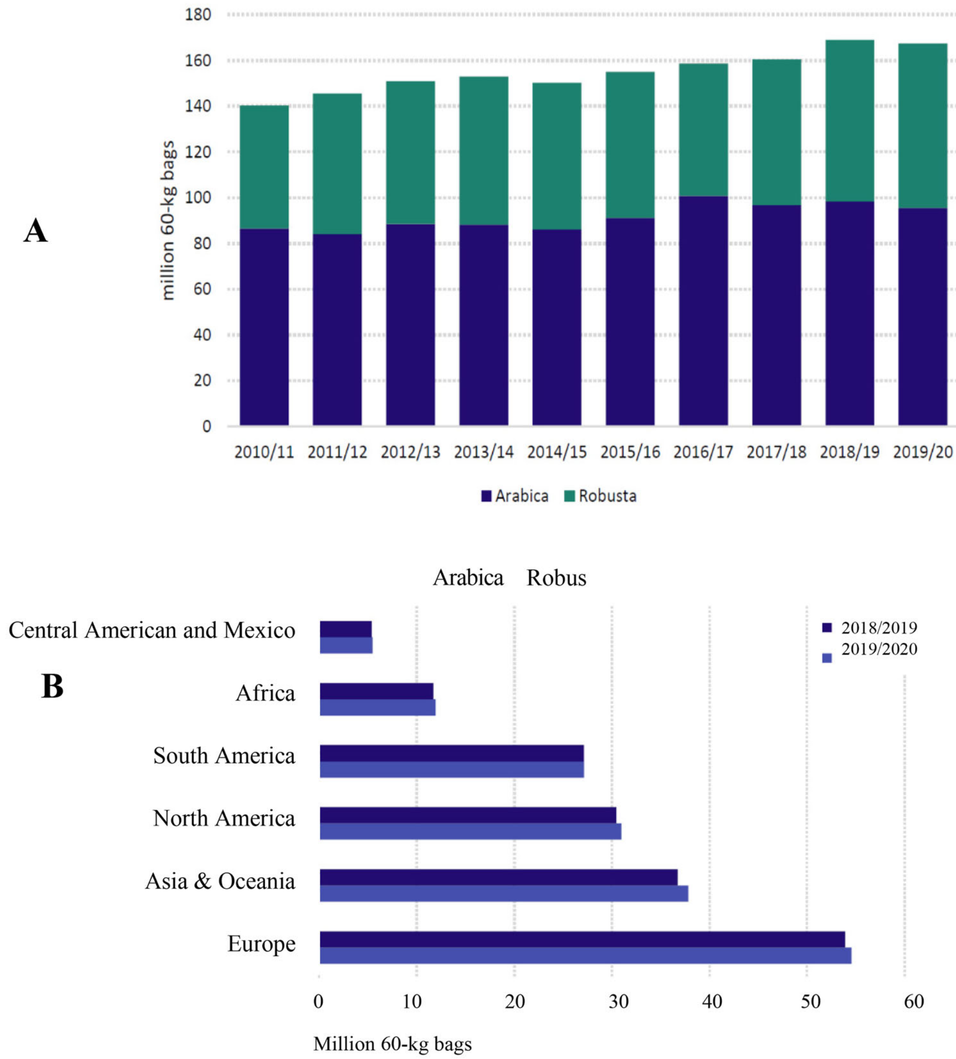

Figure 1. The yearly production (A) and consumption (B) of coffee beans around the world (ICO 2019).

a great role in the biological effect and aroma of roasted coffee (Acidri et al. 2020; Bothiraj and Vanitha 2020).

Caffeine (1,3,7-trimethyl xanthine) is considered as an outstanding nonvolatile alkaloid compound due to the specific physiological effects (e.g., increased blood circulation, improved respiration, and stimulation of the central nervous system). The caffeine content of raw Arabica coffee is lower than Robusta variety (Table 1). This compound is a white crystalline powder with a bitter taste, and among the chemical compounds of coffee, only caffeine is thermostable and exists in both green and roasted coffee beans (Belitz, Grosch, and Schieberle 2009). There are also other polyphenolic compounds in coffee beans, including tannins, anthocyanins, and lignans. Overall, polyphenols from coffee are known as excellent antioxidant, anticancer, and anti-inflammatory agents (Acidri et al. 2020; Durak, Gawlik-Dziki, and Pecio 2014; Ferrazzano et al. 2009).

Processing of the beans (before brewing)

The texture of coffee beans is porous and spongy, so these beans can be subjected to the microbial deterioration if they become too moist. Therefore, immediate coffee processing is the most important step after the coffee cherry is harvested (Ghosh and Venkatachalapathy 2014). During coffee processing, the pulp and hull of cherry are removed from the flesh and the produce is converted into green coffee beans based on two different methods; Wet and Dry.

The Dry method

This method is cheaper, older, and simpler than the Wet method. The Dry method is commonly used for Robusta coffee and results in the so-called "unwashed" or "natural coffee" (i.e., unfermented coffee). The morphological details of coffee processing using this method are depicted in Figure 2. In this process, the harvested berries are spread out in the sun for 2–3 days. After that, coffee berries are transferred to the drying rooms with a temperature of 45–60 °C for about four weeks, in order to reach a moisture content of 12%. The drying step is of importance as it affects the final quality of the green coffee. Overdried coffee beans will become brittle and break during hulling (broken beans are considered as defective beans) (Ghosh and Venkatachalapathy 2014; Kleinwächter, Bytof, and Selmar 2015; Mussatto et al. 2011).

Table 1. The chemical composition of unroasted and roasted Arabica and Robusta coffee beans. Modified from Farah (2012) and Farah and Donangelo (2006).

	Green bean		Roasted bean	
Compounds (%)	Arabica	Robusta	Arabica	Robusta
Carbohydrates/fibers				
Sucrose	6.0-9.0	4.0	4.2	NR
Reducing sugars	0.1	0.4	0.3	NR
Polysaccharides	34-44	48	31-33	41.5*
Lignin	3.0	3.0	3.0	NR
Pectin	2.0	2.0	2.0	NR
Nitrogenous				
protein	10.0-11.0	10.0-11.0	7.5-10	10
Free amino acids	0.5	0.8	ND	ND
Caffeine	0.9-1.3	2.2	1.1-1.3	2.4
Trigonelline	0.6-2.0	0.7	0.2-1.2	0.7
Nicotinic acid	ND	ND	0.016-0.026	NR
Lipids				
Coffee oil	15.0-17.0	10.0	17	11
Diterpene	0.5-1.2	NR	0.12-0.9	NR
Minerals	3-4.2	4.4	4.5	4.7
Phenolics; acids and esters				
Chlorogenic acids	4.1-7.9	10	1.9-2.7	3.1
Aliphatic acids	1.0	1.0	2.4	2.5
Quinic acids	0.4	0.4	0.8	0.8
Melanoidins	ND	ND	23-25	23-25

*This value was reported as total carbohydrate compounds. ND: not detected, NR: not reported.

The Wet method

During the Wet method, the depulping and dehulling processes are carried out while the coffee beans are still fresh. This includes further processing stages, and consequently, it requires a greater capital investment, more water consumption, and more care, as compared to the Dry method. This method involves a microbial fermentation step for removing any mucilage attached to the beans (Figure 2). Moreover, the volatile compounds generated during the fermentation lead to the beans with higher aroma quality (Mussatto et al. 2011). Overall, the green coffee beans produced by the Wet method have better intrinsic quality (a more homogeneous shape with few defective beans), so they can be sold at higher prices (Duarte et al. 2005; Ghosh and Venkatachalapathy 2014; Kleinwächter, Bytof, and Selmar 2015).

The roasting step is another important process to develop specific organoleptic characteristics of coffee beans (e.g., aromas, flavors, and color). This process is time–temperature-dependent; 160–220 °C for six (light roasted) to > nine minutes (very dark roast) (Maia et al. 2013). During this step, coffee beans undergo several chemical and biological reactions (e.g., the Maillard reaction, pyrolysis, and caramelization), which lead to special sensory properties of roasted coffee (Duarte et al. 2005; Kleinwächter, Bytof, and Selmar 2015; Mussatto et al. 2011). After roasting and cooling steps, the coffee beans may be ground, using multi-stage grinders and packed in vacuumed bags and shipped. However, for the production of instant coffee, several additional steps will be needed. First, the soluble solids and volatile compounds should be extracted from the coffee beans using hot (175 °C) pressurized water (Kleinwächter, Bytof, and Selmar 2015), and the concentrated extracts are then dried (using freeze-drying or spray drying methods). Therefore, the sensorial profile of the instant coffee product depends on several factors such as the type and quality of the beans, storage time, fermentation process, roasting condition, extraction of the soluble solids, drying method, and packaging material(s)/conditions (Mussatto et al. 2011).

Beverage preparation

There are numerous ways for preparing a cup of coffee around the world. While in most societies brewed transparent and clear coffee drink is popular, in others, a turbid drink with the sediment (such as Turkish Mocca) is preferred (Belitz, Grosch, and Schieberle 2009). Generally, there are three basic methods of brewing coffee: I) *boiling*: coffee is added to hot water, brought to the boiling temperature, and filtered; II) *steeping*: this method includes placing ground coffee in a bag, pouring hot water on it, and swirling the bag in a pot for around ten minutes; III) *percolating-filtration:* this procedure is considered as the best method for extracting the soluble compounds of ground coffee. In this method, the ground coffee is placed in a support grid (such as muslin, meshed metal, filter paper, sintered glass, or cloth filter) and is then extracted by spraying hot (but not boiling) water by gravity percolation. The extracted material can flow into a container where it will not come into contact with the grounds again (Mussatto et al. 2011). To prepare a quality cup of coffee using the percolating-filtration method, 50, 100, and 150 g/L of the coffee ground is used for the Regular, Mocca, and Italian espresso brewed coffees, respectively (Belitz, Grosch, and Schieberle 2009).

Coffee is often combined with various ingredients such as sugar, honey, milk, citrus, ice, cream, and stabilizers, amongst which milk is the most popular added ingredient. However, the production of coffee-milk drinks (especially, the canned products) requires a series of processing steps and beverage formulations (e.g., the adjustment of pH) (Kumazawa and Masuda 2003). Moreover, the bioaccessibility of bioactive compounds can be influenced by several factors such as digestive enzymes, food matrix, and host-related factors (e.g., the initial content and properties of bioactive compounds) (Quan et al. 2020). Hence, there has been an interest in the investigation of the interactions between the components of coffee and milk regarding its effect on the functionality, nutritional quality, and sensorial properties of coffee beverages. This will be discussed in detail in the following sections.

Milk; composition and functionality of its components that affect coffee properties

Although milk of different species can be added to coffee infusions, the addition of bovine milk is the most common option; thus, only the milk of this species is discussed in this paper. From a molecular composition perspective, cow's milk is a complex product. It is an excellent source of lipids, proteins (caseins and whey proteins), carbohydrates (mainly lactose – milk sugar), minerals, salts, and a long list of miscellaneous constituents such as amino acids, vitamins, immunoglobulins,

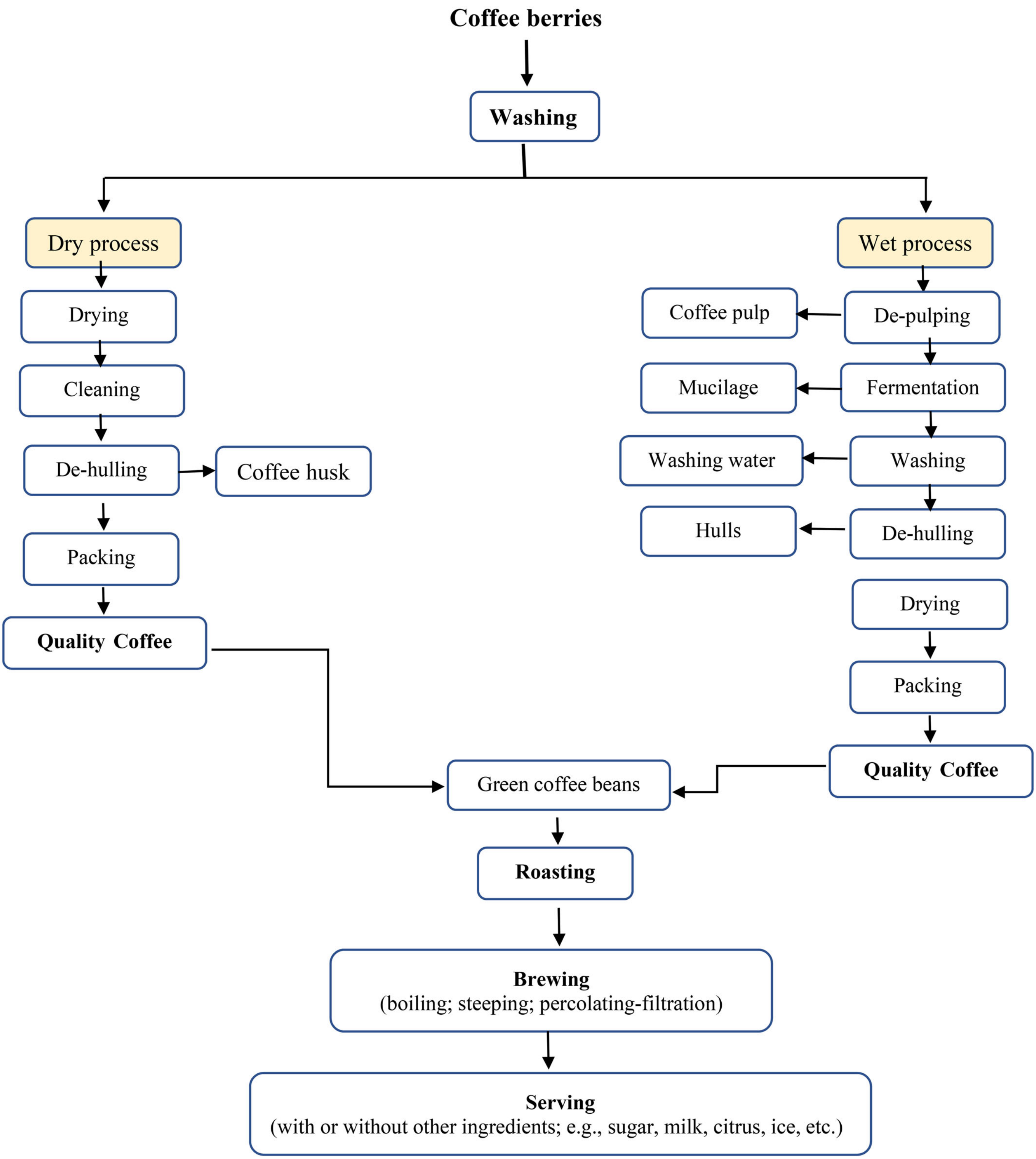

Figure 2. Coffee processing from farm to cup. Modified from Hartati, Riwayati, and Kurniasari (2012).

hormones, growth factors, cytokines, nucleotides, peptides, polyamines, and enzymes (Haug, Hostmark, and Harstad 2007). The typical concentrations of milk principal constituents are: fat 4.9%, proteins 3.4%, lactose 4.1%, and minerals (ash) 0.7% (Mehta (Mehta, 2015). Due to its high nutritional value, milk is an important part of a human's diet (Haque, Chand, and Kapila 2008; Raikos 2010). Physically speaking, milk is an emulsion of fat globules in an aqueous phase. The fat globules, ranging from 0.1 to 15 μm in diameter, are stabilized by the milk fat globule membrane (MFGM) composed of phospholipids, various glycoproteins, enzymes, and cholesterol (Singh and Gallier 2017). The aqueous phase contains dissolved and suspended components such as casein micelles, serum proteins, lactose minerals, and vitamins (Raikos 2010). As summarized in Table 2, individual milk components, such as active peptides, the components of MFGM, and immunoglobulins have been shown to exhibit positive physiological functionalities in humans.

Table 2. Milk components and their functional bioactive properties.

Compounds	Properties	References
a) Functional properties		
Proteins	Emulsifying properties, thickening, gelling, flavor binding, and foaming	Singh and Ye (2020)
Milk fat	Providing lubrication, creamy mouthfeel, and a unique flavor functionality	Chojnicka-Paszun, De Jongh, and De Kruif (2012)
MFGM*	Emulsifying and foaming properties	Dewettinck et al. (2008)
b) Bioactive properties		
Bioactive peptides: immunopeptide	Immunomodulatory activity	Meisel (1997)
α-Lactorphin	Opioid activity	
β-Casomorphin-7	Inhibition of the angiotensin-converting enzyme	
MFGM/phospholipids	Improvement of the skin elasticity of human adults	Higurashi et al. (2015)
MFGM/phosphatidylcholine	Supporting liver recovery from toxic chemical attack or viral damage	Kidd (2002)
MFGM/phospholipids	Reduction of hepatic accumulation of intestinal cholesterol and increase of fecal cholesterol excretion	Kamili et al. (2010)
MFGM/mucin 1	Protective effect against rotavirus Infection	Kvistgaard et al. (2004)
MFGM/butyrophilin	Influencing pathogenesis of autistic behavior	Vojdani et al. (2002)
MFGM/beta-glucuronidase inhibitor	Inhibition of colon cancer	Ito et al. (1993)
MFGM/MFGM antigens	Coronary atherogenic effects	Moss and Freed (2003)
Fat/conjugated linoleic acid, sphingomyelin, and butyric acid	Anti-cancer activity	Parodi (2001)
Fat/sphingomyelin	Inhibition of lipid absorption, modulating adiposity and inflammation, and altering the host response to gut microbiota; thus, preventing the development of chronic diseases	Norris et al. (2019)
Fat/oleic acid	Lowering plasma cholesterol, LDL-cholesterol, and triacylglycerol concentrations	Haug, Hostmark, and Harstad (2007)
Fat/conjugated linoleic acid	Improvement of plasma cholesterol status	Haug, Hostmark, and Harstad (2007)
Immunoglobulins, lactoferrin, lactoperoxidase, and lysozyme	Antimicrobial activity, prevention of orally mediated infections	Mehra, Marnila, and Korhonen (2006)
Lactoferrin	Immunomodulatory effects	Cross and Gill (2000)
Vitamins:		
A	The key role in vision and cell differentiation	Haug, Hostmark, and Harstad (2007)
E	Antioxidant activity	
B_{12}	The key role in folate and homocysteine metabolism	
Minerals:		
calcium	Formation and maintenance of healthy bones and teeth, prevention of hypertension	Haug, Hostmark, and Harstad (2007)
Magnesium	Maintenance of normal nerve and muscle function, supporting a healthy immune system	Öste, Jägerstad, and Andersson (1997)

*MFGM: milk fat globule membrane.

Milk proteins

Milk proteins (i.e., caseins and whey proteins) have functional properties providing the final product with favorable textural and other characteristics. Among different functional properties, emulsifying and foaming abilities, due to their unique interfacial properties, are the most important properties. However, these properties, beside thickening, gelling, and flavor binding properties, depending on the treatment conditions, can interact with other food components such as fats, sugars, polysaccharides, salts, flavors, aroma compounds, and bioactives (e.g., phenolic compounds). Moreover, any changes at the molecular level that causes the unfolding of the molecular structure (e.g., as a result of heat impact) may also have an influence on the functionality and reactivity, which can be desirable or disadvantageous. Thus, the type and the strength of various interactions determine the structure, texture, rheology, sensory properties, and shelf life of the final products (Raikos 2010; Singh and Ye 2020). It is important to note that strong associations between milk proteins and polyphenolic compounds from plant sources have been reported; such interactions may not only decrease the antioxidant activity of these compounds but also the functionality of milk proteins (Rashidinejad, Birch, Hindmarsh, et al. 2017).

Milk fat

Milk fat, which constitutes more than 98% of the total milk lipids, is predominantly composed of triglycerides, while other major lipid classes are partial glycerides (1–2 wt% total lipids), polar lipids (0.5–1.0 wt% total lipids), and sterols (0.2–0.5 wt%) (Lecomte, Bourlieu, and Michalski 2017). Functional properties supplied by milk fat relate to the contribution to flavor and texture in the perception of creaminess. Small and even-sized fat globules present in fluid products of adequate viscosity enhances the perception of creaminess (Richardson, Booth, and Stanley 1993).

MFGM, a natural emulsifying agent, blocking the flocculation and coalescence of the fat globules in milk and protecting the fat against enzymatic action, is known to possess components that have unique functional properties and health benefits (Keenan and Dylewski 1995; Singh and Gallier 2017). MFGM is a multilayer structure containing phospholipids (the most abundant ones include phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin), proteins (over 40 types, mainly glycoproteins, and phospho- and sphingolipids, including mucin 1, butyrophilin, adipophilin) (Dewettinck et al. 2008; Singh and Gallier 2017). The entire membrane, as well as the separate lipid and protein components, have great potential for new product

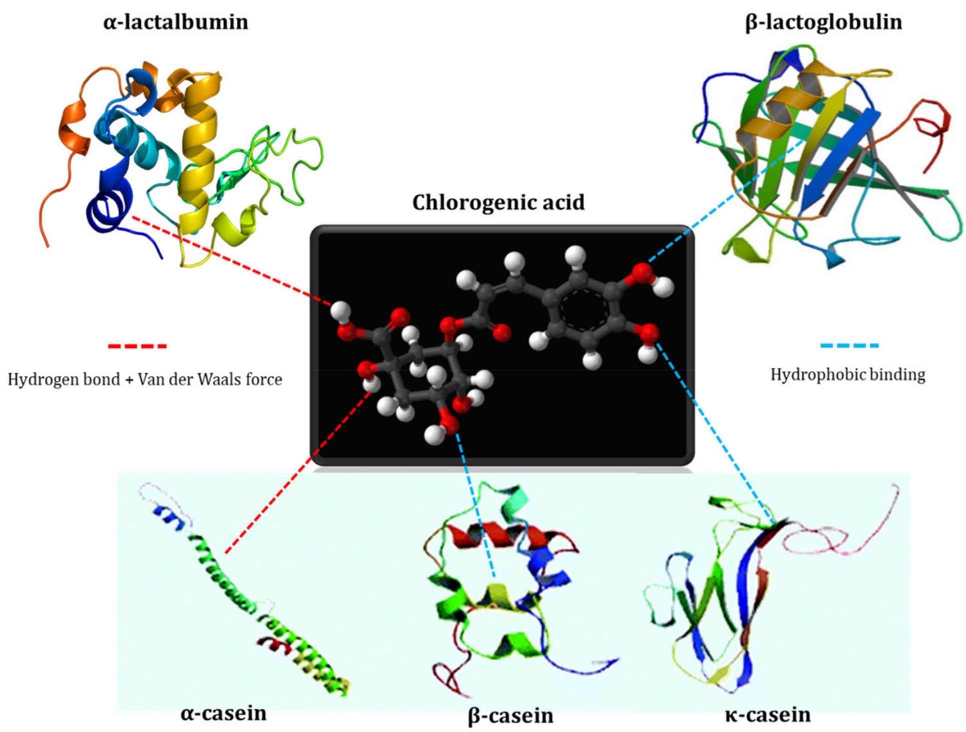

Figure 3. A schematic drawing of the possible interactions between chlorogenic acid and milk protein constitutes.

applications with exceptional nutritional and technological properties. Examples of the health effects reported for MFGM components are presented in Table 2.

Processing operations used in the industrial manufacture have an impact on the behavior of milk fat and MFG components, due to the possible changes in the structure of milk lipids, denaturation of the membrane proteins, and the association of whey proteins (*β*-lactoglobulin) with the MFGM. Also, these compounds are vulnerable to the reactivity in complex products, mainly due to interactions with milk proteins.

The role of milk fat in human nutrition is twofold. On one hand, it supplies lipid-soluble ingredients (carotenoids, liposoluble vitamins A, D, E, and K) and essential fatty acids. Some milk lipids (conjugated linoleic acid, sphingomyelin, and butyric acid) are shown to exhibit anti-cancer activity (Parodi 2001; Rodriguez-Alcala et al. 2017) and can improve plasma cholesterol status (Haug, Hostmark, and Harstad 2007). On the other hand, the saturated fatty acids and cholesterol are associated with the risk of cardiovascular disease (Lokuruka 2007). Nevertheless, when added to other food systems such as coffee infusions, such role of milk fat may be influenced, as the results of the interactions between MFGM and bioactive compounds from the plant sources such as tea that has been reported previously (Rashidinejad, Birch, and Everett 2016b; Rashidinejad, Birch, Hindmarsh, et al. 2017).

Interactions between milk components and coffee phenolics

Interaction characteristics

As depicted in Figure 3, milk components such as proteins can strongly bind/interact with phenolic compounds of coffee (especially, CGAs that can bind with different milk proteins including different types of caseins). Such interactions can significantly affect the sensorial characteristics, digestibility, and bioavailability of the components of both coffee and milk when these two are blended (Liu et al. 2016). As the main health-promoting attributes of coffee are associated with its bioactive compounds, the interaction of these compounds with milk constituents, especially proteins, has been extensively investigated in the literature. This is due to the concern related to the effect on the health-promoting properties of the numerous phenolic compounds in coffee such as CGAs (e.g., caffeoylquinic acids (CQAs), di-caffeoylquinic acids, and feruloylquinic acids), *p*-coumaric acid, caffeic acid, ferulic acid, and proanthocyanidins, which are known to present antimicrobial, antiradical, hepatoprotective, and immunostimulatory effects in humans (El-Messery et al. 2020; Quan et al. 2020; Duarte and Farah 2011; Niseteo et al. 2012). More on the effect of such interactions on various properties of coffee phenolics is discussed in the following sections.

The main possible interaction between milk proteins and coffee phenolics is the covalent binding (Ali et al. 2013). These researchers showed a concentration-dependent binding pattern of 5-CQA and various proteins (i.e., sodium caseinate, soy glycinin, spray-dried skimmed milk, and *β*-lactoglobulin). They reported that the protein:5-CQA ratio of 7:1 is the proportion in which all active sites of proteins (independent of the protein type) and 5-CQA are saturated and the thereof interaction occurred. The 5-CQA-*β*-lactoglobulin interaction is mainly generated due to their covalent interactions and thiol-disulfide interexchanges. It has been stated that protein-polyphenol affinity is strongly dependent on the polyphenol type, solubility, molecular free space, weight, and the existence of glycosylate, hydroxylate, and methylate functional groups (Yildirim-Elikoglu and

Erdem 2018). Niseteo et al. (2012) reported that the addition of milk to various coffee brews caused a drastic decrease in the concentration of CGA derivatives and their radical scavenging attributes, due to the formation of protein-polyphenol interactions (Niseteo et al. 2012). They confirmed that the higher molecular weight phenolics and the presence of more methylated functional groups lead to a further decrease in polyphenol content and antioxidant effects of coffee brews. Duarte and Farah (2011) similarly studied the in vivo changes in CGAs and their urine metabolites as a result of concurrent ingestion of milk and coffee (Duarte and Farah 2011). The CGAs and their metabolites in the milk-coffee mixture were about 28% lower than water-coffee combination. In line with these results, Dupas et al. (2006) reported that about 40% of 5-CQA attached to milk proteins via the formation of 5-CQA-casein complex and 17% of that attached to caseins and small peptides even after in vitro digestion (Dupas et al. 2006). In addition to caseins, whey proteins (especially, β-lactoglobulin) are also able to interact with coffee polyphenols. It has been noted that polyphenols can interact with free cysteine residues of β-lactoglobulin and α-lactalbumin, while employing some strategies like alkylation of cysteine residues could prohibit this interaction (Yildirim-Elikoglu and Erdem 2018).

Liu et al. (2016) studied the interactions of both whey proteins and caseins with CGA by molecular docking calculations and spectroscopic assay (Liu et al. 2016). The results showed that the binding constant of β-lactoglobulin-CGA interaction was bigger than those obtained from the interactions of CGA with α-lactalbumin, α-casein, β-casein, and κ-casein at a binding distance below 8 nm. The hydrogen bond coupled with Van der Waals forces were mainly found in CGA interactions with α-lactalbumin and α-casein, while the CGA interactions with β-lactoglobulin, β-casein, and κ-casein were mostly attributed to hydrophobic bindings (Figure 3), as approved by thermo-dynamical results (Liu et al. 2016). There is no mechanistic report on coffee-milk carbohydrate possible interaction, while there few studies have investigated the interactions between coffee ingredients and milk fat. In this regard, Bücking and Steinhart (2002) scrutinized the effects of different milk additives on the volatile compounds of Arabica and Robusta coffees (Bücking and Steinhart 2002). The results revealed that high fat content milk additives (e.g., whipping cream containing 30% fat and condensed milk containing 10% fat) could decrease the coffee-milk aroma compared to the reduced-fat milk additives (e.g., skimmed milk powder containing 1% fat and ultrahigh temperature milk containing 0.3% fat), most likely due to the dipolar interactions of milk fat and coffee aromatic compounds (in particular, 2-furfurylthiol) induced by Van der Waals forces and result in a reduced aroma and flavor coffee-milk mixture.

Factors defining the interactions between milk components and coffee phenolics

Besides structural features of coffee polyphenols, milk proteins, and other constituents, some other conditions such as temperature and pH are also important factors that can affect the binding forces, and thus, result in the alteration of bioactive potentials. The presence and the degree of these interactions between milk proteins and coffee phenolics depend on several factors such as the concentration of proteins and phenolics, type of proteins, and the structure of phenolics (Ozdal et al. 2013; Özyurt and Ötleş, 2016). For example, it was reported that the structural characteristics of the phenolic acids such as type, methylation, hydroxylation, and steric hindrance, affected their binding affinity to β-casein (Li et al. 2020). In this study, out of six different phenolic compounds that were examined for their binding affinity against β-casein, caffeic acid was found to have the highest affinity, which was followed by CGA (Li et al. 2020). On the other hand, it has also been reported that the ratio of protein and phenolic compounds and the presence of other components (e.g., minerals) in the experimental matrix may affect the level of such interactions. For example, Siebert, Troukhanova, and Lynn (1996) reported that with increasing the concentration of the polyphenol (tannic acid) at a fixed level of protein (gelatin), the observed haze (in a model system of pH 4.02 potassium phosphate) rose at first, before reaching a plateau, and finally in most cases it declined considerably. Additionally, a similar behavior was observed when the concentration of the polyphenol was kept at a fixed level and the protein concentration increased (Siebert, Troukhanova, and Lynn 1996).

Another point that should also be considered is the method used for the analysis of antioxidant activity, since different techniques may lead to different results (Dubeau et al. 2010). In some cases, the total antioxidant activity (measured using assays such as 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, and oxygen radical absorption capacity) may be highly correlated with the total phenolic content (Jacobo-Velazquez and Cisneros-Zevallos 2009; Rashidinejad 2015). However, some compounds such as most peptides, vitamins (e.g., vitamins C and E), and carotenoids, which are considered as radical scavengers in the lipid phase of milk, also show phenolic content when analyzed using total phenolic contents (Folin-Ciocâlteu) method (Gmeiner and Seelos 1994; Rashidinejad 2015). Because, Folin-Ciocâlteu technique was originally developed for the determination of total protein concentration by measuring the content of amino acids (e.g., tyrosine and tryptophan), which result in the formation of a blue color in the assay (Sánchez-Rangel et al. 2013), but it is now frequently used for the determination of phenolic properties and antioxidant activity of biological samples. Al-Ghafari et al. (2017) reported that the binding affinity of active groups of polyphenols for proteins differed, revealing lower polyphenol-hydrogen peroxide interactions and higher phenol-protein interactions. Similarly, the metal chelation effect of coffee antioxidants could be interrupted by the occupation of binding sites of phenols with milk proteins.

The effect of milk addition on properties of coffee infusions/beverages

The effect on the functional and nutritional properties

Given the fact that antioxidants are known as free radical scavengers and metal ion chelators, the antioxidant capacity of coffee polyphenols can be assessed by various measurements including total phenolic content, radical scavenging activity, reducing power assay, and metal chelating activity (Al-Ghafari et al. 2017). An increase or decrease in these activities determined by the analysis of coffee beverages prepared with milk is essentially used to evaluate the antioxidant potential of coffee polyphenols. Additionally, the absorption and bioavailability of phenolic compounds in coffee are assessed via in vitro and in vivo studies, which are well-documented in the current literature (Alongi, Calligaris, and Anese 2019; Bhagat et al. 2019; Duarte and Farah 2011; Dupas et al. 2006).

Based on the information available so far, the possible effects of milk components on the functional properties of coffee phenolics can be categorized into three groups that are discussed below. These include; (I) *masking/negative* effects, which reveal the decreased antioxidant capacity of coffee phenolics, and thus, suppressing positive health impacts of coffee brews, (II) *positive* effects, which reveal the increased antioxidant capacity of coffee phenolics, and (III) *neutral* effects.

Negative (masking) effects

As mentioned during the previous sections, the addition of milk to coffee beverages can mediate functional and nutritional changes due to the interferences between milk components (e.g., proteins and fats) and coffee constituents (e.g., CGAs, caffeine, trigonelline, and diterpenes) (Al-Ghafari et al. 2017; Komes et al. 2015; Niseteo et al. 2012; Quan et al. 2020). Some recent studies reporting the negative effects of milk added to coffee are given in Table 3. Undesirable changes are defined in some functional and nutritional properties of coffee polyphenols, especially with the predominant CGA derivatives, due to the type, amount, and content of the milk product added. Although coffee contains other beneficial properties such as antimicrobial, immune-stimulating, and hypoglycemic effects, the focus of the current review is on the antioxidant activity and bioaccessibility of its phenolic compounds.

As explained earlier, hydrophobic, covalent, and non-covalent interactions occurring among milk protein, fat, minerals, and coffee polyphenols exhibit significant masking effects on the antioxidant activity of milk-coffee beverages (Dupas et al. 2006; Stojadinovic et al. 2013; Tagliazucchi et al. 2012). Within this phenomenon, strongly occurring protein-polyphenol interactions are the well-known and most effective factors that limit the antioxidant potential of milk-coffee beverages. Besides affecting the total phenolic content of coffee, milk addition to coffee brews can also significantly influence the bioaccessibility and bioactivity of their phenolic compounds (Bhagat et al. 2019; Dupas et al. 2006; Tagliazucchi et al. 2012). It is notable that not only for coffee but also for other beverages (e.g., tea), the repressive effect of milk proteins on the antioxidant capacity of polyphenols have been reported (Rashidinejad 2015; Rashidinejad, Birch, and Everett 2016b; Rashidinejad, Birch, Hindmarsh, et al. 2017).

The fat content of milk added to coffee is also an effective factor on the antioxidant activity of coffee polyphenols. As seen in Table 4, the type of milk with various fat contents (i.e., skimmed, semi-skimmed, and whole milk), as well as the ratio of milk to coffee could affect the investigated functional properties of coffee. These include antioxidant activity, phenolics bioaccessibility, and caffeine content. For example, Quan et al. (2020) reported that the antioxidant activity of all the coffee samples upon milk addition in a ratio of 1:1.7 (milk:coffee v/v) declined or unchanged upon in vitro digestion. The bioaccessibility of phenolic compounds in coffee beverages containing no milk, skimmed milk, and whole milk was diminished by 29.2%, 21.1%, and 28.5%, respectively, after high-pressure homogenization processing (HPHP) and by 14.7%, 33.8%, and 34.2%, respectively, after pasteurization. According to these results, utilization of whole milk is more applicable in the fabrication of coffee–milk beverages than skimmed milk, which is due to higher bioaccessibility of polyphenolics than coffee.

Al-Doghaither et al. (2017) investigated the effect of low- and full-fat evaporated milks on the antioxidant properties of instant coffee and interestingly revealed that although total phenolic content increased by the milk addition, reducing antioxidant power, and metal chelation activity decreased based on the fat content (Al-Doghaither et al. 2017). The decrease in reducing antioxidant power of coffee containing milk was explained by the masking effect of milk fats and proteins on polyphenols, whereas reduced metal chelation activity was referred to as the interference via saturated metal ion chelators by milk minerals.

Niseteo et al. (2012) studied the total phenolic and flavonoid contents of coffee brews and the effect of milk addition on their antioxidant capacity. They showed that milk addition caused a significant decrease in polyphenol content and thus antioxidant capacity of coffee brews, due to the domination of phenolics by milk proteins. Since CGAs and their derivatives are the major antioxidants in coffee, they can be considered as the primary indicators of the antioxidant capacity of coffee beverages. Jeon et al. (2019) reported that milk-added to the ready-to-drink coffee infusions had relatively low CGA content (compared with caffeine), when aimed at the evaluation of CGA and caffeine content in different types of coffee beverages sold in supermarkets in the Republic of Korea (Jeon et al. 2019). In another study (Sánchez-González, Jiménez-Escrig, and Saura-Calixto 2005), the antioxidant activity of espresso coffee blended with milk in different amounts was tested based on the reducing power and radical scavenging activity assays and it was found that antioxidant activity was decreased proportionally to the increased amount of milk added.

Tagliazucchi et al. (2012) studied the effects of whole, semi-skimmed, and skimmed milks ranging from 10% to 50% (v/v) of the coffee beverages on the bioavailability of CGAs after in vitro gastrointestinal digestion (Tagliazucchi et al. 2012). The results showed that all types of milks particularly at higher ratios (50%) could considerably decrease the bioavailability of CGAs, as a result of casein-CGAs

Table 3. The masking/negative effects of milk added to coffee infusions reported in the recent literature.

Coffee type tested	Milk type added	Milk ratio (v/v %) added	The masking/negative effect reported	Reference
Instant coffee	Whole and skimmed milk	56, 40	• Skimmed milk lowered bioaccessibility of coffee phenolics, but no significant decrease in the case of whole milk.	Quan et al. (2020)
Instant, ready to drink and roasted & ground coffee	Whole milk	10	• Milk relatively lowered the CGA, but increased caffeine content due to milk added to coffee.	Jeon et al. (2019)
Instant coffee (Nescafe Red Mug)	Full-fat and low-fat evaporated milk	20	• Significantly decreased H_2O_2 radical scavenging, reducing power and metal chelation activities. • Lower radical scavenging activity in the case of low-fat content was observed. • There was a slightly lower metal chelation activity in the case of full-fat content.	Al-Doghaither et al. (2017)
Instant coffee (Nescafe Red Mug)	Whole, semi-skimmed, and skimmed milk	10, 20	• Significantly reduced H_2O_2 scavenging activity of coffee in the case of 20% milk addition. • There was observed significantly reduced metal chelating and reducing activities of coffee in the case of either 10% or 20% milk addition.	Al-Ghafari et al. (2017)
Instant coffee (Nescafe Red Mug)	Whole fat and evaporated milk	10, 20, 30	• Significantly lowered DPPH and H_2O_2 radical scavenging activity were found only in the case of evaporated milk.	Alsufiani and Omar (2017)
Coffee substitutes	Whole milk	25, 50, 75	• The bioactive properties of coffee were decreased.	Komes et al. (2015)
Espresso, Turkish/Greek, instant, filter coffee (regular and decaffeinated)	Homogenized milk	∼35, 50 100	• A significant decrease in the polyphenolic content and antioxidant capacity of brews was seen.	Niseteo et al. (2012)
Instant coffee	Whole milk	100	• Milk addition reduced CGA bioavailability in humans.	Duarte and Farah (2011)
Instant coffee	Whole, semi-skimmed, and skimmed milk	50	• An immediate decrease in the bioaccessibility of CGA in all types of milk added was observed.	Tagliazucchi et al. (2012)
Instant coffee	Whole, semi-skimmed, and skimmed milk	25	• Lowered the absorption of CGA due to the binding of milk proteins was found. • No significant effect on the antioxidant power of coffee was seen.	Dupas et al. (2006a)
Instant coffee	Semi-skimmed and skimmed milk	20	• The bioaccessibility of phenolic compounds was impaired, mainly due to the binding with milk proteins.	Dupas et al. (2006b)
Espresso	Whole milk	∼ 9, 50	• The antioxidant activity was significantly decreased when milk was added to espresso coffee.	Sánchez-González, Jiménez-Escrig, and Saura-Calixto (2005)

covalent interaction. Skimmed milk was more effective in reducing CGAs bioavailability compared to the whole and semi-skimmed milks, possibly due to the higher protein content.

Recently, Quan et al. (2020) investigated the effects of milk type (whole and skimmed) and operational processing parameters (thermal, high-pressure homogenization, and pH) on the bioavailability of coffee phenolic compounds and their antioxidant activity. High-pressure homogenization and pH adjustment of both milks indicated no significant change in the bioavailability of coffee phenolics, while using thermal treatments adversely affected the phenolics bioavailability in the case of skimmed milk (Quan et al. 2020). Based on this evidence, it is possible to keep the coffee health-promoting attributes by optimizing milk processing parameters and choosing milks with lower protein content.

Tagliazucchi et al. (2012) reported an immediate decrease in CGA bioaccessibility (∼ 20%) after milk addition, probably through binding of CGAs to milk proteins when whole, semi-skimmed, and skimmed milk were blended to instant coffee (Tagliazucchi et al. 2012). They also emphasized a direct relation between the fat content of milk and the bioaccessibility of CGAs. According to Duarte and Farah (2011), the consumption of coffee with milk may negatively influence the absorption of CGAs in the human body depending on the proportion of milk to coffee blended (Duarte and Farah 2011). These researchers investigated the bioavailability of phenolic compounds in coffee when consumed with milk by analyzing the 24-h urinary excretion of five nonsmoking subjects. Based on HPLC/LC-MS data, a consistently lowered amount of CGAs and metabolites such as caffeic and ferulic acid were detected in the urine of the all subjects consumed coffee (∼ 40%) and milk simultaneously, in comparison to that of subjects consumed coffee alone (∼ 68%). Average reduction (∼ 28%) obtained in the recovered CGAs in the case of coffee with milk consumption was considered as the indicator of quantitative alteration in CGAs absorption. Additionally, they concluded that adding high amount of milk to coffee decreased the bioavailability of those polyphenols, whereas milk addition in lower amounts did not restrict the bioaccessibility of CGAs (Duarte and Farah 2011).

In another study, Quan et al. (2020) used an in vitro gastrointestinal digestion model to investigate the effects of processing (thermal treatment and high-pressure homogenization process) and milk matrix on the antioxidant capacity and bioaccessibility of coffee polyphenols such as CGA, caffeic acid, and ferulic acid (Quan et al. 2020; Schramm et al.

Table 4. The effect of milk addition on various properties of coffee, as the result of milk type and its ratio.

Type of the milk added	Milk:coffee ratio	Investigated parameters	Main findings	References
Skimmed (1% fat w/w) and whole milk (26.5% fat w/w)	1:1.7 (w/v)	In vitro gastrointestinal digestion, the antioxidant power, and phenolics bioavailability of coffee	• The antioxidant activity of all the coffee samples declined or unchanged upon in vitro digestion. • The polyphenolic bioaccessibility of coffee, coffee with skimmed milk, and coffee with whole milk was decreased by 29.2%, 21.1%, and 28.5% from HPHP treatment Vs. by 14.7%, 33.8%, and 34.2% from TT, respectively. • Utilization of whole milk was more applicable in the fabrication of coffee–milk beverages than skimmed milk due to higher bioaccessibility of polyphenolics than coffee and even coffee-skimmed milk.	Quan et al. (2020)
Skimmed milk (0.1% fat w/w) and full-fat milk (3.6 or 7.1% fat w/w)	1:1	In vitro digestion, particle physicochemical properties, bioaccessibility of CGAs, and inhibition of α-glucosidase	• Milk with the highest fat content (7.1%) represented the smaller particle formation upon HPHP and digestion as well as lower ζ-potential and higher stability. • A 25% increment of CGAs bioavailability over milk addition and HPHP was observed. • Improved α-glucosidase inhibitory • the effect over properly milk combination and HPHP was seen. • Reduction of the Type 2 diabetes risk by a proper combination of milk and homogenization pressure was found.	Alongi, Calligaris, and Anese (2019)
Skimmed (0.1% fat w/w), semi-skimmed (1.5 fat w/w), and whole milk (3.5% fat w/w)	1:3	Phenolic composition, Maillard reaction products, polyphenol fractionation, the interactions between coffee phenolics and milk proteins, and antioxidant activity	• Bounding up to 40% of coffee chlorogenic acid to dairy proteins after 25% milk addition could be observed. • No significant effect of milk addition on the antioxidant activity of coffee infusions before and upon consumption was seen. • A slight modification of CQA absorption in the body was found in the presence of milk.	Dupas et al. (2006)
Whole milk	–	Antioxidant activity	• Decreasing the antioxidant activity of coffee as compared to its own by 62% and 95% over mixing with 17 and 100 ml milk, respectively, was observed.	Sánchez-González, Jiménez-Escrig, and Saura-Calixto (2005)
Whole milk (2.6% fat w/w)	1:1.7 (*macchiato* coffee brew) 1:1 (*latte* coffee brews) 50 ml: 7 g (instant *cappuccino* brews)	Total phenol and flavonoid content, methylxanthine composition, caffeine, and antioxidant capacity	• Both the total phenolic content and antioxidant power of brews were declined by milk addition due to probable potential interactions of milk proteins and polyphenolic compounds.	Niseteo et al. (2012)
Whole milk (2.5% milk fat)	1:19 (v/v) 1:4 1:1 2.3:1	Total free polyphenols and antioxidant capacity	• Milk addition (10–70%) decreased ferric reducing power (FRP) of drinks reflecting a high number of inactive polyphenols.	Ziyatdinova et al. (2011)
Low-fat milk (1.5% fat) and whole milk (3.5%-7% fat)	1:1 (w/w)	Particle size distribution, sensory properties, external static headspace sampling (SHS), GC/MS, and GC/olfactometry of the trapped flavor compounds	• The entire odor and the milk-typical descriptors for the retronasal odor did not differ practically upon the addition of the whole milk or twice-pasteurized, twice-homogenized low-fat milk. • The addition of milk with lower fat content was generally provided the coffee with more taste and retronasal odor descriptors similar to typical ones. • The descriptor intensity of coffee was furtherly diminished upon increasing the fat content of the recombined milk samples from 3.5 to 7%.	Parat-Wilhelms et al. (2005)
Whole milk (2.6% fat w/w)	1:3 1:1 3:1	The content of phenolic compounds, flavonoid content, flavan-3-ols, proanthocyanidins, and antioxidant capacity	• The addition of milk diminishes the bioactive properties of coffee substitute brews in accordance with the amount of added milk.	Komes et al. (2015)

*CGAs: chlorogenic acids, HPHP: high-pressure homogenization processing, TT: thermal treatment

2003). High-pressure processing did not show any significant effect on the bioaccessibility of phenolics, while thermal processing affected this parameter, in the presence or absence of milk in coffee. They reported that skimmed and low-fat milk resulted in the lowered bioaccessibility of those phenolics in coffee remarkably, when compared to the whole and full-fat milk. This was explained by lowered fat coverage and the protection around coffee antioxidants in the case of low-fat milk.

By considering the findings of the aforementioned works, it can be declared that the consumption of coffee and milk simultaneously may result in negative/masking effects on the antioxidant activity and bioaccessibility of coffee bioactive compounds including polyphenols. These masking effects are mostly attributed to the strong interactions between milk proteins and coffee polyphenols, mainly CGA and its derivatives. The amount and contents of milk (e.g., fat or protein content as dry matter) are the critical parameters influencing both antioxidant potential and bioavailability of coffee phenolics. The possible interactions between milk fat and polyphenols may also impact on the antioxidant properties and bioaccessibility of phenolic compounds in coffee. There are many contradictory pieces of evidence in the literature regarding the effect of milk components on coffee antioxidants in the case of coffee-milk beverages. Further research will be expected to make more contributions to evaluate the effects of other milk constituents such as fat, lactose, and minerals on the functionality of coffee antioxidants.

Positive effects

There are a few studies that have reported positive effects of milk addition on the antioxidant properties and bioavailability of the phenolic compounds in coffee. As discussed in the previous sections, the complexations between milk components and polyphenols can be formed through hydrophobic or hydrophilic interactions and induce the formation of soluble or insoluble aggregates. Depending on whether these complexes are reversible or irreversible, in some cases, they might enhance the antioxidant properties of phenolic compounds in coffee (Bandyopadhyay, Ghosh, and Ghosh 2012). Additionally, the increased total amount of phenolics due to milk fat may also be related to the lipid-based antioxidants existing in milk, which can lead to an increase in the antioxidant potential of the food matrix when milk is added (Ryan and Petit 2010). Some studies asserted lipid-polyphenol interactions protecting phenolics during gastrointestinal digestion, and hence, enhancing the bioaccessibility of phenolics in coffee with high-fat milk (Alongi, Calligaris, and Anese 2019; Jakobek 2015; Ortega et al. 2009; Schramm et al. 2003).

Alongi, Calligaris, and Anese (2019) investigated the addition of milk (milk:coffee, 1:1 w/w) containing different fat concentrations (0.1, 3.6, and 7.1%) and HPHP on the bioaccessibility of CGA. The bioaccessibility was determined upon in vitro digestion suggested by Minekus et al. (2014). Their results indicated that the fat concentration of milk, as well as the homogenization pressure, had a significant effect on CGAs bioaccessibility. The bioaccessibility of CGAs in all samples containing different concentrations of fat was enhanced, and the addition of milk and HPHP increased the CGAs bioaccessibility up to $> 50\%$. The coffee contained milk with 0.1% fat and 50 Mpa pressure showed the highest increase in phenolic bioaccessibility up to 112%. One reason for such an enhancement could be that the micellization of CGAs in the presence of milk fat may decrease their sensitivity to degradation. Furthermore, the complex of phenolic compounds and milk proteins may decrease their sensitivity to degradation during the first part of the gastrointestinal tract (GIT), meaning that higher concentrations of CGAs are delivered for the absorption during the second part of the GIT (Dupas et al. 2006; Lamothe et al. 2014). HPHP was used to stabilize the emulsion of coffee-milk based beverages by the formation of smaller particles (Alongi, Calligaris, and Anese 2019), and the results indicated that the particle size of the coffee beverage was decreased by increasing the pressure and CGAs bioaccessibility was enhanced by decreasing the particle size.

The mechanisms related to the positive effects of milk added to the coffee on the antioxidant properties of this beverage are presented in Figure 4. As stated earlier in this section, according to some studies, the positive effect of milk on the bioaccessibility of phenolic compounds in coffee is associated with milk fat (Al-Ghafari et al. 2017; Tagliazucchi et al. 2012). Al-Ghafari et al. (2017) investigated the effect of different types of milk (whole milk, semi-skimmed milk, and skimmed milk) on the antioxidant activity of coffee. Milk was added in the proportion of 10% and 20% (v/v) into the coffee beverage. The total phenolic and flavonoid contents were increased in the presence of different types of milk and all concentrations especially with whole milk at 10% or 20% fat content. This increase might be related to the presence of other compounds (e.g., tocopherols, lactoferrin, and tocotrienols) in milk that may show antioxidant activity (Lindmark-Månsson and Åkesson 2000; Rashidinejad, Birch, and Everett 2016a; Rashidinejad et al. 2013). The authors explained that the mechanisms of milk fat on the antioxidant properties of coffee were unclear and more investigation was needed to understand such a mechanism (Al-Ghafari et al. 2017).

Another study (Tagliazucchi et al. 2012) also indicated that in the presence of whole milk and semi-skimmed milk (50%), the bioaccessibility of coffee CGAs was increased after the pancreatic digestion, as compared to the obtained values for coffee alone. The bioaccessibility of coffee CGAs in the samples containing skimmed milk did not change after pancreatic digestion, as compared to the obtained values for coffee alone. The authors explained that the hydrophobic interactions between fat milk and CGAs could enhance CGAs stability against oxidative degradation. Furthermore, fat may increase the surfactant amount in the beverage and interfere with the formation of caffeine and CGAs aggregates, and correspondingly, prevent the precipitation of these compounds and enhance their bioaccessibility (Tagliazucchi et al. 2012).

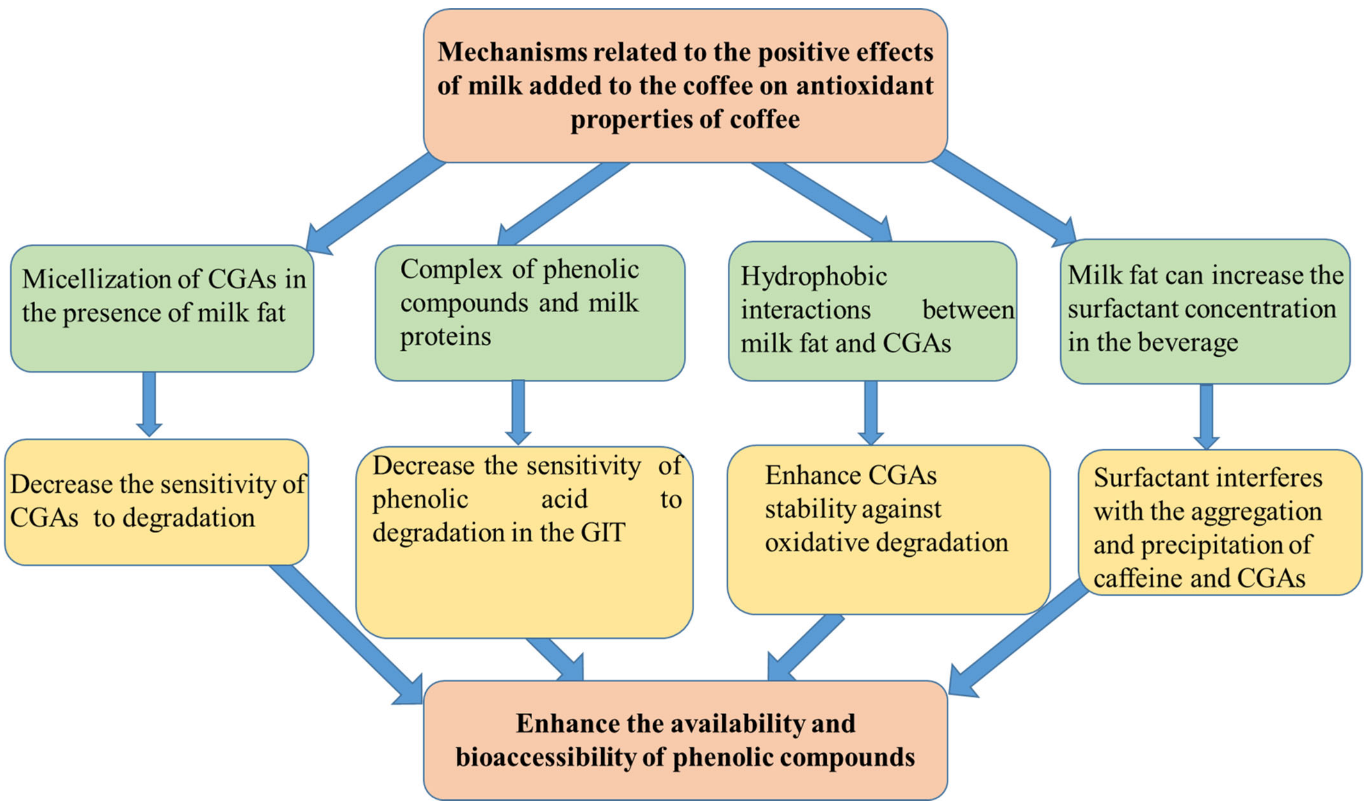

Figure 4. The mechanisms related to the positive effects of milk added to the coffee infusions and the effect on the antioxidant properties of coffee phenolics. CGAs: colorogenic acids, GIT: gastrointestinal tract.

Neutral effects

Even though the effect of milk addition to coffee beverages has been mostly reported to be negative (and to a certain extent positive), some reports are also indicating a neutral effect on the antioxidant properties of the phenolic compounds of coffee (Table 5). For example, Tagliazucchi et al. (2012) indicated that even though the addition of whole milk, semi-skimmed milk, and skimmed milk to coffee generally resulted in an immediate decrease in the total concentration of CGAs before digestion, skimmed milk addition did not affect the bioaccessibility of this compound as analyzed by using in vitro gastropancreatic digestion, compared to the control (coffee alone). Besides, it was also reported that the quantity and the fat content of the added milk were the critical factors and according to their results, the addition of 10% whole milk or 10% skimmed milk did not affect CGAs content before digestion (after mixing).

Another study (Otemuyiwa, Williams, and Adewusi 2017) also showed that the addition of milk powder at different concentrations (4.0, 8.0, 12, 16, and 20 mg milk powder/ml of the coffee solution, which was prepared by placing two g of coffee into 250 ml conical flask and made up to volume with boiling water) did not affect the in vitro bioavailability of total phenolics in coffee (Otemuyiwa, Williams, and Adewusi 2017). Similar results were also observed in an in vivo study performed by Renouf et. al (2010). These researchers (Renouf et al. 2010) investigated the effect of the addition of whole milk or sugar and a lipid-derived nondairy creamer on the bioavailability of phenolics of an instant coffee product. The nondairy creamer was composed of maltodextrin and corn sirup together with palm oil, casein, water, and some other minor components. The participants (four men, five women, aged 18–50 y, healthy, average, with regular coffee consumption of 1–5 cups/d, and non-smoker) were asked to drink black coffee, coffee with 10% whole milk, or instant coffee, sugar, and nondairy creamer already premixed. The bioavailability of phenolics in the plasma was assessed during 0 to 12 hours. According to the findings of this study (Renouf et al. 2010), it was concluded that the addition of 10% whole milk to coffee did not affect the bioavailability of coffee phenolics such as caffeic acid, ferulic acid, and isoferulic acid. On the other hand, sugar and nondairy creamer addition resulted in a decrease in the maximum plasma concentration (C_{max}) and an increase in the time required to reach C_{max} but not the appearance of coffee phenolics in the plasma (Renouf et al. 2010).

Jiang et al. (2018) also worked on the non-covalent interaction of chlorogenic acid with casein and whey protein isolate (WPI) and investigated the scavenging capacity of in vitro digesta of casein and WPI-treated with different concentrations of CGA (20, 120, and 240 μmol/g protein). The results indicated that although the radical scavenging capacity of CGA (measured with $ABTS^{+\bullet}$ radical scavenging assay) increased significantly as the concentration of CGA increased, no difference was observed in the radical scavenging activity of the protein-phenolic mixture containing the lowest concentration of CGA (20 μmol/g protein).

In a study by Ziyatdinova, Nizamova, and Budnikov (2011), the effect of milk proteins on the antioxidant

Table 5. A summary of the previous studies on the neutral effect of milk added to coffee infusions.

Type of coffee	Type of milk	Milk:coffee ratio	Interaction conditions	Analyses carried out	Main conclusion	References
Ground and roasted (Arabica and Robusta) coffee	Milk (not specified)	1:10 (v/v)	Coffee was prepared as instructed for the consumer; 1.7% soluble coffee per 220 ml with and without 10% milk.	LDL* oxidation assay	Milk did not affect the antioxidant activity of coffee.	Richelle et al. (2001)
Instant coffee	Whole milk, semi-skimmed milk, and skimmed milk, and their spray-dried forms	1:4 (w/v)	Samples were 50 g/L instant coffee solution, coffee containing 25% (w/v) skimmed milk/semi-skimmed milk/whole milk, and their spray-dried counterparts.	Antioxidant activity (DPPH, AAPH, and TAC assays)	Any type of milk did not significantly affect the antioxidant activity of coffee.	Dupas et al. (2006)
Instant coffee	Whole milk	1:10 (v/v)	Coffee was prepared to dissolve 4 g soluble instant coffee in 400 mL water, and for milk treatment 40 ml whole milk was used instead of water.	Participants were made to drink coffee and the contents of phenolics (including caffeic acid, ferulic acid, and isoferulic acid) in their blood plasma were analyzed.	Adding 10% of whole milk did not affect the bioavailability of phenolic acids in coffee.	Renouf et al. (2010)
Instant coffee, and roasted and ground coffee beans	Pasteurized milk (2.5% of fat)	0.5:10, 2:10, 5:10, 7:10 (v/v)	Instant coffee prepared by 2 g coffee in 150 ml hot water. Ground coffee was prepared as 4 g coffee per 150 ml water and left for 4 min.	Antioxidant activity (FRAP)	Milk proteins did not affect the antioxidant activity until 20% of milk was added.	Ziyatdinova et al. (2011)
Instant coffee	Whole milk, semi-skimmed milk, and skimmed milk	1:1 or 1:10 (v/v)	Coffee was prepared by dissolving 2 gr instant coffee in 100 ml boiling water. Then mixed with different concentrations (%50 and %10) and types of milk. Water was added to the control sample.	CGA content	Neither skimmed milk nor whole milk (up to 10%) affected the content of chlorogenic acid before digestion (after mixing).	D. Tagliazucchi et al. (2012)
Coffee (not specified)	Powdered milk (not specified)	2/4/6/8/10:125 (w/v)	Coffee was prepared by boiling (2 g coffee per 250 ml of water), then milk powder (with a final concentration of 4.0, 8.0, 12, 16, 20 mg milk powder/ml of the coffee solution) was added, and in vitro analysis was performed immediately.	In vitro bioavailability of phenolics	Milk addition did not affect the in vitro bioavailability of coffee phenolics.	Otemuyiwa, Williams, and Adewusi (2017)
Chlorogenic acid	WPI, (purity >90%) and CS (from bovine milk, purity > 90%)	1:2, 1:12, 1:24 (protein w/ μmol CGA)	WPI and CS solutions (10 mg/ml in phosphate buffer) were prepared and mixed with different concentrations of CGA (20, 120, and 240 μmol/g protein).	Antioxidant activity (ABTS)	No effect was observed on the antioxidant activity in the lowest concentration of CGA (20 μmol/g protein).	Jiang et al. (2018)

*LDL: low-density lipoprotein, AAPH: 2-2-azobis (2-amidinopropane) dihydrochloride, ABTS: [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], CGA: chlorogenic acid, CS: casein, DPPH: 2,2-diphenyl-1-pycrylhydrazyl, FRP: Ferric reducing power, TAC: total antioxidant capacity, WPI: whey protein isolate.

properties of several tea and coffee samples was studied. The antioxidant activity of the samples was investigated by measuring FRAP, and the results indicated that until 20% of milk addition, the antioxidant power of coffee beans was not significantly affected. In another investigation, Richelle et al. (2001) explored the relative antioxidant activity of tea, coffee, and cocoa using an in vitro low-density lipoprotein oxidation model, and concluded that the addition of milk did not affect the antioxidant activity of any of these samples. Dupas et al. (2006) too studied the effect of milk addition on the phenolics and antioxidant activity of instant coffee and investigated the changes in the phenolic profile and antioxidant activity before and after in vitro gastrointestinal digestion. Their results indicated that although CGA was bound with proteins, no significant difference was observed on the chain-breaking activity of this antioxidant compound. On the other hand, the effect of interactions on the antioxidant activity differed depending on the compounds formed and the antioxidant activity method used for the corresponding analysis. In general, the results indicated that the addition of milk to instant coffee did not significantly affect the antioxidant power of the coffee samples. The researchers also concluded that to get a better overview of the real antioxidant potential of coffee samples, different test methods

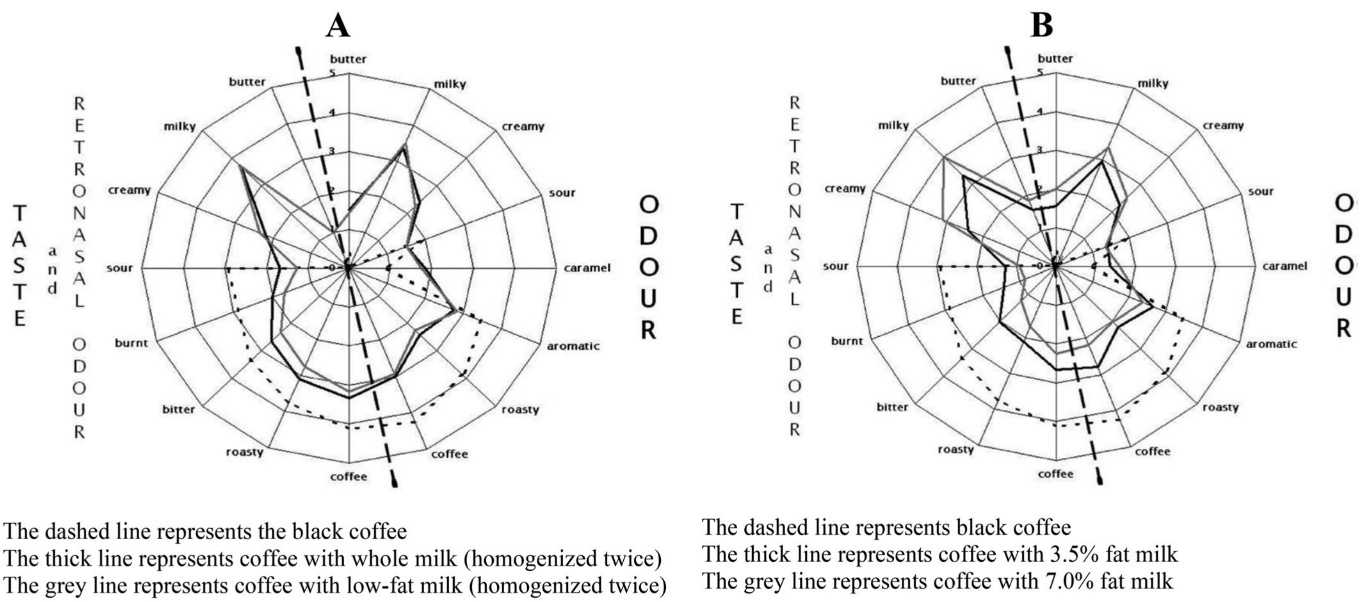

Figure 5. Aroma profile, odor, and taste intensities (0; no smell/taste, to 5; dominant, very strong smell or taste) of coffee beverages affected by the addition of different milk types (whole and low-fat; A, and 3.5 and 7.0% fat; B). Reproduced with permission from Parat-Wilhelms et al. (2005).

with different mechanisms should better be used. In another study of the same group (Duarte and Farah 2011), CGA absorption and its bioavailability were studied using an in vitro Caco-2 cell model coupled with an in vitro digestion process, and an in vivo rat model. Both experiments showed that CGA absorption was not modified by the addition of milk proteins. The researchers concluded that there were some possible interactions between coffee phenolics and milk proteins, but these had no significant effect on the bioavailability of CGA in the rats (Duarte and Farah 2011).

Besides coffee, some other studies also revealed an insignificant change in the antioxidant capacity of tea (Kyle et al. 2007; Leenen et al. 2000; Reddy et al. 2005) and blackberry juice (Hassimotto et al. 2008), when treated with milk addition. It should also be noted that all these changes are dose-dependent, meaning that based on the concentration of the phenolics and/or proteins in a coffee-milk beverage system, the results obtained may change from a negative effect to a neutral (insignificant change) or even positive effect.

The effect on the sensorial attributes

The interactions between milk fat or milk proteins and the bioactives in coffee, as well as industrial production parameters of milk and coffee products, could be important for the fabrication of a good coffee beverage (Parat-Wilhelms et al. 2005). Aroma release from coffee could be affected by the fat content levels of milk (Parat-Wilhelms et al. 2005). The hydrophobic and/or hydrophilic interdependency is used to express the effect of fat on coffee flavor. As it was stated earlier, reversible van der Waals interactions may occur between the fat molecules and the lipophilic flavor/aromatic compounds of coffee (Plug and Haring, 1993). Furthermore, it has also been well established that proteins have the potential to make covalent and reversible hydrogen bonds with the flavor substances, which is the reason for the decrease of the flavor release in food/beverage matrix containing proteins (Parat-Wilhelms et al. 2005). The interactions between polyphenols of coffee and proteins of milk can modify the taste of the final coffee drink, as well as the structure of the proteins. As shown by Gallo et al. (2013), phenolic compounds have an affinity to globular proteins and tend to agglomerate (i.e., to form complexes with proteins) with a higher probability as the molecular size of the polyphenol increases. When ingesting a product rich in polyphenols like coffee, the consumer's first perception is probably the astringency. This taste results from the interaction of the polyphenols of the coffee and the protein of the saliva (mainly, proline), resulting in the formation of an insoluble complex that is responsible for the astringency sensation (Jöbstl et al. 2004).

In a comprehensive study carried out by Parat-Wilhelms et al. (2005), the effect of pasteurized milk addition (with 1.5–7% fat) on the flavor of white coffee beverages was investigated. A more drastic coffee-related retronasal odor sensation was observed upon the addition of the low-fat milk with significantly smaller fat globules, obtained from double homogenization (each 250/50 bar), while the milk-related perception was approximately the same. Despite enhancing the milky and creamy retronasal odor, the perception of coffee after the addition of casein, the coffee-related taste, and the retronasal odor were also weakened (Parat-Wilhelms et al. 2005).

Aroma profile, odor, and taste intensities (0; no smell/taste, up to 5; dominant, very strong smell or taste) of coffee beverages as affected by the addition of different milk types are represented in Figure 5. With the triangular tests, it was observed that the entire odor and the milk-typical descriptors for the retronasal odor did not vary practically upon the addition of the whole milk as compared to the addition of double-pasteurized, double-homogenized low-fat milk (Figure 5A); i.e., the thick line that represents coffee with double homogenized whole milk is almost identical to the gray line that represents coffee with double-homogenized low-fat milk. However, the addition of the milk with lower fat content provided a coffee with a better taste and retronasal odor descriptors, similar to the standard coffee beverages. In addition, the descriptor intensity of coffee was furtherly diminished upon increasing the fat content of the recombined milk samples from 3.5 to 7% (Figure 5B). Even though the whole milk could provide better retention of the flavor compounds due to its higher fat content, more volatile compounds were released from the coffee beverages containing whole , double-pasteurized, or double-homogenized low-fat milk than the beverage containing once-homogenized low-fat milk. The probable scenario was attributed to the interactions of the flavor compounds with only fat globule membrane constituents instead of their dissolution in fat.

In another study, Komes et al. (2015) evaluated the effect of the addition of whole milk (2.6% milk fat) to coffee at different milk to coffee ratios (1:3, 1:1, and 3:1) on the sensory characteristics (taste, flavor, and overall acceptability) (Table 4). According to their results, the milk addition to all prepared coffee substitute brews positively enhanced the corresponding sensorial characteristics. All in all, based on the information available in the literature so far, the sensory characteristics of coffee can be positively enhanced upon the addition of milk, depending on its fat content and the ratio of milk to coffee, besides its preparation method. Nevertheless, black coffee without the addition of any type of milk (or any other additives), although stringent and bitter, is still a pleasurable choice for some other consumers.

Conclusions

Considering the findings available to date, there is enough evidence that the simultaneous consumption of milk and coffee may have a negative/masking effect on the nutritional quality and functionality of the phenolic compounds (especially, CGA) in coffee, while it can improve the sensorial attributes of this popular beverage. Nevertheless, there is also enough evidence reporting a positive or an insignificant effect of milk addition on the nutritional and functional properties of coffee brews. Therefore, the effect of milk addition on the nutritional, functional, and sensorial properties of coffee seems to be dependent on several factors such as the proportion of milk to coffee, the temperature of the infusion/beverage before and after the addition of milk, type of the milk added, and the methods of assessing the antioxidant properties. Such an effect may also vary based on the concentration of both milk proteins and coffee phenolics. The dose-response studies that have evaluated the bioavailability of CGA in human subjects are scarce, meaning that more studies are required for the determination of the minimum proportion of milk to coffee that may result in the impairment of CGA bioavailability after the consumption of coffee with milk.

Although drinking coffee is associated with some various traditional habits, drinking this beverage for the sake of its health benefits (originated from phenolic compounds) is becoming more popular these days; however, there is no robust evidence to support any recommendation for the appropriate portion of milk to be added to a specific volume of coffee infusions. Additionally, it appears that most of the information around the addition of milk to coffee infusions so far has focused on the interactions between milk proteins and phenolic compounds of coffee. However, milk (especially, whole and full-fat types) also contains a considerable amount of fat in form of milk fat globules, the role of which has been neglected or less regarded, while their interactions with the phenolic compounds in other types of drinks (e.g., catechins in tea) has been reported (Rashidinejad, Birch, and Everett 2016b; Rashidinejad, Birch, Hindmarsh, et al. 2017).

Another important aspect is that majority of coffee drinkers may drink coffee for pleasure or the immediate benefits of its caffeine content. Although the negative effects of milk addition on the bioefficacy of phenolics such as CGAs in coffee have been reported to an acceptable extent, there is a scarcity of information about the effect of milk addition on the activity or bioavailability of caffeine in coffee beverages. Thus, further research is required to systematically evaluate the effect of the addition of various types of milk on the bioaccessibility and bioavailability of coffee phenolic compounds and milk components. Lastly, although the habits of drinking coffee and the use of milk addition in various coffee formulations, or even between different individuals, can be different, the current scientific evidence suggests that polyphenols such as CGA in coffee may play a substantial role in the prevention of several chronic diseases and disorders in the individuals who are regular coffee drinkers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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