Chapter

Recent Development in Antioxidant of Milk and Its Products

Fouad M.F. Elshaghabee, Ahmed A. Abd El-Maksoud and Gustavo M. Ambrósio F. de Gouveia

Abstract

Free radicals are produced in humans through natural metabolism or the external environment, such as diet. These free radicals are neutralized by the antioxidant system, whereas enzymes, for example, catalase, superoxide dismutase, and glutathione peroxidase, play an important role in preventing excessive free radicals. Food antioxidants give a good hand in enhancing the human antioxidant system; high consumption of a diet rich in natural antioxidants protects against the risk of diseases such as cardiovascular, cancer, diabetes, and obesity. Milk and its products are popular for a wide range of consumers. Milk contains casein, whey protein, lactoferrin, milk lipid and phospholipids, vitamins, and microelements, for example, selenium (Se), which have antioxidant properties. Furthermore, probiotication of milk either sweet or fermented could enhance the antioxidant capacity of milk. This chapter focuses on presenting recent review data on milk components with antioxidant activity and their health benefits, probiotics as antioxidant agents, and methods for enhancing the antioxidant capacity of dairy products. The key aim of this chapter is to focus on major strategies for enhancing the antioxidant capacity of milk and its products.

Keywords: essential oils, plant extracts, probiotication, dietary management, metabolic diseases, antioxidant capacity of milk

1. Introduction

Reactive species are formed during different cellular process, especially during mitochondrial respiratory chain. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major reactive species that act as second messengers to regulate biological processes. However, they could cause oxidative stress and protein and DNA damage, which may cause different diseases such as atherosclerosis, diabetes, accelerated aging, and cardiovascular diseases [1].

Milk as a natural product is the first food for humans, and dairy products represent approximately 25–30% of an individual's diet. It also contains different components with antioxidant activity such as casein, whey protein, sulfur-containing amino acids cysteine, conjugated linoleic acid, and catalase that could restore the antioxidant system of the host [2]. Supplementation of milk with natural sources represents a dietary strategy in order to enhance the antioxidant capacity of milk and its products. Essential oils (EO) are volatile hydrophobic liquids that are extracted from a wide range of plants. They also possess different therapeutic effects, for example, anti-inflammatory and anti-microbial activities [3]. Supplementation of butter oil/ghee with different concentrations of essential oils (glove, garden cress, and jojoba) enhances its antioxidant capacity and shelf life [4, 5]. Furthermore, addition of ethanol extraction of pomegranate peels to ghee enhances the oxidative stability [6].

Probiotication means addition of probiotics (beneficial microbes for the host) to food products. It was considered as a dietary strategy for enhancing the antioxidant capacity of different fermented milk products through the ability of different probiotic strains, for example, *Lb. casei* shirota strain, to produce different metabolites from lactose fermentation or milk protein hydrolysis [7, 8]. This chapter aims to present the recent knowledge on the antioxidant potential of milk and major methods for enhancing the antioxidant capacity of dairy products.

2. Milk component with antioxidant activities: an overview

The antioxidant components in milk could be classified as non-enzymatic compounds, for example, milk proteins, and enzymatic antioxidants, for example, superoxide dismutase (SOD). **Figure 1** shows both selected antioxidant categories in milk. Casein is a major milk protein, accounting for 80% of the total protein in cow milk, and it presents in macromolecule aggregates because of the phosphorus content of casein [9]. Furthermore, the primary structure of casein has free radical scavenging activity [10]. Casein-derived phospho-peptide and phosphoserine residues can bind the non-heme iron [11]. Results obtained by Çekiç et al. [12] showed that β-casein fraction exhibited high antioxidant activity due to the presence of proline residues.

Whey protein as an antioxidant agent was used for inhibiting lipid peroxidation. The antioxidant activity of whey protein is due to its content of sulfur-containing amino acids. Addition of whey protein to soybean and salmon oils increased the oxidative stability of these products [13, 14]. The antioxidant activity of lactoferrin is due to iron-chelating activity and inhibits pro-oxidant effect and release of ROS by leucocytes [15, 16].

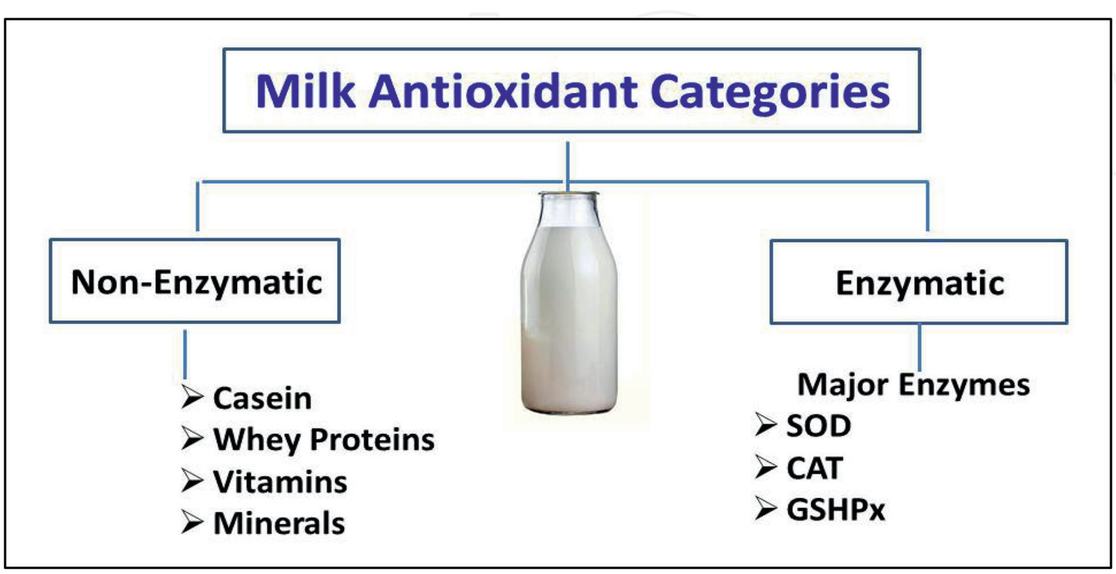

Figure 1.
The major two antioxidant categories in milk. SOD: super oxide dismutase, CAT: catalase, GSHPx: glutathione peroxidase.

Vitamins (soluble in either milk fat or milk serum) and minerals play an essential role as antioxidant factors. The antioxidant capacity of vitamins E (α-tocopherol), A, and C (ascorbic acid) as well as carotenoids is due to their ability to scavenge free radicals (mainly oxygen, hydroxyl, and peroxyl radicals), inhibit lipid peroxidation, and protect DNA from damage [17, 18]. Supplementation of milk with ascorbic acid in light-exposed milk enhanced the antioxidant capacity of milk and inhibited the degradation of riboflavin [19]. Moreover, fortification of cheddar cheese with vitamin E and selenium (Se) enhanced the oxidative stability of cheddar cheese and its shelf life [20].

Feeding strategies of dairy animals has a potential impact on levels of polyphenols, changes in amino/fatty acid composition in milk, and its overall antioxidant capacity [21]. In this respect, feeding dairy cow with carrot results in increased levels of β-carotene and α-tocopherol in milk [22]. Also, supplementation of animal feeds with fish oil and grazing improved the antioxidant capacity of cow and sheep milk, respectively [23, 24]. Recently, supplementation of grazing with tannin for dairy cow has enhanced the status of antioxidant capacity of blood plasma and cheese [25].

Enzymatic antioxidant in milk includes super oxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT). SOD safeguards cells from superoxide free radicals and lipid peroxidation [26]. Levels of SOD in cow milk range from 0.15 to 2.4 mg/L. However, the content of SOD in human milk is higher than (2.0–2.3 times) in cow milk [27]. GSHPx (Se encompassing enzyme) plays an important role in protection from lipid peroxidation [28]. Also, human milk has a higher concentration of GSHPx than caprine and cow milk [29]. A decrease in levels of selenium content and antioxidant activity could be detected with the progression of lactation [30]. Catalase (CAT: heme protein with molecular weight = 200KDa) has a dismutation effect against hydrogen peroxide [31]. The concentration of CAT in human milk is 10 times more than in cow milk, whereas the content of CAT in cow milk is approximately 1.95 U/mL [32].

3. Natural plant extracts for enhancing the antioxidant capacity of milk and its products

In recent years, there has been high focus toward the field of antioxidants and the reduction of free radicals. Milk and dairy products are essential components of human nutrition, and they are considered the carriers of several bioactive compounds that are important for a variety of biochemical and physiological functions. Milk and dairy products (yogurt and cheese), accounting for approximately 25–30% of the average human diet, are undoubtedly a rich source of compounds exhibiting antioxidant properties. Additionally, it is worth emphasizing that regular consumption of natural dairy antioxidants minimizes the risk of development of civilization diseases (e.g., cardiovascular disease, cancer, or diabetes). It also slows down the aging process in the organisms [33].

On the other hand, the consumption of natural antioxidant-rich foods improves an organism's antioxidant status by protecting it from oxidative stress and damage. Consumption of food products that are rich in natural antioxidants improves the antioxidant status of an organism through protection against oxidative stress and damage [34]. The antioxidant status of milk and dairy products can be improved with the use of natural additives in animal nutrition or at the stage of milk processing. Herbal mixtures, seeds, fruits, and waste from the fruit and vegetable industry are used most commonly [35]. Commercially, cheddar cheese was fortified by chili and red pepper by Monterey Jack Co., California, USA. Also, Khaled Khoshala for industry and trading Co., Obour city, Egypt, manufactured Egyptian soft cheese (Gebna Bida) and processed cheese fortified with green and red pepper that enhanced the shelf life of final products.

Numerous studies have tried to enhance antioxidant activities of foods by mixing them with phenolic components [36, 37]. Examples constitute the non-covalent complexes of polyphenols and proteins in foods [38, 39]. However, these types of interactions have been shown to alter the structure, function, stability, and nutritional properties of the complex [40, 41]. Though these methods are relatively cheaper, they are largely ineffective due to the reversible nature of the interactions between proteins

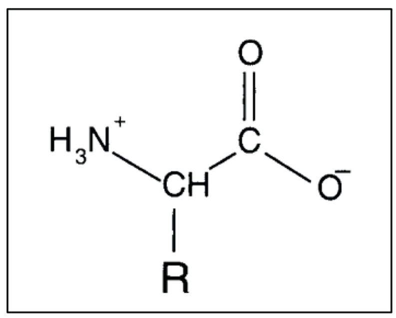

Figure 2.
Individual amino acids consist of a primary amine, a carboxylic acid group, and a unique side-chain structure (R).

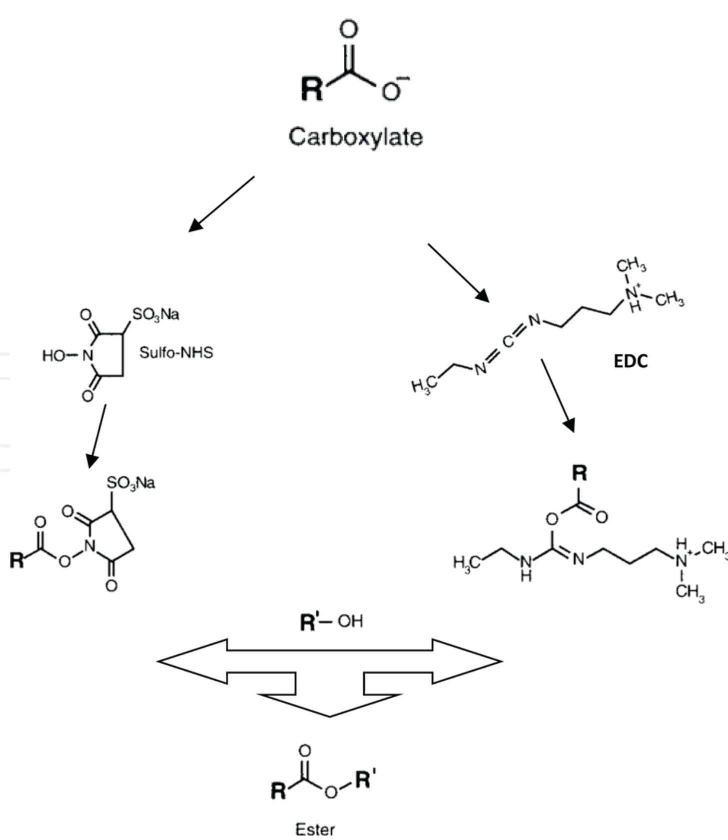

Figure 3.
Derivatives of carboxylic acids can be interacted through the use of active intermediates that react with target functional groups, NHS: N-hydroxysuccinimide; EDC: N-(3-imethylaminopropyl)-N′-ethylcarbodiimide hydrochloride.

and phenolic acids, which leads to an unstable complex for food processing conditions. Thus, covalently linking phenolic acids to proteins might be a way to generate a more stable antioxidant for food [42].

Bioconjugation involves the linking of two or more molecules to form a novel complex having the combined properties of its individual components. Natural or synthetic compounds with their individual activities can be chemically combined to produce unique substances possessing multifunctional characteristics. A protein that can bind discretely to a target molecule through the functional groups (**Figure 2**) within a complex mixture can thus be crosslinked with another detectable molecule to form a traceable conjugate. The conjugation techniques are dependent on the functional groups present on the target macromolecules to be modified. Protein molecules are the most common targets for modification with natural antioxidants such as phenolic acids (**Figure 3**) [43, 44].

4. Dairy products fortified with essential oils as antioxidant promoters

The control of free radicals, prooxidants, and oxidation intermediates is used to protect the protein and lipid components of food from oxidation [45]. In addition to oxidative damage and death of cells, tissue damage and various pathological conditions may be the consequence of oxidative stress. Deleterious changes in dairy products caused by lipid oxidation include not only flavor loss or the development of off-flavors but also color loss, nutrient value loss, and the accumulation of compounds that may be harmful to consumers' health. One of the most effective ways of reducing the lipid oxidation in dairy products is to incorporate antioxidants [46].

Free radical scavengers (FRS) inhibit lipid oxidation by reacting faster than unsaturated fatty acids with free radicals. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used to prevent lipid oxidation (BHA). However, large amounts of these synthetic ingredients have been linked to carcinogenic and cytotoxic effects. Therefore, the focus has shifted toward the use of natural antioxidants such as essential oils and phenolic acids [43]. Essential oils are liquid aromatic substance, and they are extracted from plants that have been proven to be good sources of bioactive compounds with antioxidative and antimicrobial properties. Essential oils play a high role as good free radical scavengers. Also, natural essential oils have to be given a lot of interest for enhancing overall well-being, in the prevention of diseases and in the incorporation of health-promoting substances into the diet [47]. Additionally, the use of essential oils as natural antioxidants in dairy products can reduce the rate of lipid oxidation and hydrolysis and may be beneficial in increasing the shelf life of these products [46]. Marjoram, frankincense, thyme, myrtle, lemon, oregano, and lavender essential oils are commonly used as food additives. These supplementations will move the dairy products into the functional food area as healthy dairy products.

Different essential oils extracted from plant sources such as cumin, rosemary, and thyme and their mixtures have been studied for their effect on physicochemical, microbial, rheological, and sensorial attributes of ultra-filtrated (UF)-soft cheese. The results revealed that the different essential oils had remarkable antimicrobial effect on the growth of pathogenic bacteria (i.e., *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, and *Aspergillus niger*) [48].

5. Impact of probiotication of dairy foods on enhancement of their antioxidant capacity

Probiotics are *live microorganisms, which when administered in adequate amounts confer health benefits to the host* [49]. A recent definition of probiotics by Elshaghabee [50] was probiotics are live microbial strains with health impact on host when they consumed daily with enough amounts (not less than 10^6–10^8 CFU/g) and incorporated into the gut micro-biome. The main two genera of probiotics are *Lactobacillus* (*Lb.*) and *Bifidobacterium*. Different studies had led to a renewed interest in probiotics as antioxidant agents. Isolated *Lb. fermentum* from GIT mucosa could scavenge free radicals using *in vitro* model and enhance the antioxidant status and health of pigs [51]. Probiotic yeast *Sacch. cerevisiae* DSMZ strain had higher antioxidant capacity than *Lb. casei* 01 and bifidobacteria B-12 in either viable or non-viable form [52].

A mixture of probiotic bacteria containing *Lb. acidophilus* W70, *Lb. casei* W56, *Lb. salivarius* W24, *Lactococcus lactis* W58, *Bifidobacterium* (Bif.) *bifidum* W23, and *Bif. lactis* W52 enhanced de novo synthesis of GSH under severe acute pancreatitis in a rat model [53]. Furthermore, Spyropoulos et al. [54] reviewed that several probiotic species, for example, *L. lactis* and *Lb. plantarum*, could produce SOD, resulting in a protective effect against radiation-induced enteritis and colitis. Also, some species of probiotic bacteria could produce folate, which could enhance the antioxidant capacity [55].

Feeding mice with engineered *Lb. casei* BL23-producing SOD could significantly decrease the intestinal inflammation in mice with Crohn's disease [56]. Feeding boiler with spore-forming probiotics *Bacillus coagulans* could enhance the antioxidant capacity, immunity, and gut function [57]. In a human experiment, the status of total antioxidant capacity of type 2 diabetic patients was enhanced when they received yogurt containing *Lb. acidophilus* La 5 and *Bif. lactis* Bb-12 [58]. All health benefits of spore-forming probiotics with their future prospects were reviewed by Elshaghabee et al. [59].

Gut microbiota, including probiotics, has a protective effect against pathogens by competitive exclusion [60]. Imbalance in the composition of gut microbiota resulted in increased levels of ROS and could affect redox homeostatic in the host [61]. Probiotics can regulate positively the composition of gut microbiota through different mechanisms, for example, producing a wide range of organic acids, mainly lactic and acetic. Propionic and butyric acids produced from cross feeding of lactate by other gut microbiota resulting in lowered the pH of colon and inhibiting the growth of a wide range of pathogens as well as other harmful bacteria [62, 63].

Probiotic *Lb. johnsonii* BS15 could attenuate high fat diet that induced oxidative stress and modulated the ratio of *Firmicutes*/*Bacteroidetes* in mice model [64]. Also, supplementation of probiotic ABT-fermented milk with heat-treated *Sacch. cerevisiae* could significantly enhance the antioxidant capacity of the product [65]. Recently, lased-treated *Lb. casei* had higher free radical scavenging activity than non-treated cells [66, 67].

Akkermansia (A.) muciniphila represents a new generation of probiotics; it is an intestinal mucin-degrading bacterium, and it could regulate blood pressure, and it could release the endogenous hydrogen sulfide (H2S), which has been considered a potential regulator of vascular homeostasis, possibly through the regulation of vascular tone and inflammation, antioxidant mechanism, vascular cell proliferation, and apoptosis [68]. Data in **Figure 4** conclude different possible mechanisms of antioxidant activity of different probiotic genera.

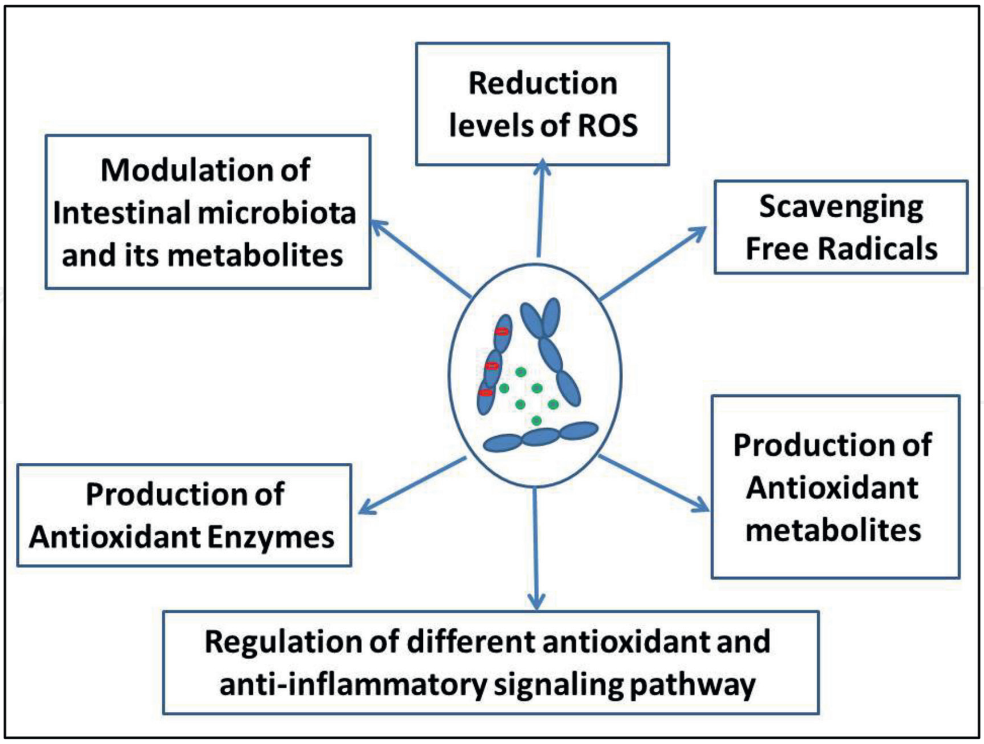

Figure 4.
Selected different possible mechanisms of probiotics as antioxidant food supplements.

6. Conclusion

In the past few years, there has been an increasing demand for natural products with antioxidant activity as well as dairy foods. Milk is the first food for mammalians. It contains different antioxidant components that cleared in this chapter. The use of different plants or herbs has been was in practice from the ancient time. Fortification of different dairy products with either plant extracts or essential oils enhanced the antioxidant capacity and quality parameters including shelf life of these products. Recently, different species of probiotics could be used also for enhancing the antioxidant capacity of fermented milks. This chapter reveals that consumers could use different methods for enhancing the antioxidant status of dairy products resulting in an enhancement the health status of consumers which serve the sustainable development goals SDG 3 (good health and wellbeing).

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