

## REVIEW



# Milk Protein–Stabilized Emulsion Delivery System and Its Application to Foods

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## Abstract

Milk proteins, such as casein and whey protein, exhibit significant potential as natural emulsifiers for the preparation and stabilization of emulsion-based delivery systems. This can be attributed to their unique functional properties, such as the amphiphilic nature, GRAS (generally recognized as safe) status, high nutritional value, and viscoelastic film-forming ability around oil droplets. In addition, milk protein has been used as a coating material in emulsion-based delivery systems to protect bioactive compounds during food processing and storage owing to its unique functional properties. These properties include the ability to bind lipophilic bioactive compounds and antioxidant activity. In this review, we present the use of milk proteins as emulsifiers for the formation of emulsions and food applications of milk protein-stabilized emulsion delivery systems.

## Keywords

milk protein, emulsion, delivery system, emulsifier, food application

## Introduction

Emulsions can be defined as the mixture of two immiscible liquids with one dispersed liquid phase in the form of fine droplets within the other [1]. Oil-in-water (O/W) emulsions are mixtures of dispersed oil phase within continuous water phase while water-in-oil (W/O) emulsions are dispersion of water phase in the continuous oil phase [1]. Milk, creams, mayonnaise, and salad dressings are typical O/W emulsions whereas butter and margarine are classified into W/O emulsions [2,3]. More recently, multiple emulsions, such as water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O) emulsions, have received much attention due to their unique properties that can solubilize both hydrophilic and lipophilic compounds in dispersed phase [4,5]. Moreover, there is a growing interest in nano-sized emulsions, such as nanoemulsions [6,7] and multiple nanoemulsions [5] for the delivery of bioactive compounds since a reduction in the droplet size of emulsions could lead to an increase in interfacial area between oil and water, which could increase the bioavailability of bioactive compounds [3,7,8].

Since emulsions are thermodynamically unstable, emulsifiers have been used to promote the formation and enhance the stability of emulsions [9]. Most emulsifiers are composed of both hydrophilic and hydrophobic moieties which make them bind at oil and water interfaces and reduce the interfacial tension stabilizing emulsions [3,9]. Although various synthetic (e.g., monoglyceride, sucrose esters, polyglycerol esters) polymers have been used as emulsifiers in food industry, there has been an increase

in demand for 'clean' label food, which can make food manufacturers use natural emulsifier for food application [2,3]. Among natural emulsifiers, milk proteins, such as casein and whey proteins, have several benefits for the formation of emulsion-based delivery system due to their amphiphilic characteristics, GRAS status, excellent nutritional value, and various functionalities [10-12].

Although there are numerous methods to form emulsion-based delivery system prepared with milk proteins, the application of milk protein-stabilized emulsion delivery systems to foods has not been extensively studied. In this review, we focused on the functional properties of milk protein as a delivery material of emulsion delivery system and their applications to foods.

## Milk Proteins as Emulsifiers

Low molecular-weight emulsifiers, such as monoglyceride, sucrose esters, and polyglycerol esters, have been widely used to form and stabilize emulsions because they have a capability to adsorb at oil and water interface rapidly and high diffusivity. Compared to low molecular-weight emulsifiers, high molecular-weight emulsifiers, typically food proteins, have lower diffusivity than low molecular-weight emulsifiers. However, food proteins have other functional properties including the ability to produce a thick film around oil droplets [1,9]. Among various food proteins, milk proteins are the most commonly used natural emulsifiers in food industry due to their amphiphilic nature, GRAS status, high nutritional value, and good sensory properties [11,12]. In addition, binding abilities of milk proteins to lipophilic bioactive compounds and ions and antioxidant activity make them suitable for the encapsulate and protect bioactive compounds efficiently [11,12]. Caseins and whey proteins are two major milk proteins and they exist in bovine milk at the ratio of 80:20 [12]. Caseins, such as  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , and  $\kappa$ -caseins, have flexible conformations due to proline-rich structure while the conformation of globular whey proteins, such as  $\alpha$ -lactalbumin ( $\alpha$ -LA),  $\beta$ -lactoglobulin ( $\beta$ -LG), bovine serum albumin (BSA), immunoglobulins and lactoferrin, are rigid [9,11-13]. Surface activity of each milk proteins are reported as following order:  $\beta$ -casein > casein micelles > BSA >  $\alpha$ -LA >  $\alpha_s$ -caseins =  $\kappa$ -casein >  $\beta$ -LG [12,14].

Table 1 summarizes the previous studies on the preparation of milk protein-stabilized emulsions. Milk proteins by alone or with other emulsifiers and polymers have been used to form and stabilize various types of emulsions, such as W/O, O/W, W/O/W, O/W/O, and oil-in-water-in-water (O/W/W), with various size including micro and nano-sized applications [5-7,15-29].

### 1. Emulsion stabilization by milk proteins

Due to the inherent thermodynamic instability of emulsions, the use of appropriate emulsifiers is needed to enhance the stability of emulsions. During formation of emulsions, milk proteins as emulsifiers adsorb to the surface of oil droplets (interfaces between oil and water) after disruption by mechanical forces (e.g., homogenization) [30,31]. The insufficient coverage of milk proteins on the oil droplet causes desta-

**Table 1.** Summary of studies on the milk protein-stabilized micro- and nanoemulsions

Milk proteins	Droplet size	Emulsion type	References
Caseins			
Caseinate	Micro-sized	O/W	[15–17]
	Nano-sized	O/W	[18]
		W/O/W	[19]
$\alpha_{s1}$ -Caseins	Micro-sized	O/W	[20,21]
$\beta$ -casein	Micro-sized	O/W	[20,21]
Whey proteins			
WPI	Micro-sized	O/W	[16,18,22,23]
		W/O/W	[24]
		O/W/O	[25]
		O/W/W	[26]
		W/O	[27]
		O/W	[28–31]
WPC	Micro-sized	O/W	[5]
	Nano-sized	W/O/W	[16]
$\beta$ -Lactoglobulin	Micro-sized	O/W	[22]
	Nano-sized	O/W	[6,7]

O/W, oil-in-water; W/O, water-in-oil; W/O/W, water-in-oil-in-water; O/W/O, oil-in-water-in-oil; O/W/W, oil-in-water-in-water; WPI, whey protein isolate; WPC, whey protein concentrate.

bilization including coalescence and/or flocculation [30,31]. On the other hand, the sufficient coverage of amphiphilic milk proteins on oil droplets leads to the reduction in surface tension and form viscoelastic film at oil and water interfaces, which makes emulsions stable over time [3,13]. Milk protein-stabilized emulsions have exhibited excellent stability against aggregation due to the long-range electrostatic repulsion and short-range steric repulsion [3,9,32]. The adsorption of milk proteins on oil and water interfaces are affected by their conformational properties, pH, ionic strength and temperature [12]. Moreover, these factors also important to determine the physical and chemical properties of emulsions including the droplet size and surface charges, which play a key role to stabilize milk protein-stabilized emulsions [13].

## 2. Antioxidant activity of milk proteins

It has been reported that milk proteins have antioxidant activity and metal chelating properties, which make them beneficial to enhance the oxidative stability of oils (e.g., fish oil [5], salmon oil [16,22], and walnut oil [27]) and lipophilic bioactive compounds (e.g., curcumin [18] and  $\omega$ -3 fatty acids [23]).  $\beta$ -LG, a major whey protein, can act as a free radical scavenger due to its antioxidant activity owing to the free sulfhydryl residues and metal chelating property [11,12,22,27,33]. Caseins are also known to chelate oxidizing metal ions [12,34]. Therefore, milk protein-stabilized emulsions are beneficial to deliver oxidation-sensitive oils and lipophilic bioactive compounds.

## 3. Barrier and protective properties of milk proteins

The use of milk proteins as delivery material is beneficial to encapsulate and protect encapsulated bioactive compounds against severe conditions encountered in food

processing and storage and gastrointestinal tract (e.g., light, temperature, pH and oxygen) due to its ability to bind various lipophilic bioactive compounds [11,12] and magnificent buffering capacity, and barrier effect [11,12,33,35]. The formation of physical barrier by milk proteins in various types of delivery systems, such as nanoparticles and nanoemulsions, could reduce the diffusion and escape of bioactive compounds [12,33,35] and efficiently protect bioactive compounds from the exposure of UV light [36] and acidic pH [37], which make them ideal as delivery materials.

## Food Application of Milk Protein–Stabilized Emulsions

There is high demand for dairy industry to enhance the application of milk protein-stabilized emulsions to dairy foods since those emulsions as a delivery system can provide several benefits to dairy foods. It has been known that the use of milk protein-stabilized emulsions can be a promising strategy to increase the protection of bioactive compounds against chemical degradation during storage and digestion [9,38]. Emulsion delivery systems also have positive effects to enhance the bioavailability of encapsulated bioactive compounds [7,11,38].

### 1. Protection of bioactive compound

Various bioactive compounds have been used to give health benefits to consumers. However, the use of lipophilic bioactive compounds, such as omega-3 fatty acids, for dairy food applications can be limited due to their poor chemical stability during food storage and gastrointestinal digestion [11]. Milk protein-stabilized emulsion contains potential benefits as a delivery system of polyunsaturated fatty acid including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Those polyunsaturated fatty acids are vulnerable to autoxidation, which can negatively affect the nutritional value and flavor. It was reported that the application of W/O/W multiple nanoemulsion contributed to a decrease in the autoxidation of fish oil [5]. In this research, whey protein was applied as a surface coating component in outer water phase of multiple emulsion. A decrease in the autoxidation of fish oil incorporated in the oil phase of multiple nanoemulsion could be attributed to the antioxidant activity of whey protein as a free radical scavenger and barrier effect at oil and water interfacial area in emulsion [5].

Dairy food fortification with other lipophilic bioactive compounds, such as resveratrol and coenzyme Q<sub>10</sub>, can be hard due to their poor aqueous solubility. Using O/W conventional emulsion can be a useful way to enhance the solubility and food applicability of lipophilic bioactive compounds since those lipophilic bioactive compounds are directly incorporated into liquid dairy food, such as milk [6,39]. However, those conventional emulsions with a size of  $> \sim 1 \mu\text{m}$  are often exposed to physical instability under food processing and storage because of their inherent thermodynamic instability [39]. Because a reduction in the droplet size of emulsion results in an increase in physical stability, nanoemulsion delivery system, which has a submicron size ( $< \sim 200 \text{ nm}$ ) of droplet, contains much higher physical stability against coalescence and



flocculation during food storage compared with conventional emulsion delivery system [5,8]. It was reported that nanoemulsion prepared with milk protein as a protective material exhibited excellent physical stability as well as chemical stability during food storage [5,11,38]. In those studies, milk proteins could act as emulsifiers, which can adsorb on oil and water interface and form thick film enhancing emulsion stability while the antioxidant ability of milk protein enhanced oxidative stability [5,11,38].

## 2. Enhancing the bioaccessibility of lipophilic bioactive compounds

Since several lipophilic bioactive compounds including coenzyme Q<sub>10</sub>,  $\beta$ -carotene, and resveratrol have limited aqueous solubility, permeability, and high molecular weight, they contain poor bioaccessibility [7,40]. It is a hurdle that dairy food manufacturers could overcome when they use those lipophilic bioactive compounds for food formulation. This poor bioaccessibility issue can be resolved by solubilizing lipophilic bioactive compounds in emulsion-based delivery system. The use of nanoemulsion delivery system, which has smaller droplet size (<~200 nm) than that of conventional emulsion, led to increased bioaccessibility of lipophilic bioactive compounds [7,18,41]. It was reported that a significant ( $p < 0.010$ ) negative correlation with  $r$  value of  $-0.76$  was observed between the droplet size of emulsion and bioaccessibility of lipophilic bioactive compound, such as coenzyme Q<sub>10</sub>. As the droplet size of emulsion was decreased, the available surface region of oil droplet where pancreatic lipase can hydrolyze was increased. It can enhance the lipid digestion and production of more absorbable forms of lipophilic bioactive compounds leading to increased bioaccessibility [7,18].

## Conclusion

Milk proteins including caseins and whey proteins can be utilized as natural emulsifiers to form and stabilize emulsion-based delivery system because of their excellent emulsifying activity because of the amphiphilic structure and an ability to form viscoelastic film on oil and water interfaces. Moreover, the antioxidant activity and barrier and protective properties of milk proteins make them highly beneficial to efficiently encapsulate, protect, and enhance the bioaccessibility of lipophilic bioactive compounds. Although milk protein-stabilized emulsions with various types and size were successfully produced, there are major challenges ahead for food applications. Future studies should focus on evaluating the application of milk protein-stabilized emulsions to various types of foods, since the complicated networks of foods make it difficult to predict the sensory attributes of foods containing milk protein-stabilized emulsion, such as color, flavor, texture, and taste. Therefore, understanding of interactions between milk protein-stabilized emulsion and food components are needed.

## Conflict of Interest

The authors declare no potential conflict of interest.

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