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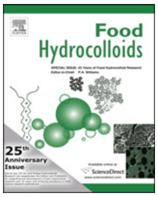

# Aspects of milk-protein-stabilised emulsions

Harjinder Singh

*Riddet Institute, Massey University, Private Bag 11222, Palmerston North, New Zealand*

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Milk proteins are widely used as ingredients in prepared foods, in which they perform a wide range of key functions, including emulsification, thickening, gelling and foaming. An important functionality of milk proteins in food colloids is their ability to facilitate the formation and stabilisation of oil droplets in emulsions. The ability of milk proteins to adsorb at the oil–water interface and to stabilise emulsions has been exploited by the food industry in the manufacture of nutritional products, specialised medical foods, dietary formulations, cream liqueurs and dairy desserts. This article provides an overview of the properties and functionalities of food emulsions formed with milk proteins, focusing on the structure and composition of adsorbed protein layers, competition between proteins and the physical and chemical stability of emulsion droplets. Of particular importance is the understanding of the behaviour of milk-protein-based emulsions under the conditions relevant to digestion in the human gastrointestinal tract. Recent relevant research in this area is reviewed and discussed.

## 1. Introduction

There are two main classes of milk proteins: caseins and whey proteins. Caseins can be fractionated into four distinct proteins, $\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-caseins, all of which are phosphoproteins (Fox, 2009). The caseins are very flexible molecules and have been referred to as rheomorphic (Holt & Sawyer, 1993); the most unusual feature is the amphiphilicity of their primary structure. The hydrophobic residues and many of the charged residues, particularly the phosphoserine residues, in the caseins are not uniformly distributed along the polypeptide chain. Because the casein monomers cannot sufficiently remove their hydrophobic surfaces from contact with water, the caseins tend to associate with themselves and with each other. In addition, all caseins are able to bind calcium, with the extent of binding being proportional to the number of phosphoserine residues in the molecule. $\alpha_{s1}$-Casein and $\alpha_{s2}$-casein are most sensitive to calcium followed by β-casein, whereas κ-casein is insensitive to calcium. κ-Casein is capable of stabilising other caseins against calcium-induced precipitation and allows the formation of colloidal sized aggregates (Horne, 1998, 2003).

In normal milk, these caseins exist mainly as colloidal particles, called casein micelles, with diameters ranging from 80 to 300 nm (average ~150 nm). In addition to caseins, the micelles also contain small amounts of calcium, phosphate, magnesium and citrate, commonly referred to as micellar calcium phosphate (see Fox, 2009). Most of the κ-casein is present on the surface of casein micelles, and the hydrophilic C-terminal part of κ-casein protrudes from the micelle surface into the surrounding solvent, giving it a 'hairy' appearance. The highly charged flexible 'hairs' physically prevent the approach and interactions of hydrophobic regions of the casein molecules (Horne, 1998).

Whey proteins can be fractionated into β-lactoglobulin, bovine serum albumin, α-lactalbumin and immunoglobulins. β-Lactoglobulin represents about 50% of the total whey protein in bovine milk, has a molecular weight of 18,000 Da and contains two disulphide bonds and a single free thiol group. In contrast to caseins, the whey proteins possess high levels of secondary, tertiary and, in most cases, quaternary structures. For instance, β-lactoglobulin is built up of two β-sheets, formed from nine strands converging at one end to form a hydrophobic calyx or pocket, and a flanking three-turn α-helix (Edwards, Creamer, & Jameson, 2009; Kinsella & Whitehead, 1989).

Because of their nutritional importance and physico-chemical properties, milk proteins are used in a wide range of prepared foods. Various types of caseins and caseinates, whey-protein concentrates and isolates, milk-protein concentrates and isolates, hydrolysed proteins and milk-protein fractions are manufactured by the dairy industry (Mulvihill & Fox, 1989). These products have applications in dairy products, meat products, beverages, baked products and infant foods. The important functional properties of protein products include water binding, emulsification, foaming and whipping, gelation and nutritional properties (Singh, 2010). In this chapter, only emulsification aspects are considered.

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*E-mail address:* h.singh@massey.ac.nz.

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Emulsion-type products, e.g. coffee whiteners, whipped toppings, cream liqueurs, dietary formulatons, liquid nutritional products and medical foods, are an important application of caseinates and whey proteins in the food industry. The basis for the emulsifying ability of milk proteins is the amphiphilicity of their primary structure, which determines their ability to adsorb to the oil–water interface. Major advances have been made in understanding the adsorption process, the composition and structure of adsorbed layers of proteins and how they influence the physical and chemical properties of emulsions (Dickinson, 1998, 1999, 2008; McClements, 1999; Singh & Ye, 2009). In recent years, there has been considerable research activity on understanding how the adsorbed layers and the physical structures of food emulsions influence the rates of lipid digestion (Golding & Wooster, 2010; Le Révérend, Norton, Cox, & Spyropoulos, 2010; McClements, Decker, & Park, 2009; Singh, Ye, & Horne, 2009). This research activity is aimed at developing novel foods that regulate calorie intake, control satiety responses, provide controlled lipid digestion and/or deliver bioactive molecules.

This chapter provides an overview of the properties of emulsions formed with milk proteins, and discusses latest advances in understanding and controlling the behaviour of milk-protein-based emulsions under physiological conditions.

## 2. Properties of milk-protein-stabilised emulsions

Extensive studies have been carried out on purified milk proteins, in particular β-casein and β-lactoglobulin. Two major caseins, $\alpha_{s1}$-casein and β-casein, are distinctly amphiphilic and have strong tendencies to adsorb at oil–water interfaces and stabilise oil-in-water emulsions. β-Casein has been shown to adsorb with an extensive hydrophobic region anchored directly at the surface and a hydrophilic region (40–50 residues at the N-terminus) protruding extensively into the aqueous phase. For $\alpha_{s1}$-casein, a loop-like conformation has been predicted as it does not have such a pronounced inequality in the distribution of hydrophobic and hydrophilic residues in its primary structure (Dickinson, 1992). It has been suggested that $\alpha_{s1}$-casein adsorbs to the oil–water interface via peptides towards the middle of its sequence, rather than at the end, as in β-casein, and it may be this that causes the protein to form a thinner adsorbed layer than does β-casein (Dalgleish, 1996; Dickinson, 1992). Because of its relatively higher surface activity, β-casein can displace $\alpha_{s1}$-casein from the oil–water interface (Dickinson, Rolfe, & Dalgleish, 1988; Dickinson & Stainsby, 1988).

Because of its amphiphilic nature, β-lactoglobulin readily adsorbs at the oil–water interface, where it partially unfolds. In contrast to caseins, a closely packed, dense and rather thin (2–3 nm at neutral pH) adsorbed layer of β-lactoglobulin is formed (Dalgleish, 1996). Additionally, the partial unfolding of the globular whey-protein structure following adsorption causes exposure of the reactive sulphydryl group, leading to slow polymerisation of the adsorbed protein in the aged adsorbed layer via sulphydryl-disulphide interchange (Dickinson & Matsumura, 1991; McClements, Monahan, & Kinsella, 1993).

Milk proteins manufactured by the dairy industry are complex mixtures of individual proteins. Moreover, the processes of the isolation and conversion into protein powder modify the native protein structures and consequently influence their functional properties, including emulsification. The most commonly used commercial milk-protein product, i.e. sodium caseinate, is made using the following procedure. Casein is extracted from skim milk via isoelectric precipitation by the addition of mineral acid. The casein curd is then heated to 50–55 °C, washed with water, followed by a dewatering process to reduce the moisture content to 50–60%. Drying of casein curd is most commonly carried out on horizontal vibrating fluid bed driers. Sodium caseinate is usually prepared by solubilising acid casein with NaOH by increasing the pH to 6.7–7.0 to produce a solution of about 23% solids. This solution is then spray dried. In calcium caseinate manufacture, a similar process is used except that $Ca(OH)_2$ is used to neutralise the acid casein curd. The type of commercial heating equipment and the drying conditions affect the extent of protein–protein interactions, resulting in different levels of aggregation in caseinate products. For instance, it has been shown that casein molecules in aqueous dispersions of commercial sodium caseinate exist as a polydisperse mixture of monomers, complexes and aggregates (Lucey, Srinivasan, Singh, & Munro, 2000).

The most important commercial whey-protein products are whey-protein concentrates (WPCs) (up to about 85% protein) and whey-protein isolates (WPIs) (approximately 95% protein). The manufacture of whey-protein products involves combinations of several processes such as ultrafiltration, diafiltration, ion exchange, evaporation and drying (see Singh, 2010). Depending on the process used, whey-protein products tend to contain various levels of lipids, lactose and minerals, as well as different extents of whey-protein denaturation and aggregation. These variations in composition and denaturation level have a major influence on the emulsifying and other functional properties of whey proteins.

Because of their importance in food emulsion applications, several studies have been carried out to understand the adsorption behaviour of caseinates and whey proteins in model oil-in-water emulsions and its relationship to emulsion stability. Both sodium caseinate and whey proteins (particularly WPI) show excellent emulsifying ability; generally a protein surface coverage of 1–2 mg/$m^2$ is sufficient is produce a fine stable emulsion (Singh, 2005).

The composition of the interfacial layer is determined by the quantities and structures of the proteins present at the moment the emulsion is formed (Dalgleish, 1997), although rapid exchanges between adsorbed protein and unadsorbed protein could occur after emulsion formation. Emulsions formed with sodium caseinate show that the casein composition of the adsorbed layer is different from that of the original material. When the ratio of protein to oil is very low (about 1:60), β-casein is preferentially adsorbed at the emulsion droplet surface but, when the total amount of protein is greatly in excess of the amount needed for full surface coverage, $\alpha_{s1}$-casein is adsorbed in preference to the other caseins. At all concentrations, κ-casein from sodium caseinate appears to be less readily adsorbed (Srinivasan, Singh, & Munro, 1999a, 1999b).

Addition of $CaCl_2$ at above a certain critical concentration to a sodium-caseinate solution before homogenisation increases the surface protein coverage and alters the casein composition of the adsorbed layer; the adsorption of $\alpha_{s1}$-casein at the droplet surface is markedly enhanced whereas the adsorption of β-casein is hardly affected (Ye & Singh, 2001). The effects of calcium and protein concentrations on the composition of the adsorbed layer reflect the state of aggregation of the casein molecules in a sodium-caseinate solution prior to homogenisation. The binding of calcium to the phosphoserine residues of caseins reduces electrostatic repulsions between the protein molecules and increases the potential for intermolecular associations. Similarly, the possibility of interaction between casein aggregates/complexes becomes more pronounced at higher caseinate concentrations. Therefore, under a given set of homogenisation conditions, the composition of the adsorbed layer in emulsions formed with sodium caseinate is determined by the surface activities and flexibilities of the casein aggregates and complexes that exist at the time of emulsification. The underlying physics of the self-association of caseins in complex systems, such as sodium or calcium caseinate, under different environmental conditions (particularly at high concentrations) is not well understood.