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**INFLUENCE OF CALCIUM CHLORIDE ADDITION  
ON THE PROPERTIES OF EMULSIONS FORMED  
WITH MILK PROTEIN PRODUCTS**



**MASSEY  
UNIVERSITY**

**A THESIS PRESENTED IN PARTIAL FULFILMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
MASTER OF TECHNOLOGY**

**BY**

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**DEDICATION**

**TO MY PARENTS**

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**ABSTRACT**

The objective of this study was to investigate the effects of added  $\text{CaCl}_2$  on (i) the adsorption behaviour of caseinate and whey protein concentrate (WPC) at the oil-water interfaces and (ii) the stability of emulsions formed with caseinate or WPC. The relationship between aggregation state of protein, due to  $\text{Ca}^{2+}$  binding, and emulsifying properties is discussed. The effects of addition of NaCl to the emulsions containing various concentrations of  $\text{CaCl}_2$  were also explored.

Protein solutions and 30% soya oil, at pH 7.0, were mixed and homogenized at 207/34 bar and 55°C to form emulsions.  $\text{CaCl}_2$  was added to protein solutions prior to emulsion formation or to the emulsions after they were made. The average particle size ( $d_{32}$  or  $d_{43}$ ), the surface protein concentration, the composition of protein adsorbed layer at the interface and the creaming stability of emulsions were determined. The microstructure of emulsions was observed using the confocal laser microscopy.

The droplet sizes of emulsions made with sodium caseinate or WPC were similar and were independent of the protein concentration at concentration above 0.5%. The surface protein concentration of emulsions made with sodium caseinate, WPC or calcium caseinate generally increased with increase in the protein concentration, although the trends were different. The emulsions made with calcium caseinate had higher  $d_{32}$  and surface protein concentration than that of sodium caseinate or WPC.

In emulsions made with sodium caseinate at low protein concentrations, the adsorption of  $\beta$ -casein occurred in preference to  $\alpha_s$ -casein, whereas  $\alpha_s$ - ( $\alpha_{s1}$ - +  $\alpha_{s2}$ -) casein was found to adsorb in preference to  $\beta$ -casein at high protein concentrations. In calcium caseinate emulsions, the  $\alpha_s$ -casein was adsorbed in preference to  $\beta$ -casein at all concentrations. In emulsions made with WPC,  $\beta$ -lactoglobulin adsorbed slightly in preference to  $\alpha$ -lactalbumin. In emulsions made with mixtures of sodium caseinate and WPC (1:1), the adsorption of whey

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proteins occurred in preference to caseins at low concentrations (< 3%), whereas caseins were adsorbed in preference to whey protein at high concentrations.

In emulsions made with calcium caseinate or WPC, the creaming stability of emulsions followed mainly the changes in particle size of emulsions. However, the creaming stability of emulsions made with sodium caseinate decreased markedly as the caseinate concentrations were increased above 2.0%. This was attributed to depletion flocculation occurring in these emulsions. Whey proteins did not retard this instability, due to depletion flocculation, in emulsions made with mixtures of caseinate and WPC

When  $\text{CaCl}_2$  was added prior to or after emulsion formation, the  $d_{43}$  and surface protein concentration increased with increasing  $\text{CaCl}_2$  concentration in emulsions made with 0.5 and 3.0% sodium caseinate. The adsorption of  $\alpha_s$ -casein increased with increase in the concentration of  $\text{CaCl}_2$ , with a corresponding decrease in the adsorption of  $\beta$ -casein. The creaming stability of emulsions made with 0.5% caseinate decreased with increasing  $\text{CaCl}_2$  concentration. However, the creaming stability increased with  $\text{CaCl}_2$  concentration in 3.0% caseinate emulsion. The destabilising effects of  $\text{CaCl}_2$  in emulsions made with sodium caseinate were reduced by the addition of 200 mM NaCl.

Addition of  $\text{CaCl}_2$  to protein solutions prior to emulsion formation increased the  $d_{43}$  and surface protein concentration in emulsions made with 0.5 or 3.0% WPC. In this case, the adsorption of  $\beta$ -lactoglobulin occurred slightly in preference to  $\alpha$ -lactalbumin. The creaming stability of emulsions decreased with increase in the concentration of  $\text{CaCl}_2$ . The addition of  $\text{CaCl}_2$  to emulsions after emulsion formation also resulted in increases in  $d_{43}$  and surface protein concentration of emulsions made with 0.5% WPC and formation of gel-like network structure at high  $\text{CaCl}_2$  concentrations. However, the stability of emulsion made with 3.0% WPC was not affected by the addition of  $\text{CaCl}_2$ .

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Different aggregation mechanisms are involved depending upon whether  $\text{Ca}^{2+}$  is added to protein solution before emulsification or to the emulsion after its formation. Addition of  $\text{Ca}^{2+}$  to protein solution may lead to decrease in emulsifying capacity and subsequently result in protein bridging flocculation between emulsion droplets.  $\text{Ca}^{2+}$  bridging flocculation between emulsion droplets may be formed in emulsions that have  $\text{Ca}^{2+}$  added. The change in aggregation state of caseinate due to  $\text{Ca}^{2+}$  binding could retard the instability of emulsion due to depletion flocculation. The protein unfolding at the surface of emulsions made with low whey protein concentrations may promote the protein-Ca-protein bridges forming between protein-coated emulsion droplets, consequently forming gel-like network structure in emulsions.

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## Chapter 1

### INTRODUCTION

Milk proteins are well known surfactants and hence are used as ingredients in a wide range of formulated food systems (Mulvihill, 1992). They stabilize emulsions and foams because they are amphiphiles. They adsorb onto the oil-water interface and stabilize emulsions by a combination of charge repulsion and steric stabilization (Dickinson and Stainsby, 1982). The stability and rheological properties of emulsions stabilized by milk proteins are largely determined by the interactions between the droplets. The nature and strength of the interactions between droplets are dependent on the structure and composition of adsorbed layer at the oil-water interface (Dickinson, 1998).

In an aqueous environment, the proteins are held in their conformations by a number of different interactions, such as hydrophobic interactions, hydrogen bonding, ion-pair interactions (attractive and repulsive), coordination of metal ions, and van der Waals interactions (Dalgleish and Hunt, 1995). The major proteins in milk, the caseins ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -), are generally considered to have disordered and flexible structures. Sodium caseinate, a widely used food ingredient produced from milk casein, exists in aqueous solution at neutral pH as a mixture of casein monomers (Mulvihill, 1992) and small casein aggregates (so-called 'sub-micelles') (Pepper and Farrell, 1982). In contrast to the caseins, there are the whey proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, bovine serum albumin and immunoglobulins), which are characterized by well defined three-dimensional structures held together by disulfide bridges; these proteins are much more rigid than the caseins (Kinsella, 1984).

The milk proteins whether individual molecules or in the form of aggregates becomes rapidly adsorbed at the new oil/water interface during emulsification (Walstra and Smulders, 1997). However, the state of protein in bulk solution does influence its adsorption behaviour and composition of protein interfacial

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layer, which subsequently influences the stability of emulsions (Dalglish, 1995, Srinivasan *et al.*, 1996).

Milk proteins exist in bulk solution as aggregates in many situations, for example 'casein micelles', aggregated caseins in sodium caseinate after heating, in the presence of  $\text{Ca}^{2+}$ , high ionic strength and pH close to pl (Dalglish and Hunt, 1995). However, they have received comparatively little research attention relative to other model emulsions. There are still many gaps in our understanding of the adsorption behaviour of aggregated proteins at the oil/water interface and their influence on stability of emulsions.

The binding of calcium ions to milk proteins have been studied under a variety of environmental conditions (Dalglish and Parker, 1980; Parker and Dalglish, 1981; Pappas and Rothwell, 1991). The binding of  $\text{Ca}^{2+}$  to protein reduces the electrostatic repulsion and interaction of the hydrophobic domains leads to formation of large aggregates (Swaisgood, 1992). This property will inevitably influence the adsorption behaviour of milk protein at the oil/water interface and the stability of emulsions formed by milk proteins.

Little information is available on the influence of  $\text{Ca}^{2+}$  on the adsorption behaviour of milk proteins at the oil/water interface and stability of milk protein-stabilized emulsions. No previous work has been reported on the effects of  $\text{Ca}^{2+}$  on the composition of protein interfacial layer and creaming stability of emulsions stabilized by milk proteins. Srinivasan *et al.* (1996) reported that addition of  $\text{Ca}^{2+}$  to sodium caseinate solution prior to emulsion formation caused the increases in the droplet size and the surface protein concentration of emulsions. Mulvihill and Murphy (1991) reported that highly-aggregated calcium caseinate had higher protein load at the interface of emulsion droplets. When  $\text{Ca}^{2+}$  is added to emulsions, the association of  $\text{Ca}^{2+}$  with adsorbed protein between the protein-coated oil droplets also results in larger particle size due to the aggregation of emulsion droplets (Dickinson *et al.*, 1992; Agboola and Dalglish, 1995).

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The main objective of this study was to investigate the effects of addition of  $\text{CaCl}_2$  to the protein solution prior to making emulsions or to emulsions made in the absence of  $\text{Ca}^{2+}$  on the adsorption behaviour of proteins in oil-water emulsions. The effects on the stability of emulsions made with sodium caseinate (Chapter 5) or WPC (Chapter 6) were also studied. The relationship between aggregation state of protein, due to  $\text{Ca}^{2+}$  binding, and emulsifying properties is discussed. In addition, It is known that ionic strength influences the binding of  $\text{Ca}^{2+}$  to milk proteins (Dalgleish and Parker, 1980; Parker and Dalgleish, 1981; Baomy and Brule, 1988). Therefore, the effects of addition of  $\text{NaCl}$  to the emulsions containing various concentrations of  $\text{Ca}^{2+}$  were also explored. The emulsifying properties of sodium caseinate, WPC and calcium caseinate as well as of the binary protein mixtures are discussed in Chapter 4. The confocal microscopy was used to observe the microstructure of emulsions for examining the relationship between structure and creaming stability of emulsions.

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