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EMULSIFYING PROPERTIES OF SODIUM CASEINATE

A THESIS
PRESENTED IN PARTIAL FULFILMENT OF THE REQUREMENTS
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BY

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DEDICATION

TO MY BROTHERS

ABSTRACT

The main objectives of this study were to determine the influence of compositional and processing parameters on: (i) the protein surface coverage and protein surface composition and (ii) the creaming stability, for emulsions stabilized by sodium caseinate.

Emulsions were usually prepared from 2.5% (w/w) protein solution and 30% soya oil. In some cases, emulsions were made with varying concentrations of caseinate or soya oil. The mixture was usually homogenized at 102/34 bar at 55°C and in some cases the mixture was homogenized at varying pressures.

Surface coverage of protein in freshly prepared emulsions was determined from analysis of the aqueous phase, using Kjeldahl. SDS-PAGE was used to identify the unadsorbed protein components in the aqueous phase.

As the concentration of caseinate was increased from 0.5 to 7.5% (w/w) the protein load increased; the protein load attained a plateau value of 1.3 mg/m^2 when the caseinate concentration was in the range 2 - 4% (w/w). Further increases in caseinate concentration markedly increased the protein load with a value of 3.55 mg/m^2 at 7.5% caseinate concentration. At low concentrations of caseinate (below 2%), β-casein adsorbed at the surface of oil droplets in preference to other caseins while at higher concentrations of caseinate, no distinct preference of any caseins was observed.

As the fat concentration was increased from 5 to 20% (w/w), the protein load decreased from ~ 9.9 to 3.7 mg/m^2, but further increases in fat concentration caused only slight decreases in the protein load. At high fat concentration (50%) β-casein was adsorbed in preference to other caseins.

As the homogenization pressure was increased from 34 to 340 bars, the protein load decreased from ~ 2.2 mg/m^2 to ~ 1.5 mg/m^2. β-Casein was preferentially adsorbed at the surface in emulsions homogenized at pressures above 204 Bar.

Variations in the pH of sodium caseinate solution prior to emulsification from 6.0 to 8.5 caused a slight decrease in the protein load (from ~ 1.8 to 1.6 mg/m^2). However, the sodium caseinate solutions adjusted to pH 2.0 or 3.0 prior to emulsification showed considerably greater protein loads (~ 2.7 mg/m^2). There was no preferential adsorption of any of the caseins in different pH emulsions.

Addition of calcium chloride to sodium caseinate solutions above 0.08% w/w, resulted in large casein particles/aggregates which subsequently adsorbed on to the oil surface resulting in higher protein loads (~ 5.8 mg/m^2). Addition of calcium chloride increased the adsorption of α_s-casein at the interface.

The protein loads of different emulsions prepared from sodium caseinate manufactured under different processing conditions deceased in the order of freeze-dried laboratory made sodium caseinate (~ 2.1 mg/m^2) > sodium caseinates made under mild manufacturing conditions (~ 1.4 mg/m^2) > sodium caseinate manufactured under normal conditions (~ 1.2 mg/m^2) = freeze dried sodium caseinate manufactured under severe heat treatments (~ 1.2 mg/m^2) = spray dried sodium caseinate manufactured under severe heat treatment (~ 1.2 mg/m^2) = commercially made sodium caseinate (~ 1.2 mg/m^2). β-Casein was preferentially adsorbed in freeze-dried laboratory made sodium caseinates and sodium caseinate made under mild manufacturing conditions. There was no significant preferential adsorption

for the other sodium caseinates.

A stability tube was designed to study the extent of creaming under gravity at 20°C for 24 hours in these sodium caseinate-stabilized emulsions. The results were expressed as stability rating, defined as per cent change in fat in lower aqueous phase after creaming.

In general, the stability rating increased with an increase in caseinate concentration. Emulsions containing caseinate concentrations of 4 and 5% (w/w) showed little fat separation under these conditions. The stability rating also increased with an increase in fat concentration in the emulsions indicating that high fat emulsions were more stable than emulsions containing low fat concentrations. As expected the stability rating increased with increase in homogenization pressure (i.e., decrease in oil droplet diameter). Emulsions prepared from sodium caseinate solutions, adjusted to pH 2.0 and 3.0, were found to be more stable than those prepared at pH 6.0, 7.0 and 8.5. Addition of calcium chloride to the sodium caseinate solution at 0.02 and 0.04% (w/w) had no effect on the stability rating of emulsions, but further additions of calcium chloride caused a marked increase in stability rating with no visible fat separation. Variations in the processing conditions (i.e. pasteurization temperatures, cooking temperatures, washing temperatures) during the manufacture of sodium caseinate had no significant effect on the stability of emulsions.

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INTRODUCTION

Commercial milk protein products represent an ever-increasing source of functional ingredients for use by the food industry. For example, annual world casein and caseinate production is estimated at about 250,000 tones. New Zealand exports 50,000 to 80,000 tonnes of casein and caseinate annually and about 75% of this amount is used in formulated food products. Caseinates and caseins provide fat emulsification in coffee creamers, whipped toppings, soups and meat emulsions; foam expansion and stabilization in whipped topping; emulsification and control of melting in cheese analogs; and contribute to viscosity in various food products. Emulsification is an important functional property of the product for about 50% of the casein and caseinates produced in New Zealand.

It is well known that caseinates possess excellent emulsifying properties. Various studies have been reported on the adsorption behaviour of sodium caseinate at oil/water interfaces (Tornberg,1978b, Robson and Dalgleish, 1987, Hunt and Dalgleish, 1994) and its ability to stabilize emulsions (Tornberg, 1978a). Caseinate is a composite of four different proteins (α_{s1}-, α_{s2}-, β- and κ-caseins, in weight proportions of approximately 4:1:4:1) which are co-precipitated at pH 4.6. During emulsion formation, all of the casein types are adsorbed at the interface and decrease the interfacial tension. β-Casein adsorbs most rapidly and causes the greatest decrease in surface tension. It is also the most hydrophobic of the caseins (Bigelow, 1967). This provides stability to the resultant emulsion with respect to coalescence and flocculation. Previous studies have concentrated mainly on the adsorption of pure caseins and mixtures of isolated proteins (Benjamins *et al.* 1975; Dickinson *et al.* 1988a,b; Hunt and Dalgleish, 1994). Studies on the adsorption behaviour of

sodium caseinate, especially commercially produced, are limited. The influence of various compositional parameters, eg. fat concentration, protein concentration, homogenization pressure etc. has not been fully explored. The influence of various parameters during caseinate manufacture (eg. pasteurization temperature, cooking temperature, washing temperature) on the adsorption behaviour of sodium caseinate-stabilized emulsions has not been reported.

Emulsion stability is another important aspect of emulsifying properties. Food emulsions comprise a wide variety of products where either fat or water or both are dispersed in a state of macroscopic homogeneity (Friberg, 1976). Because the contact between fat and water is energetically unfavourable, emulsions are thermodynamically unstable, and stabilizing agents are necessary to improve the shelf life (Das and Kinsell, 1989). Emulsion instability can appear visually as creaming or fat separation or a mixture of both. Various studies have been reported on the creaming behaviour of caseinate-stabilized emulsions (Leman *et al.* 1988; Halling, 1981; Acton and Saffle, 1971; Walstra, 1975; Walstra and Oortwijn, 1975). Very few studies have been reported on the creaming stability of sodium caseinate stabilized emulsions, as influenced by compositional parameters (eg. protein concentration, fat concentration, pH) and no studies to date have reported on the influence of method of caseinate manufacture (eg. pasteurization temperature, cooking temperature, washing temperature).

CHAPTER 2.

LITERATURE REVIEW

2.1 General characteristics of milk proteins

Because the native conformation of proteins greatly influences their functional behaviour, a detailed knowledge of the inherent structural features of proteins is required in order to understand their functional properties. Bovine milk contains ~ 3.5% milk protein which, apparently like the proteins of all other milks, falls into two categories: caseins and whey proteins; the caseins represent ~ 80% of the total proteins.

Several reviews covering aspects of the chemistry of milk proteins have been published (Schmidt, 1980; Swaisgood, 1982; Whitney, 1988; Fox, 1989; Swaisgood, 1992).

2.2 Caseins

Casein, the major protein of milk, isolated from skimmed cows milk by a variety of commercial processes, finds specialized use in edible foods, animal feed and certain non-food technical applications. The casein protein, which comprises approximately 3% of fluid cows milk, may be distinguished from the so called "whey proteins" (0.6%) by their insolubility and tendency to precipitate and coagulate at the isoelectric point (pH 4.6).

Bovine casein consists of four distinct proteins: α_{s1}-, α_{s2}-, β and κ-caseins in the approximate proportions 38%, 10%, 36% and 13% (Davies and Law, 1980; Barry and Donnelly, 1980, 1981). In addition to these four caseins, which are gene products, bovine milk contains several derived caseins, known as γ-caseins, resulting from the action of indigenous milk proteinases, especially plasmin, on the main caseins.

Table 2.1: Properties of caseins.

(Adapted from Walstra and Jenness, 1984).

Property	Caseins			
	α_{s1}-B	α_{s2}-A	β-A	κ-B
Molecular weight (Da)	23,614	25,230	23,983	19,023
Residues/Molecule				
Amino acids	199	207	209	169
Proline	17	10	35	20
Cysteine	0	2	0	2
Disulphide	0	1	0	1
Phosphoserine	8	11	5	1
Isoionic point	4.96	5.19	5.19	5.43
Hydrophobicity (kJ/residue)	4.9	4.7	5.6	5.1

Accurate compositional data are now available for all the caseins (Swaisgood, 1992). Physical-chemical characteristics of the caseins are summarized in Table 2.1.

The primary structures of the four principal caseins have been established (Swaisgood, 1992). A number of interesting and technologically important features are apparent from the primary structures:

1. The four caseins contain high levels of proline: α_{s1}-, α_{s2}-, β and κ- contain 17, 10, 35 and 20 moles proline/mole respectively, compared with 8 and 2 for β-lactoglobulin and α-lactalbumin. The proline residues are fairly uniformly distributed throughout the polypeptide which creates a stable poly-L-proline-type helix but which precludes long segments of α-helical or β-sheet

structures. Thus, the caseins have a relatively open, "denatured" structure which renders them readily hydrolyzable by proteinases, which may be advantageous or disadvantageous, and also allows them to spread readily at interfaces.

2. The caseins are relatively hydrophobic proteins: α_{s1}-, α_{s2}-, β- and κ- caseins have hydrophobicities on the Bigelow scale of 4.89, 4.64, 5.58 and 5.12 kJ/residue, respectively. However, the hydrophobic residues are not uniformly distributed along the polypeptides.

3. Many of the polar residues in the caseins are clustered: this is particularly marked for the phosphoseryl residues, the clustering of which is due to the sequence adjacent to the seryl residue required to catalyse phosphorylation, i.e. an acid residue (glutamic and aspartic) second next to the serine toward the carboxyl teriminal (Mepham *et al.* 1982).

Thus, all four caseins have a distinctly amphipathic character with separate hydrophilic and hydrophobic domains, which renders them particularly well structured to function as emulsifiers at lipid/water or air/water interfaces. α_{s1}- and β-Caseins have no cysteine or cystine residues, while α_{s2}- and κ-caseins contain 2 cysteines which appear to form intermolecular rather than intramolecular disulfide bonds giving rise to variably-sized, covalently linked polymers.

Caseins are prone to association due to their high hydrophobicity and peculiar charge distribution. The major interactions involved in these processes are of an electrostatic and hydrophobic nature. A delicate balance between these forces, depending on experimental conditions, such as pH,

temperature and ionic strength, determines the type and extent of association.

All casein components are able to bind different divalent metal ions. Because of its relevance to structure and stability of casein micelles, the binding of calcium to the different casein components has been studied extensively (Dickson and Perkins, 1971; Waugh *et al.* 1971; Dalgleish and Parker, 1980; Ono *et al.* 1980; Parker and Dalgleish, 1981; Paynes, 1982).

2.3 General methods of manufacturing caseins

Casein manufacture involves precipitation, dewheying, washing, dewatering and drying processes as shown in Figure 2.1 (Southward and Walker, 1980; Mulvihill, 1989). All these steps are discussed below.

2.3.1 Isoelectric precipitation

The pH of the skim milk may be reduced to the isoelectric point (pH 4.6) of the caseins by converting some of the lactose in milk to lactic acid through the action of an added culture (usually referred as lactic casein) or by the addition of dilute mineral acids (usually referred to as mineral acid casein).

2.3.2 Precipitation with mineral acid

It is well known that casein in milk exists as a calcium caseinate phosphate complex. When acid is added to milk, this complex is dissociated. At pH 5.3, the casein begins to precipitate from the solution and at the isoelectric point of casein maximum precipitation occurs. In initial methods casein was precipitated by adding acid to pasteurized skim milk at ~ 44°C to give a pH of 4.3 (Muller and Hayes, 1962). Difficulty was experienced in controlling the pH and the resultant casein had a very high ash content (King *et al.* 1962). To achieve low ash content, either low pH values during precipitation or the

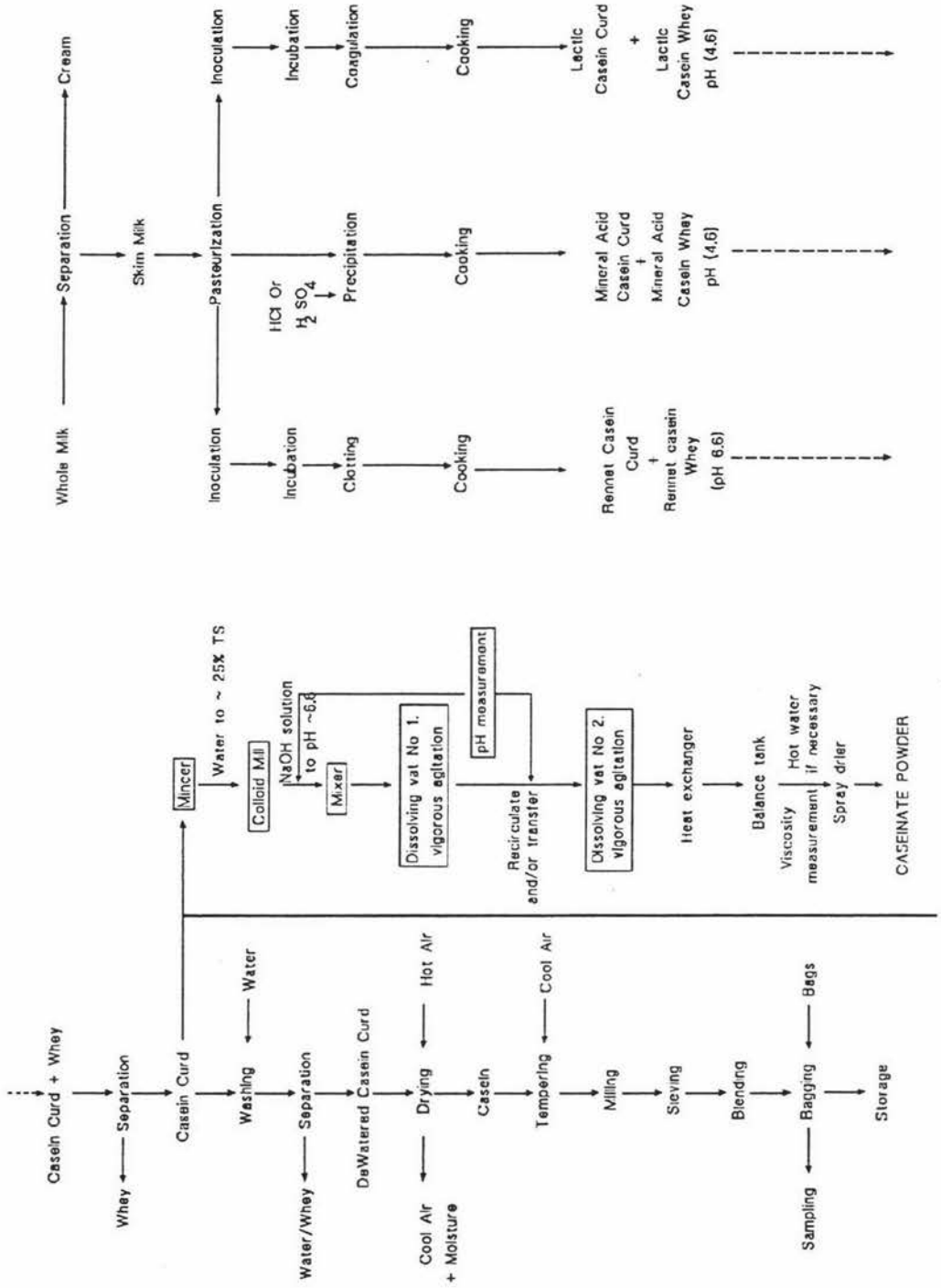


Figure 2.1: General methods of casein and caseinate manufacture.

addition of extra acid during acidulation was necessary. Low pH values during precipitation resulted in high fines losses in the whey. To overcome this problem Muller and Hayes (1962) recommended thorough and rapid mixing of milk and acid using dilute acids (1-2M) injected under pressure into milk flowing in the opposite direction and that the rate of coagulum formation could be reduced by lowering the mixing temperature to 30 - 35°C. Steam was then injected to heat the acidified milk to the required precipitation temperature.

Particle size distribution of the casein curd is an important physical characteristic since it influences the efficiency of solid-liquid separation steps, mass transfer from the particles during washing and drying and fines losses. Jablonka and Munro, (1985) found that on precipitation of reconstituted skim milk at 65°C, the curd particles formed were very small and soft due to co-precipitation of whey proteins. Calcium binding to casein also increased markedly with pH above 4.4 and therefore both calcium content and particle size followed similar trends as precipitation temperature or pH were altered, particularly above the isoelectric point. Calcium binding is an important factor affecting particle charge and hence the precipitation. The effect of temperature was more pronounced at high pH, because calcium binding to casein increases with temperature at high pH values (Jablonka and Munro, 1985).

Jablonka and Munro (1986a) developed an objective plate extrusion method for assessing the mechanical strength of casein curd. Two parameters were defined from force-distance curves: Cohesiveness, defined as the force exerted on the curd at the beginning of extrusion and Firmness, defined as the maximum force exerted on the curd during extrusion.

In a study on casein precipitated in the laboratory using H_2SO_4, Jablonka and Munro (1986b) found that curd cohesiveness varied only slightly with precipitation pH at 25 and 35°C but increased markedly with pH at 45 and 53°C. At any precipitation pH, cohesiveness increased with increasing temperature. Curd solids content, mean curd particle size and curd calcium content followed similar trends.

2.3.3 Lactic casein

Precipitation in the manufacture of lactic casein is accomplished by inoculating pasteurized skim milk with mixed or multiple defined starter cultures (Heap and Lawrence, 1984) and incubating at 22-26°C (Southward and Walker, 1980). During an incubation period of 14 - 16 hours, the starter ferments some of the lactose in milk to lactic acid. As the pH of the milk slowly falls, the colloidal calcium phosphate in the casein micelles progressively dissolves (Pyne, 1962) and a casein gel network or coagulum with good water holding capacity is formed as the isoelectric pH of the casein is approached. The mechanism of acid gelation and the factors influencing gelation have been reviewed extensively by a number of workers including Kalab *et al.* 1976; Davis *et al.* 1978; Heertje *et al.*1985; Roefs *et al.* 1985; Jablonka *et al.* 1988; Fox and Mulvihill 1990.

The coagulum is then cut with cheese knives before steam injection and during cooking, the curd is agitated gently to promote syneresis (Muller, 1982). When the syneresis is complete, the whey is drained off and the casein curd is washed in vats. It was proposed by Johnston (1974) and Roeper and Elston (1976) to replace direct steam injection by a plate heat exchanger.

2.3.4 Rennet casein

In the manufacture of rennet casein, calf rennet or microbial rennet (Southward and Elston, 1976) is added to skim milk (1:7500) at a temperature of 29°C. When rennet casein is intended for technical purposes, it is important that the skim milk should not be pasteurized for it has been observed (Munro *et al.* 1980) that the rennet casein plastic produced from pasteurized milk has a darker colour than casein plastic from unpasteurized milk.

Any number of proteinases can coagulate milk in a two-stage process; the first stage involves the specific hydrolysis of κ-casein to yield para-κ-casein and glycomacropeptides, while the second stage involves the coagulation of the rennet-altered casein micelles by Ca^{++} at temperatures above 20°C. When a coagulum of this nature is formed from skim milk, it can be further processed to yield rennet casein in a manner similar to that used for the manufacture of lactic casein following quiescent acid coagulation (Mulvihill, 1989).

2.3.5 Dewheying

Following destabilization of the casein by any of the methods described and the treatments (cooking, acidulation, etc.) employed to optimize properties of curd for further processing, the curd is separated from whey prior to washing. It is well known that production of high quality casein requires efficient separation of curd from whey and thorough washing to reduce the lactose content in the final product (Muller, 1971). The efficiency of the dewheying step is of the utmost importance in determining the volume of whey recovered for further processing, the efficiency of the washing operation and quality of the final casein produced. The equipment used to achieve separation includes (a) vibratory, moving or stationary inclined screens made

of nylon or fine-mesh stainless steel (Muller, 1960; King, 1970) or inclined screens made of polyester fabric laid in a cascade-like profile which subjects the curd to turning and rolling as it travels down the slope and (b) mechanical devices.

Studies by Hobman and Elston (1976a) showed that the efficiency of an inclined screen for separation of curd from whey was a function of the angle, the length of the screen and the aperture size. Continuous roller presses can also be used for removing whey from the curd, provided the temperature and pressing conditions are controlled so as to avoid excessive matting of the curd; the curd moisture content can be reduced to about 65%, compared with about 85% from inclined screens (Hobman and Elston, 1976b). About 20% more whey is recovered and less wash water is needed. Similar considerations have led to the use of horizontal centrifuges for 'dewheying' (Higgs *et al.* 1973).

2.3.6. Washing

The removal of lactose, salts and free acid from casein by thorough washing is an essential feature of the manufacture of a high-quality product. These impurities diffuse from the curd during washing and the diffusion rate depends on the size and permeability of the curd particles, and on the purity, amount, temperature and rate of movement of the wash water (Muller, 1959).

The temperatures used in washing are chosen so as to maintain the curd in a suitable condition for agitation, pumping as necessary and separation at each stage from the wash water. With lactic casein, higher temperatures are needed during washing to reduce to low numbers the bacteria which multiply during incubation of the milk with starter (Roeper and Elston, 1976).

However, even with acid-precipitated casein it is normal, as a safeguard against bacterial contamination, to apply temperatures of 70°C or more at the penultimate washing stage. The final washing stage is normally at 40-45°C to minimize matting of the curd during its separation from wash water by a continuous press or horizontal centrifuge. An efficient press or centrifuge and the maintenance of optimum curd characteristics and temperatures during washing are necessary to reduce the moisture content of the curd to 55-60% and so minimize the amount of water to be evaporated during drying (Muller, 1982).

2.3.7. Dewatering

When washing is complete, casein curd is mechanically dewatered to produce a curd of minimum moisture content to minimize the quantity of water to be evaporated and thus minimize the energy required during the subsequent thermal drying operation. The properties of the casein curd following washing should be such as to allow for maximum dewatering under the conditions of operation of the dewatering machine while at the same time maintaining the curd in a suitable condition for subsequent drying (Southward, 1971).

Vu and Munro (1981) performed constant-pressure expression experiments on casein curd at room temperature (17-20°C), and found that at high applied pressures (greater than 104 kPa) the appearance of the drainage surface of the casein curd changed from opaque white to translucent creamy-brown. They referred to the phenomenon as surface sealing or plasticization, which has a detrimental effect on dewatering and on subsequent drying since the plasticized curd layer at the drainage surface acts as a relatively impermeable barrier to further moisture flow (Vu and Munro, 1981 and Espie *et al.* 1984).

The physical properties of the curd were also influenced by dewatering temperature and each dewatering machine had a characteristic temperature below which curd plastization did not occur under the operating conditions used, i.e. ~ 30°C for the screw press and ~ 39°C for the roller press and decanter centrifuge (Munro *et al.* 1983).

2.3.8. Drying

There have been a number of types of equipment developed for drying casein (Muller, 1971). To produce a stable, storable product that meets the internationally recognized compositional standards for edible grade product recommended by the IDF standard number 45, (1969), the casein curd is dried to less than 12% moisture in any one of the variety of drier types. Traditionally driers were a semi-fluidized, vibrating type in which casein curd passed along vibrating perforated stainless steel conveyors while warm air was forced up through the perforations, partially fluidizing the curd which was normally minced to reduce it to evenly-sized particles before entering the drier (King, 1970). Energy requirements for a number of driers of this type have been compared by Patchett (1968, 1969). The drying characteristics of a number of casein curd types have been determined in a laboratory-scale fluidized bed drier by Espie *et al.* (1984).

Dried casein is relatively hot as it emerges from the drier and the moisture content of the individual particles varies. Therefore, it is necessary to temper and blend the dried product to achieve a cooled final product of uniform moisture content (Muller, 1960; King, 1970). This is usually achieved by pneumatic circulation of the curd between a number of holding bins.

Following drying, tempering and blending, the casein is ground in roller or

pin-disc mills to produce the small-size particles required by users of casein (Hobman, 1976).

2.4 General methods of caseinate manufacture

Soluble caseinates of importance have sodium, ammonium, potassium or magnesium as the cations. Sodium caseinate is by far the most commonly used in food systems. To obtain a bland flavoured caseinate, it is normal to use a fresh acid casein curd, dissolved in the appropriate alkali and spray dried (Muller, 1971). The main difficulties in the manufacture of spray dried caseinate are related to the logarithmic increase in the viscosity of caseinate solutions as their concentration increases and the tendency for the reaction of the alkali to be impeded by relatively impervious gels which form on the surface of casein particles in the presence of alkali. A dissolving technique which was found satisfactory in practice involves adding fresh wet curd and alkali progressively to water in a vat equipped with a powerful agitator and use of a large capacity centrifugal pump to apply shearing forces to curd particles during recirculation. Towards the end of the process it becomes very difficult to dissolve freshly added curd so the mixture is then passed through a colloid mill.

A detailed study by Towler (1976) regarding the factors affecting the rate of conversion of curd to sodium caseinate led to the proposal that the fresh curd and water be passed first through a colloid mill to give slurry of about 25% solids content, and then mixed with NaOH solution before passing into an agitated vat with recirculation facilities. Careful control of pH is essential. The minimum viscosity for sodium caseinate is in the pH range of 6.6 - 7.0 (Hayes and Muller, 1961). In practice it is better to maintain a pH below this value during dissolving and make any adjustment when the curd is finally in

solution (Towler, 1976). It is also important to avoid exposing casein to high pH and temperature during dissolving as this can lead to loss of lysine and serine and the production of degradation products, such as lysinoalanine (Creamer and Matheson, 1977).

The viscosity of the soluble caseinates limits the concentration at which they can be spray dried, normally to only about 20% solids at 90 - 95°C. There is an optimum viscosity for spray drying, depending on the atomization system used. As the viscosity of casein may vary throughout the year, it is better to standardize the viscosity and temperature of the solution to be spray dried rather than to standardize on solids content (Towler, 1976).

2.5 Functionality of caseins

Functional properties denote physicochemical properties which affect the processing and behaviour of protein in food systems and other application systems, eg. technical. These functional properties are related to the interactions between the physicochemical properties of proteins, and their composition, structure, conformation etc. General classes of functional properties of proteins are flavour, texture, solubility, dispersibility, viscosity, gelation, emulsification, foaming, elasticity etc. These functions vary with pH, temperature, protein concentration, protein species, ionic strength and dielectric constant of the medium. They are also affected by macromolecules in the medium and by processing treatments.

Caseins, have exceptional water binding capacity, fat emulsification properties and whipping ability and are viscous and soluble in neutral or alkaline conditions (Jonas *et al.* 1976). Caseins are currently used in a variety of products, including coffee whiteners, cheese analogs and meat products. The

approximate composition of commerical casein and caseinate products is given in Table 2.2.

Protein functionality in general has been reviewed by a number of workers, including Friberg (1976), Kinsella (1976, 1981, 1982), Schmidt (1980), Halling (1981), Whitney (1988), Fox (1989) and Swaisgood (1992).

Table 2.2 : Approximate composition of commercial casein and caseinate products. (Adapted from Morr, 1982).

Component	Sodium caseinate	Calcium caseinate	Acid casein	Rennet casein
Protein N x 6.38 (min)	94	93.5	95	89
Ash (max)	4.0	4.5	2.2	7.5
Sodium	1.3	0.05	0.1	0.02
Calcium	0.1	1.5	0.08	3.0
Phosphorus	0.8	0.8	0.9	1.5
Lactose (max)	0.2	0.2	0.2	--
Fat (max)	1.5	1.5	1.5	1.5
Moisture (max)	4.0	4.0	10.0	12.0
pH	6.6	6.8	--	7.0

2.6 Emulsification

When comparing fat emulsification by proteins, one is faced with a wide range of apparatus types and environmental conditions (pH, ionic strength, temperature and protein concentration) that have been used to prepare emulsions in model and pilot scale experiments. The methods used to measure the functional properties of milk proteins have been reviewed by Mulvihill and Fox (1989) and Patel and Fry (1987). The influence of

procedural factors on the emulsifying efficiency of soya protein, sodium caseinate and whey protein concentrate has been studied by a number of workers, including Tornberg and Hermansson (1977), Tornberg (1978a,b), Dalgleish and Fang (1993) and Hunt and Dalgleish (1994).

2.6.1 Emulsion formation

Emulsion manufacture is a highly energetic and dynamic process. Coarse emulsions can be made by vigourous stirring. Fine emulsions are normally made using high-pressure homogenizers. Other techniques for emulsification are sonication and colloid milling. Tornberg (1978a) has compared the various methods for making protein-stabilized emulsions using laboratory-scale equipment.

Dickinson *et al.* (1987) had developed a high pressure mini-homogenizer which produces emulsions of similar particle size distribution to that of emulsions made with commercial laboratory-scale valve homogenizer. The main experimental difficulty in using valve homogenizers is that small volumes cannot be homogenized (Dickinson and Stainsby, 1988). This new method of emulsification was called 'microfluidization'. The important features of this microfluidization were that there were no moving parts and the energy of the moving streams was released extremely rapidly; the process was nevertheless gentle enough, apparently, for enzymes to remain native. It is claimed by the manufacturer that very fine emulsions can be obtained with a narrow particle size distribution. Castle *et al.* (1988) have used a similar experimental arrangement to produce coarse protein-stabilized oil-in-water emulsions.

The high-pressure valve homogenizer is still a widely used method for

manufacturing food emulsions. Droplets are disrupted by a combination of intense laminar and turbulent flow (Walstra, 1983; Phipps, 1985; Davies, 1985). The main factor affecting the emulsions droplet-size distribution is pressure drop across the homogenizer valve, and increased turbulence on the low-pressure side of the valve favours the formation of finer emulsions. If the premix viscosity is high, only a coarse emulsion can be formed unless the operating pressure is raised substantially (Dickinson and Stainsby, 1988). Under conditions of turbulent flow, casein micelles are adsorbed at the oil-water interface more rapidly than the monomeric caseins (Walstra, 1980). In the same way, one expects the less voluminous aggregates of caseinate or globular proteins to adsorb preferentially over their monomeric constituents (Dickinson and Stainsby, 1988).

2.7 Proteins as emulsifiers

Extensive studies of protein films have been conducted both at the air/water and oil/water interface. Studies on the adsorption of proteins at the air/water interface have relevance to the stabilization of food emulsions, as many food emulsions, e.g., ice cream, may contain dispersed air bubbles which are stabilized by adsorbed films of proteins (Friberg, 1976). However, most studies have not been done in emulsion systems; rather they have mostly been done on flat, undisturbed constant area interfaces. Emulsion droplets have higher curvature, but, nevertheless, it is believed that the general behaviour of proteins at a flat interface and a highly curved interface is not significantly different (Dickinson and Stainsby, 1988; Tadros and Vincent, 1983).

Furthermore, only relatively simple systems, using pure proteins, have been investigated for surface properties at air/water and oil/water interfaces.

However, understanding the behaviour of proteins at the interface and the interfacial interactions involved in stabilizing adsorbed films could be useful in manipulating and controlling complex food emulsions.

Many soluble proteins are effective emulsifiers. The conformation of proteins and the internal bonding are important (Phillips, 1981). Proteins usually contain both positively and negatively charged regions. These attract one another and cause the protein to fold on itself giving a more compact structure. The functional properties of proteins largely depend on the "opening up" of this compact structure. Unfolding of the protein gives access to the hydrophobic core, which allows the use of proteins as an emulsifier.

Thus proteins are usually surface active and have a strong tendency to adsorb at oil-water interfaces, with the hydrophobic regions orientating in the oil phase and the hydrophilic regions in the water phase. After adsorption they stabilize the resulting emulsion by forming a mechanical "steric" barrier which protects the oil droplets against flocculation and coalescence. In considering the adsorption behaviour of proteins in food colloids, it is important to distinguish between (i) role of protein at the instant of emulsification (ii) role of protein in relation to the long term stability of the resulting emulsion. In role (i) a protein like β - casein is favoured which is highly disordered and hydrophobic and is able to reduce the interfacial tension rapidly at the newly formed interface. Role (ii) is favoured by a protein like β-lactoglobulin which has a more ordered globular structure and is, therefore, able to form a tightly packed viscoelastic structure at the oil-water interface (Dickinson *et al.* 1989).

Proteins that are good emulsifiers include caseinate, whey, egg albumen, egg yolk, serum albumin and gelatin. An important property of emulsions is heat

stability; whey and egg form a gel on heating but caseinate does not. Therefore, caseinate emulsions can be heat-treated.

2.7.1 Behaviour of proteins at an interface

Protein molecules are surface active because of their amphipathic nature. The change in conditions accompanying adsorption can be sufficient to cause denaturation. When a protein is adsorbed at an air/water or oil/water interface, an alternative way of folding the molecule to minimize the hydrophobic free energy becomes possible; thus polar side chains can be located in the oil or air phase and the tails of residues protrude in the bulk or aqueous phase. The adsorption behaviour of proteins at the air/water or oil/water interface is generally monitored by interfacial tension measurements, which give a measure of the thermodynamic driving force for the formation of protein films or lipid/protein films around air cells or fat globules in foams and emulsions. One such technique for measuring surface tension is the Wilhelmy plate method. With this technique, it is possible to obtain static information on adsorption from the bulk phase or dynamic information from surface pressure versus area plots corresponding to compression of the adsorbed monolayer film.

There is general agreement that β-casein forms dilute monolayers at air-water interfaces whereas lysozyme forms concentrated films. Dilute protein monolayers are supposed to contain completely unfolded molecules, whereas the structures of concentrated films are not clear. These two molecules represent the extremes of tertiary structure for soluble proteins; lysozyme is rigid and globular, whereas the hydrophobic β-casein molecule is a flexible, 'almost random coil' chain.

At low protein concentration (C_p), the rate of adsorption is diffusion controlled, but at high C_p there is an activation energy barrier to adsorption because the protein molecules create a space in the existing film and penetrate and rearrange in the surface. Adsorption kinetics depend upon the flexibility of the native protein. As the C_p for β-casein is increased to 10^{-4} wt%, adsorption proceeds with a concomitant rise in π (surface pressure) to a maximum value of about 24 mN/m. Lysozyme is less surface active in that it always shows lower film pressure for the same C_p, and surface pressure (π) does not attain maximum steady value until $C_p = 10^{-2}$ wt% (Philips 1981).

Table 2.3: Selected properties of β-casein, BSA and lysozyme. (Phillips, 1981).

Property	β-casein	BSA	Lysozyme
Native conformation	flexible coil	globular	globular
Spread monolayers	dilute	dilute	concentrated
Molecular weight	24,000	65,000	14,000
α-Helix content (%)	< 10	47	23
Disulphide bridges	0	17	4
Hydrophobicity (Cal/mole residue)	1,320	1,120	970

The adsorption isotherm at 22°C for β-casein and lysozyme behaves differently (Philips, 1981). The surface concentration (Γ) *vs* C_p for β-casein shows a well defined plateau over the C_p range of 10^{-4} to 10^{-2} wt%. In contrast, Γ for lysozyme increases steadily with C_p despite the influence on π at 8 mN/m. The adsorption of β-casein is irreversible over the region where

π changes with C_p because the removal of excess protein remaining in the substrate after adsorption causes no desorption of the interfacial protein. In this region, protein can only be removed from the interface by compression with a barrier. Lysozyme exhibits a similar property except when $C_p > 10^{-3}$ wt%. (Phillips, 1981).

The behaviour of proteins is qualitatively similar at oil/water and air/water interfaces (Graham and Phillips, 1979). β-Casein forms a similar structure except that loop formation occurs more readily at oil/water interfaces because the oil molecules can solvate the hydrophobic residues. The increased loop formation leads to lower film pressure at the oil/water interface than air/water interface at a given Γ. Opposite behaviour is observed with BSA and lysozyme, in that π is higher at an oil/water interface; this presumably arises because disruption and unfolding of the native structure is greater when oil molecules can solvate the polar residues and reduce the van der Waals attraction between them.

At all surface concentrations, β-casein films are considerably less viscous than those of lysozyme (Table 2.4). The molecules in films formed from proteins which are originally globular have more residual structure than those formed from β-casein, and there is more cross-linking or entanglement of chains, giving greater resistance to shear.

Castle *et al.* (1987) tabulated the steady-state interfacial tensions for the α_{s1}-, β-, and κ- caseins adsorbing at a hydrocarbon oil-water interface (Table 2.5). The differences between the tensions for the individual caseins are rather small as compared with the differences between the casein values and those for other food proteins (e.g., α-lactalbumin or gelatin).

Table 2.4: Some physical parameters of β-casein, BSA and lysozyme films adsorbed at the air/water and oil/water interfaces from solution of final protein concentration = 10^{-3} wt%.
(Phillips 1981)

Property	β-Casein		B S A		Lysozyme	
	A/W	O/W	A/W	O/W	A/W	O/W
Film pressure mN/m	24	22	17	17	12	17
Surface concentration Γ mg/m^2	2.7	3.0	2.6	2.5	3.3	3.5
Thickness h (Å)	50	--	90	--	70	--
Shear viscosity coeff. η (mN sec/m)	< 1	< 1	50	10	10^3	$5x10^3$
Shear elastic modulus G′ (mN/m)	< 0.1	< 0.1	0.5	2	12	14
Dilatational modulus (mN/m)	10	--	160	--	270	--

There are significant differences in surface viscosities of three caseins at the oil-water interface as indicated in the Table 2.5. Surface viscosities for α_{s1}- and β-caseins are lower than those for most other food proteins. The value for β-casein is especially low. The value for κ-casein, however, is of the same order of magnitude as those for α-lactalbumin or gelatin, possibly due to the formation of intermolecular disulphide linkages at the interface. The relative efficiency of adsorbed proteins in preventing coalescence were found to lie in the order: lysozyme > κ-casein > β-casein. This represents a positive correlation between coalescence stability and viscoelasticity of the adsorbed protein film.

Table 2.5: Interfacial tension (γ) and shear viscosity (η) for adsorbed protein films at n-hexadecane-water interface.
(Castle *et al.* 1987)

Property	γ mN m^{-1}	η mNm^{-1}
α_{s1}-casein	24.0	5
β-casein	22.4	0.5
κ-casein	23.0	200
sodium caseinate	22.4	7.5
α-lactalbumin	28.5	300
gelatin	35.2	120

Tornberg (1979) compared the concentration-dependence of the interfacial tension of three proteins i.e., caseinate, whey protein concentrate and soya proteins.

The surface pressure at 40 mins (π_{40}) was plotted against the initial subphase concentration (wt%).

$$\pi_t = \gamma_0 - \gamma_t$$

where γ_0 is the initial interfacial tension.

At high concentration, the surface activity of all the proteins was high and almost equal, whereas at low concentration, differences in surface behaviour of the proteins became very evident. Caseinate was the most effective surface active agent and was more or less independent of concentration in the concentration range of 10^{-1} to 10^{-3} wt %. The opposite behaviour was observed in soya protein which actually loose their surface activity with

decreasing subphase concentration. The increase in lowering of the interfacial tension with the addition of salt were also observed (Tornberg, 1979).

Dickinson *et al.* (1983) suggested that the components of sodium caseinate, consisting of α_s-, β-, κ- casein, seem to adsorb independently and not competitively. Moreover, the interfacial activity of the casein micelle dispersed in synthetic milk serum and studied under same conditions did not show any appreciable divergent behaviour. The different colloidal states of the casein molecules did not give rise to a marked difference in interfacial activity.

The small, but significant spread of steady-state tensions for α_{s1}-, β-, and κ-caseins adsorbing at the oil-water interface implies that the protein film adsorbed from a mixture of caseins (e.g., sodium caseinate) will not have the same composition, as that of the bulk aqueous phase with which it is in equilibrium. In particular, one is led to expect that the most surface-active component, β-casein will be adsorbed preferentially (Dickinson *et al.* 1985; Castle *et al.* 1987).

2.8. Protein load at the oil-water interface

Since most adsorbed proteins form an interfacial film around oil droplets, it is important to know how much protein is present at the interface. The amount of protein present at the interface per unit surface of dispersed phase is termed protein load, usually expressed as milligrams of protein per unit area of the dispersed phase. Protein load may determine the amount of protein required to make an emulsion with desired oil volume and droplet size (Walstra, 1984). If the protein forms large aggregates, a higher amount of protein is required to form a stable emulsion, which also suggests that the

protein is a poor emulsifier. The protein load is also related to coalescence stability; low protein load enhances the coalescence process (Walstra, 1987).

The determination of protein load requires knowledge of the total surface area of the emulsion (Walstra, 1987). This, in turn, implies a means of determining the complete droplet diameter distribution. The different methods for determining the droplet diameter distribution are summarized below.

Droplet size is the most important fundamental property for characterization of emulsions. Most research work with food emulsions requires a knowledge of the droplet size distribution of the emulsions, because it influences properties such as stability, viscosity, texture and mouthfeel. Droplets ranging from 0.1 to 100 μm in diameter may occur in food emulsions (Friberg, 1976).

Several methods are used to determine droplet size distribution of emulsions. The microscopic method is one of the oldest methods still in use. Since the maximum magnification (with acceptable resolution) with an optical microscope is limited to about 1 mm under the microscope, because of the lack of resolution and contrast, quantitative measurement of droplet diameters of less than 0.5 μm is not practicable. Since the small droplets have greater motion, it is difficult to measure accurately diameters of less than 1 μm. The ultramicroscope improves the visibility of small droplets by reducing the back ground intensity (Mcfayden and Smith, 1973). When the refractive index of the dispersed phase is close to that of the dispersion medium, the droplets may appear transparent. In such cases, phase-contrast techniques may prove helpful in providing greater contrast (Pluta, 1975). High speed photomicrography can be used to freeze the motion of the droplets in the

picture (Das and Chattoraj, 1980; Das and Kinsella, 1989).

Klemaszewski *et al.* (1989) described an electronic imaging system where microscopic views are transferred electronically to a video monitor with magnification. However, problems of poor contrast and droplet flocculation can cause image distortion and artifacts.

Electron microscopy has also been applied to the study of emulsions (Liboff *et al.* 1988; Reddy and Fogler, 1981), but because of difficulty in sample preparation, reproducibility, and artifacts, it is difficult to use it as a routine method, although the method may be useful for specific studies, such as fat crystallization in emulsions or formation of liquid crystal phases (Liboff *et al.* 1988). Reddy and Fogler (1981) determined droplet size distribution by the freeze-fracture technique, in which a frozen emulsion is sectioned and observed under a scanning electron microscope.

Techniques for automatic sizing of droplets from optical and electron microscopes have been reviewed by Rosen (1984) and by Herrmann and Krahl (1984). For automatic counting and sizing, the picture is stored in digital form in a computer. Digitization is done with respect to both position and light intensity. The continuous picture formed in the image plane of the microscope is replaced by a set of pixels. Automated microcopy can separate objects of interest from the background and perform statistical analysis on the computer. The coulter counter is a device that electronically counts large numbers of particles and classifies them into different size ranges.

Spectroturbidimetric methods are also used widely for droplet size measurement. One such method, widely used in emulsion studies, was

developed by Walstra (1965, 1968). In this method light transmission through a diluted sample of emulsion is measured with a spectrophotometer which is modified to have a small angle of acceptance (1.5°) for transmitted light. An experimental turbidity spectrum is produced by plotting reduced turbidity, Z, against the values of

$$\log \frac{[2\ \pi\ (n_1 - n_2)]}{\lambda}$$

Reduced turbidity is defined as

$$Z = 0.2443\ A\ \lambda\ /\ l\ \phi\ (n_1 - n_2)$$

where A is absorbance, λ wave length, l path length of cuvet, ϕ volume fraction of the droplets, and n_1 and n_2 are the refractive indices of oil and water respectively. There are some practical problems that limit the extensive use of this method. The method requires knowledge of the refractive index of the oil and water as a function of wavelength, a modified spectrophotometer, and a good match between the experimental and theoretical turbidity spectra. The method fails when a good match is not obtained. Often, the experimental turbidity spectrum is limited by the available maximum wavelength of the spectrometer, which is typically around 1000 nm in most cases. In order to get both sides of the maximum in the experimental turbidity curve, adsorbance needs to be taken at least up to λ = 2000nm which is seldom possible.

Pearce and Kinsella (1978) used some simple equations to calculate droplet size from turbidity measurements at one wavelength. The equations used are

over simplified and assume no wavelength dependence of turbidity and are valid strictly for large monodisperse droplets, which are rarely found in actual emulsion. It is not clear what type of average diameter (e.g. number average, volume average, volume-surface average) can be obtained from these equations. One should not attempt to calculate absolute diameter of droplets using these equations, although in certain systems, droplet size as determined by Pearce and Kinsella's method was found to be in good agreement with those from turbidimetric or electron microscopic methods (Haque and Kinsella, 1989; Klemaszewski *et al.* 1989). This method is operationally simple, practical and useful for comparison of relative surface area produced by different proteins under different conditions.

The measurement of droplet diameter using the Malvern Mastersizer was described in chapter 3.

The chemical composition of the adsorbed protein layer in an emulsion is usually determined by inference using the so-called 'depletion method'. In this method, the interfacial composition is calculated from measurements of the continuous phase composition before and after emulsification (Oortwijn and Walstra, 1979; de Feijter *et al.* 1987). The results are usually represented as the protein load. The method may not be satisfactory when most of the protein emulsifier is not adsorbed and the depletion is low (Dickinson and Stainsby, 1988). In such cases, direct measurement on the interfacial protein layer is essential. Direct measurements on the interfacial protein layer were recently made by Sharma (1993) and Hunt and Dalgleish (1994). Whatever method is used, there are always some practical difficulties in the separation of the aqueous phase layer from the emulsion.

Protein loads values obtained for different proteins by various workers are shown in Table 2.6.

Table 2.6: Protein load values at air-water and oil-water interfaces. (Adapted from Sharma, 1993)

Protein type	Type of interface	(mg/m^2)	Reference
Egg albumin	air/water	1.80	Bull (1972)
Lysozyme	air/water	3.00	Phillips *et al.* (1975)
	air/water	4.50	Yamashita & Bull (1968)
α_s-Casein	polystyrene/water	4.20	Dalgleish *et al.* (1985)
α_s- or β-Casein	tetradecane/water	3.00	Dickinson *et al.* (1988b)
β-Casein	air/water	2.70	Phillips *et al.* (1975)
	polystyrene/water	5.80	Dickinson *et al.* (1983)
	tetradecane/water	1.75	Courthaudon *et al.* (1991a)
	soya oil/water	1.05	Courthaudon *et al.* (1991a)
κ-Casein	polystyrene/water	4.20	Dalgleish *et al.* (1985)
Casein micelles	oil/water	20.0	Oortwijn and Walstra (1979)
Sodium	oil/water	2.60	Oortwijn and Walstra (1979)
caseinate	oil/water	2.20	Dickinson *et al.* (1984)
β-Lactoglobulin	tetradecane/water	1.70	Courthaudon *et al.* (1991b)
Whey proteins	oil/water	2.50	Oortwijn and Walstra (1979)
	oil/water	3.60	Vaitkus and Ziberkaite (1978)
Skim milk	oil/water	12-16	Vaitkus and Ziberkaite (1978)
proteins	oil/water	10.0	Oortwijn and Walstra (1979)
	oil/water	23.35	Dalgleish and Robson (1985)
	oil/water	6-8	Sharma (1993)

If the protein load is ~ 1 mg/m^2, it suggests that the protein molecules are fully unfolded or there is adsorption of an extended polypeptide chain. If the protein load is ~ 3 mg/m^2, there may be adsorption of a monolayer of globular proteins or unfolded molecules that are adsorbed in the conformation of trains, loops and tails. Protein load values over 5 mg/m^2 suggest adsorption of aggregates of proteins or multilayers of proteins, although some proteins of higher molecular weight may also give higher protein loads (Walstra, 1988).

The amount of protein adsorbed on the interfaces in o/w emulsions and the conformation of the adsorbed layer, depend on the concentration and type of protein as well as the nature of the interface (Dickinson *et al.* 1988a,b, 1989, 1991; Dalgleish and Fang, 1993; Hunt and Dalgleish, 1994).

2.9. Factors affecting protein load

The factors which may influence the protein load at the oil/water interface include volume of oil, protein concentration, homogenization pressure, aggregation state of protein etc.,

The volume fraction of oil before emulsification directly affects the droplet diameter and the specific surface area of the dispersed phase. A high volume fraction may result in larger-sized droplets due to coalescence during emulsification, which may consequently lead to an increase in protein load (Oortwijn and Walstra 1979, Tornberg, 1980).

The influence of protein concentration on the oil droplet size of emulsions is rather contradictory. For whey protein-stabilized emulsions prepared using a valve homogenizer, Pearce and Kinsella (1978) reported that the oil droplet

size decreased as the protein concentration increased from 0.5 to 5.0%. In contrast, Oortwijn and Walstra (1979) found that the droplet size of emulsions prepared from milk fat and whey were relatively unaffected by increasing protein concentration in the range 0.1 - 2.0%. For emulsions of soya oil and whey proteins or sodium caseinate, dispersed in 0.2 M NaCl at pH 7.0, Tornberg *et al.* (1990) reported that increasing protein concentration from 5 to 37.5 mg protein/g oil decreased oil droplet size to a certain level, and then the droplet size remained constant. In general, the protein load at oil/water interface increases with increase in the bulk protein concentration. For most protein-stabilized emulsions, the protein load reaches a maximum value and thereafter it remains constant (Walstra, 1988).

The formation of multilayers at the interface is favoured when adsorption occurs from a solution of high bulk protein concentration. According to Dickinson *et al.* (1988a) the protein layer close to the oil surface (primary protein layer) is strongly bound to the oil surface compared to the subsequent protein layer (secondary protein layer) which can be desorbed easily. In experiments with radio-labelled proteins at air/water and oil/water interfaces, Graham and Phillips (1979) found that the maximum protein loads of β-casein, lysozyme and bovine serum albumin were in the range of 4 - 9 mg/m^2 and these could be reduced to ~ 3 mg/m^2 by washing the interface with buffer solution. Dickinson *et al.* (1988a) suggested that the irreversibly adsorbed protein molecules may exist in a primary layer directly at the interface probably in unfolded configurations, with the remainder stacked on the aqueous side in a conformation close to those existing in the bulk solution.

The homogenization conditions during emulsification are quite critical in

determining the protein load at the interface. Increasing the homogenization pressure decreases the oil droplet size as long as there is enough protein to cover the increased fat surface area in such a way that coalescence is prevented. This results in increased surface area of the dispersed phase and lower protein loads at the interface. Generally, the ratio of fat surface area to protein concentration is a crucial factor in determining the protein load (Walstra, 1988; Tornberg *et al.* 1990). The protein load is also affected by the type of homogenizer (Todt, 1976).

Murphy and Fox (1991) measured the protein load as a function of power input and fat surface area. Protein load decreased slightly with increasing power input or fat surface area for the α_s-/κ-casein-enriched caseinate stabilized emulsion indicating the adsorption of protein from the bulk phase was dominant. Sodium caseinate showed highest protein load at highest power inputs and also at highest fat surface areas. The sharp decrease in protein load in the power input range 15 to 50 W (fat surface area range from 1 to 2.5 m^2/ml) indicated that newly formed surface of the sodium caseinate stabilized emulsion was due to a combination of protein adsorption from the bulk phase and rearrangement of protein molecules already adsorbed. At higher power inputs, adsorption from the bulk phase dominated, as for the α_s-/κ-casein enriched caseinate stabilized emulsions.

In the case of the β-casein enriched caseinate, rearrangement of adsorbed protein dominated at low power inputs/fat surface area, with adsorption from the bulk phase dominating at fat surface areas between 1.0 and 3.0 m^2/ml. Above a fat surface area of 3.0 m^2/ml emulsion, the sharp increase in protein load relative to fat surface area may be due to increased association of the β-casein by hydrophobic bonding above a critical micelle concentration.

The aggregation state of protein in the bulk phase is an important factor in determining protein load at the interfaces (Oortwijn and Walstra, 1979). For emulsions stabilized by soluble protein, for example, whey protein and caseinates, the adsorption occurs to some degree as a monolayer, depending on the protein/fat ratio. A spread monolayer of proteins should result in a protein load of little higher than 1 mg/m^2. Dalgleish and Fang, (1993) reported that when the protein load was less than 1 mg/m^2, there was insufficient casein to stabilize emulsions. Studies by Mulvihill and Murphy (1991) found that the protein loads were dependent on the aggregation state of the emulsifying protein. The protein loads were highest for highly aggregated micellar casein-stabilized emulsions, lower for less aggregated ethanol precipitated casein and high calcium caseinate-stabilized emulsions, and lowest for the least aggregated low calcium, sodium and ammonium caseinate-stabilized emulsions.

Oortwijn and Walstra (1979) also observed similar relationships between protein loads and state of aggregation of milk protein used in emulsion preparation and they reported the protein loads of 2.5, 2.6, 10 and 20 mg/m^2 in emulsions of relatively similar globule size stabilized by whey proteins, sodium caseinate, skim milk or washed casein micelles, respectively.

Todt (1976), claims that the protein load is affected by mechanical factors, because when emulsifying milk fat in skim milk with a valve homogenizer, a protein load of about 12 mg/m^2 was attained compared to approximately 5 mg/m^2 obtained in a blender. This difference in protein load could be due to adsorption of larger particles (casein micelles) on the smaller droplets which results when making emulsions in a valve homogenizer as opposed to a blender. Walstra (1983), pointed out that during the strong turbulence

prevailing in the valve homogenizer, the transport of proteins to the interfaces is convection controlled. In contrast to diffusion controlled adsorption, the larger protein molecules are then preferentially adsorbed and this effect is mostly stronger for the small droplets, i.e., when the size of the protein aggregates is of the order of the droplet size. Walstra and Oortwijin (1982) have shown a higher protein load for the smaller droplets when there is a large spread in the protein particle size.

2.10 Competitive adsorption

A food emulsion may contain more than one protein or it may contain a combination of several proteins and low-molecular weight surfactants. In such cases, there is competition between different proteins/emulsifiers in occupying the interface. Therefore, the interface may be occupied by more than one emulsifier in different amounts. The composition of the interface may change with time, bulk concentration of surface active materials, mode of addition, etc. Thus, knowing interfacial composition is very important from a practical point of view and recently a number of studies on competitive adsorption have been carried out by Dickinson and associates using model systems (Dickinson, 1986; Castle *et al.* 1987; Dickinson and Rolfe 1989; Dickinson *et al.* 1989; Courthaudon *et al.* 1991a-c).

In general, studies of two different types of competitive adsorption have been carried out: competition between protein and small-molecule surfactants and competition between two different types of proteins. Recent studies on competition adsorption in model emulsion systems stabilized by pure milk proteins have shown that the addition of water-soluble non-ionic surfactant after emulsification leads to complete removal of protein from the droplet surface at high surfactant/protein ratios (Courtahudon *et al.* 1991a-c). It has

also been demonstrated that the presence of an oil-soluble non-ionic or zwitterionic surfactant during emulsification leads to a reduction in protein load of the resulting emulsion (Courthaudon *et al.* 1991a-c).

In a study of oil/water emulsions, made by binary mixtures of pure α_{s1}- and β-casein (Dickinson *et al.* 1988b), it was found that β-casein predominates at the oil/water interface during or shortly after homogenization. At higher concentrations of α_{s1}-casein in the bulk phase, replacement of β-casein with α_{s1}- casein was observed. However, in emulsion systems where at least one of the proteins is globular (e.g. β-lactoglobulin), the ability of one protein to competitively displace another from the oil/water interface is slow and limited to some extent (Dickinson *et al.* 1989).

Various studies reporting the existence of competitive adsorption of proteins in dairy emulsions and foams have been published (Mulder and Walstra, 1974; Darling and Butcher, 1978; Robson and Dalgleish, 1987; Anderson and Brooker, 1988; Britten and Giroux, 1991). In dairy emulsions, for instance, there is competition at the oil/water interface between the intact casein micelle, casein micelle fragments, and casein molecules, as well as between casein and whey proteins. Generally, although complete dominance of one or more proteins over the others does not occur, it appears that the caseins tend to adsorb in preference to whey proteins, and β-casein in preference to other caseins (Dickinson *et al.* 1989). The prediction of the surface composition of adsorbed protein films in dairy systems is not a straight forward matter, since a number of factors influence the adsorption.

Although sodium caseinate is mainly composed of a mixture of α_{s1}- and β-caseins, it behaves rather differently at the oil-water interface from a simple

binary mixture of two major caseins. Robson and Dalgleish (1987) reported no clear preference for either α_{s1}- or β-casein. However, on aging the emulsions for several hours, some partial replacement of α_{s1}-casein by β-casein was observed. This result can be explained by the fact that the protein molecules in sodium caseinate are substantially aggregated. Both proteins go to the interface in the form of proteinaceous aggregates and the strong interaction between the two casein components at the interface then greatly reduces the kinetics of competitive adsorption.

2.11 Emulsion Stability :

It is a matter of everyday experience that two immiscible liquids rapidly separate into two distinct phases, oil and water. But there are many exceptions to this rule. We know that butterfat does not spontaneously separate from milk and cream and milk can be mixed back by gentle agitation. The cream does not separate immediately and after homogenization it never separates. An essential part of these apparently stable emulsions is another ingredient which may be called a stabilizer or emulsifying agent.

Therefore, in addition to emulsion formation, the capacity of proteins to stabilize emulsions is the most important criterion in most food applications. A stable emulsion is one that undergoes changes rather slowly. Among other functions, including reduction of interfacial tension, an emulsifier reduces the tendency of dispersed droplets to coalesce. This is enhanced if the emulsion possesses a net repulsive force. The forces involved in stabilizing and destabilizing emulsions include van der Waals attractive forces, electrostatic interactions, and steric factors related to surface active agents, osmotic effects, hydration forces and viscosity of the continuous medium (Parker, 1987).

Since different emulsions exhibit different types of instability, the choice of method for measuring instability depends on the type of stability to be measured. There are, however, a number of methods in the literature that measure emulsion stability without specifying the kind of instability involved in the determination procedure. These include the emulsion stability index (ESI) method of Pearce and Kinsella (1978), the stability rating of Acton and Saffle (1970, 1971), and the emulsifying activity test. These methods, in both original and modified form, have been extensively used throughout the literature (Halling, 1981). These tests are empirical "functional tests", and interpretation and valid comparison of the results of these tests are not possible because of the lack of information of mechanisms involved in the methods.

Emulsion stability may be categorized in a number of ways that reflect the different mechanisms involved i.e., creaming, flocculation, coalescence and phase inversion.

2.11.1 Creaming

Creaming refers to the gravitational separation of emulsified droplets to form a densely packed phase without changes in droplet size. Creaming is usually a reversible process because gentle shaking can redisperse the creamed layer. Extensive creaming may be controlled by reducing the droplet size, which can be achieved by high homogenization pressure provided sufficient emulsifier is present (Walstra, 1983; Leman and Kinsella, 1989). Tornberg and Hermansson (1977) observed that the creaming stability of protein-stabilized emulsions was improved by increasing emulsification intensity and time. Leman *et al.* (1988) compared the stability of emulsions stabilized by whole milk protein, β-lactoglobulin, whey protein and casein micelles under various

conditions. They found that creaming stability improved with increasing energy input and protein concentration. Upon heating the emulsions (70 - 80°C) the creaming stability improved. The creaming stability was also influenced by the pH. As the pH was raised from 6 to 9, the rate of creaming decreased.

Rate of creaming can be measured by keeping the emulsions undisturbed in long glass cylinders and measuring the changes in the fat content of the lower emulsion phase. Several methods can be used to estimate the fat content, such as gravitational estimation and measurement of viscosity, density, turbidity (Leman *et al.* 1988, Haque and Kinsella, 1989), particle size, conductivity or dielectric properties (Leman and Kinsella, 1989).

A number of methods are based on gravity-induced separation. Smith and Dairiki (1975) measured gravity-induced separation of emulsions after keeping them undisturbed for a period of 24 h. The stability index was expressed as the percentage of separated phase. However, the rate of creaming in emulsions is slow, unless the droplets are large (greater than 5 μm). Therefore, accelerated tests are usually employed. The most common accelerated technique is centrifugation and instruments used range from a low-speed table centrifuge (Tornberg and Hermansson, 1977; Leman *et al.* 1988) to the high-speed ultracentrifuge (Vold and Mital, 1972).

Usually, a low gravitational speed is employed to avoid breaking the interfacial film. Tornberg and Hermansson (1977) used 15 min of centrifugation at 180 g to determine the cream stability. After centrifugation, aliquots (5 ml) of the lower phase were removed and the fat content was determined by solvent extraction (Leman *et al.* 1988; Haque and Kinsella,

1988). Emulsion stability was calculated as the percentage of fat remaining in the emulsion after centrifugation. Some methods employing an ultracentrifuge have actually measured the amount of separated oil (Vold and Mital, 1972) where the processes involved are a combination of creaming and coalescence. If the centrifugal field applied is not sufficient to coalesce all droplets in the creamed layer, the measured rate may not be a true reflection of creaming rate.

Emulsions are often heated to accelerate the creaming process (Wang and Kinsella, 1976; Wu and Sexson, 1976). However, heating may cause additional changes, such as changes in protein conformation, desorption and coagulation and may affect film stability, and the measured stability may not be attributed to creaming alone (Leman and Kinsella, 1989).

Gunning *et al.* (1988) followed the rate of rise of the creamed layer meniscus to measure rate of creaming. One problem with the method was that it did not provide any information for the initial period before the meniscus become apparent. Walstra and Oortwijn (1975) removed samples of emulsions at different heights and determined the fat content to determine creaming rate.

Reddy and Fogler (1981) employed a freeze-fracture electron microscopic method to determine droplet concentration at different heights of the frozen emulsion.

An ultrasonic interferometric method of determining creaming stability was described (Howe *et al.* 1986). The velocity of ultrasound through the oil and continuous phase is different. The method measures ultrasound velocity through an emulsion sample. If the droplet size is smaller than the

wavelength of ultrasound, the velocity of sound depends only on the volume fraction of dispersed oil. Therefore, changes in the ultrasound velocity during creaming can be correlated to the change in the oil concentration profile.

2.11.2 Flocculation

Flocculation denotes the aggregation of globules to come together to form clusters. This generally modifies the physical properties of the emulsion, but the particle size distribution remains unchanged and the floccule can be dispersed because the interactions are weak. Flocculation of emulsion depends on the nature of protein stabilizing the emulsion and on the solution conditions, such as pH and ionic strength. Tornberg and Ediriweera (1987) studied the flocculation behaviour of protein-stabilized emulsions. The degree of flocculation varied with type of protein and increased with increase in ionic strength. Both in creaming and flocculation, slight agitation can redisperse the droplets.

There are very few studies that measure flocculation in food emulsions. However, flocculation can provide valuable information about the interactions between droplets. Srivastava and Haydon (1964) used a microscopic method to count the change in the number of doublets or triplets with time to find the flocculation rate constant (K_f) according to them

$$\frac{1}{N_t} - \frac{1}{N_o} = K_f\, t$$

Where N_o and N_t refer to the number density of droplets at the time t = 0 and t = t, respectively.

The same method was used by Eley *et al.* (1976). Flocculation kinetics can also be determined using a Coulter counter. However, care must always be exercised to avoid coalescence of droplets that may result in a decrease in the number of droplets. Tornberg and Ediriweera (1988) used ionic detergents to disperse the flocculi and based on the amount of detergent required to disperse the emulsion into single droplets, they arranged the emulsions in a arbitrary scale according to their degree of flocculation.

Darling (1982) evaluated the degree of clustering of emulsion from the measurement of the change of viscosity at constant shear with time. A number of light scattering techniques have also been used to measure flocculation kinetics (Lips and Willis, 1973).

2.11.3 Coalescence

Coalescence refers to an increase in the droplet size by joining two droplets, which may eventually result in separation of the oil and aqueous phases. This process is irreversible. When two droplets approach each other, a thin film of the continuous water phase is trapped between the droplets. The behaviour of the thin film determines the degree of stability or instability of the emulsion, and the rate of thinning of the film determines the time required for the two drops to make contact. When the film has thinned to a critical thickness, it may rupture and the two drops may unite and coalesce into a large drop.

Various factors, such as solubility of the emulsifier, pH, salts, emulsifier concentration, phase-volume ratio, temperature and properties of the film affect coalescence stability of emulsions. Before the formation of an emulsion, the solubility of the emulsifier is an important parameter in forming the

emulsion and in controlling the coalescence phenomenon (Halling, 1981, Kinsella, 1984). Limited solubility is desirable once the emulsion is formed in order to prevent film loss and desorption. Many proteins display poor emulsifying properties and coalescence stability at or near their isoelectric point because of reduced solubility and low net charge. Das and Kinsella (1989) found that β-lactoglobulin-stabilized emulsions showed maximum rate of coalescence around pH 5.0 which is close to the isoelectric point.

Coalescence of emulsion droplets causes several changes in an emulsion with time; these include increase in average droplet size and volume of separated phase and decrease in the number of droplets, turbidity, emulsion viscosity, etc. Therefore, coalescence stability of emulsions can be determined experimentally by following the changes over time in any one of the above properties. Changes in droplet size and number can be followed by microscopy (Das and Chattoraj, 1980), Coulter counter (Walstra, 1968), or turbidity measurements (Walstra, 1968; Pearce and Kinsella, 1978; Das and Kinsella, 1989). Measurement of the volume of separated phase to determine coalescence stability of emulsions has been employed by Tornberg and Ediriweera (1987). Viscosity measurements have been used by Shioya *et al.* (1981) to determine coalescence.

2.11.4 Phase Inversion

Phase inversion involves a change from an oil-in-water to a water-in-oil emulsion or vice versa. This may occur when the phase volume of the oil is sufficiently high.

2.12 Factors Affecting Emulsion Stability

The apparent stability of dispersions, such as emulsions, results from the existence of interparticle forces between dispersed droplets. Therefore, it includes van der Waals forces, electrical double layer interactions, etc (Leman and Kinsella, 1989). The main physical factors affecting food emulsion stability are summarized in the Table 2.7.

Table 2.7: Physical factors affecting food emulsions. (Dickinson, 1988).

Attributes	Creaming	Flocculation	Coalescence
Droplet size	***	**	*
Droplet size distribution	***	**	--
Volume fraction of dispersed phases	***	***	***
Density difference between phases	***	--	--
Viscosity (rheology) of continuous phase	***	***	**
Visosity (rheology) of the adsorbed layer	--	--	***
Thickness of the adsorbed layer	--	**	***
Electrostatic interaction between droplets	*	**	**
Macromolecular interaction between droplets	--	***	**
Fat crystallization	--	--	***
Liquid crystalline phases	*	*	**

*** - Generally important, ** - often important, * - sometimes important,

-- Not important

The stability of emulsions is affected by a number of factors, including composition of continuous phase, droplet size and net charge, ionic strength, viscosity of the continuous phase, density of the droplet, temperature, nature of the emulsifying agent, and mechanical and physical properties of the adsorbed film (Graham and Phillips, 1976).

2.12.1 Droplet size

Larger sizes usually result in more rapid creaming and coalescence. The size of the droplets formed depends on the type of equipment used and on the energy intensity applied during emulsion formation. By using high homogenization pressure the size of the droplet can be reduced (Walstra, 1984). The type of surfactant and its concentration also affect the droplet size.

2.12.2 Nature of the interfacial film

The stability of an emulsion depends on the nature of the interfacial film. Surface viscosity is a measure of the susceptibility to deformation of the interface. A possible relationship between surface elasticity, surface viscosity and emulsion stability was emphasised in the past. The higher the surface viscosity and elasticity the lower is the rate of coalescence. These observations have been supported by recent experimental data obtained with protein and non-protein emulsifiers (Rivas and Sherman, 1984). Surface viscosity plays a major role in controlling the rate of flow of film away from the point where globules make contact, whereas surface elasticity helps to prevent coalescence on impact by accommodating deformation. According to Boyd *et al.* (1972), both surface viscosity and surface elasticity are involved in controlling the coalescence. Coalescence is caused by the force pressing adjacent globules together, giving rise to a compressive stress. With time the stress causes breakdown of the film that results in coalescence (Boyd *et al.* 1972).

The coalescence stability cannot be explained on the basis of surface viscosity alone. Casein forms highly stable emulsions and gives very small droplets, although surface viscosity of a casein film is very low (Graham and Phillips, 1980 a,b ; Tornberg, 1978a). Graham and Phillips, (1980 a,b) did not find any positive correlation between surface viscosity and coalescence stability. If surface viscosity is the only determining factor in coalescence stability, emulsions with high surface load and greater film thickness of the absorbed layer should be more stable than emulsions containing droplets with thinner films. Studies of β-lactoglobulin-stabilized emulsions (Das and Kinsella, 1989) indicate that emulsions with a thicker absorbed layer around the isoelectric point were in fact least stable against coalescence. The most stable emulsions were obtained at pH 9.7. Tornberg and Ediriweera (1988) made similar observations on studies with casein and whey protein.

2.12.3 Temperature

Increasing temperature both directly and via a reduction in viscosity and surface rigidity, tends to increase all the breakdown processes in emulsions. But in the case of proteins, thermal denaturation may cause an increase in viscosity of the continuous aqueous phase above certain temperatures and may even result in gelation. This may decrease the rate of emulsion breakdown since droplet mobility is reduced (Halling, 1981).

2.12.4 Effect of low molecular weight surfactants

Small amphiphilic molecules can displace protein films, cause disruption of the film, and result in destabilization of emulsions. Monoglycerides and fatty acids can destabilize emulsions by displacing the surfactant protein film (Halling, 1981).

2.12.5 Viscosity

The mobility of emulsified droplets affects emulsion stability by influencing the rate and extent of collisions. This is determined by the viscosity of the continuous phase. In packed food emulsions the protein covered oil droplets are separated by an aqueous layer. The viscosity of the continuous phase determines the rate at which adjacent globules approach. Generally, an increase in stability is observed with increasing viscosity of the continuous fluid (Walstra, 1984 and Menon and Wasan, 1985).

2.12.5 Effect of pH

The pH affects the stability of food emulsions in many ways (Halling, 1981). The pH affects the net charge of the absorbed layer and hence the conformation of protein molecules that affects protein load and electrostatic interactions at the oil/water interface, and determines the film cohesiveness and interfacial rheology.

MATERIALS AND METHODS

3.1 Materials

Sodium caseinate (Alanate 180) and milk fat (FFMR) were obtained from New Zealand Dairy Board, Wellington, New Zealand. Soya Oil was purchased from the local supermarket.

All the chemicals used were of analytical grade obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co.(St Louis, MO, USA) unless specified otherwise.

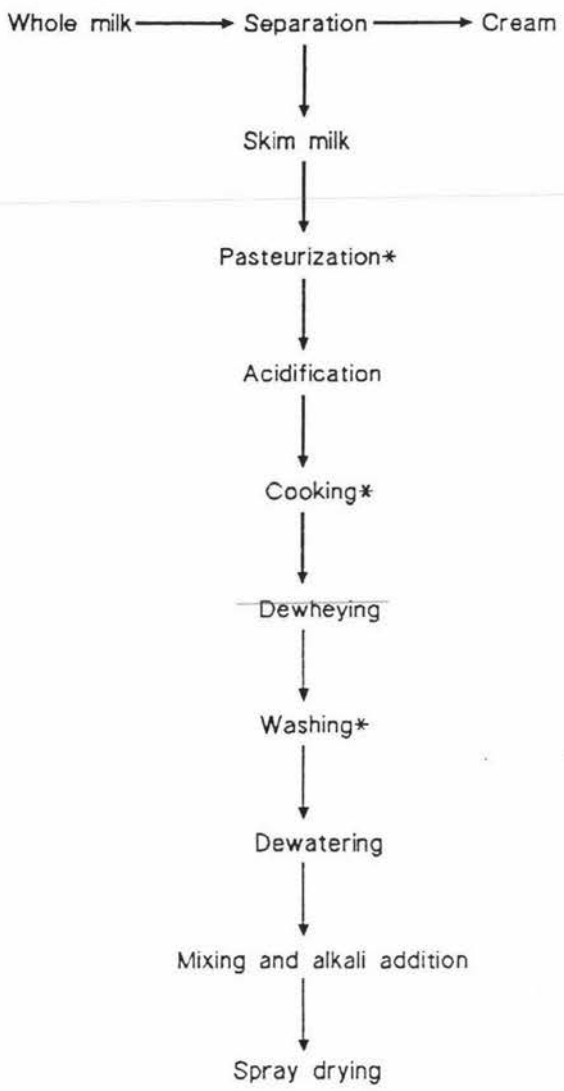
3.2 Methods

3.2.1 Preparation of Sodium Caseinate

Sodium caseinates were prepared in the laboratory and on a pilot scale.

The laboratory sodium caseinate was prepared from whole milk, obtained from Massey University Dairy Farm Number 1, preheated to 55°C, and fat removed by centrifugation at 5150 *g* for 25 minutes (Du Pont Instruments, Sorvall SS-3). Skim milk at room temperature was adjusted to pH 4.6 by the addition of 2M HCl with gentle stirring. The curd/whey mixture was warmed to 35°C and held for 10 minutes. The mixture was then filtered through a cheese cloth. The curd was washed twice with distilled water at 22°C. After dewatering, the curd was dispersed in distilled water (twice the amount of curd) and the pH of the mixture was adjusted to 7.0 with slow addition of 2M NaOH to vigorously stirred curd slurry. The mixture was stirred for 2 hours and pH maintained at 7.0. The dispersion was dialysed against large volumes of distilled water at 5°C for 30 hours. Later, the dispersion was freeze dried using Cuddon Laboratory Freeze Drier.

For the preparation of pilot scale sodium caseinate, skim milk was pasteurized using a Spiral flow Laboratory Ultra High Temperature plant (Spira flow, Australia, Marketed by Alfa-laval) at different temperatures as shown in Table 3.1. Dilute (0.5 M) sulphuric acid was rapidly mixed into the skim milk to obtain the desired pH of 4.6. The curd/whey mixture was gently stirred for 10 minutes using a stainless steel spoon at different cooking temperatures. The mixture was then drained on a stainless steel mesh (0.7 mm apertures). The curd was given four washes with tap water at different washing temperatures. During washing, the curd was gently stirred for about 10 minutes. The curd and the wash water were drained on a stainless steel mesh screen (0.7 mm apertures). The curd was then weighed and an equal amount of distilled water was added. The mixture was then passed through a colloid mill (Premier Colloid Mill Ltd., 3" Multipurpose Mill). Using 2.5 M NaOH the pH of the slurry was raised to 7.0 with vigorous agitation. Immediately the temperature of the slurry was raised to 90°C. The slurry was then dried using an Anhydro - Laboratory Spray Drier Number 1 (Anhydro, Denmark, Model Number LAB. S1) with inlet and outlet temperatures of 180° and 80°C, respectively. The details of the processing conditions used for making these three types of sodium caseinate are given below.



* Pasteurization, Cooking, Washing temperatures were varied as shown in Table 3.1.

Figure 3.1: Preparation of sodium caseinate on pilot scale.

Table 3.1: Preparation methods of sodium caseinates under different processing conditions

Manufacturing Variables	Heat Treatment Conditions		
	Mild	Normal	Severe
Pasteurization Temp. °C	None	72	90
Pasteurization time (seconds)	None	15	5
Cooking Temperature °C	40	55	80
First Wash Temperature °C	55	55	55
Second Wash Temperature °C	55	65	65
Third Wash Temperature °C	55	75	90
Fourth Wash Temperature °C	35	35	35

3.3 Preparation of Emulsions

Emulsions were usually prepared from 2.5% (w/w) protein solution and 30% (w/w) soya bean oil. In some cases, emulsions were made with varying concentrations of protein or soya oil. The mixture was heated to 55°C, and then passed through a two stage valve homogenizer without applying any pressure (Rannie, model LAB, type 12.50 H, capacity 100 l/h, Rannie a/s, Roholmsvej 8 DK - 2620, Albertslund, Denmark). This produced a temporary oil-water emulsion. The mixture was then homogenized at the desired pressure, usually 102/34 bars for the first and second stages respectively. The resulting homogenized emulsion was stored at 20°C.

3.4. Emulsion Characterization

3.4.1 Droplet Size Distribution

The droplet size distribution in the emulsions was determined using MasterSizer (Model MS 20, Malvern Instruments Ltd., Malvern,

Worcestershire, UK) at the New Zealand Dairy Research Institute, Palmerston North.

In this method low power laser beam is diffracted by a diluted dispersion of the emulsion and is collected over a range of scattering angles by a series of semicircular photo-electric diodes. The volume size distribution is calculated from the intensity of light diffracted at each angle using Lorenz-Mie theory. To calculate successfully this size data, the MasterSizer requires the refractive index and absorbance of the particle being measured and the refractive index of the medium in which the particles are dispersed.

The parameters which were used in analysing the particle size are

Presentation : (2NAD) 1.330, 1.456 + i0.00000 polydispere model

Relative Refractive index = 1.095

Absorbance = 0.001

$$\frac{\textit{Refractive Index of the particle}}{\textit{Refractive Index of water}} = 1.33$$

The MasterSizer divides the size distribution into 22 classes across the sub-micron range (0.1 - 1.0 microns). The parameters obtained from the MasterSizer are shown below:

D_{32} : The volume-surface mean diameter (also known as the Sauter mean diameter or D_{vs})

$$D_{32} = \frac{\int_{D_1}^{D_2} D^3 N(D)\, dD}{\int_{D_1}^{D_2} D^2 N(D)\, dD}$$

The D_{32} is the surface weighted mean diameter (i.e., the diameter of the particle having the sample mean surface area) where N is the number of particles with diameter D. D_1 is the minimum diameter in the distribution and D_2 is the maximum distribution diameter.

3.4.2. Specific Surface Area

The specific surface area was also obtained from the MasterSizer results, and was used for the protein load calculations.

A typical analysis of an emulsion obtained using the Malvern Mastersizer including size distribution of the globules is shown in Figure 3.2.

3.5. Protein Load determination

The amount of protein adsorbed onto the oil surface was determined by measuring the protein content of the subnatant after separation of the dispersed and aqueous phase by centrifugation. The details of separation of cream and subnatant are described below.

3.5.1. Separation of Aqueous phase and Cream

The emulsion (30 g) was centrifuged at 45,000 *g* for 40 min at 20°C in a temperature controlled centrifuge (Sorvall RC5C, DuPont Company, USA) and the subnatant was carefully removed using a syringe. The cream layer was dispersed in deionized water (purified by reverse osmosis followed by treatment with a Milli-Q apparatus, Millipore Corp., Bedford, MA, USA) and recentrifuged at 45,000 *g* for 40 min. Again, the subnatant and cream layer were collected carefully.

STABILITY –6, :Run Number 51

Emulsion made with 50% fat and 2.5% protein homogenization
pressure 1500 psi / 500 psi.
Stability AT THE SEPTUM LEVEL –3.

Sample File Name: EMUL2 , Record: 66 Source: Analysed
Measured on: Tue, Dec 14, 1993 1:23PM Last saved on: Tue, Dec 14, 1993 1:23PM

Presentation: (2NAD) 1.330, 1.456 + i 0.00000
Polydisperse model Volume Result Focus = 45 mm.

Residual = 0.308 % Concentration = 0.008 % Obscuration = 19.56 %
d (0.5) = 1.17 µm d (0.1) = 0.39 µm d (0.9) = 2.61 µm
D [4, 3] = 1.57 µm Span = 1.91
Sauter Mean (D[3,2]) = 0.81 µm Mode = 1.56 µm
Specific Surface Area = 7.4078 sq. m. / gm Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.10	0.00	0.12	0.00
0.12	0.40	0.15	0.40
0.15	0.90	0.19	1.30
0.19	1.48	0.23	2.79
0.23	2.20	0.28	4.98
0.28	3.08	0.35	8.06
0.35	4.14	0.43	12.20
0.43	5.39	0.53	17.59
0.53	6.79	0.66	24.38
0.66	8.24	0.81	32.63
0.81	9.60	1.00	42.23
1.00	10.64	1.23	52.87
1.23	11.37	1.51	64.24
1.51	11.57	1.86	75.81
1.86	9.72	2.30	85.53
2.30	6.68	2.83	92.21

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
2.83	3.92	3.49	96.13
3.49	1.99	4.30	98.12
4.30	0.83	5.29	98.95
5.29	0.21	6.52	99.16
6.52	0.00	8.04	99.16
8.04	0.00	9.91	99.16
9.91	0.00	12.21	99.16
12.21	0.02	15.04	99.19
15.04	0.08	18.54	99.27
18.54	0.14	22.84	99.41
22.84	0.20	28.15	99.61
28.15	0.22	34.69	99.83
34.69	0.16	42.75	99.99
42.75	0.01	52.68	100.00
52.68	0.00	64.92	100.00
64.92	0.00	80.00	100.00

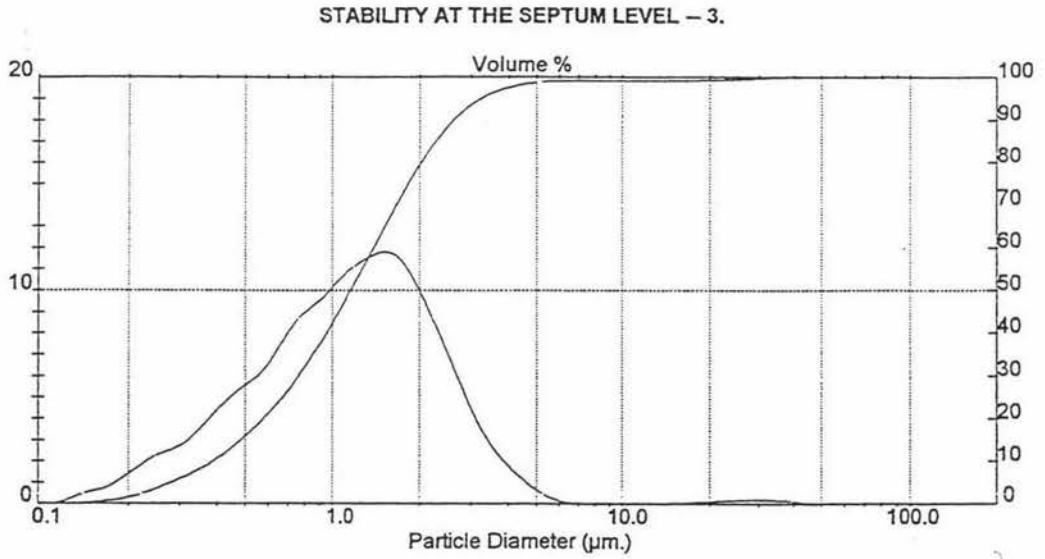


Figure 3.2: The typical Malvern MasterSizer output.

The subnatants were then filtered through 0.45μm and 0.22μm filter paper (Millipore Corporation, Bedford, MA 01730) using Nalgene filtering unit (Nalge Company, Rochester, New York 14602-0365). The filterates were analyzed separately for total protein and individual proteins.

3.5.2 Protein Load calculations

% Protein of the subnatant was obtained from Kjeldahl.

$$\textit{Adsorbed Protein} = \textit{Total Protein (g) taken for making an emulsion} - \textit{Protein (g) present in the subnatant}$$

$$\textit{Protein Load } (mg / m^2) = \frac{\textit{Total mg protein adsorbed}}{\textit{Total Fat Surface area}}$$

3.6. Viscosity measurement

The viscosities of caseinate solutions at concentration ranging from 0.1 to 7.5% (w/w) were determined at 20°C using Haake RV_3 Viscometer (Type- MK 50) with rotor and stator types NV. The effects of $CaCl_2$ addition and pH on viscosity were determined using 2.5% w/w, sodium caseinate solution.

3.7 Chemical Analysis

Total protein was measured by determining the total nitrogen by macro-Kjeldahl method (AOAC, 1974) and multiplying it by the factor 6.38. The samples were digested using a Kjeltec digester (Kjeltec 1007 Digester, Tecator, Sweden) and distilled using a Kjeltec system (Kjeltec 1026 Distilling Unit, Tecator, Sweden).

The total fat in the emulsion was determined using the Rose-Gottlieb gravimetric method for cream (IDF 16C : 1987).

3.8 Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method of Laemmli (1970), as described by Singh and Creamer (1991).

3.8.1. Preparation of stock solutions

Acrylamide/Bis (30% T, 2.67%C)

Acrylamide (30 g) and N, N-bis acrylamide (0.8 g) were dissolved in deionised water to give a final volume of 100 ml, and stored at 4°C in a dark bottle.

1.5 M TRIS-HCl buffer, pH 8.8

TRIS (tris hydroxymethyl aminoethane, 18.15 g), obtained from USB (United States Biochemicals Corp. Cleveland, OH, USA), was dissolved approximately in 60 ml of deionised water, the pH adjusted to 8.8 with 1 M HCl and the volume made up to 100 ml with deionised water. The buffer was stored at 4°C.

0.5 M TRIS-HCl Buffer, pH 6.8

TRIS (6 g) was dissolved in about 60 ml of deionised water. The pH of the solution was adjusted to 6.8 with 1 M HCl, and then the volume made up to 100 ml with deionised water. The buffer was stored at 4°C.

10% SDS

SDS (10 g) was dissolved in deionised water with gentle stirring and the volume made up to 100 ml.

SDS-reducing buffer (sample buffer), 100 ml

To 50 ml of deionised water, 0.5 M Tris HCl buffer(12.5 ml), glycerol (10 ml), 10% (w/v) SDS (20 ml), β-mercaptoethanol (5 ml) and 0.05% (w/v) bromophenol blue (2.5 ml) were added. The pH of the sample buffer was adjusted to 6.8.

5X Electrode Buffer

Electrode buffer (5X) was prepared by dissolving TRIS (15 g), glycine (43.2 g) and SDS (3.0 g) in deionised water. After adjusting the pH to 8.3, the volume of the buffer was made up to 1 l and the buffer was stored at 4°C. For each electrophoresis run 60 ml of this electrode buffer was diluted with 240 ml deionised water.

Preparation of resolving gel (16%, w/v, 0.375 M TRIS, pH 8.8)

To prepare 10 ml of resolving gel buffer, the following solutions were mixed: deionised water (2.02 ml), 1.5 M Tris-HCl buffer (2.50 ml), 10% SDS (100 µl) solution and acrylamide/bis mixture (5.3 ml). The mixture was then degassed for 15 minutes. Immediately 5 µl of TEMED (tetramethylethylenediamine) and freshly prepared 10% (w/v) ammonium persulphate (50 µl) were added. After gentle mixing, 3.3 ml of the contents were poured between the electrophoresis casting plates (Bio-Rad Mini Protean, Bio-Rad, Richmond, CA, USA). A small quantity of deionized water was added to form an upper layer and the acrylamide solutions allowed to polymerize at 20°C for about 45 minutes. The water was then drained and dried with filter paper before

pouring the stacking gel.

Preparation of stacking gel

To prepare 10 ml of stacking gel, the following solutions were added to 6.1 ml of deionised water, 0.5 M Tris-HCl buffer (2.5 ml), 10% SDS (100 µl) solution and 30% acrylamide/bis mixture (1.3 ml). The mixture was degassed for 15 minutes. Immediately TEMED (10 µl) and freshly prepared ammonium persulphate (50 µl) were added and 2.2 ml of this mixture was then poured on top of the resolving gel. The slot former (plastic comb) was immediately placed at the top of the stacking gel (between the plates) to form appropriate slots for the samples. Polymerisation was carried out at 5°C overnight. Next day the plastic comb was removed and the slots were rinsed with deionised water. The excess water was then removed with filter paper. Gel plates were then placed in the electrode chamber and samples were applied to the gel slots.

3.8.2. Sample preparation and running gels

Samples were dispersed in SDS sample buffer and the mixtures were heated in a water bath at 90°C for 5 minutes and then cooled to room temperature. The dilution of the sample (subnatant) with the sample buffer was dependent on the amount of protein present in the subnatant. The subnatants dispersed in sample buffer (5 µl) were applied to the slots of the SDS gel. The gels were run on the a Mini-Protean system (Bio-Rad, Richmond, CA, USA) at 200 V using a Bio-Rad power supply unit (Model 1000/500, Bio-Rad, Richmond, CA, USA) until the tracking dye moved out of the gel. The approximate running time was about 60 min after which the gels were removed from the plates and transferred gently to staining solution.

Staining

The gels were put into plastic boxes and about 50 ml of Coomassie Blue R solution (1 g brilliant blue R was dissolved in 500 ml of isopropyl alcohol and 200 ml acetic acid and the contents made to 2 l with distilled water) was added. The gel boxes were put on a rocking table (made at the Food Technology Department workshop) so that the gels were uniformly stained with the staining solution.

Destaining

The staining solution was drained carefully after 1 h and replaced with destaining solution which contained a mixture of 100 ml isopropyl alcohol and 100 ml acetic acid diluted to 1 l. The destaining soluion was again changed after 1 hour with fresh destaining solution. Then the plastic box is left on the rocking table for 19 hours. After that the destaining solution is replaced with distilled water and the gel is scanned using the densitometer.

3.8.3. Densitometry

Quantitative determination of the component of the mixture separated by SDS-PAGE was performed by densitometry using a laser densitometer (LKB Ultroscan XL, LKB Produkter, AB, Sweden). In the densitometer, the protein bands on the stained gel are scanned with a narrow beam of laser light and the absorbance at 522 nm is plotted as a function of track distance. The output from the densitometer was quantified by measuring the areas under individual peaks. The scanning procedure involved cleaning the densitometer gel plate, placing the gel on the densitometer gel plate, defining the tracks and scanning each individual band. The printout resulted in the form of a graph of individual peaks and table of individual peak areas. Standard protein solutions were run in conjunction with samples to aid identification

of unknown protein bands in the sample.

3.9. Creaming Stability

Immediately after preparation, the emulsions were transferred into the stability tubes and maintained at 20°C for 24 hours. The stability tube is a silicon tube of length 199 mm with septums attached to it at four different places (made at Food Technology Department workshop). The distance between each septum is kept constant (see Figure 3.3 and 3.3a). After 24 hours 5 ml samples were drawn using a syringe from each septum of the tube as well as from the top of the tube. The samples were analyzed for the droplet size distribution and the fat content.

The creaming stability or stability rating was based on the percent change in fat in the sample drawn to percent fat in the original emulsion.

$$\%\ \textit{Stability Rating} = \frac{\%\textit{Fat in sample}}{\%\ \textit{Fat in original emulsion}} \times 100$$

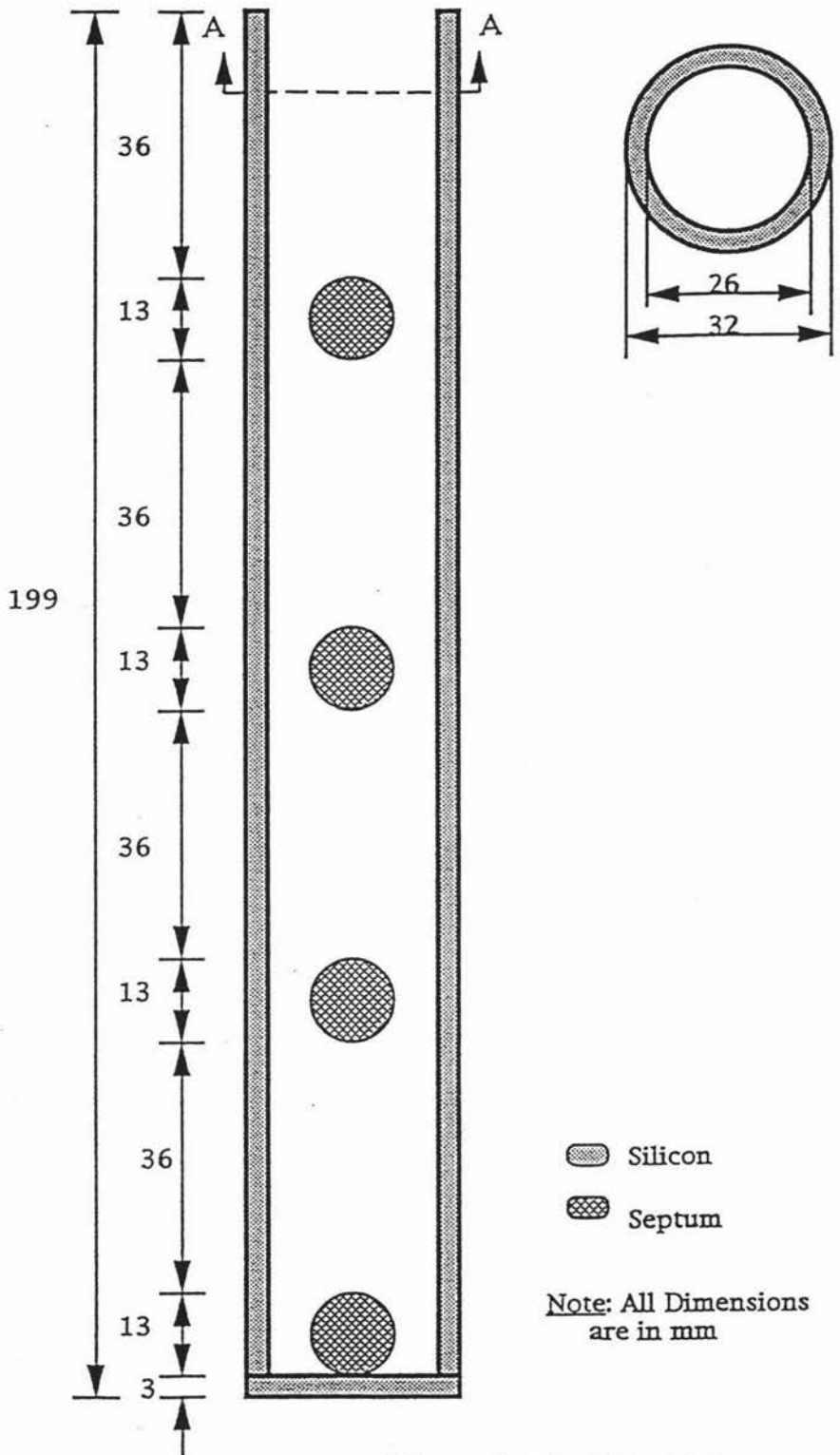


Figure 3.3: Stability Tube.

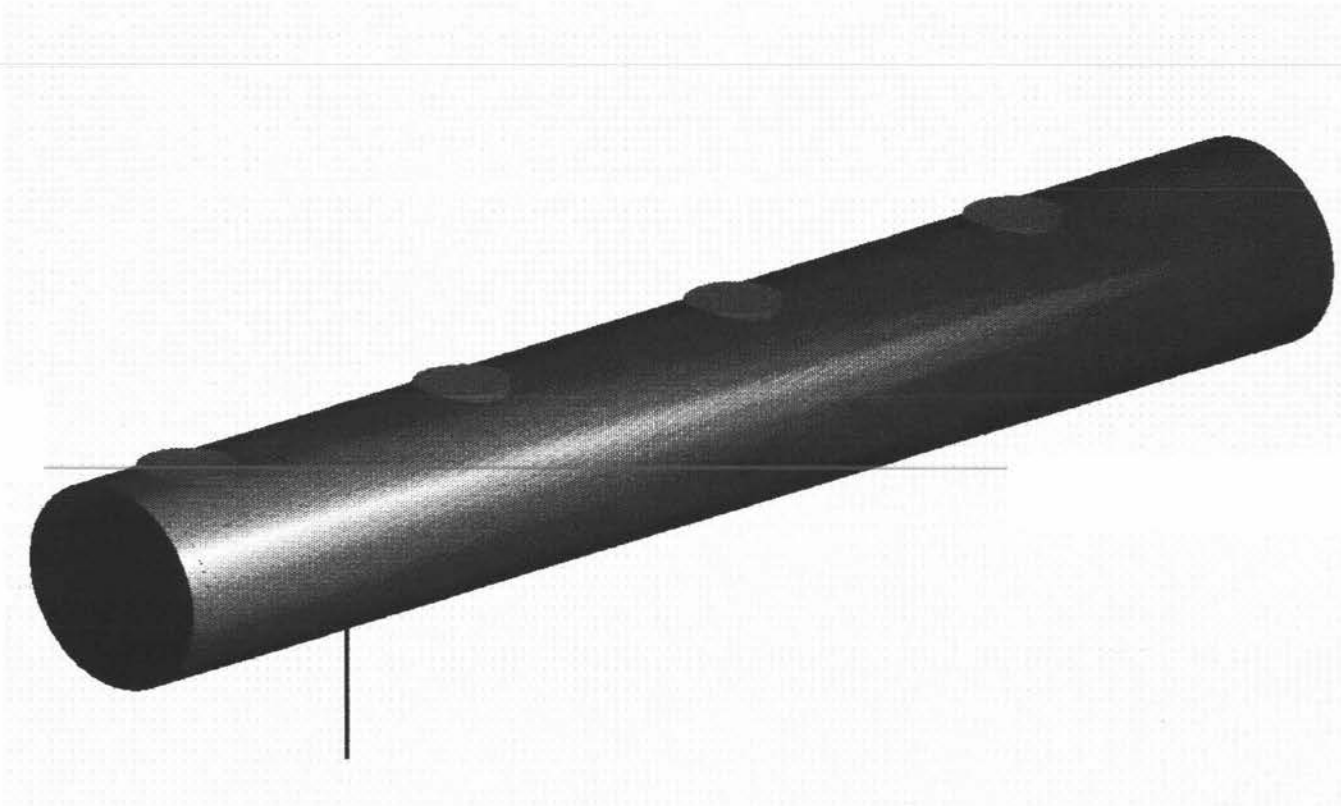


Figure 3.3a: An Isometric view of stability tube.

OBJECTIVES

1. To determine the adsorption behaviour of sodium caseinate (Alanate 180) in soya oil-in-water emulsions.

2. To determine the influence of some compositional and processing parameters (eg. protein concentration, fat concentration, homogenization pressure) on the adsorption behaviour and creaming stability (under gravity) of sodium caseinate-stabilized emulsions.

3. To determine the influence of method of caseinate manufacture (eg pasteurization temperature, cooking temperature, washing temperature) on the adsorption behaviour and creaming stability (under gravity) of sodium caseinate-stabilized emulsions.

5.0 ADSORPTION BEHAVIOUR OF SODIUM CASEINATE IN OIL-IN-WATER EMULSIONS

5.1 Introduction

Sodium caseinate is an excellent emulsifying agent and is a commonly used ingredient in a wide range of emulsions, for example coffee whiteners, cream liqueurs and whipped toppings. Caseinate is a composite of four different proteins α_{s1}-, α_{s2}-, β- and κ-casein in weight proportions of approximately 4:1:4:1. All caseins, especially α_{s1}- and β-caseins, readily adsorb at oil/water interfaces and decrease the interfacial tension. This provides stability to the resultant emulsions with respect to coalescence and flocculation. Studies to date tended to concentrate on the adsorption of pure caseins and mixtures of isolated proteins (Benjamins *et al* 1975; Dickinson *et al* 1988a,b; Hunt and Dalgleish, 1994). Studies on the adsorption behaviour of sodium caseinate, especially that commercially produced are limited. The effects of various compositional parameters, such as fat concentration, protein concentration, pH and salt concentration on adsorption have not been fully explored.

The present chapter describes the effects of some compositional and processing variables on the adsorption of sodium caseinate at oil/water interfaces in emulsions. Factors such as fat concentration, protein concentration, homogenization pressure, pH, and addition of salts have been investigated. Emulsions were characterized by (i) volume-surface droplet diameter, d_{32}, (obtained from Malvern MasterSizer), (ii) protein load (calculated by the "depletion method") and (iii) proportions of individual proteins at the oil droplet surface (using SDS-PAGE).

5.2 Effect of protein concentration

Protein concentration was varied by dissolving different quantities of sodium caseinate in water and mixing with 30% (w/w) soya oil. Homogenization was carried out at ~ 102/34 bars and 55°C as explained in Materials and Methods.

5.2.1 Droplet diameter and specific surface area

The effect of protein concentration on average droplet diameter, d_{32} (μm), is shown in Figure 5.1. When the concentration of protein was increased from 0.5 to 7.5%, w/w, there was no significant change in d_{32}. The changes in specific surface area (m^2/g oil) were also not significant as the protein concentration was increased (Figure 5.1). The specific surface areas were in the range of 8.11 to 7.82 m^2/g oil.

These results are essentially in agreement with those reported by Dalgleish and Fang (1993) who reported that d_{32} of emulsions containing 20% soya oil was independent of casein concentration above 1%, but considerably large particles were formed at lower casein concentrations. Hunt and Dalgleish (1994) reported a slight decrease in d_{32} (approximately 0.1 μm) as the casein concentration was increased from 0.5 to 1.0%.

5.2.2 Adsorbed protein and protein load

The amount of protein adsorbed (mg/g oil) at the interface increased from ~ 2.73 to 28.05 mg/g fat with increase in protein concentration from 0.5 to 7.5% (w/w) (Figure 5.2). When the protein concentration was between 2 and 4%, the increase in adsorbed protein at the interface was not very significant. Between 0.5 and 2.0% concentration, the amount of adsorbed protein increased markedly from ~ 2.7 to ~ 10.5 mg/g fat. Above 5%, the adsorbed protein increased from ~ 12.36 to 28.05 mg/g fat as the protein concentration

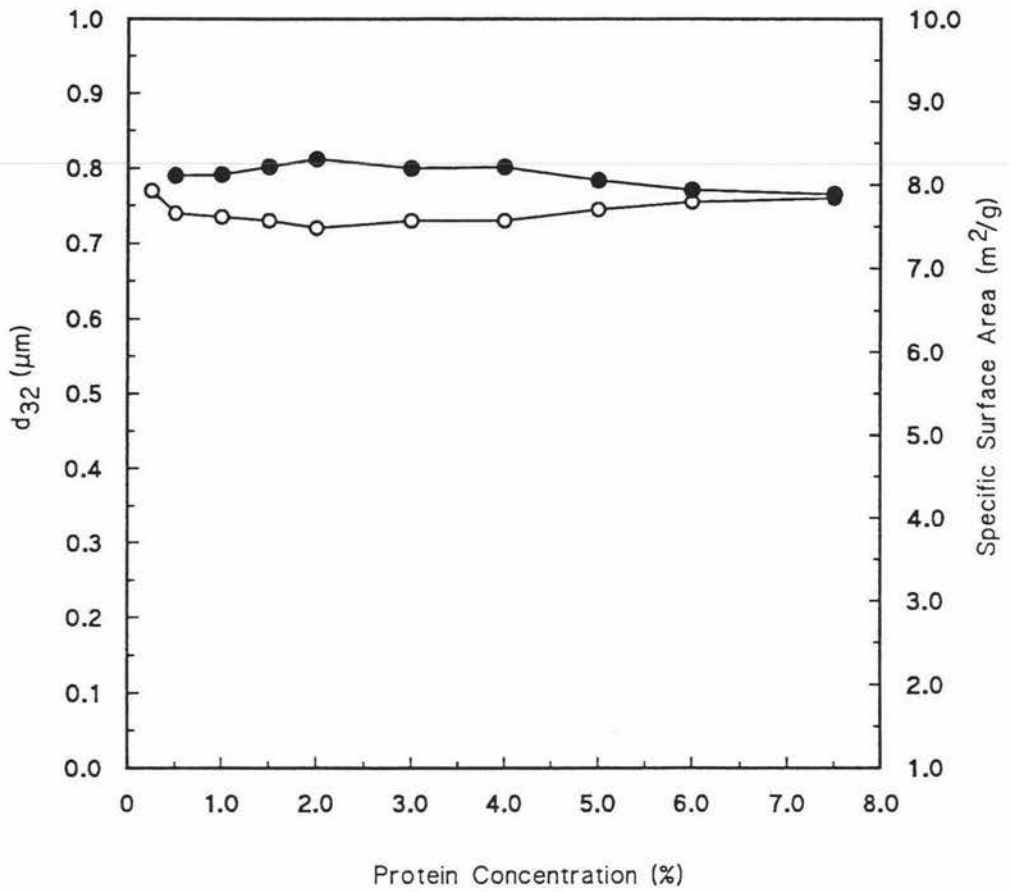


Figure 5.1: Changes in average droplet diameter, d_{32} (O) and specific surface area (●)in emulsions containing 30% oil and different amounts of casein.

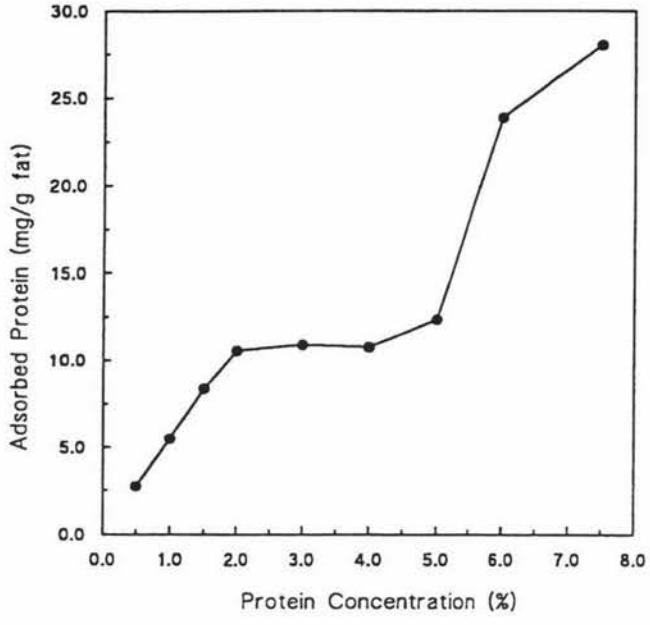


Figure 5.2: Changes in adsorbed protein (mg/g fat) in emulsions containing 30% soya oil and different amounts of casein.

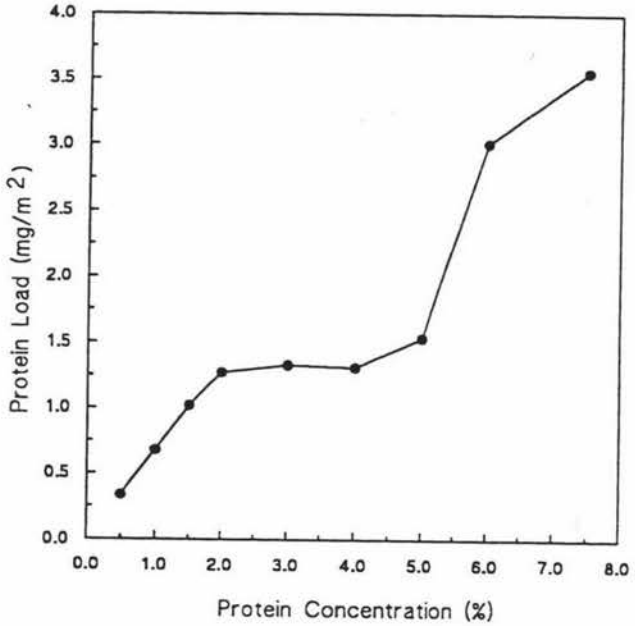


Figure 5.3: Changes in protein load (mg/m^2) in emulsions containing 30% soya oil and differents amounts of casein.

was increased up to 7.5%.

The changes in protein load (mg/m^2) with increase in protein concentration are shown in Figure 5.3. As the protein concentration was increased, the protein load (mg/m^2) also increased. The changes in protein load followed the same trend as that for the adsorbed protein at the interface (Figure 5.2). When the protein concentration was between 2.0 and 4.0% the change in protein load was not very significant. Further increases in protein concentration from 5 to 7.5% increased the protein load dramatically from ~ 1.53 to 3.55 mg/m^2. Decrease in protein concentration from 2.0 to 0.5% gradually decreased the protein load from ~ 1.27 to ~ 0.33 mg/m^2.

It is clear from these results that when the casein concentration is in the range of 2 - 4%, surface coverage attains a plateau value of ~ 1.3 mg/m^2. This probably corresponds to saturated monolayer coverage of adsorbed casein molecules. The addition of more caseinate (up to 5%) increases the surface coverage slightly which may be attributed to closer packing of the adsorbed caseins in the monomolecular layer. The sharp increase in surface coverage with further increase in caseinate concentration (up to 7.5%) may suggest the formation of secondary or multilayers around the oil droplets. Similar behaviour has been observed for β-casein, bovine serum albumin and lysozyme adsorption on to a planar oil-water interface by Graham and Phillip (1979).

The adsorption behaviour of sodium caseinate observed in this study is different from that reported by Dalgleish and Fang (1993) and Hunt and Dalgleish (1994). Dalgleish and Fang (1993) reported that a surface coverage in caseinate-stabilized emulsions increased to a maximum of about 3 mg/m^2

as the casein concentration was increased to 2%. However, the present study showed surface coverage of 1.3 mg/m^2 at 2% casein concentration. In addition, the present study showed a region (between 2 - 4%) where the surface coverage does not change with casein concentration, whereas Dalgleish and Fang (1993) found gradual increases in surface coverge with casein concentration. These differences could be attributed to the type of homogenizer and/or caseinates used to prepare emulsions. Dalgleish and Fang (1993) used a microfluidizer for making emulsions thereby obtaining a very small droplet diameter, i.e. ~ 0.3 μm at a protein concentration of 0.5 to 2.0%. They used a homogenizer pressure drop of 28 M Pa during the homogenization step and in the present study emulsions were homogenized in a valve homogenizer in which fairly large particle sizes were obtained. Dalgleish and Fang (1993) used freeze-dried caseinate prepared in a laboratory under relatively mild conditions, compared with commercial caseinate in the present study.

5.2.3 Proportions of individual caseins in subnatant (unadsorbed protein)

The effect of varying protein concentration on the proportions of the individual proteins in the subnatant is shown in Table 5.1.

Table 5.1: Effect of protein concentration on the individual proportions of caseins in the subnatant.

% Protein (%, w/w)	Proportions of individual proteins(%)		
	α_s-casein	β-casein	κ-casein
0.5	75.0	12.5	12.5
1.0	69.7	24.2	6.1
2.0	49.4	41.8	8.9
3.0	44.2	44.2	11.6
4.0	40.6	46.9	12.5
5.0	41.9	41.9	16.3
6.0	40.6	43.8	15.6
7.5	42.9	38.1	19.1
Original protein solution	45.05	40.05	14.9

As the concentration of protein in the emulsion was increased, the proportion of α_s-casein decreased dramatically from ~ 75 to 40%, but that of β-casein increased ~ 12.5 to 43% and the proportions of κ-casein were quite variable with no consistent trend. The changes in individual proteins at lower concentrations of protein (below 3%) were more marked than at higher concentrations of protein.

It can be concluded that at low protein concentration (i.e. at 0.5 and 1.0%) β-casein adsorbs at the interface in preference to α_s-casein. As the concentration of protein is increased, preference for β-casein adsorption diminishes. It is not clear why the preferential adsorption of β-casein was influenced by initial protein concentration. Pepper and Farrell (1982), using gel permeation

chromatography, observed that increasing protein concentration in the range of 0.1 to 2.94 wt % caused formation of casein aggregates in soluble whole casein. It is possible that the formation of these aggregates at protein concentrations > 1.0 wt % affects the adsorption behaviour of different casein components. Another possibility is that higher concentration of protein favours the formation of β-casein micelles (Swaisgood, 1982), which are not adsorbed as effectively as the monomeric β-casein (Euston *et al.* 1995). In emulsions containing above 3% protein, there appears to be no clear preference for any of the caseins.

Robson and Dalgleish, (1987) showed that there was no preference for adsorption of β-casein in sodium caseinate-stabilized emulsions immediately after homogenization. However, on ageing these emulsions, β-casein displaced some of the adsorbed α_{s1}-casein. Robson and Dalgleish (1987) used two passes through a Manton-Gaulin two stage homogenizer at a pressure of 27.2 M.Pa to prepare sodium caseinate stabilized emulsions. Earlier studies on pure caseins have also shown that β-casein is the most surface active of the caseins and adsorbs most readily at an oil-water interface (Benjamins *et al.* 1975). Dickinson *et al.* (1988b) showed that in emulsions stabilized by a mixture of pure α_{s1}- and β-caseins, β-casein was adsorbed in preference to α_{s1}-casein. Hunt and Dalgleish (1994) reported no preference for any casein at more than 1.5 wt%.

5.3 Effect of fat concentration

Fat concentration was varied by adding different quantities of soya oil or milk fat (AMF) to the protein solution (2.5%, w/w) prior to homogenization. Homogenization was carried out as described in Materials and methods.

5.3.1 Droplet diameter and specific surface area

The effect of fat (soy oil and milk fat) concentration in sodium caseinate stabilized emulsions on the average droplet diameter, d_{32} (μm) is shown in Figure 5.4 (A). As the fat concentration was increased from 5 to 50%, both soya oil and milk fat containing emulsions showed an increase in the d_{32}. The increase was somewhat greater in the case of soya oil.

The changes in specific surface area (m^2/g oil) with increase in fat concentration are shown in Figure 5.4 (B). The specific surface area decreased gradually from ~ 10.05 to 7.39 m^2/g with increase in soya oil concentration whereas in the case of milk fat the specific surface area decreased from 9.79 to 7.96 m^2/g oil.

The changes in specific surface area with increasing fat concentration are consistent with the results of earlier studies (Tornberg *et al.* 1990).

This increase in d_{32} with the increase in fat concentration of emulsions could be due to the recoalescence of fat globules as the fat concentration was increased (Tornberg *et al.* 1990). The probability of bridging between oil droplets is also greater at higher fat concentrations (Tornberg *et al.* 1990).

Recoalescence of the newly formed droplets depends on the time available for the interfaces to be covered by proteins in such a way that coalescence is prevented. This mainly depends on the fat content, homogenization pressure, homogenization temperature and probably the temperature at which emulsions were analyzed etc. It also depends on the rate at which the protein molecules cover the interface, protein concentration to fat surface area ratio and conditions of emulsification (Tornberg, 1980).

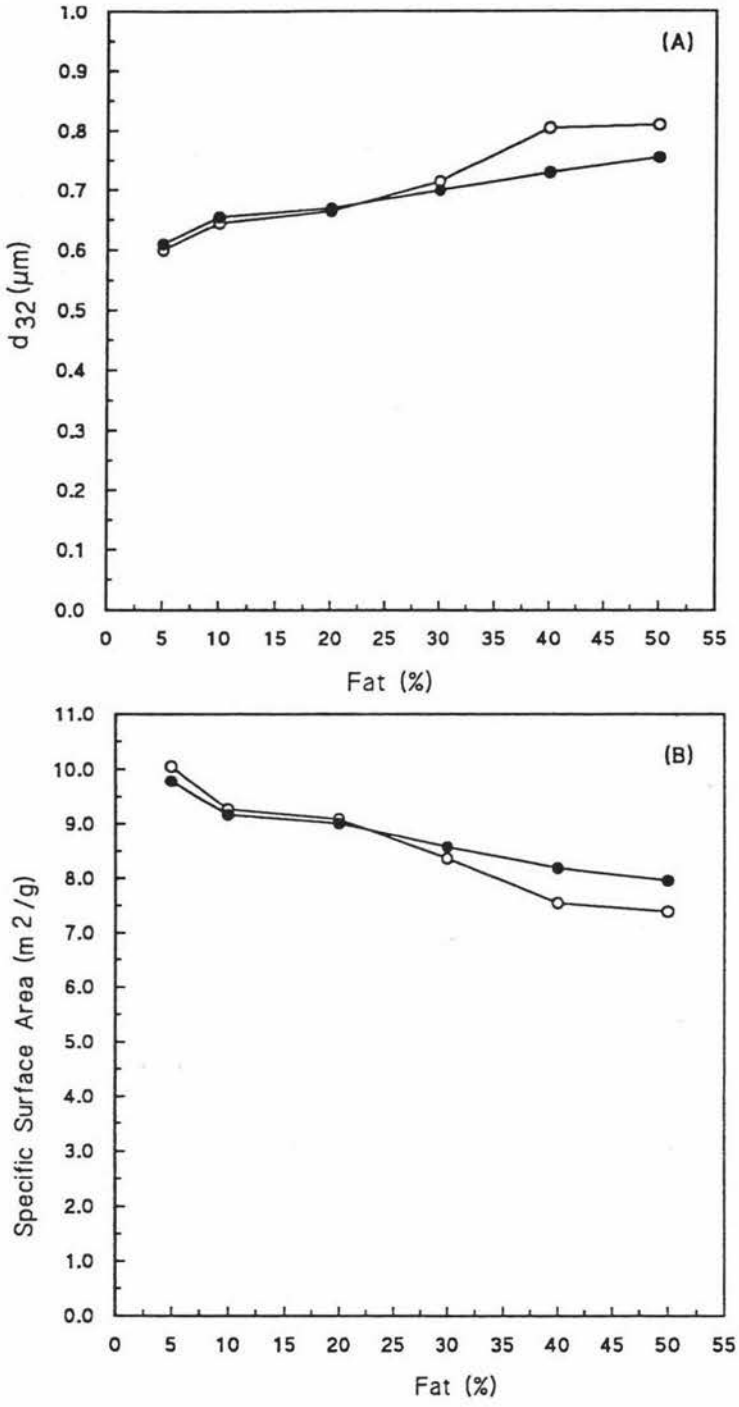


Figure 5.4: Average droplet diameter (d_{32})(A) and specific surface area (B) in emulsions containing 2.5% protein and different amounts of fat. (O) soya oil (●) Milkfat.

5.3.2 Adsorbed protein and protein load

The amount of protein (mg/g oil) adsorbed at the interface decreased from ~ 99.8 to 7.0 mg/g oil in soya oil containing emulsions as the fat concentration was increased from 5 to 50% (Figure 5.5). The changes in adsorbed protein with increasing milk fat concentration also showed similar trend, except at 5% fat concentration where the value was ~ 50% lower.

In the case of soya oil emulsion, the protein load (mg/m^2 fat) decreased markedly (i.e. from ~ 9.9 to 2.0 mg/m^2 fat), as the fat concentration was increased from 5 to 20% but further increases in fat concentration caused only a slight decrease in protein load (Figure 5.6). Similar trends were observed with milk fat; the protein load decreased from ~ 3.4 to 0.87 mg/m^2 as the concentration of milk fat was increased from 5 to 50% (Figure 5.6).

This decrease in protein load may be largely attributed to decrease in protein to fat surface area ratio at higher concentrations of fat, which probably results in increased protein unfolding/spreading at the interface resulting in lower protein load.

The high protein loads at low fat concentrations may be mainly due to the formation of multilayers because of high protein to fat ratio; this situation is similar to that observed in Figure 5.3.

5.3.3 Proportions of individual caseins in subnatant (unadsorbed protein)

The effect of fat (soya oil) concentration on the proportions of individual proteins in the subnatant is shown in Table 5.2.

At 5 and 50% fat concentration, the proportion of α_s-casein in the subnatant

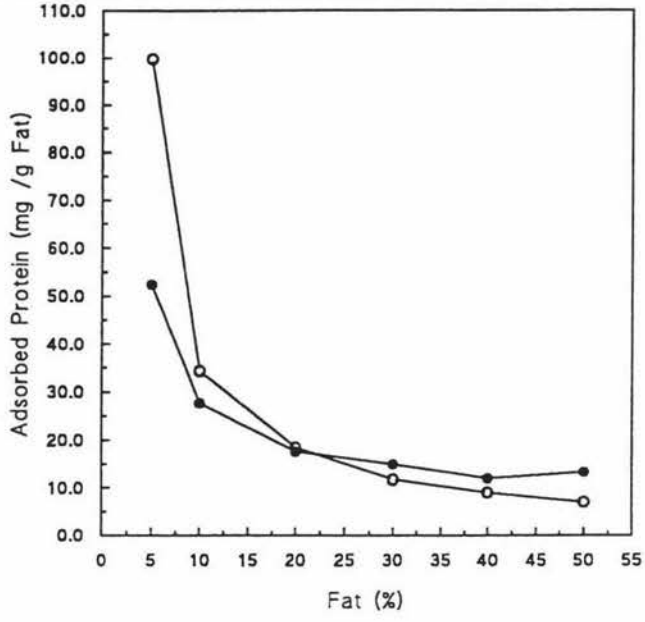


Figure 5.5: Changes in adsorbed protein in emulsions containing 2.5% protein with varying fat concentration

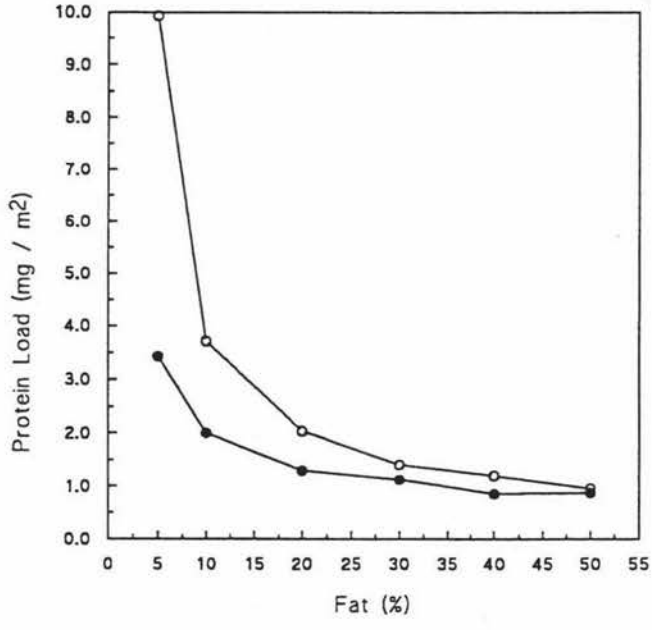


Figure 5.6: Changes in protein load in emulsions containing 2.5% protein with varying fat concentration.
Soya oil (O) Milk fat (●).

was slightly higher than that of β-casein, suggesting that slightly more β-casein was adsorbed at the interface. At fat concentrations between 10 to 40%, the proportions of α_s-, β and κ-caseins showed slight variations with no consistent trends.

Table 5.2: Effect of fat (soya oil) concentration on the proportions of individual caseins in the subnatant.

% Fat	Proportions of individual proteins (%)		
	α_s-Casein	β-Casein	κ-Casein
5	45.7	37.1	17.1
10	42.4	40.9	16.7
20	42.6	43.6	13.8
30	41.3	41.3	17.5
40	43.8	43.8	12.5
50	46.4	37.5	16.1

5.4 Effect of homogenization pressure

Emulsions made with soya oil (30%, w/w) and sodium caseinate (2.5% w/w, pH 7.0) were homogenized in a two stage valve homogenizer at different pressures, ranging from ~ 34 to ~ 340 bars at 55°C.

5.4.1 Droplet diameter and specific surface area

The effect of homogenization pressure on the average droplet diameter, d_{32} (μm), is shown in Figure 5.7 (A). The d_{32} decreased from 0.97 to 0.33 μm with increase in pressure from ~ 34 to ~ 340 bars.

A linear relationship (r^2 = 0.99) was found between pressure (P) and the log of fat droplet diameter (d_{32}) (Figure 5.8 B). The relationship could be

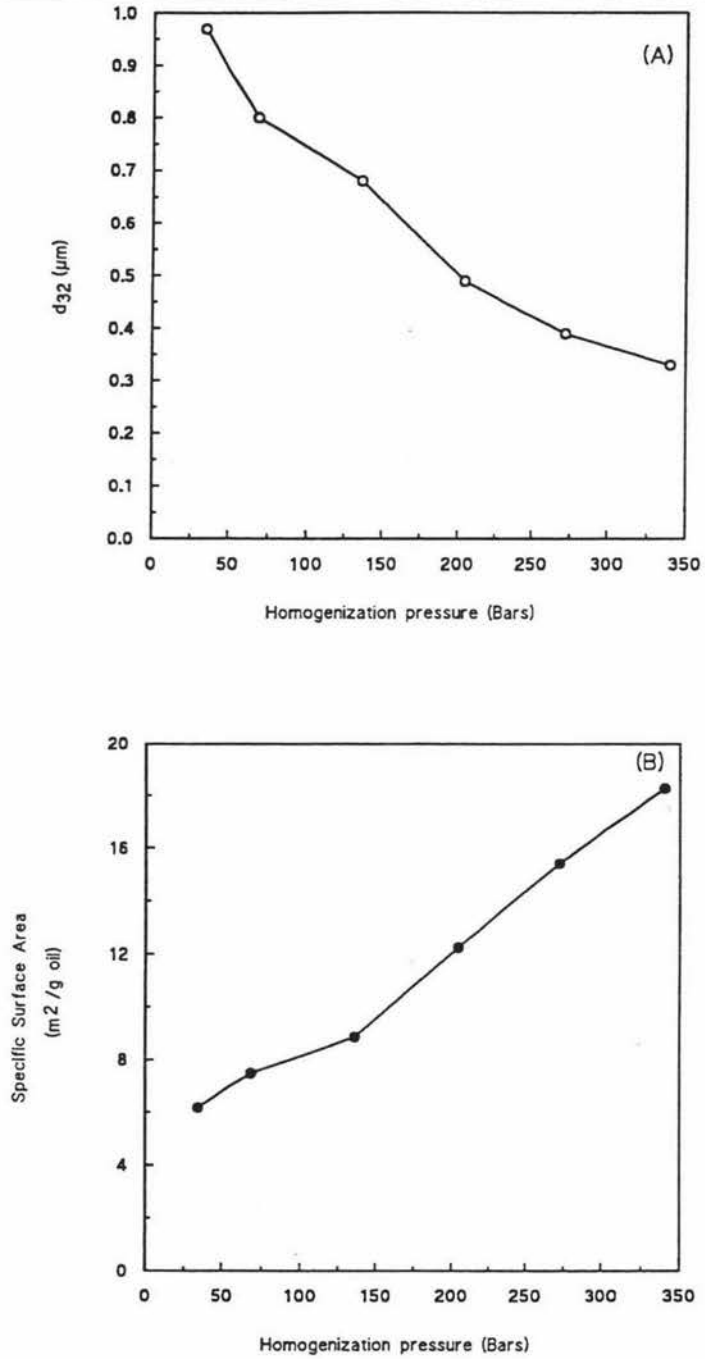


Figure 5.7: Changes in average droplet diameter, d_{32} (A) and specific area (B) in emulsions containing 30% soya oil and 2.5% casein concentration at different homogenization pressures.

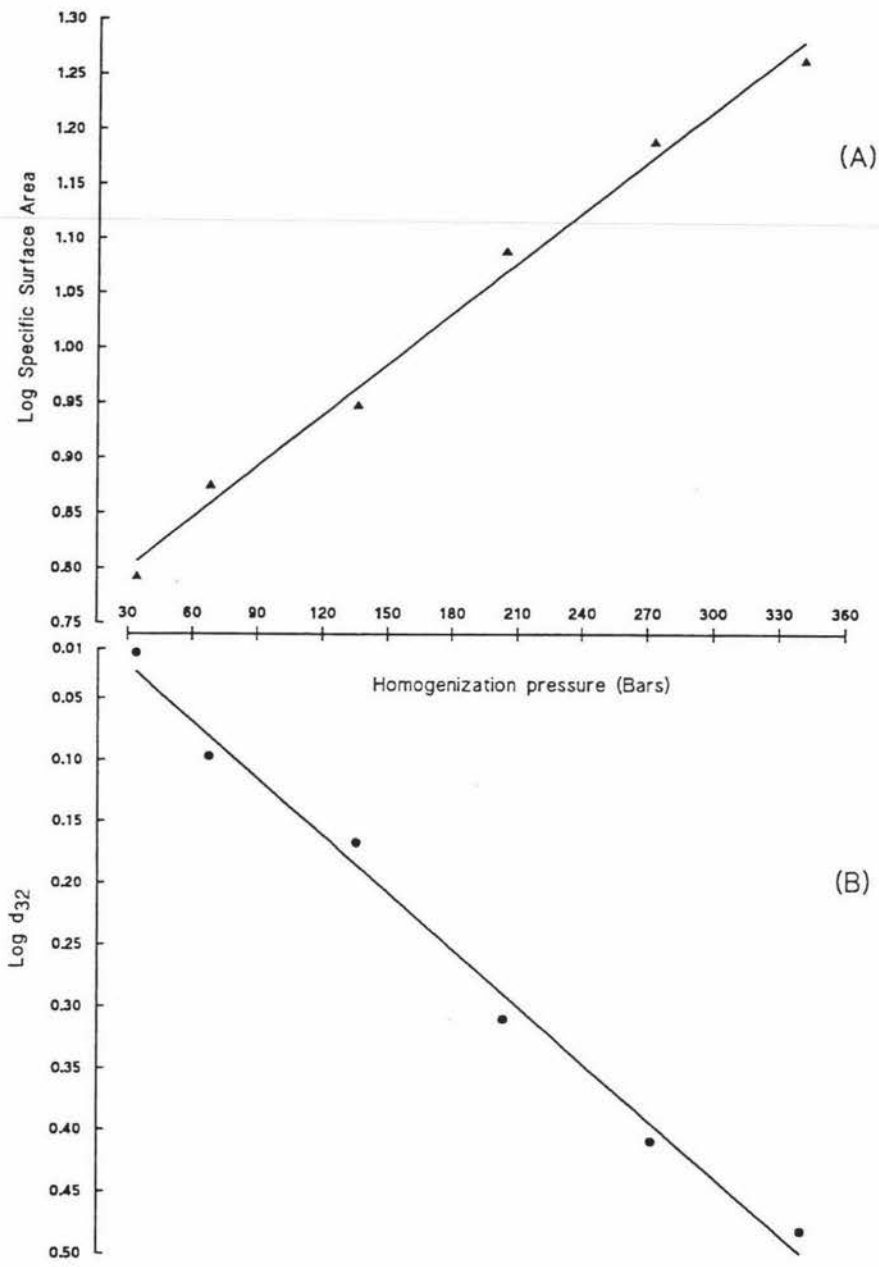


Figure 5.8: Relationship between homogenization pressure (P) and the log of fat globule size (d_{32}) (B) and log specific surface area (A) in sodium caseinate stabilized emulsions.

represented by the following equation

$$\log d_{32} = 0.02435 - 0.00154\ P$$

The changes in specific surface area (m^2/g oil) are also shown in Figure 5.7. The specific surface area increased almost linearly (from ~ 6.2 to 18.3) with increase in homogenization pressure from 34 to 340 bars.

The relationship between pressure and log of specific surface area (A) could be represented as ($r^2 = 0.99$) (Figure 5.8 A)

$$\log A = 0.001546\ P + 0.7535$$

The results, as expected, showed an increase in specific surface area with an increase in homogenization pressure as smaller droplets were formed.

5.4.2 Adsorbed protein and Protein load

The amount of protein adsorbed (mg/g oil) at the interface as a function of homogenization pressure is shown in Figure 5.9. The amount of protein adsorbed at the interface showed no significant changes as the pressure was increased from ~ 34 to ~ 136 bars, but above 136 bars the amount of protein adsorbed at the interface increased almost linearly with increase in pressure. The amount of protein adsorbed increased from ~ 13 to ~ 25.5 mg/g oil as the pressure was increased from 136 to 340 bar.

Protein load (mg/m^2) decreased sharply (from ~ 2.2 to ~ 1.5 mg/m^2) with increase in pressure from 34 to 136 bars (Figure 5.10), but further increases in pressure had no significant effect on protein load.

The changes in protein load (mg/m^2) as a function of specific surface area are

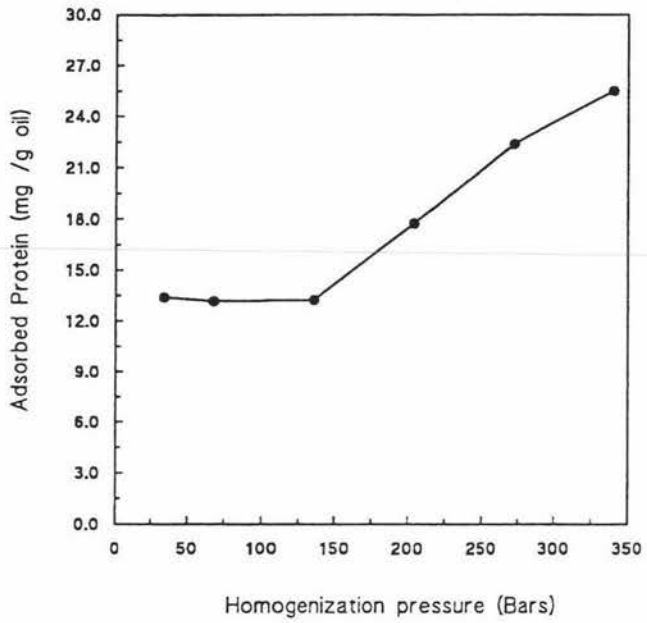


Figure 5.9: Changes in adsorbed protein (mg/g oil) in emulsions containing 30% soya oil and 2.5% homogenization pressures

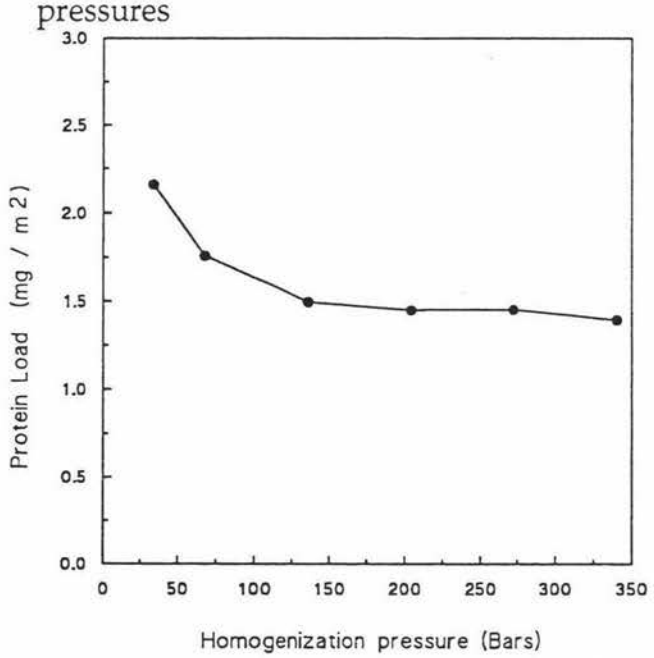


Figure 5.10: Changes in protein load (mg/m^2) in emulsions containing 30% soya oil and 2.5% protein at different homogenization pressures.

shown in Figure 5.11. The protein load showed a sharp decrease from 2.2 to 1.5 mg/m^2 as the specific surface area increased from ~ 6.2 to 8.8 mg/g oil, but further increases in specific surface area caused no significant change in protein load.

The results indicate that although the amount of protein adsorbed at the interface increased with increase in homogenization pressure between 136 and 340 bars (Figure 5.9), the increase was not sufficient to result in higher protein loads (Figure 5.10). Similar results have been reported by Tornberg (1978a), Murphy and Fox (1991), and Mulvihill and Murphy (1991), using a valve homogenizer incorporated into a recirculating emulsifying system in which the power input could be varied, as described by Tornberg and Lundh (1978). The decrease in protein load on increasing homogenization pressures may be attributed to increase in surface area, and thus lower protein to surface area ratio. Increase in surface area may cause greater spreading or unfolding of the adsorbed protein molecules at the interface resulting in decreased protein loads. High protein loads at low homogenization pressure, i.e., smaller surface areas, might indicate that multilayer of proteins were formed at the interface, whereas at high homogenization pressure the layers of protein might be thinner and probably approaching a monolayer.

5.4.3 Proportions of individual caseins in subnatant (unadsorbed protein)

The changes in the proportions of the individual caseins in the subnatant as a function of homogenization pressure are shown in Table 5.3. As the homogenization pressure was increased from 34 to 136 bars, the relative proportions of α_s-, β- and κ-caseins in the subnatant did not change significantly. At higher pressures i.e., 204 and 272 bars, the proportion of β-casein decreased markedly (by ~ 40%) with a corresponding increase in the

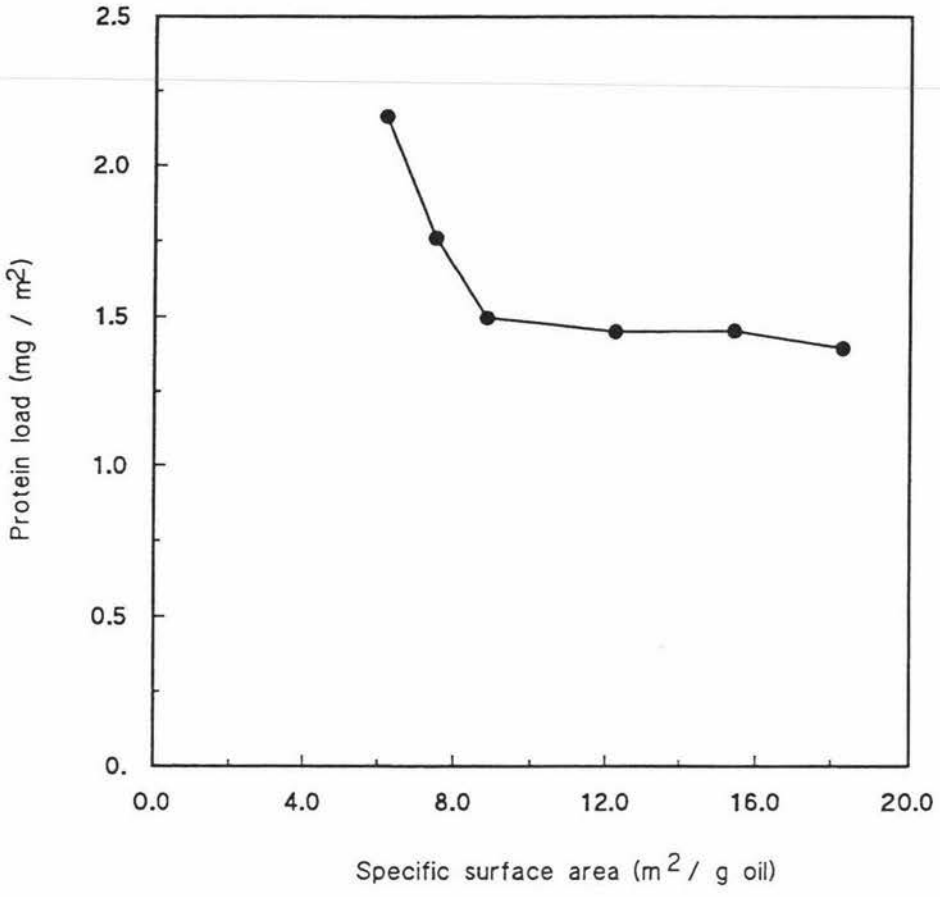


Figure 5.11: Changes in protein load as a function of specific surface area in emulsions containing 30% soya oil and 2.5% protein at different homomgenization pressures.

proportion of α_s-casein. The proportions of κ-casein decreased slightly with increase in pressure in the range, 34 - 272 bars.

It can be concluded from Table 5.3 that at high pressures where protein to surface area ratio is low β-casein is preferentially adsorbed. At low pressures, it appears that there was a slight preference for α_s-casein adsorption.

Table 5.3: Effect of homogenization pressure on the proportions of individual caseins in the subnatant.

Pressure (Bars)	Proportions of individual proteins (%)		
	α_s-casein	β-casein	κ-casein
34	32.0	44.0	24.0
68	33.3	44.4	22.2
136	34.6	42.4	23.1
204	52.4	28.6	19.0
272	56.2	25.0	18.8

5.5. Effect of pH

Emulsions were made with soya oil (30%, w/w) and sodium caseinate (2.5%, w/w) using a homogenization pressure of ~ 102/34 bars. Before adding soya oil, the pH of the sodium caseinate solutions was adjusted in the range of 2.0 - 8.5 using 1 M HCl or 1 M NaOH. For making low pH emulsions, protein solutions were cooled to 5°C in ice water and the pH adjusted to 2.0 or 3.0 by 1 M HCl with vigorous stirring. The pH values of the protein solutions were maintained at the desired pH for about 30 minutes and readjusted if necessary. Sodium caseinate solutions adjusted to pH values 4 and 5 showed visible coagulation prior to homogenization.

5.5.1 Droplet size and Specific surface area

The changes in the average droplet diameter, d_{32} (μm) as a function of pH are shown in Figure 5.12. The d_{32} values of pH 7.0 and 8.5 emulsions were slightly lower (~ 0.75 μm) than emulsions at pH 2.0, 3.0 and 6.0 (~ 0.81 μm).

The changes in specific surface area (m^2/g oil) as a function of pH are also shown in Figure 5.12. The specific surface area values of pH 7.0 and 8.5 emulsions were slightly higher (~ 8.04 m^2/g oil) than emulsions at pH 2.0, 3.0 and 6.0 (~ 7.41 m^2/g oil).

5.5.2 Adsorbed protein and Protein Load

The amount of protein adsorbed (Figure 5.13) at the interface was not significantly affected with pH in the range of 6.0 to 8.5 (~ 13 mg/g oil). In contrast, the amount of protein adsorbed was considerably higher in low pH emulsions (~ 21 mg/g oil).

The changes in protein load (mg/m^2 fat) with changes in pH are shown in Figure 5.14. Protein load decreased slightly with increase in pH in the range 6.0 to 8.5. However, the protein load was higher in low pH (2.0 and 3.0) emulsions.

The greater protein load values at low pH (2.0 and 3.0) may suggest that larger casein particles or aggregates were present in the sodium caseinate solution which may subsequently become adsorbed on to the oil surface.

5.5.3 Proportions of individual caseins in subnatant (unadsorbed protein)

The effect of varying pH on the proportions of individual caseins in the subnatant obtained using quantitative SDS-PAGE is shown in Table 5.4.

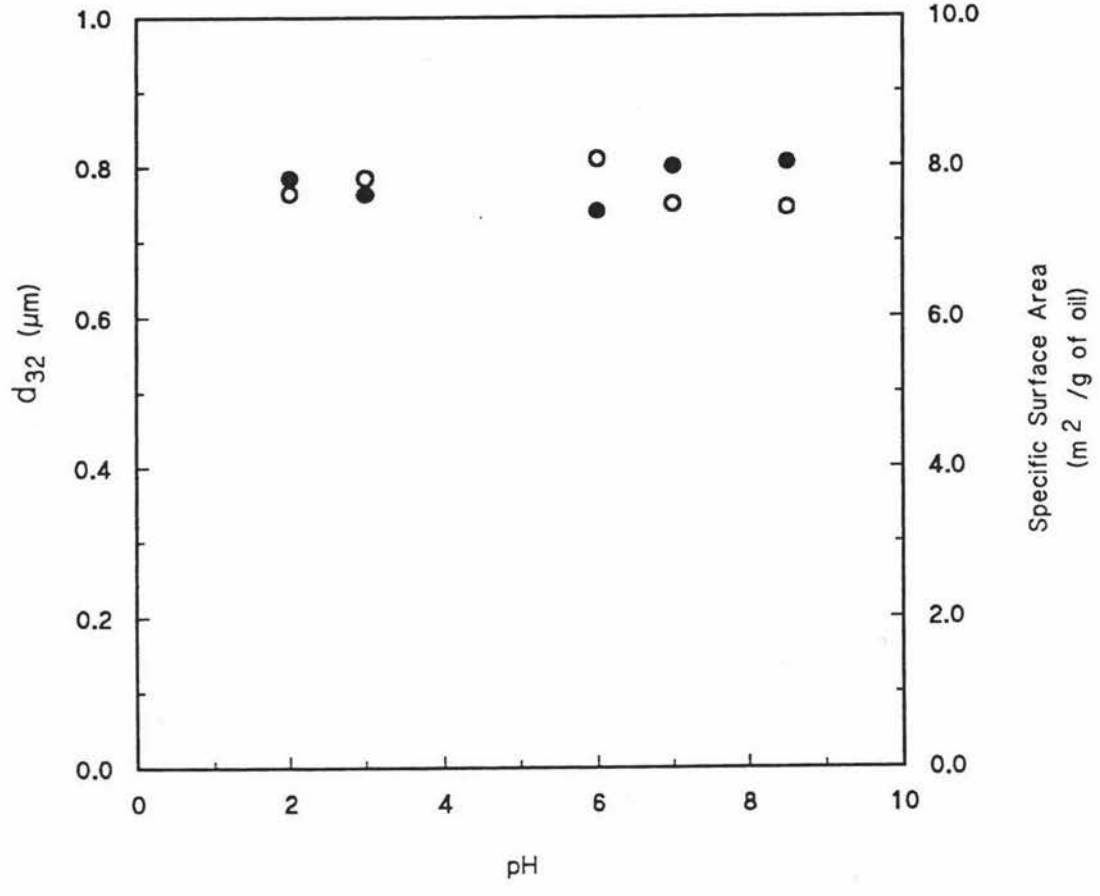


Figure 5.12: Changes in average droplet diameter, d_{32} (O) and specific surface area (●) in emulsions containing 30% soya oil and 2.5% protein at varying pH.

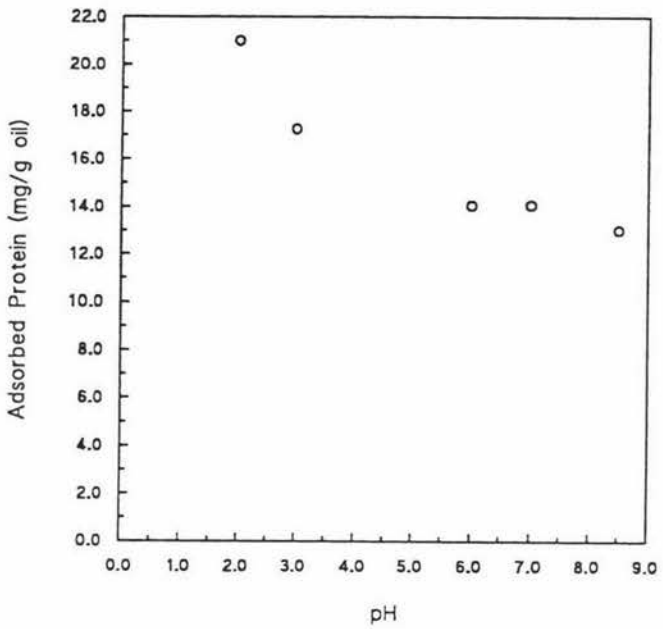


Figure 5.13: Changes in adsorbed protein (mg/g oil) as a function of pH in emulsions containing 30% soya oil and 2.5% protein.

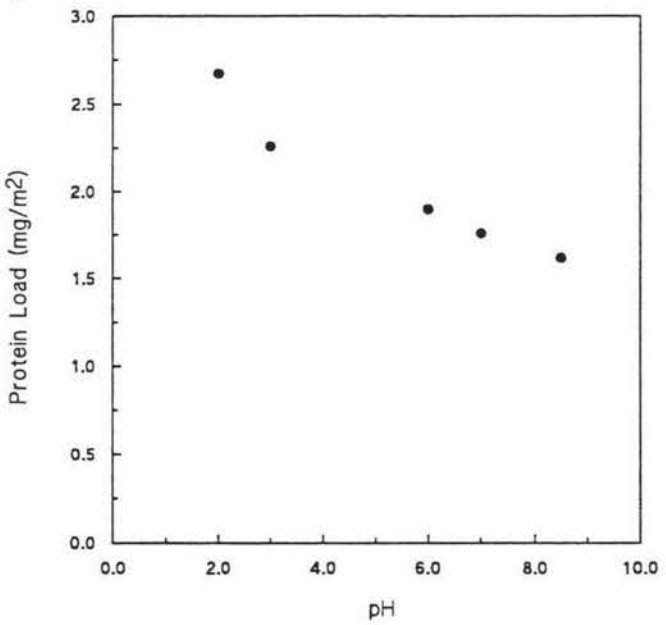


Figure 5.14: Changes in protein load (mg/m^2) as a function of pH in emulsions containing 30% soya oil and 2.5% protein.

Table 5.4: Effect of pH on the proportions of individual caseins in the subnatant.

pH	Proportions of individual proteins (%)		
	α_s-casein	β-casein	κ-casein
2.0	35.7	47.6	16.7
3.0	30.6	49.0	20.4
6.0	37.5	41.7	20.8
7.0	41.6	44.4	14.0
8.5	43.5	44.0	12.5

As the pH of protein solutions was increased from 2.0 to 3.0, the proportions of α_s-casein decreased slightly from ~ 35.7 to ~ 30.6 while those of β and κ-caseins increased slightly. On further increase in pH from 6.0 to 7.0, the proportions of α_s-, and β-caseins increased slightly with corresponding decreases in the proportions of κ-casein. At pH 7.0 and 8.5, the proportions of α_s- and β-casein were similar.

5.6 Effect of calcium chloride

A known amount of $CaCl_2$ (% w/w) was added to the sodium caseinate prior to making the protein solution. Emulsions made with soya oil (30%, w/w) and sodium caseinate (2.5%, w/w, pH 7.0) were homogenized in a two stage valve homogenizer as described in Materials and Methods.

5.6.1 Droplet diameter and specific surface area

Addition of $CaCl_2$ to sodium caseinate affected the average droplet diameter of the emulsion (Figure 5.15). Increase in concentration of $CaCl_2$ from 0.02 to 0.2% (w/w) gradually increased the d_{32} from 0.75 to 1.09 μm.

The changes in specific surface area (m^2/g) with change in $CaCl_2$ concentration are shown in Figure 5.15. The specific surface area (m^2/g) decreased from ~ 7.98 to ~ 5.56 m^2/g with increase in $CaCl_2$ concentration from 0.02 to 0.20% (w/w).

These results are consistent with those of Mulvihill and Murphy (1991) who showed that the extent of increase in fat surface area as a function of homogenization pressure was lower in calcium caseinate-stabilized emulsions than in sodium caseinate-stabilized emulsions.

5.6.2 Adsorbed protein and Protein load

The changes in the amount of protein adsorbed at the interface (mg/g oil) with increase in the $CaCl_2$ concentration are shown in Figure 5.16. There was no significant change in the adsorbed protein at the interface as the concentration of $CaCl_2$ was increased from 0 - 0.08%. However, addition of $CaCl_2$ in the range of 0.08 to 0.20% caused a dramatic increase in the amount of adsorbed protein i.e., from ~ 13.60 to 32.51 mg/g oil.

The changes in protein load with increasing $CaCl_2$ concentration showed a trend similar to the adsorbed protein. At $CaCl_2$ concentration up to 0.08%, (w/w) there was no significant change in protein load but at higher concentrations of $CaCl_2$, the protein load increased markedly from ~ 1.85 to ~ 5.8 mg/m^2 (Figure 5.17).

Addition of $CaCl_2$ to sodium caseinate solutions above a certain concentration results in the formation of large casein particles/aggregates which subsequently may be adsorbed on to the oil surface, resulting in a higher protein load. Turbidity results (measured using spectrophotometer at a wave

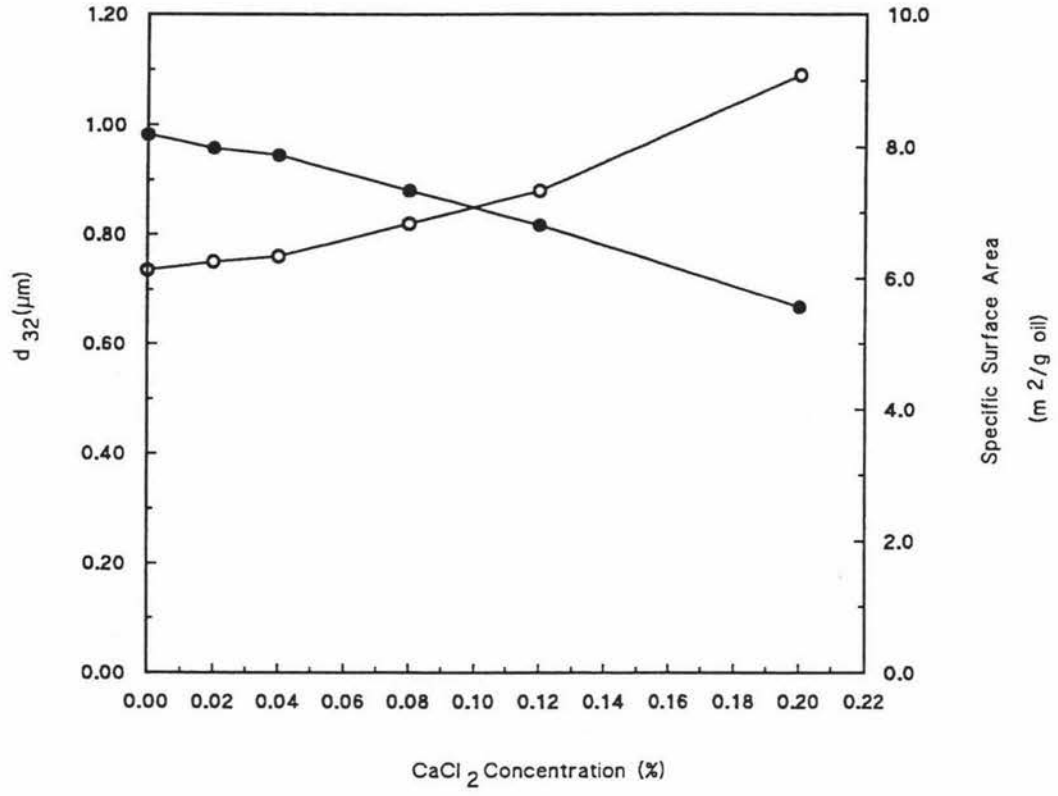


Figure 5.15: Changes in average droplet diameter, d_{32} (O) and specific surface area (●) as function of calcium chloride concentration in emulsions containing 30% soya oil and 2.5% protein.

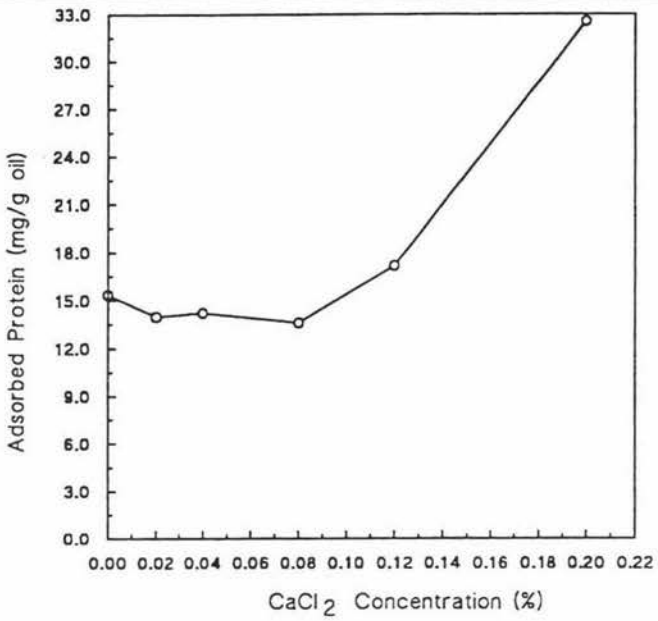


Figure 5.16: Changes in adsorbed protein (mg/g oil) as a function of calcium chloride concentration in emulsions containing 30% soya oil and 2.5% protein.

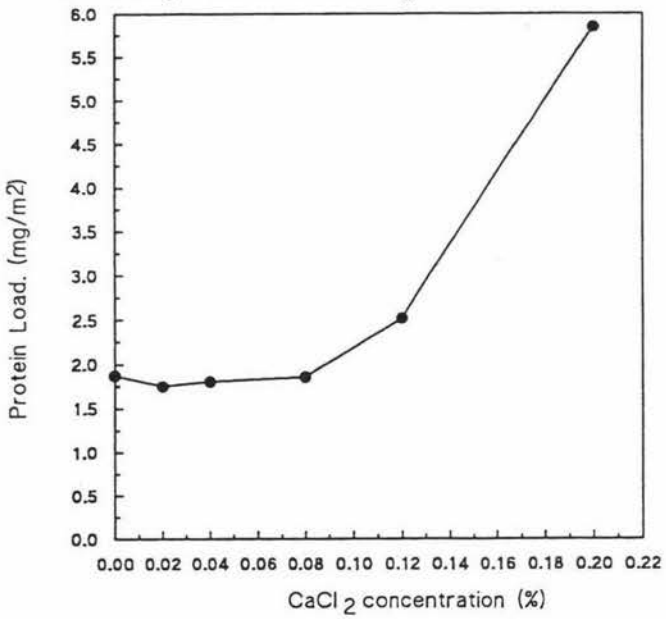


Figure 5.17: Changes in protein load (mg/m^2) as a function of calcium chloride concentration in emulsions containing 30% soya oil and 2.5% protein.

length of 650 nm) showed clearly that increase in calcium chloride concentration increased the cloudiness in the original protein solution indicating the formation of large casein particles/aggregates (Figure 5.18). In fact the turbidity *vs* $CaCl_2$ curve was almost identical to protein load *vs* $CaCl_2$ curve. Mulvihill and Murphy (1991) and Oortwijn and Walstra (1979) showed a relationship between protein load and state of aggregation of milk proteins in emulsions. For example, Mulvihill and Murphy (1991) found that the protein load in emulsions stabilized by calcium caseinate (which contains large casein aggregates) was considerably higher than in sodium caseinate-stabilized emulsions.

5.6.3 Proportions of individual caseins of subnatant (unadsorbed protein)

Comparison between the control sample (no $CaCl_2$ added) and the samples with added $CaCl_2$ showed that the proportions of α_s-casein in the subnatant decreased gradually with increase in $CaCl_2$ concentration, while the proportions of β-casein showed an increase (above 0.08%) with the increase in the $CaCl_2$ concentration (Table 5.5). The proportions of κ-casein varied between 15 - 22% with no consistent trend.

From these results, it appears that as the concentration of calcium chloride is increased the adsorption of α_s-casein at the interface increases (Table 5.5), i.e., α_s-casein is adsorbed in preference. This may be due to the adsorption of casein aggregates, rich in α_s-casein.

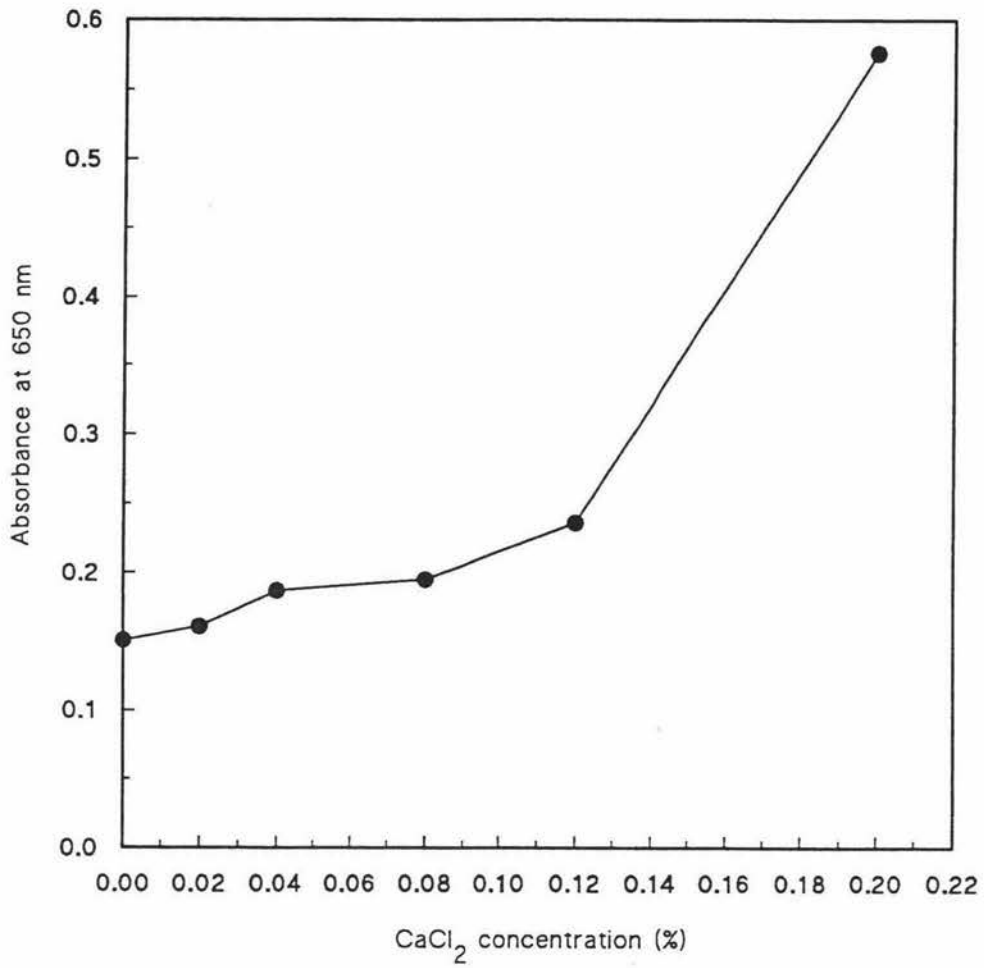


Figure 5.18: Changes in absorbance at 650 nm with change in calcium chloride concentration in 2.5% protein solution.

Table 5.5: Effect of Calcium chloride concentration on the proportions of individual caseins in the subnatant.

$CaCl_2$ Concentration (%)	Proportions of individual Composition (%)		
	α_s-casein	β-casein	κ-casein
0.00	39.6	45.3	15.1
0.02	37.5	46.4	16.1
0.04	34.9	46.5	18.6
0.08	32.6	46.5	20.9
0.12	32.5	51.2	16.3
0.20	27.3	50.0	22.7

5.7. Effect of Method of Caseinate Preparation

Sodium caseinates were prepared in the laboratory and on a pilot scale under different processing conditions as described in Materials and Methods. Emulsions were made from these sodium caseinates (2.5% w/w, protein and 30% w/w soya oil). The pH values of these protein solutions was adjusted to 7.0. The mixture was then homogenized as described in Materials and Methods.

5.7.1. Droplet diameter and specific surface area

The average droplet diameter, d_{32} (μm) and specific surface area (m^2/g oil) were in the range of 0.69 to 0.75 μm and ~ 8.02 to ~ 8.69 m^2/g oil, respectively (Figure 5.19).

5.7.2. Adsorbed protein and protein load

The amount of protein adsorbed at the interface (mg/g oil) for different

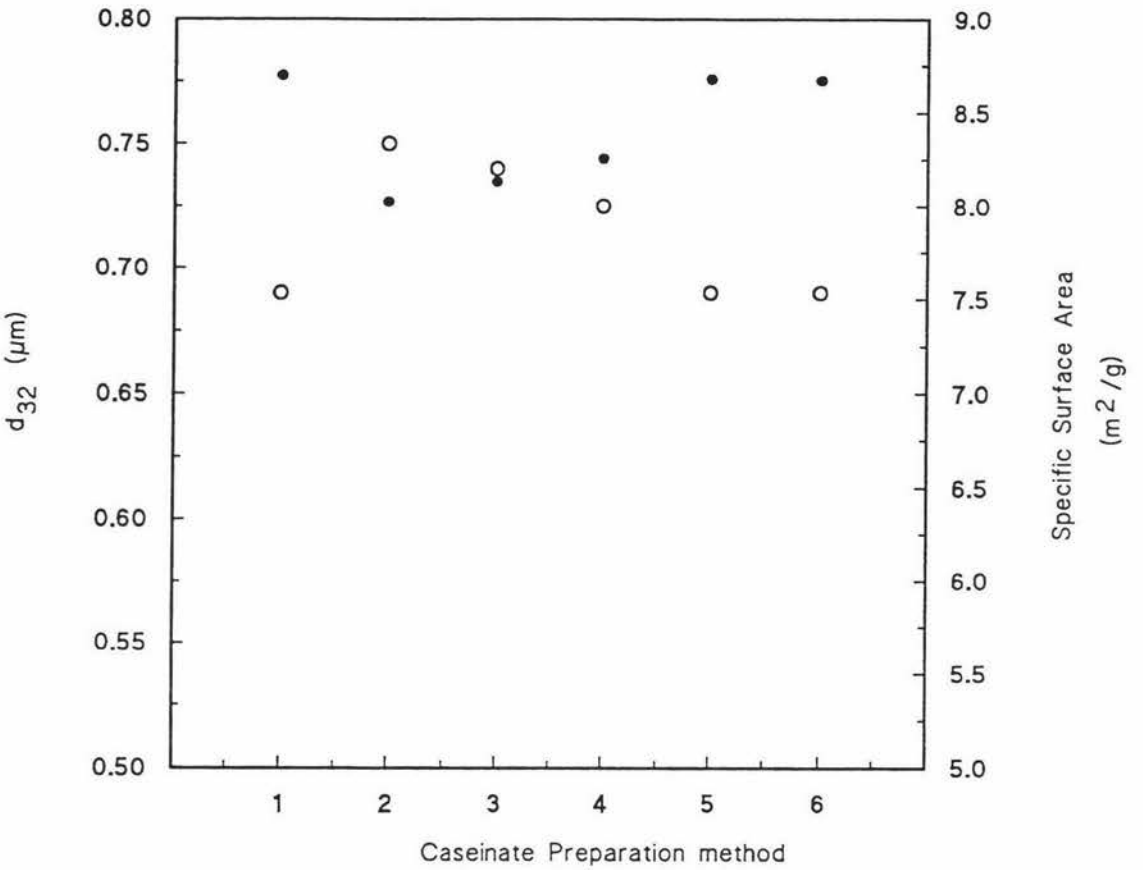


Figure 5.19: Average droplet diameter, d_{32} (O) and specific surface area (●) in emulsions containing 30% soya oil and sodium caseinates prepared using different methods.

1 - Lab made
2 - Mild
3 - Normal
4 - High (Freeze Dried)
5 - High (Spray Dried)
6 - Commercial.

emulsions is shown in Figure 5.20. The amount of protein adsorbed was found to be highest (~ 18.66 mg/g oil) for the freeze-dried lab made sodium caseinate followed by sodium caseinate made under 'mild' conditions (~ 11.51 mg/g oil). There were no significant differences in the adsorbed protein for other sodium caseinates.

The protein loads (mg/m^2) of the sodium caseinate-stabilized emulsions are shown in Figure 5.21. The protein loads of the freeze-dried lab made sodium caseinate and the sodium caseinate made under mild conditions were found to be higher compared to other sodium caseinates. The protein loads of the freeze-dried sodium caseinate and sodium caseinate made under mild conditions were ~ 2.14 mg/m^2 and ~ 1.43 mg/m^2 respectively. There were no significant differences in the protein loads of other sodium caseinates.

5.7.3. Proportions of individual caseins in subnatant (unadsorbed protein)

The proportions of individual proteins obtained from the subnatant of different sodium caseinate-stabilized emulsions are shown in Table 5.6. The proportions of α_s-casein in the lab made caseinate and sodium caseinate made under mild conditions, were greater compared to other sodium caseinates, while the proportions of β-casein in these caseinate were found less compared to other sodium caseinates. It was also observed that the proportion of β-casein was slightly more than the proportion of α_s-casein for the sodium caseinate made under high processing conditions (Spray Dried). The proportions of κ-casein for these different sodium caseinate preparations varied from 19 - 23%.

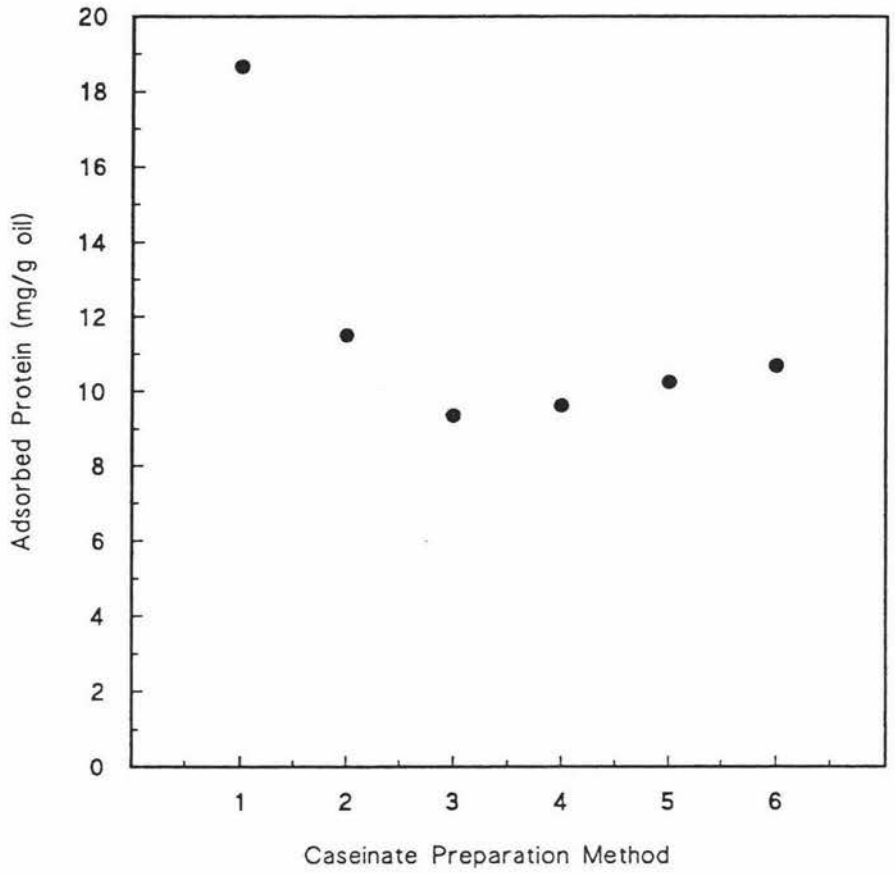


Figure 5.20: Adsorbed protein (mg/g oil) in emulsions containing 30% soya oil and 2.5% caseinates prepared using different methods

1 - Lab made
2 - Mild
3 - Normal
4 - High (Freeze Dried)
5 - High (Spray Dried)
6 - Commercial.

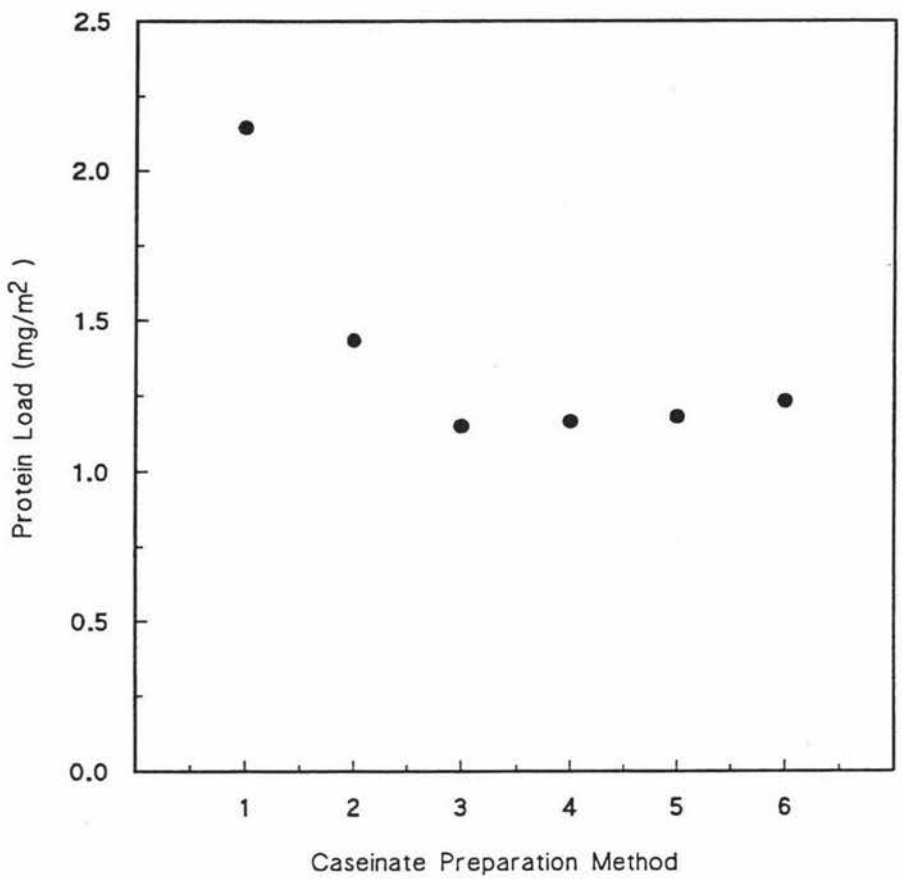


Figure 5.21: Protein Load (mg/m^2) in emulsions containing 30% soya oil and 2.5% caseinates prepared using different metthods.

1 - Lab made
2 - Mild
3 - Normal
4 - High (Freeze Dried)
5 - High (Spray Dried)
6 - Commercial.

Table 5.6: Effect of sodium caseinate preparation method on the proportions of individual caseins in the subnatant.

Preparation method of sodium caseinate	Proportions of individual proteins (%)		
	α_s-casein	β-casein	κ-casein
Lab made	47.6	29.5	22.9
Mild	44.3	32.0	23.8
Normal	40.2	37.2	22.6
High FD**	40.9	39.8	19.4
High SD**	38.1	42.1	19.8
Commercial	43.9	39.2	16.9

SD** – Spray Dried, FD** – Freeze Dried

Thus it appears that there was a preferential adsorption of β-casein in the lab made sodium caseinate and sodium caseinate made under mild conditions. However, in other sodium caseinates made using more severe processing conditions, there was no clear preferential adsorption of either α_s- or β-casein.

These results indicate that β-casein was preferentially adsorbed when caseinates were prepared under relatively mild conditions. Use of severe conditions during processing affected this behaviour with a tendency towards more α_s-casein adsorption. This may be related to changes in the aggregation state or hydrophobicity of casein molecules during processing.

As it is evident from the above results that the lab made sodium caseinate had a higher protein load and different interfacial composition than the commercial sodium caseinate, further experiments were conducted at varying protein concentrations of these caseinates.

5.7.4. Droplet diameter and specific surface area

The changes in droplet diameter and specific surface area with changes in the protein concentration of the lab made sodium caseinate and commercial sodium caseinate are shown in Table 5.7. Lab made sodium caseinate showed higher d_{32}, especially at low protein concentrations (0.5%) than the commercially made sodium caseinate. The specific surface area also showed larger differences in the low protein concentration emulsions than higher protein concentration emulsions (Table 5.7).

5.7.5. Adsorbed protein and protein load

The changes in adsorbed protein with changes in protein concentration of these two caseinates are shown in Table 5.8. At protein concentrations of 0.5 and 1.0%, the amount of protein adsorbed at the interface was slightly higher for commercial sodium caseinate than for lab made caseinate. But at a protein concentration of 2.5% the amount of protein adsorbed at the interface for the commercial sodium caseinate was lower than for the lab made caseinate (~ 19.28 mg/g oil *vs* 14.71 mg/g oil).

The changes in protein load (mg/m^2) with change in protein concentration of the lab made and commercial sodium caseinates are shown in Table 5.8. The protein loads of the commercial sodium caseinate at low protein concentrations (0.5 and 1.0%) were slightly lower than for the lab made sodium caseinate. But at a protein concentration of 2.5% the protein load of the lab made sodium caseinate was found to be higher (~ 2.46 mg/m^2) than the commercial sodium caseinate (~ 1.84 mg/m^2).

5.7.6. Proportions of individual caseins of subnatant (unadsorbed protein)

The changes in the proportions of individual caseins in the subnatant, obtained using quantitative SDS-PAGE, with change in protein concentration of the lab made and commercial sodium caseinate are shown in Table 5.9.

As the protein concentration was increased from 0.5 to 2.5%, the relative proportion of α_s-casein dropped considerably and the relative proportion of β-casein was increased significantly. κ-Casein showed no significant change at all protein concentrations.

With the increase in protein concentration of both the sodium caseinates the preferential adsorption of β-casein at the interface decreased. This is consistent with the results discussed earlier (section 5.7.3).

Table 5.7: Changes in droplet diameter and specific surface area with protein concentration of Commerical and Lab made sodium caseinate.

S.No	Protein concentration (%)	d_{32} (μm)	Specific surface Area (m^2/g)
1	0.5 C**	0.77	7.83
2	1.0 C**	0.78	7.72
3	2.5 C**	0.75	7.98
4	0.5 L**	0.82	7.31
5	1.0 L**	0.78	7.70
6	2.5 L**	0.77	7.83

Table 5.8: Changes in adsorbed protein and protein load with protein concentration of Commerical and Lab made sodium caseinate.

S.No	Protein concentration (%)	Adsorbed Protein (mg/g fat)	Protein Load (m^2/g)
1	0.5 C**	4.48	0.57
2	1.0 C**	8.41	1.09
3	2.5 C**	14.72	1.85
4	0.5 L**	1.63	0.45
5	1.0 L**	7.48	0.97
6	2.5 L**	19.28	2.46

C** for Commercially made sodium caseinate

L** for Freeze dried lab made sodium caseinate

Table 5.9: The proportions of individual caseins in the subnatant at various protein concentrations in emulsions stabilized by commercial and lab made sodium caseinate.

Protein concentration	Proportions of individual proteins (%)		
	α_s-casein	β-casein	κ-casein
0.5% C**	78.3	8.4	13.3
1.0% C**	80.0	5.7	14.3
2.5% C**	35.9	47.4	16.7
0.5% L**	78.8	6.1	15.2
1.0% L**	78.4	5.9	15.7
2.5% L**	54.9	29.2	15.9

C** for Commercially made sodium caseinate

L** for lab made lab made sodium caseinate

5.8 Overall Discussion

Overall, it is apparent from these results that the surface coverage and composition of emulsion droplets in sodium caseinate-stabilized emulsions depend on the total amount of casein present, the total area of the interface and the state of aggregation of the protein. When the casein concentration is increased (from 2.0 to 4.0%) surface coverage attains a plateau value (~ 1.3 mg/m^2) which corresponds to saturated monolayer coverage. Under these conditions β-casein is adsorbed in preference to other caseins. Further increase in casein concentration (up to 7.5%) cause a sharp increase in surface coverage which may be due to formation of secondary layers. The preferential adsorption of β-casein diminishes at these high concentrations.

Total area of interface is influenced by homogenization pressure and the volume fraction of oil. Surface coverage decreased with increase in total area of interface in an unpredictable manner. When total area of the interface is large (i.e. low ratio of protein to interfacial area), β-casein appears to adsorb in preference to other caseins.

Thus, it can be concluded that under conditions when the oil interface is protein 'starved' i.e. low protein concentration, high volume of oil, high homogenization pressures, β-casein is adsorbed in preference to other caseins. However, other caseins are also present at the interface under these conditions.

It is known that protein molecules in sodium caseinate are substantially aggregated; the nature and size of aggregates is dependent on protein concentration, presence of ions and processing conditions used in its manufacture. The adsorption of caseins from sodium caseinate solution could

therefore, involve transport of protein aggregates to the interface and strong interactions between casein components may even persist at the interface. From the present study, it is clear that additions of $CaCl_2$, low pH and severe processing conditions alter the surface coverage and composition via increasing the extent of protein aggregation in solution prior to emulsification.

It might be possible to make a range of emulsions with different compositions and coverage of adsorbed proteins by manipulation of certain compositional and processing parameters. These emulsion systems may therefore have different functional properties. Further studies will need to be carried out to relate composition of adsorbed layer with functionality of the emulsions.

It may also be possible to produce caseinates with controlled aggregation states, e.g. by addition of ions or alteration of processing conditions. This will provide opportunity to make emulsions with controlled protein coverage. Relationships between the aggregation state and functionality will need to be explored.

6.0 CREAMING UNDER GRAVITY OF SODIUM CASEINATE STABILIZED OIL-IN-WATER EMULSIONS.

Sodium caseinate-stabilized emulsions were transferred into the stability tubes (described in Materials and Methods chapter) and maintained at 20°C for 24 hours. Samples (~ 5 ml) were drawn from different heights of the tube and analyzed for droplet diameter and fat content. Stability or instability of the emulsions was expressed as the stability rating (refer chapter 3) of the emulsions at different heights of the tube.

6.1 Effect of protein concentration

The protein concentration in the emulsions (30% soya oil) was varied from 0.5 to 5.0% (w/w). Creaming profiles (stability rating *vs* height) are shown in Figure 6.1. In general, the stability rating of these emulsions increased with increase in protein concentration. Emulsions containing protein concentrations of 4 or 5% (w/w) showed little fat separation at different heights of the stability tube. In contrast, emulsions containing 0.5 or 1.0% protein concentration showed clear fat separation as indicated by an increase in stability rating values at 15 and 19.9 cm. Emulsions containing 2 or 3% protein concentration showed fat separation at 0.3 cm.

The changes in droplet diameter d_{32} (μm) with change in height of the stability tube are shown in Figure 6.2. There were no significant changes in the droplet diameter of emulsions containing 4 or 5% protein concentrations with the height of the stability tube. However, there was a significant change in the droplet diameter of the emulsions containing lower protein concentrations. The droplet diameter varied from ~ 0.29 to 0.825 μm as the concentration of protein in the emulsions was varied between 0.5 to 5.0%.

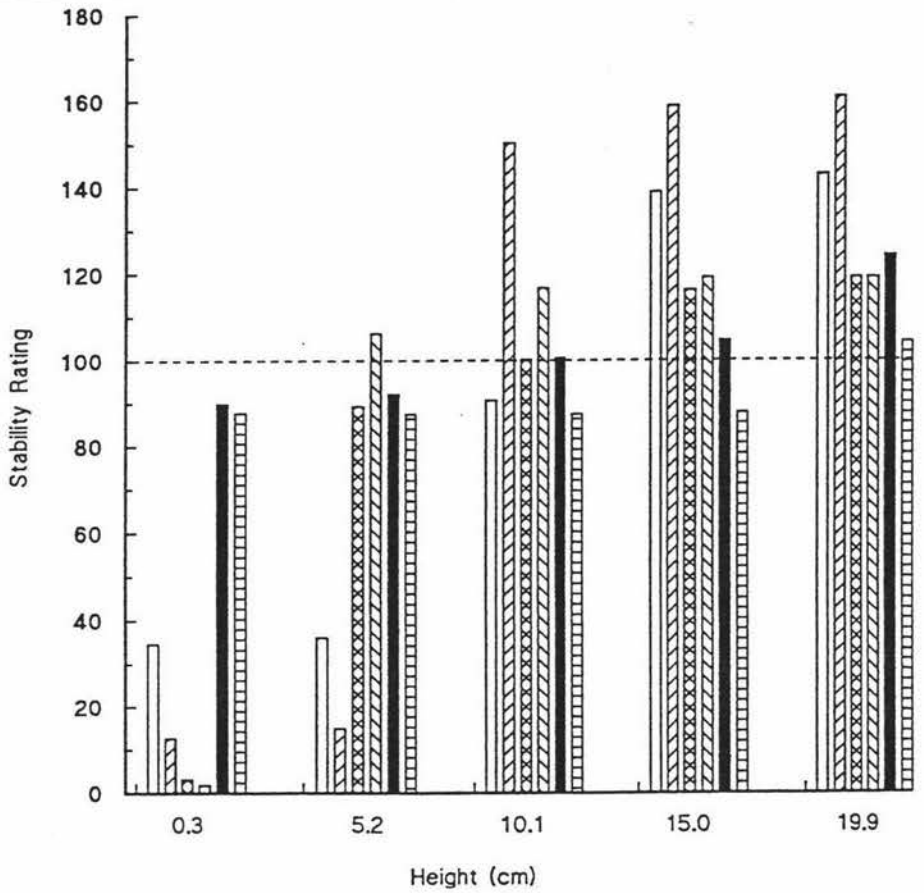


Figure 6.1: Changes in stability rating with change in height in the stability tube in emulsions containing 30% soya oil and varying amounts of casein. Dotted line (stability rating = 100) means no fat separation i.e. completely stable systems.

- 0.5% Protein
- 1.0% Protein
- 2.0% Protein
- 3.0% Protein
- 4.0% Protein
- 5.0% Protein

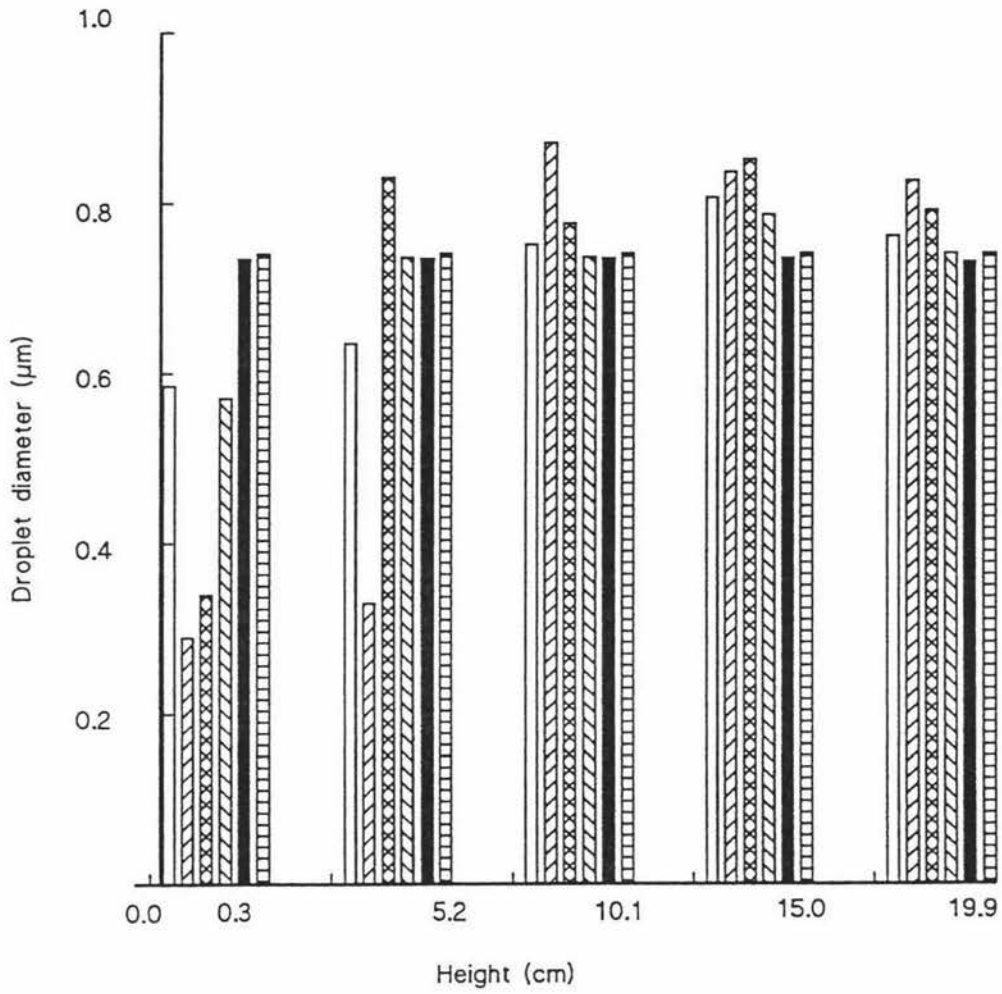


Figure 6.2: Changes in droplet diameter (d_{32}) with change in height in the stability tube in emulsions containing 30% soya oil and varying amounts of casein.

- 0.5% Protein
- 1.0% Protein
- 2.0% Protein
- 3.0% Protein
- 4.0% Protein
- 5.0% Protein

The present results generally agree with the findings of Leman *et al.* (1988), who observed that increasing protein concentration decreased the extent of creaming in peanut oil emulsions stabilized by skim milk, micellar casein, whey protein isolate or β-lactoglobulin. However, the present results disagree with those of Acton and Saffle (1971) who observed no change in the stability rating of emulsions made with sodium caseinate (0.5 to 2.5%) and corn oil (30%). In the present study, emulsions were prepared with soya oil (30%) using a two stage valve homogenizer and the stability was determined at 20°C, whereas Acton and Saffle (1971) prepared emulsions with corn oil (30%) and the stability was measured at 37°C. These differences may have contributed to the different results obtained in the two studies.

The increase in creaming stability of emulsions prepared from soya oil and sodium caseinate could be at least partly attributed to the increase in viscosity of the continuous phase with increase in protein concentrations (Figure 6.3). From Figure 6.3, it is clear that increase in protein concentration increases the viscosity of the sodium caseinate solution. Mohanty *et al.* (1988) also reported that the relative viscosity of acid casein increased with increase in protein concentration in the range of 1 - 4% w/v. Konstance and Strange (1991) observed a linear increase in viscosity with increase in sodium caseinate concentration. Earlier experiments showed that increase in protein concentration increases the protein load (refer chapter 5) which may also influence the creaming behaviour of oil droplets.

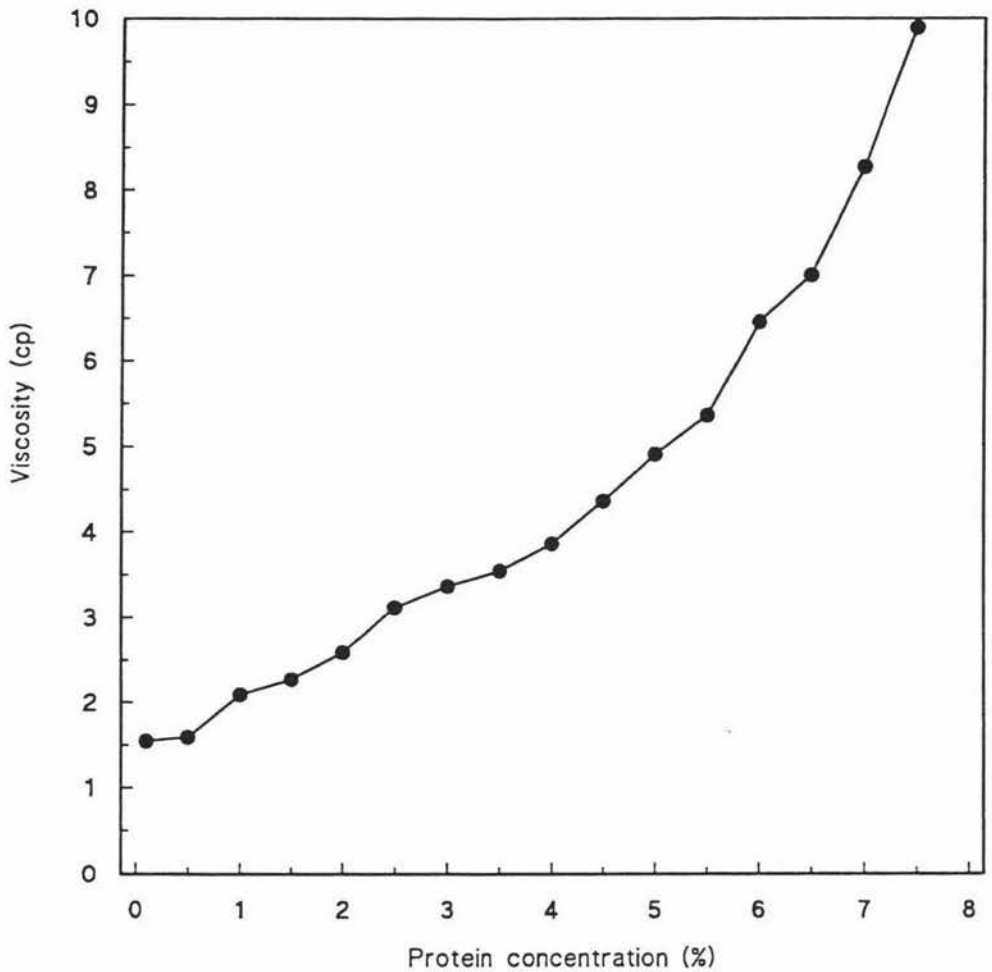


Figure 6.3: Change in viscosity of protein solutions (fat-free) with change in protein concentration

5.2 Effect of Fat concentration

Emulsions were made with varying quantities of soya oil or anhydrous milk fat (AMF) and 2.5%, (w/w) sodium caseinate, and transferred into the stability tubes.

The creaming profiles of emulsions made using either soya oil or milkfat after storage at 20°C for 24 hours are shown in Figures 6.4 and 6.5. In general, the stability of the emulsions increased with increase in fat concentration. In both soya oil and AMF, in emulsions containing fat concentrations between 5 - 20%, w/v, almost all of the fat became concentrated at the top (i.e., at 15 and 19.9 cm) of the tube; the fat and the serum phases could visually be distinguished.

Emulsions containing 40% fat concentration were stable, except in case of soya oil at 0.3 cm where the stability rating was very low (~ 7). In both soya oil and AMF, stable emulsions were formed at 50% fat concentration.

The changes in droplet diameter, d_{32} (μm) with change in soya oil or AMF concentration are shown in Figures 6.6 and 6.7. In general, d_{32} values at the bottom of the tube were smaller than those at the top of the tube. The d_{32} of the original emulsion increased with increase in fat concentration (refer chapter 5).

The above results indicate that the emulsions containing high fat were more stable to creaming than the low fat emulsions. This can be attributed to a number of factors discussed by Darling (1987). As the volume of dispersed phase increases the rate of particle rise is retarded. Particle-particle collisions also retard creaming rate, since the small particles collide with faster moving

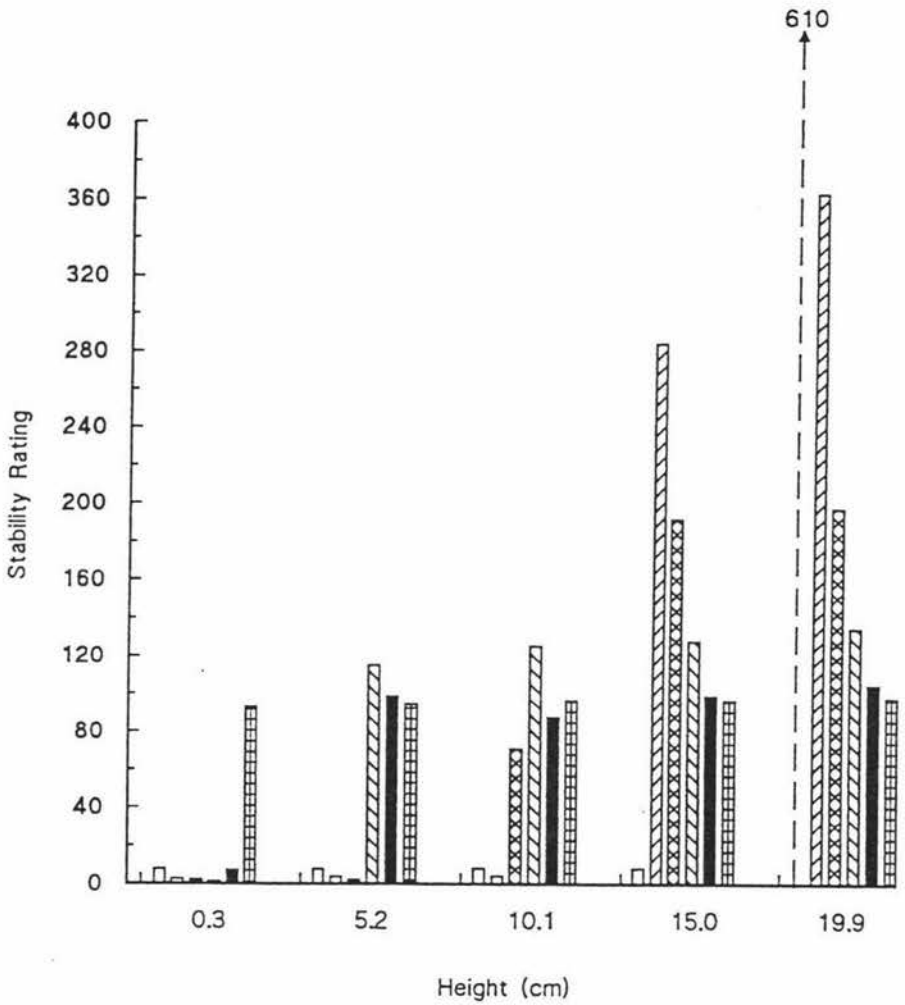


Figure 6.4: Change in stability rating with change in height in the stability tube in emulsions containing 2.5% protein at varying fat (soya) concentration.

- 5% Fat
- 10% Fat
- 20% Fat
- 30% Fat
- 40% Fat
- 50% Fat

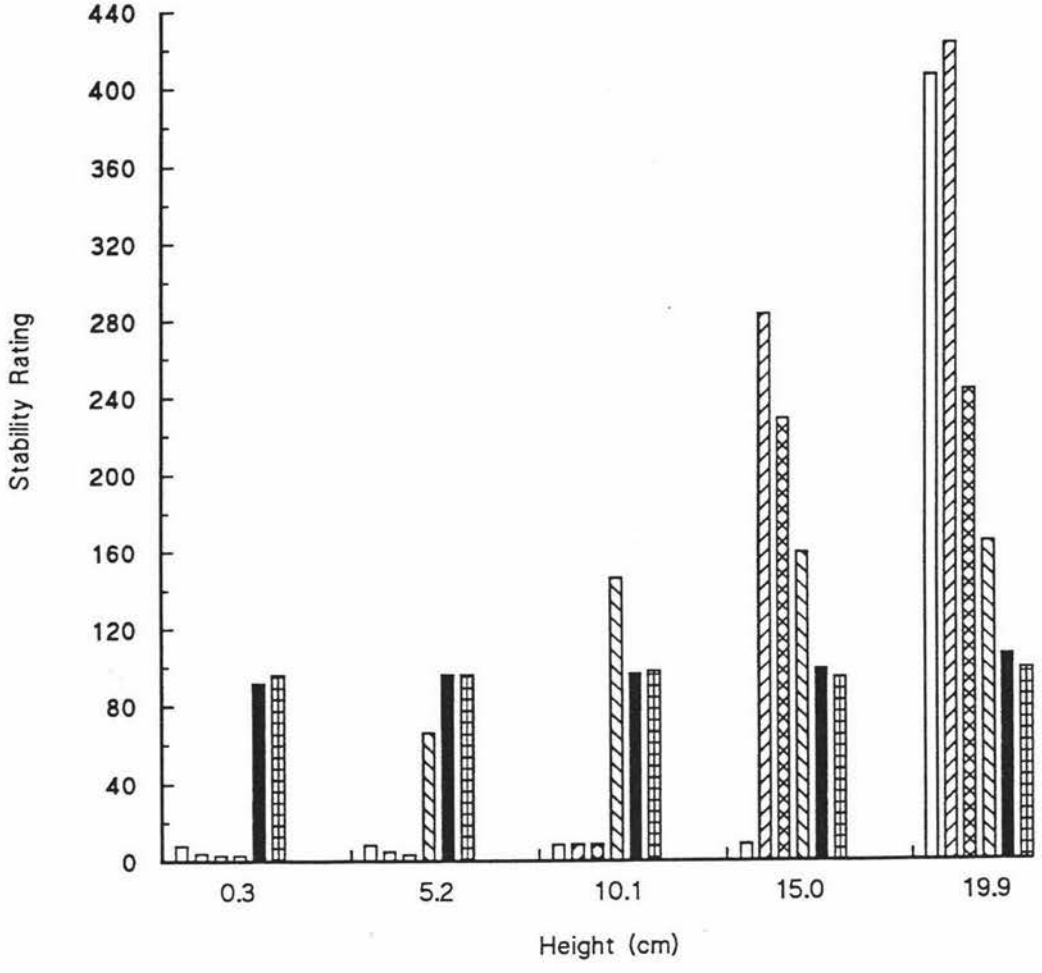


Figure 6.5: Change in stability rating with change in height in the stability tube in emulsions containing 2.5% protein at varying fat (milkfat) concentration.

- ☐ 5% Fat
- ▨ 10% Fat
- ⊠ 20% Fat
- ▧ 30% Fat
- ■ 40% Fat
- ▦ 50% Fat

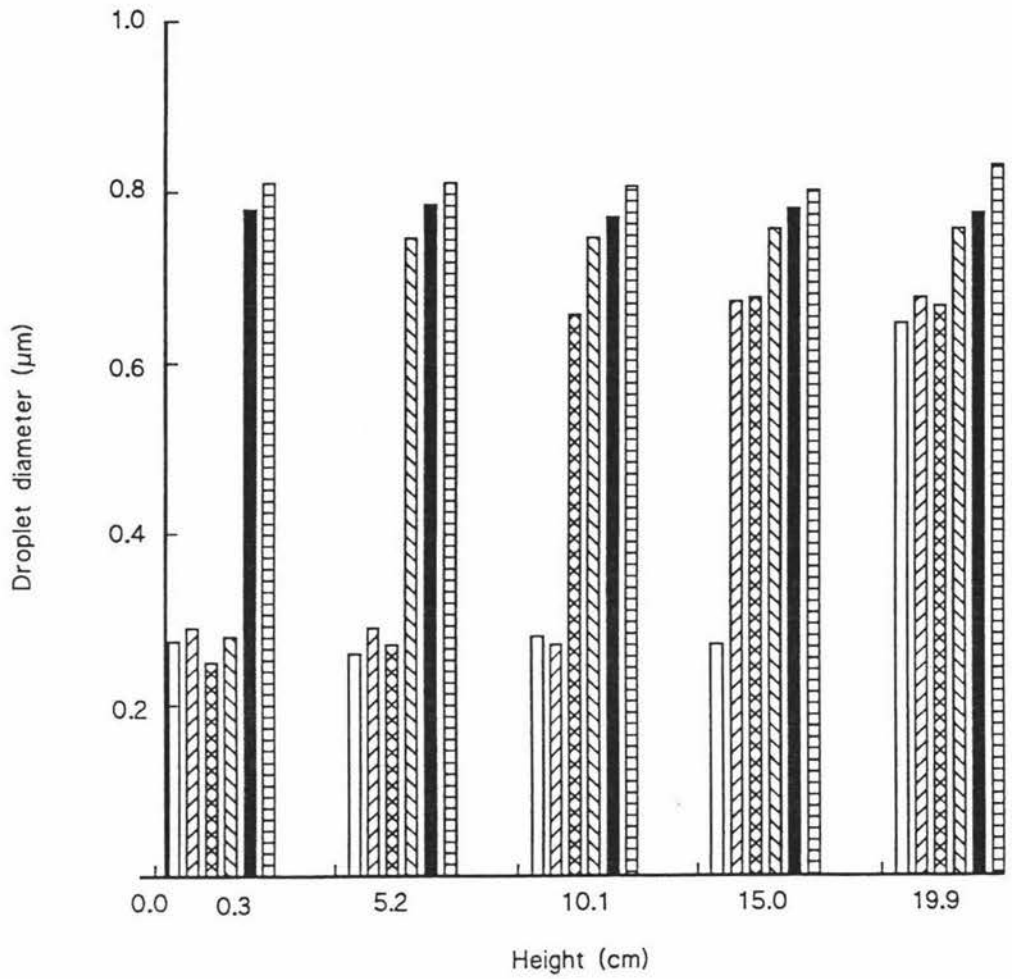


Figure 6.6: Change in droplet diameter, d_{32} with change in height in the stability tube in emulsions containing 2.5% protein at varying fat (soya) concentration.

- 5% Fat
- 10% Fat
- 20% Fat
- 30% Fat
- 40% Fat
- 50% Fat

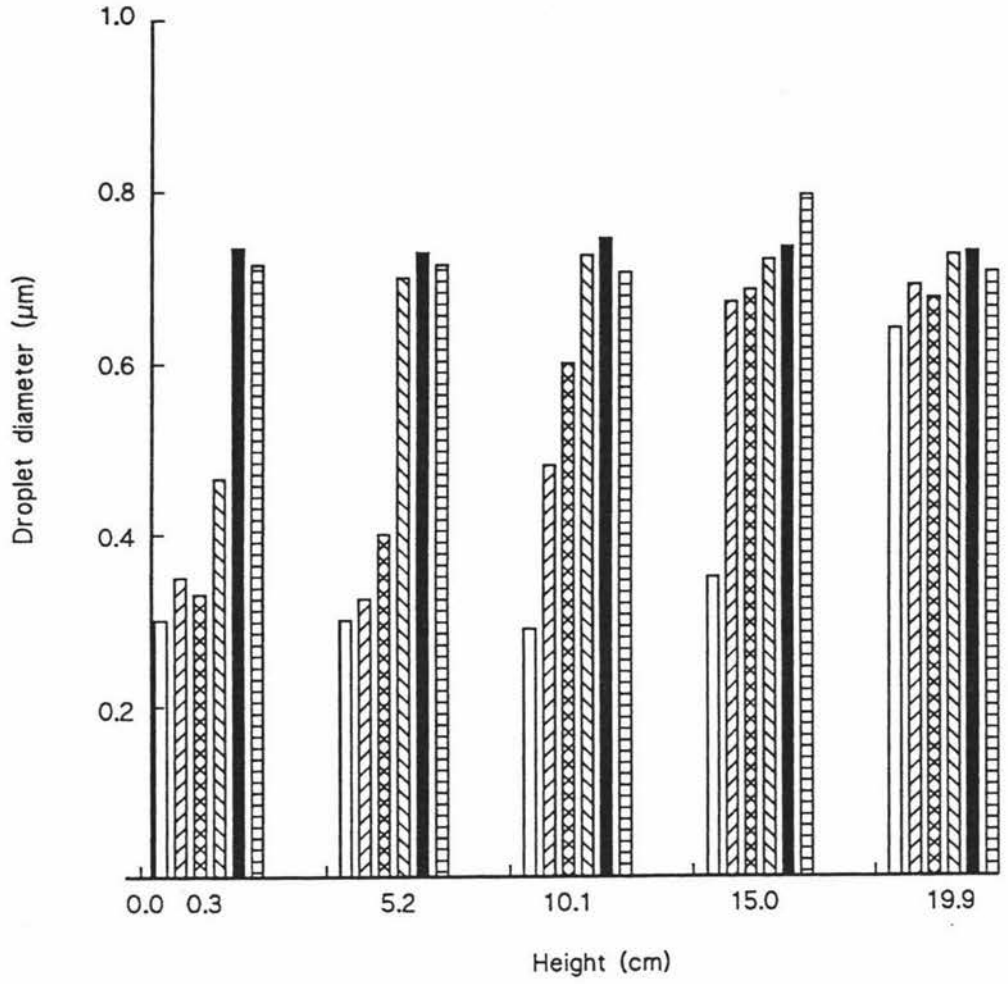


Figure 6.7: Change in droplet diameter, d_{32} with change in height in the stability tube in emulsions containing 2.5% protein at varying fat (milkfat) concentration.

- 5% Fat
- 10% Fat
- 20% Fat
- 30% Fat
- 40% Fat
- 50% Fat

large particles. Probably at high fat concentration the system was very polydisperse and the particle-particle hindrance reduced the rate of creaming.

6.3 Effect of homogenization pressure

Emulsions were made using sodium caseinate (2.5%, w/w) and soya oil (30%, w/v) and homogenized at pressures in the range ~ 34 - 340 / 34 bar in a two-stage homogenizer at 55°C. Figure 6.8 shows the creaming profiles of the emulsions after storage at 20°C for 24 hours. There was no apparent change in the stability of emulsions made at a homogenization pressures of 340 bar. In emulsions made at a pressure ~ 34 bar a concentrated cream layer emerged at the top half (i.e. between 10.1 and 19.9 cm) of the tube. At the same time, a serum layer (fat content ~ 0.2%) was developed at the bottom of the tube. Samples homogenized at pressures in the range ~ 68 - 272 bar also showed some fat separation at the top of the tube.

As expected the droplet diameter of the emulsions decreased with increase in homogenization pressure (refer section 5.4). At a homogenization pressure of 34 bar the changes in droplet diameter with change in height of the tube were not very significant. Samples obtained from the top of the tube (19.9 cm) contained relatively large droplets and there was a gradual decrease in the average size with decrease in height (Figure 6.9).

Generally, it is considered that greater energy input during emulsification i.e., higher homogenization pressure enhances the creaming stability of the emulsions (Walstra, 1975). This effect has been largely attributed to the fact that the globule size becomes smaller and the size distribution becomes narrower. Leman *et al.* (1988) investigated a number of emulsions stabilized by different milk proteins and found that in all emulsions, increase in energy

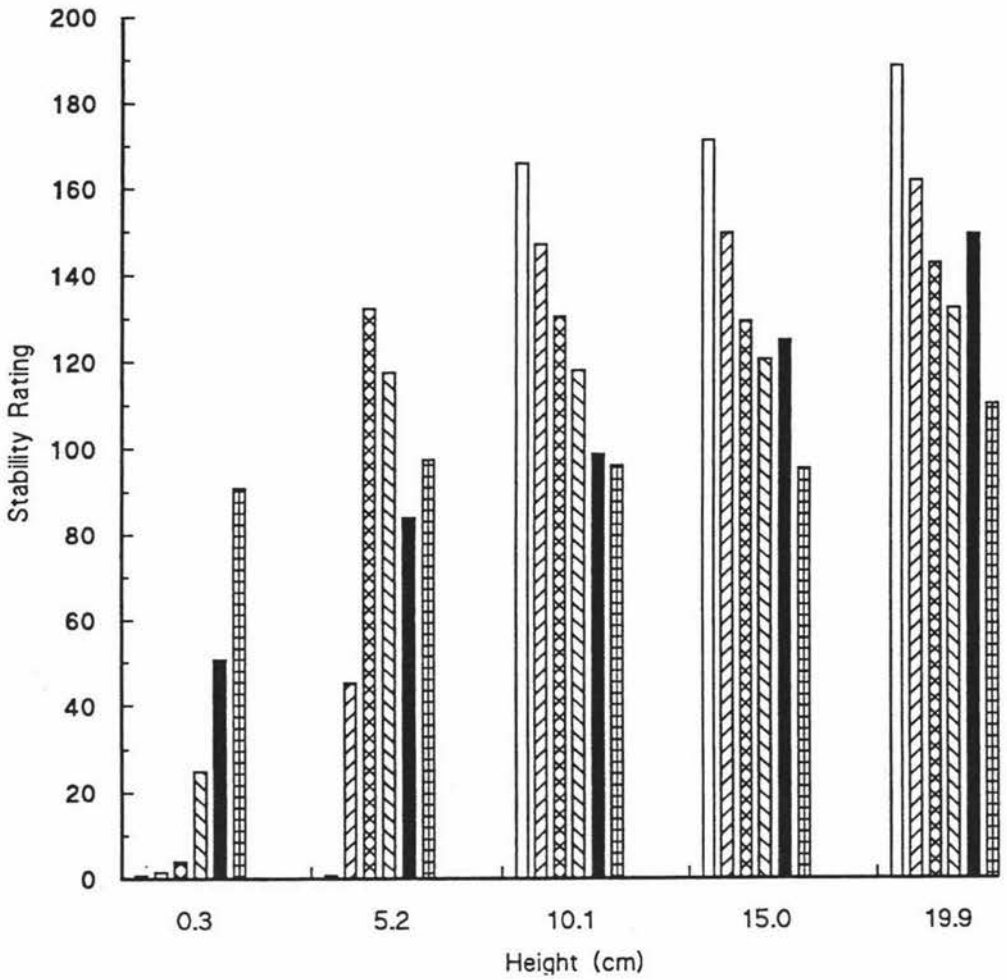


Figure 6.8: Changes in stability rating with change in height in the stability tube in emulsions containing 30% soya oil and 2.5% protein at varying homogenization pressure.

- ☐ 34 Bars
- ▨ 68 Bars
- ⊠ 136 Bars
- ▧ 204 Bars
- ■ 272 Bars
- ▦ 340 Bars

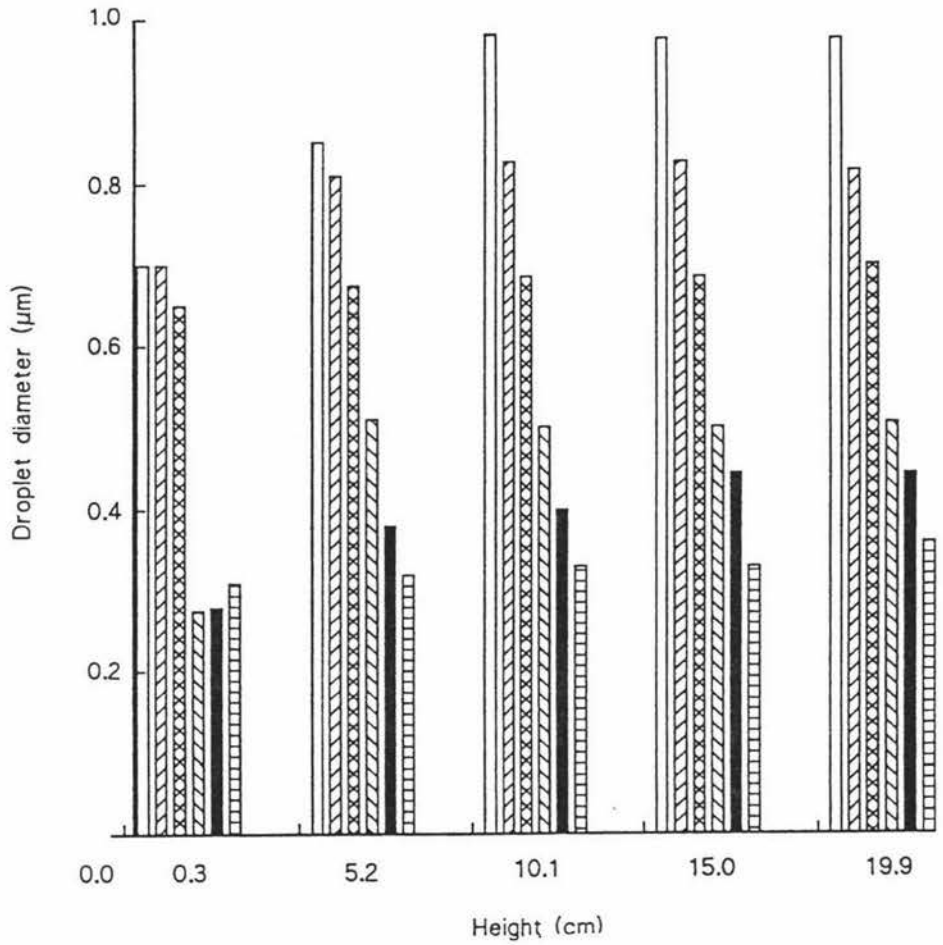


Figure 6.9: Changes in average droplet diameter (d_{32}) with change in height in the stability tube in emulsions containing 30% soya oil and 2.5% protein at varying homogenization pressure.

- 34 Bars
- 68 Bars
- 136 Bars
- 204 Bars
- 272 Bars
- 340 Bars

input caused an increase in creaming stability of the emulsions.

6.4 Effect of pH

The pH of the protein solutions was adjusted in the range 2.0 - 8.5 and the emulsions were prepared as described previously.

It was found that low pH emulsions (pH 2.0 and 3.0) were more stable than high pH emulsions (Figure 6.10). There was no significant change in the stability rating of low pH emulsions (pH 2.0 and 3.0) with the change in height in the tube. Emulsions at pH in the range 6.0 to 8.5 showed significant changes in the stability rating at different heights in the tube.

The changes in droplet diameter, d_{32} (μm) with change in height in the stability tube are shown in Figure 6.11. There was no significant change in the droplet diameter of the low pH (pH 2.0 and 3.0) emulsions with change in height in the tube. On the other hand, in all other emulsions the droplet diameter was smaller at the bottom of the tube (0.3 cm height).

It is not clear why low pH emulsions were more stable than high pH emulsions. The viscosity of the protein solution (2.5%, w/w) at pH 2.0 and 3.0 was not significantly different from that at pH 6.0, 7.0 or 8.5 (data not shown). Differences in protein loads for low and high pH emulsions may be related to the creaming behaviour (see chapter 5); low pH emulsions had higher protein loads than high pH emulsions.

No data is available in the literature on the creaming properties of caseinate stabilized emulsions at low pH. Leman *et al.* (1988) showed that in peanut oil emulsions stabilized by different milk proteins, the % fat separation decreased

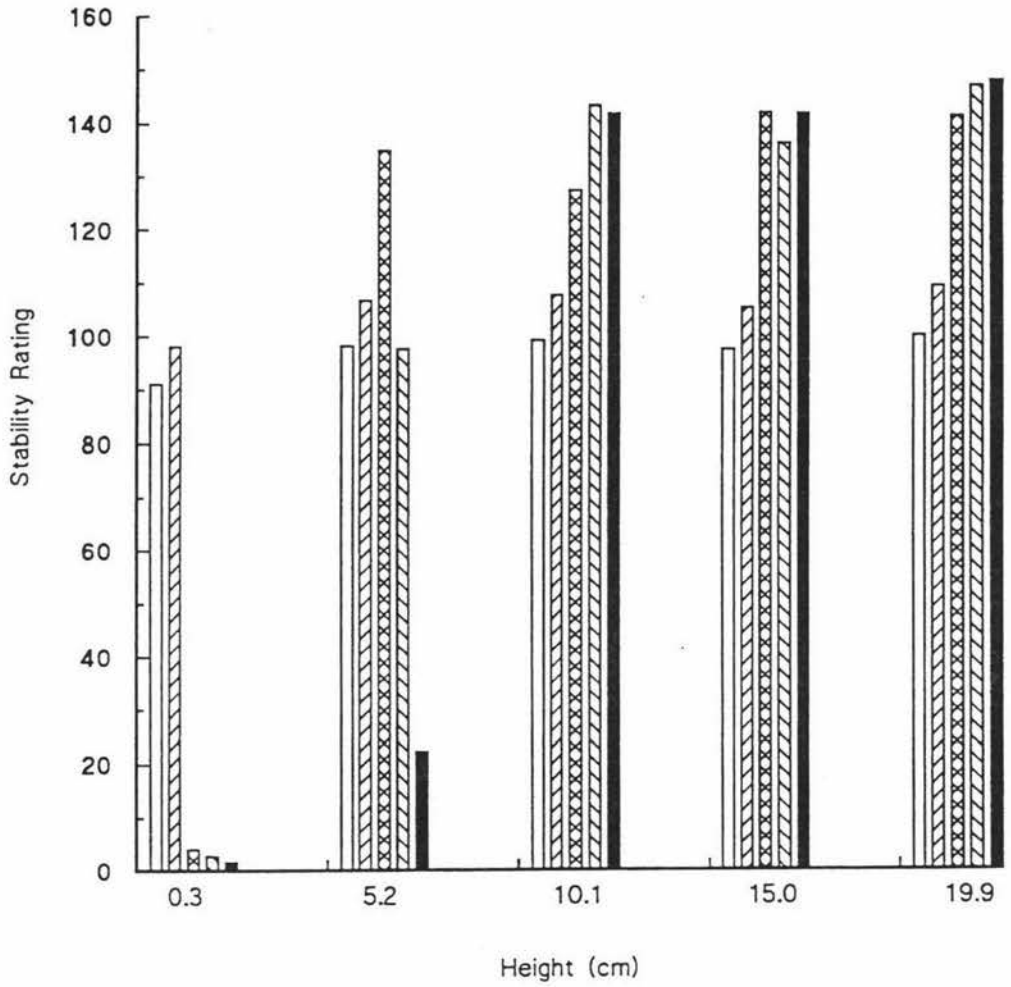


Figure 6.10: Change in stability rating with change in height in the stability tube at varying pH in emulsions containing 30% soya oil and 2.5% protein.

- □ pH 2.0
- ▨ pH 3.0
- ⊠ pH 6.0
- ▧ pH 7.0
- ■ pH 8.5

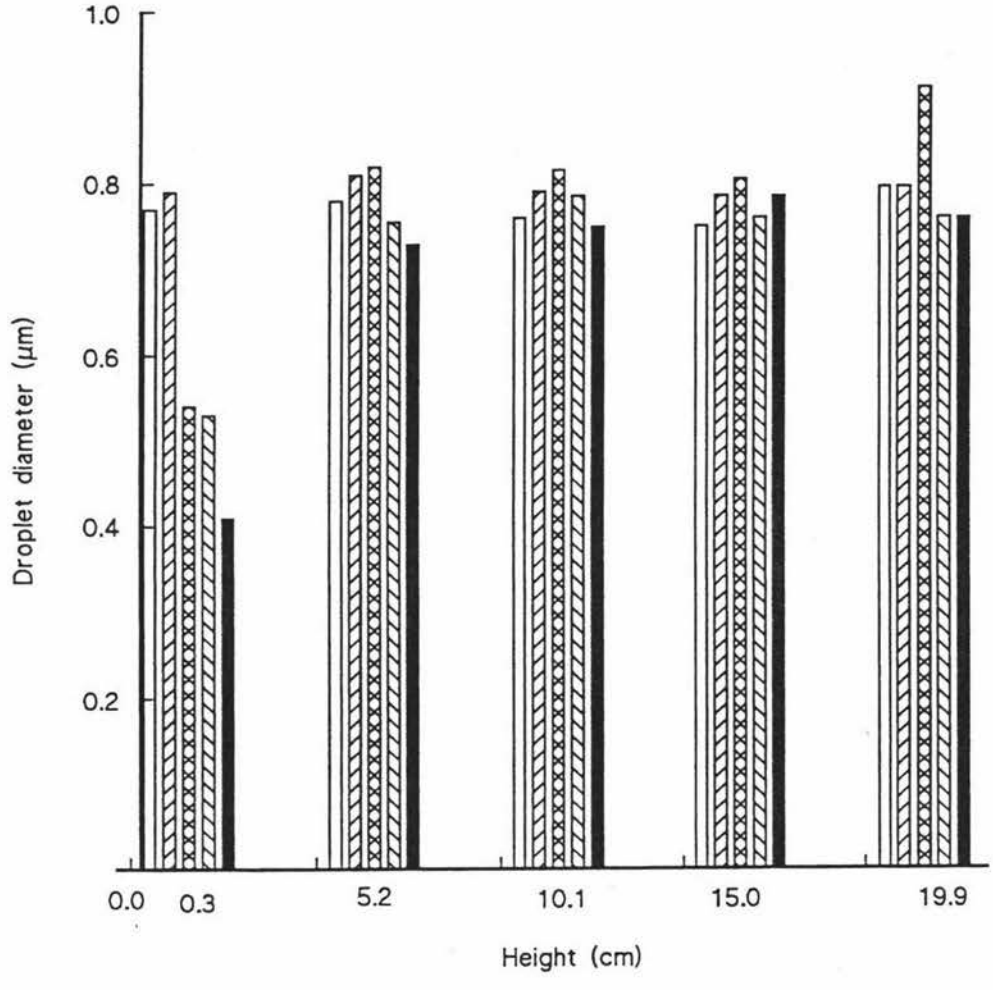


Figure 6.11: Change in average droplet diameter (d_{32}) with change in height in the stability tube at varying pH in emulsions containing 30% soya oil and 2.5% protein.

□ pH 2.0
▨ pH 3.0
▩ pH 6.0
▧ pH 7.0
■ pH 8.5

with increase in pH from 6.0 to 9.0.

6.5 Effect of $CaCl_2$

A known amount of $CaCl_2$ was added to sodium caseinate solutions prior to mixing with soya oil (30%, w/v) and homogenization.

Addition of $CaCl_2$ at 0.02 and 0.04% (w/w) had no significant effect on the stability rating of emulsions. These emulsions showed clear separation of fat at the top of the tube (19.9 cm). A further increase in $CaCl_2$ concentration caused a marked increase in the stability rating with no apparent separation at all heights (Figure 6.12).

The changes in d_{32} (μm) with change in $CaCl_2$ concentration of the emulsions are shown in Figure 6.13. At low $CaCl_2$ concentrations (0.02 and 0.04% w/w), the d_{32} of the emulsions increased slightly with height in the tube, but at higher $CaCl_2$ concentrations (i.e., 0.12 to 0.20%, w/w) the increase in d_{32} was not significant.

Viscosity experiments showed that increase in calcium chloride concentration gradually decreased the viscosity of the protein solution (Figure 6.14). As the $CaCl_2$ concentration increased from 0.02 to 0.20% (w/w), the viscosity decreased from ~ 2.54 to 1.99 cp. Therefore, increase in stability rating with $CaCl_2$ addition could not be attributed to changing viscosity of the continuous phase.

It was observed that the addition of $CaCl_2$ at concentrations above 0.08%, (w/w) increased the protein load (refer chapter 5). Protein loads were related to the state of aggregation of caseinates used in emulsion preparations.

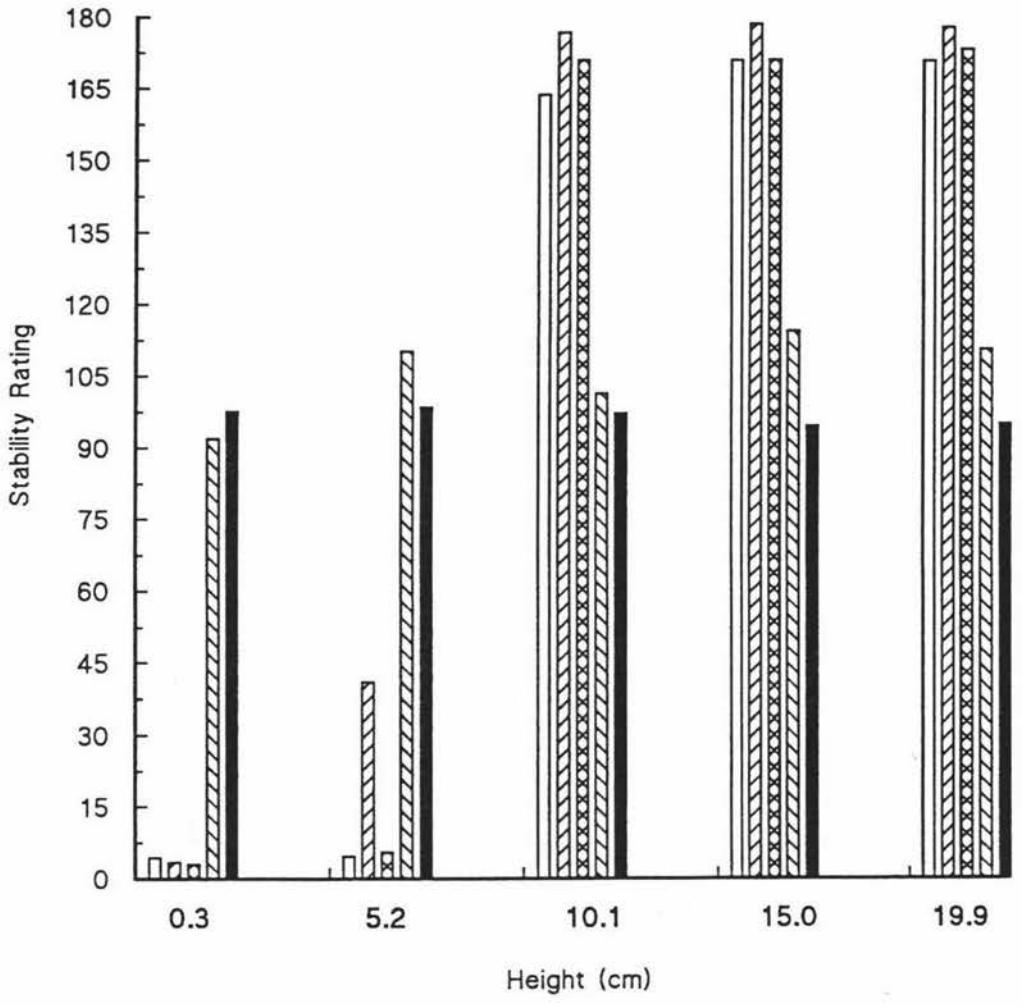


Figure 6.12: Changes in stability rating with change in height in the stability tube in emulsions containing 30% soya oil and 2.5% protein with change in $CaCl_2$ concentration.

- □ 0.00% $CaCl_2$
- ▨ 0.02% $CaCl_2$
- ⊠ 0.04% $CaCl_2$
- ▧ 0.12% $CaCl_2$
- ■ 0.20% $CaCl_2$

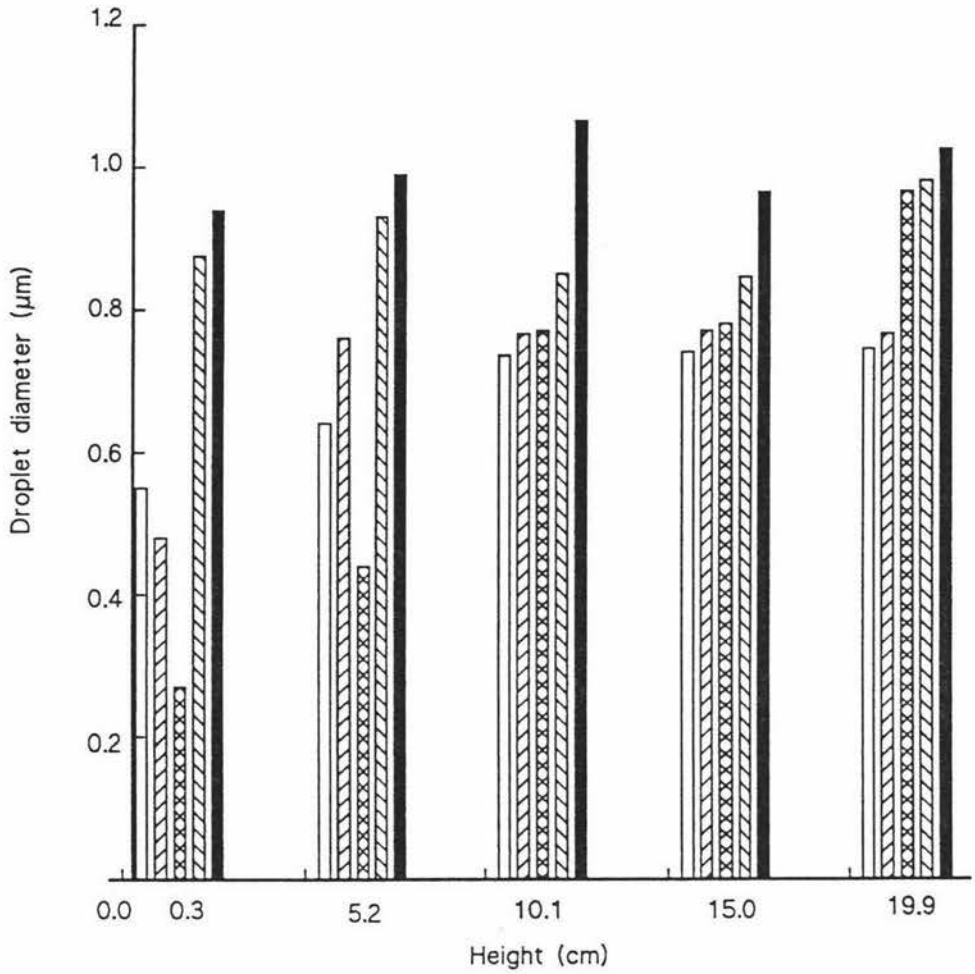


Figure 6.13: Changes in average droplet diameter (d_{32}) with change in height in the stability tube in emulsions containing 30% soya oil and 2.5% protein with change in calcium chloride concentration.

- □ 0.00% $CaCl_2$
- ▨ 0.02% $CaCl_2$
- ⊠ 0.04% $CaCl_2$
- ▧ 0.12% $CaCl_2$
- ■ 0.20% $CaCl_2$

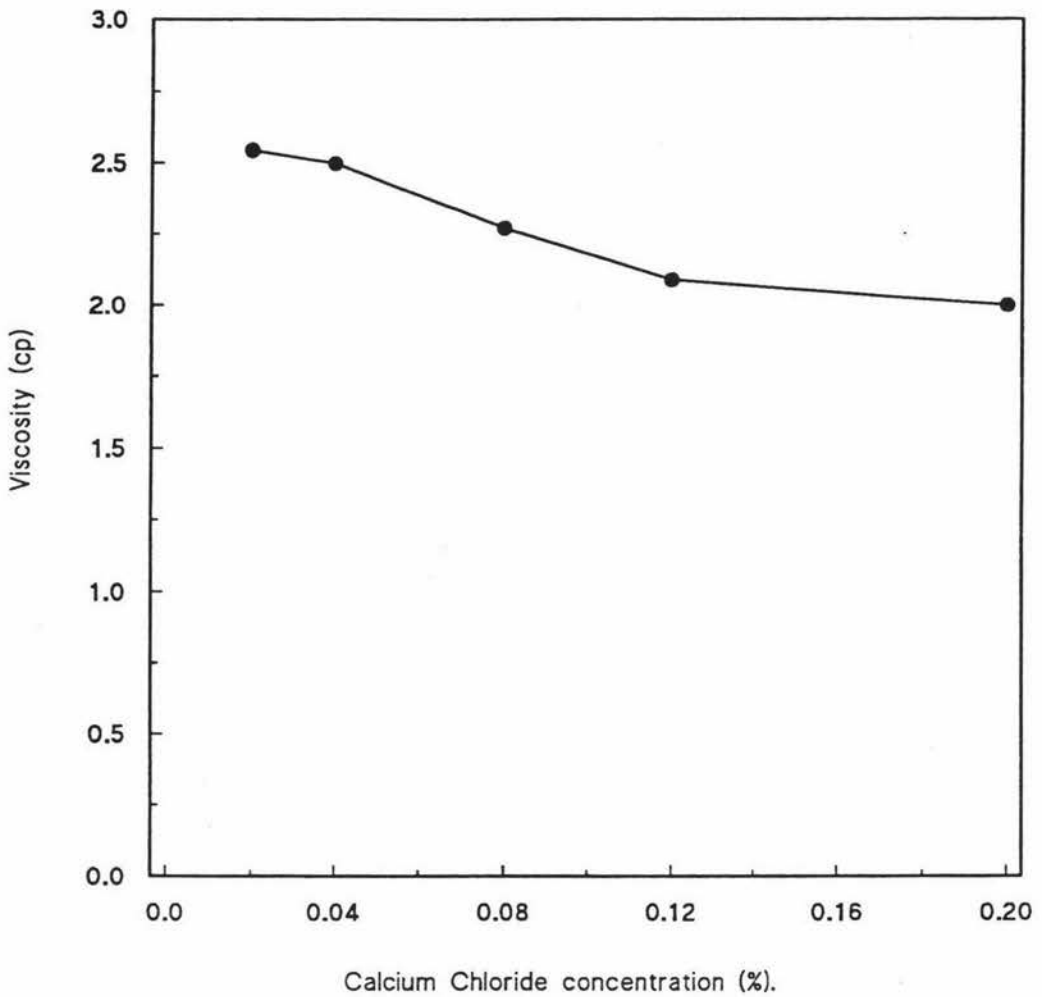


Figure 6.14: Influence of calcium chloride addition on the viscosity of 2.5% caseinate solution.

Addition of $CaCl_2$ causes the caseins to aggregate in solution and these aggregates are subsequently adsorbed at the oil-water interface. The presence of casein aggregates at the surface is likely to increase the density of oil droplets which probably retards the rate of creaming.

5.6 Effect of method of caseinate preparation on stability rating

As explained in the Materials and Methods, sodium caseinates were manufactured on a pilot scale under different processing conditions.

Emulsions were made from these sodium caseinates (2.5%, w/w) with soya oil (30%, w/w) followed by homogenization at 104/34 bar at 55°C. Emulsions were then transferred into the emulsion stability tube and changes in fat separation (under gravity) were observed at 20°C after 24 hours.

The creaming profiles of all emulsions with the change in height in the tube followed similar trends (Figure 6.15) i.e., all the emulsions showed separation of a cream layer at the top of the tube (19.9 cm). There were no significant differences in the stability of the different sodium caseinates.

The change in droplet diameter, d_{32} (μm) with the change in height in the stability tube is shown in figure 6.16. In all emulsions the average droplet diameter at the bottom (h = 0.3 cm) of the tube was considerably lower than at other heights.

It is concluded that the variations in the processing conditions during the manufacture of sodium caseinate had no significant effect on the stability of emulsions.

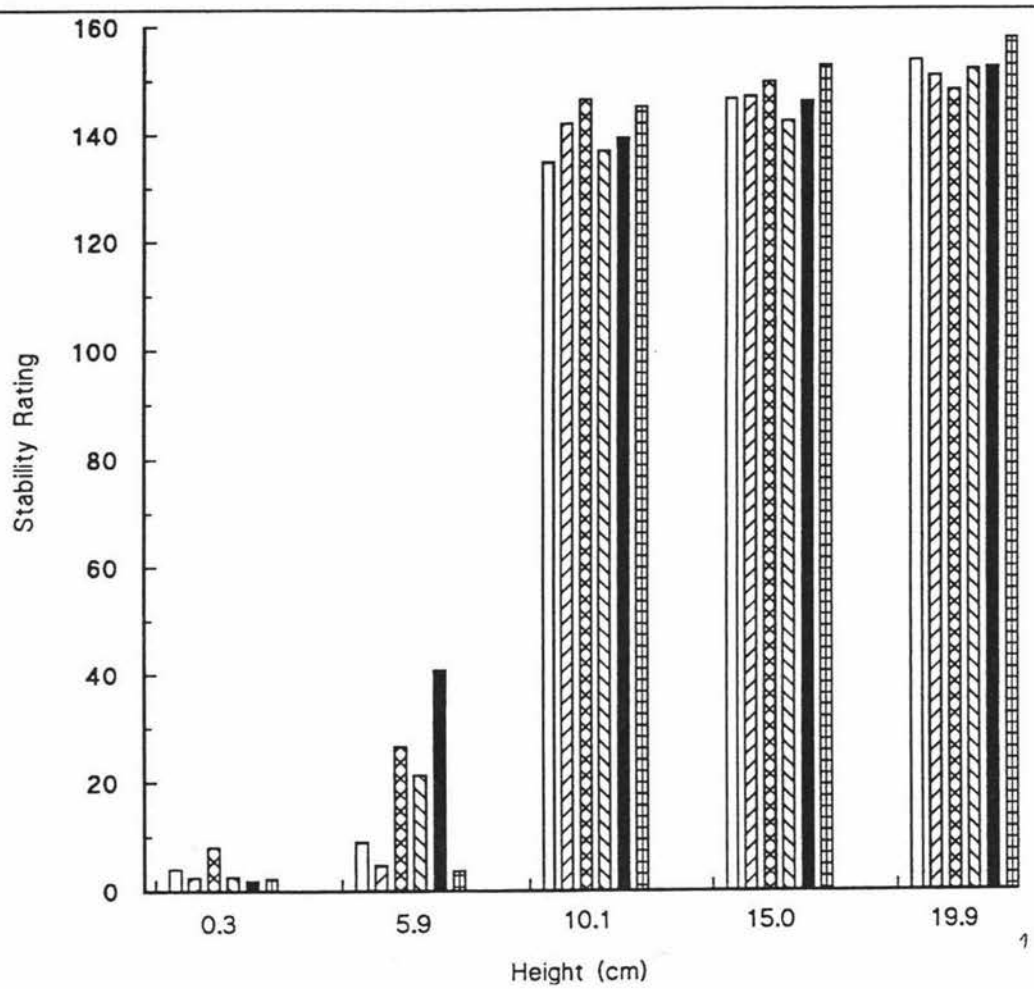


Figure 6.15: Changes in stability rating with change in sodium caseinate preparation method in emulsions containing 30% soya oil and 2.5% casein at different heights in the stability tube

- □ Lab made Freeze dried
- ▨ Mild
- ⊠ Normal
- ▧ High Freeze dried
- ■ High Spray dried
- ▦ Commercial

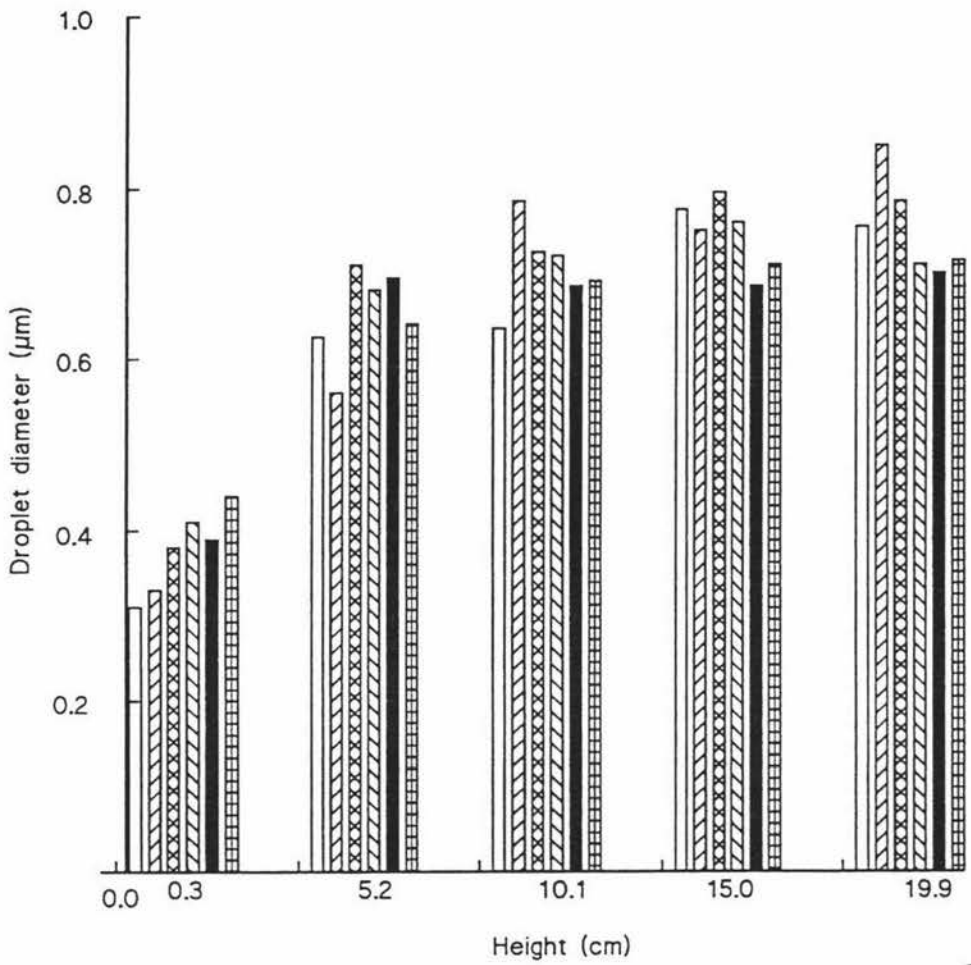


Figure 6.16: Changes in average droplet diameter (d_{32}) with change in sodium caseinate preparation method in emulsions containing 30% soya oil and 2.5% casein at different heights in the stability tube

- Lab made Freeze dried
- Mild
- Normal
- High Freeze dried
- High Spray dried
- Commercial

6.7 Overall Discussion

An oil-in-water emulsion creams because the droplets are of lower density than the continuous phase. An emulsion droplet surrounded by a stabilizing adsorbed layer behaves hydrodynamically like a rigid sphere. So, its creaming rate in a highly dilute emulsion can be described by Stoke's Law, since the presence of electrical charge on the droplet surface changes its velocity by only a few per cent relative to that of the equivalent uncharged sphere.

$$V_s = \frac{a\,(\rho_p - \rho_o)\,d^2}{18\,\eta}$$

Where d is the diameter of the droplet, a is the acceleration due to gravity, ρ_p and ρ_o are the densities of dispersed phase or particle and continuous phase respectively and η is the viscosity of the continuous phase.

Stoke's law tells us that there are three ways of increasing stability with respect to creaming, i.e. reducing the droplet size, matching the droplet density to that of the aqueous phase and increasing the viscosity of the continuous phase. Stoke's law is strictly applicable only to unaggregated particles at infinite dilution. Particle aggregation leads to an increased effective hydrodynamic diameter and enhanced creaming rate. Stoke's law does not apply at high concentrations of the dispersed phase, i.e. high fat concentrations.

The density of the stabilizing layer around a droplet (ρ_s) is usually different from the density of the internal phase (ρ_p), and for a protein stabilized oil-in-

water emulsion we typically have $\rho_s > \rho_o > \rho_i$. Thus, in emulsions of high protein load (i.e., thicker adsorbed layers), oil droplets, especially smaller ones, may have an effective density which closely matches or is greater than that of continuous phase; these droplets can never be creamed even in a centrifuge.

The results presented in this section show that creaming stability is influenced by a number of compositional and processing factors, including protein concentration, oil phase volume and homogenization pressure (i.e. oil droplet size). Creaming stability was enhanced with increase in the concentration of casein in the emulsion. This effect may be attributed mainly to increase in the viscosity of the continuous phase, although changes in protein load with casein concentration may also be involved.

Oil phase volume was an important factor in the formation of stable emulsions. As the concentration of oil was increased, higher stability was observed, which seemed to be due to enhanced mutual hindrance of oil droplets at high oil phase volume. As the oil phase volume increases, the droplets get closer together and begin to interfere with each other. Mutual hindrance of droplets occurs when the regions of liquid flow caused by droplet motion overlap. In a closed container, what makes creaming so dependent on volume fraction is that for every droplet that moves up an equivalent volume of continuous phase must move down, thereby retarding the motion of the other droplets (Dickinson, 1988).

Oil droplet size influenced the creaming stability in a predictable manner (as by Stoke's equation), i.e. increase in stability with decrease in droplet size.

The emulsion pH was an important variable. Creaming stability of the emulsion was greater at pH 2.0 or 3.0 than at pH between 6.0 and 8.5. There is no obvious explanation for this effect; higher protein loads of low pH emulsions which may influence effective droplet density and the nature of the interactions between droplets could be important.

Creaming stability was enhanced by the addition of $CaCl_2$ to the emulsion, although the viscosity of the emulsion decreased with $CaCl_2$ addition. The increase in stability in these emulsions could be attributed to a marked increase in protein load with $CaCl_2$ addition.

Overall it can be stated that the factors which influence protein load, droplet size and viscosity of the continuous phase are likely to influence creaming stability. Quantitative relationships will need to be developed between these parameters which could allow prediction of emulsion stability in food systems.

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