PROPERTIES OF MILKS CONCENTRATED BY REVERSE OSMOSIS

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TO MY GRANDMOTHER
GUNAWATI

ABSTRACT

Reverse osmosis (RO) is an energy efficient way of concentrating milk that can be operated at ambient temperatures, avoiding the product damage associated with thermal processes, and resulting in concentrates with better functional and nutritional properties. The objectives of this study were to examine in detail the effects of RO concentration on the rheological properties of milks, the states of proteins and fat globules in milk, the stability of RO concentrates towards ultra high temperature (UHT) processing, and changes in the UHT sterilized product during storage.

Whole milk, homogenized milk and skim milk were concentrated to 1.5X, 2.0X, 2.5X and 3.0X by RO, and rheological properties were measured at 5, 15, 25, 40 and 60 °C before and after 48 h storage at 5 °C, using a Bohlin VOR rheometer. The values of k (the consistency index) and n (the flow behaviour index) were obtained by analysis of the flow curves using the power law model. These values indicated that the samples were generally very slightly shear thinning (i.e. pseudoplastic). k increased with decreasing temperature and increasing concentration. A shift factor approach was used to develop a relationship between k, temperature and total solids concentration (as n remained virtually constant). The equation can be used to predict the flow behaviour of RO concentrated milk at various total solids contents and temperatures. The data was also analysed using the Fernandez-Martin approach (1972); this was found to be more successful at modelling the effects of concentration and temperature on k.

The viscosity data was also analysed using a hydrodynamic approach by means of Eilers' equation. A closer relationship was found between experimental values of basic viscosity and those predicted values by Eilers' equation when lactose was included in the equation as a component contributing to the volume fraction of the dispersed phase, rather than as a component contributing to the continuous phase viscosity.

Concentration by RO results in inevitable homogenization of whole milk, by the back pressure device needed to maintain the pressure driving force for concentration in the RO plant. Analysis of RO milks by particle size distribution measurement and electron microscopy revealed that the original fat globules were broken up into new globules in the size range 0.02-0.3 μm; these globules were smaller overall than in milks homogenized with conventional valve homogenizers.

The microstructures of protein membranes around the fat globules in RO concentrated milks were very different from those in milks homogenized conventionally. There were very few intact or semi-intact casein micelles at the surfaces of fat globules or in the serum. There were numbers of small fat globules grouped together, apparently held together by quantities of protein. Some unique particles were observed, which appeared to be similar to casein micelles, but had a number of very small fat globules embedded within their structure.

Particle size measurements and electron micrographs of samples clearly showed that UHT treatment of RO concentrated milks resulted in the formation of large aggregates of intact fat globules. This aggregation was protein-mediated and no evidence of fat globule coalescence was observed. When milks containing native fat globule membrane (non-homogenized reblended concentrated milks) were UHT treated, the large aggregates formed consisted of protein alone with no inclusion of fat globules. In RO concentrated milks, the extent of formation of aggregates, as well the aggregate size, decreased with increase in milk pH from 6.3 to 7.2 or with the addition of disodium phosphate (DSP) prior to UHT treatment.

UHT treated RO concentrated milks were examined for physico-chemical and structural changes during storage at 5, 20 and 37 °C. The effects of various processing variables (forewarming prior to concentration, post-UHT homogenization and the addition of phosphates prior to UHT treatment) on the storage-induced changes were also investigated. The results showed that irrespective of processing treatment, there was a decrease in pH with storage time, the extent of pH decrease being greater at higher storage temperatures. Gel formation, determined by a penetration test, correlated with the particle size distribution determined by light scattering. Samples stored at higher temperature showed greater quantities of large particles and gelled more quickly than those stored at lower temperature. The viscosity (measured at storage temperature) increased with storage time, the greater rate of increase being observed at the lowest storage temperature of 5 °C. The non-protein nitrogen content of all samples increased with storage time (indicating protein breakdown), the greatest rate of increase being observed at the highest storage temperature of 37 °C.

Homogenization of concentrates after UHT treatment had no appreciable effect on gel formation during storage for pasteurized milk concentrates, but it decreased the

rate of gel formation for concentrates that had been made from milk forewarmed prior to concentration. While forewarming decreased the rate of gelation for homogenized milks, an opposite trend was observed for concentrates that had not been homogenized.

Results from electrophoresis analysis and electron microscopy showed minimum changes in the samples stored at 5 °C, whereas storage of samples at 37 °C for 25 weeks resulted in an increase in non-disulphide covalent cross links, and increases in fibre-like material and proteinacious particles in the serum.

The addition of sodium hexametaphosphate (SHMP) resulted in the formation of smaller aggregates during storage, whereas the addition of DSP resulted in the formation of larger aggregates, suggesting that SHMP provides some stabilization of the concentrates. Electron microscopy and electrophoresis results did not show any significant differences between the samples with added SHMP or added DSP. Based on these results, a gelation mechanism in RO concentrated milks is proposed.

RO concentrated milks have unique structures; recommendation are made for further work aimed at a better understanding of such milks, and at the development of new or improved dairy products that utilize their unique functional properties.

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1. INTRODUCTION

Membrane separation processes are based on the principle of selective permeability of a membrane barrier to one or more components of a liquid mixture. These processes can be used as concentration or fractionation processes to produce two liquid streams of different compositions. They proceed without phase change (Pal and Cheryan, 1987). In the dairy industry today, the four membrane separation processes being used are: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). MF allows passage of proteins but retains fat globules, micro-organisms and somatic cells. UF allows passage of lactose and minerals but retains milk proteins, fat and micro-organisms. NF is used to separate mineral ions; it also called ultra-tight UF or loose RO. RO is a concentration process based on the removal of water molecules only.

The energy requirements of the RO process are low compared with other water removal processes, especially evaporation. RO requires between 9 and 19 kWh/1000 kg water as compared 626 kWh/1000 kg water for thermal evaporation (Marshall, 1985). Moreover, the RO process operates at 30-50 °C compared with the temperatures of up to 80 °C required for evaporation in conventional evaporators. These lower temperatures reduce the extent of thermal damage to proteins and result in a product with minimal cooked flavour (Pal and Cheryan, 1987). RO is a very compact process and requires less building space than an evaporator, which might require up to five-storey building. Overall, the concentration of milk by RO saves approximately 45% energy and about 25% overall cost as compared to conventional evaporators (Marshall, 1985).

In many countries, milk required for consumption in urban areas is transported from rural areas (centres of production) to processing plants in major cities. As milk contains about 87% water, it is obvious that considerable saving could be made by partially concentrating milk on the farm or at milk collection depots in rural areas. Concentration at these locations can reduce transport costs as well as result in savings in chilling and heating costs, storage space and packaging material (Cox and Langton, 1985; Gupta and Pal, 1993). The RO concentrate can be used as a raw material for the manufacture of various dairy products, e.g. cheese, yoghurt, milk powders.

To date, the main applications of RO in the dairy industry have been the concentration of milk in order to increase the capacity of existing conventional evaporators or the concentration of whey or permeate to reduce transport costs. Much of the previous research on the properties of milk products prepared from RO concentrated milk has been carried out on cheese products (Barbano and Bynum, 1984; Bynum and Barbano, 1984). Relatively little information is available on the use of RO concentrate for the manufacture of fresh milk products.

A number of preliminary studies (Dixon, 1985; Kocak, 1985; Mayes, 1985; Versteeg, 1985) indicate that there is a potential for RO not only to play an important role in reducing the costs of transporting milk (Cox and Langton, 1985), but also in offering possibilities for new or improved products such as UHT products (Kocak, 1985), liquid milk, butter, skim milk powder, yoghurt (Dixon, 1985), and cheese (Mayes, 1985, Barbano and Bynum, 1984; Bynum and Barbano, 1984). Several aspects which require careful study (for concentration of milk by RO), include the low flux rates of water through the membranes, possible damage to casein and fat, effects on flavour, the demanding level of bacteriological control required, and, lastly, regulatory aspects with regard to meeting the consumer's expectations of high quality.

Ultra-high temperature (UHT) processing of concentrated milk products is gaining increased acceptance, mainly because these products have great marketing convenience and show minimal flavour changes during storage at ambient temperature. The beneficial aspects of the RO process and UHT treatment can be reaped if the two processes are combined together to produce a concentrated product, which has a long shelf-life and has been produced economically. However, there is very little information on RO concentrates with respect to their stability towards UHT processing, and changes in UHT treated RO concentrated product during storage.

The purpose of the present study was to examine in detail the effects of RO concentration on the state of proteins and fat globules in milk, the stability of RO concentrates to UHT processing, and changes in the UHT sterilized product during storage. In addition, the rheological properties of RO concentrates and UHT treated concentrates at various concentration factors were also to be investigated.

2. REVIEW OF LITERATURE

This chapter provides a brief overview of the composition of milk and general characteristics of milk proteins, followed by an extensive review on the RO process and its application in the dairy industry, rheological properties of milk concentrates, effects of homogenization and heat treatment on milkfat globules and storage-induced changes in heat treated milks.

2.1 Milk composition

Milk is a complex fluid containing many components in several states of dispersion. The main components of milk are fat, proteins , lactose and minerals. These contribute to ~ 13% total solids content of milk. The general composition of raw milk is shown in Table 2.1. The fat component of milk is mainly triglycerides (98%), present as an emulsion of fat globules stabilised by a phospholipid and glycoprotein membrane. The protein content of milk can be divided into casein and whey proteins. In milk, caseins are present in colloidal suspension, whereas whey proteins are present in colloidal solution. Lactose, a sugar found only in milk, is present in solution. Lactose is a disaccharide, with a molecule containing the monosaccharides, glucose and galactose. The minerals of milk occur either in solution or are associated with the proteins, as either undissolved salts or bound ions.

Table 2.1 Typical raw milk composition.

Component		**Level in milk (%w/w)**
Water		87.3
Fat		3.90
Protein		3.25
	Casein	2.60
	Whey protein	0.75
Lactose		4.60
Minerals		0.65

2.2 General characteristics of milk proteins

The proteins of milk greatly influence the behaviour and properties of dairy products. The milk protein falls into two principal categories: caseins and whey proteins. Caseins represent ~ 80% whereas whey proteins represent the rest (~ 20%) of the total milk proteins. Several reviews covering chemistry of milk proteins have been published. (e.g.Whitney, 1988; Swaisgood, 1992; Creamer and McGibbon, 1996).

2.2.1 Caseins

Caseins are defined as a group of phosphate containing milk-specific proteins that precipitate on acidification to pH 4.6. Almost all casein in milk is present in casein micelles, which are large spherical complexes containing ~ 92% protein and ~ 8% inorganic salts, principally calcium phosphate (Whitney, 1988). Caseins comprise of four kinds of polypeptide chains, designated as α_{s1}-, α_{s2}-, β- and κ-casein, together with some derivatives formed by the proteolysis of these chains. All of the casein polypeptide chains have at least one ester-bound phosphate group per molecule; none of the whey proteins has any.

2.2.2 Casein micelle

Caseins unlike many globular proteins (whey proteins), are not present in milk as individual molecular structures, but rather as large protein complexes that also incorporate milk salts, particularly calcium and phosphate salts. Thus, the 'native' structure is actually a protein complex, resulting from interaction of individual caseins, known as casein micelle. The micelles appear as more or less spherical particles with a relatively wide size distribution of 50-300 nm as shown by electron microscopy (Schmidt *et al.*, 1973). They consist of ~ 92% protein (casein) and 8% inorganic salts, mainly calcium and phosphate. Casein micelles are highly hydrated and sponge-like colloidal particles containing about 3.7 g water/g protein.

The exact structure of casein micelle has not been fully resolved with several models having been proposed in the past three decades (Rose, 1969; Waugh *et al.*, 1971; Slattery and Evard, 1973; Schmidt, 1982; Walstra and Jennes, 1984 and Holt, 1992).The submicellar structure of casein micelle as proposed by Schmidt (1982) is shown in Fig.

2.1. According to this model, casein micelles are composed of discrete subunits linked through colloidal calcium phosphate. The protein composition of the subunits varies; those containing higher amounts of κ-casein are oriented towards the outer surface while others containing little or no κ-casein are buried inside the micelle. This model was later modified by Walstra (Walstra and Jenness, 1984; Walstra, 1990), who incorporated the concept of steric stabilization of the micelle by κ-casein. In the proposed model (Fig. 2.2), most of the κ-casein is located at the outside, and the protruding chains of its C-terminal end give the micelle a hairy surface. The hairs are flexible and show perpetual Brownian motion. The effective thickness of the hairy layer is at least 5 nm. A small part of κ-Casein is in the interior. The evidence of such structure also comes from hydrodynamic studies (Walstra, 1979 ; Holt and Dalgleish, 1986; Horne, 1989) and from proton nuclear magnetic resonance (NMR) in D_2 0 (Griffin and Roberts, 1985; Rollema *et al.*, 1988) which show that part of κ-casein has considerable freedom of motion. κ-Casein hairs at the surfaces of casein micelles provide stabilization to the micelles toward flocculation (Walstra, 1990).

Holt (1992) suggested that the sub-structure of casein micelles may be depicted without requiring the existence of submicelles (Fig. 2.3). He claimed that the sub-structure consists of nanoclusters of calcium phosphate which are incorporated in a protein matrix which does not resemble a sub-micelle. The outer region of the micelle shows hairy layers which are considered to provide steric stability to the micelle (Holt, 1992; Holt and Horne, 1996).

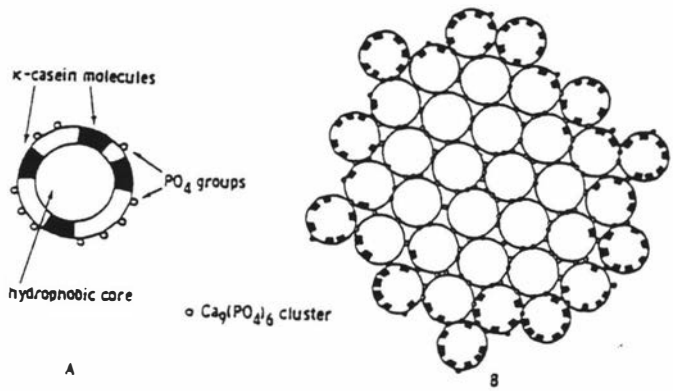

Fig. 2.1 Submicelle model proposed by Schmidt (1982) : (a) submicelle; (b) casein micelle

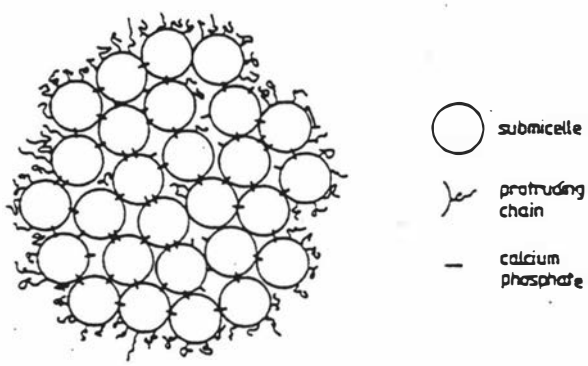

Fig. 2.2 Submicelle model showing the protruding C-terminal parts of κ-casein as proposed by Walstra (Walstra and Jenness, 1984; Walstra, 1990)

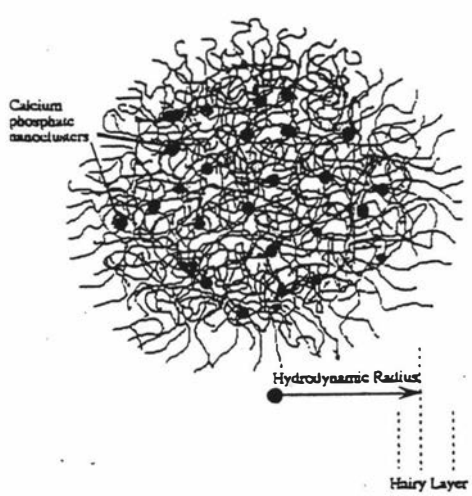

Fig. 2.3 Hairy casein micelle model, proposed by Holt (1992), shows a fairly open structure of polypeptide chains crosslinked by calcium phosphate nanoclusters in the core.

2.2.3 Whey proteins

The proteins remaining in solution after precipitation of the caseins at pH 4.6 are called whey proteins (or milk serum proteins); they consist of a rather diverse group, including β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins. In addition, milk serum contains a number of so-called minor proteins and a number of enzymes.

β-Lactoglobulin, the major protein of whey, is the most extensively characterized and best described of all food proteins (Swaisgood, 1992). Seven genetic variants (A, B, C, D, E, F, and G) have been identified with the A and B variants being predominant in most breeds of Western cattle. The primary amino acid sequence of β-lactoglobulin B consists of 162 amino acid residues with a calculated molecular weight of 18,227 (Braunitzer *et al.*, 1972). Native β-lactoglobulin possesses two disulphide bonds (Cys 66 - Cys 160 and Cys 106 -Cys 119), and a free thiol group (Cys 121) which is inaccessible to solvents at or below neutral pH. At 65 °C, β-lactoglobulin undergoes denaturation, which is accompanied by extensive conformational transitions (molecular expansion) that expose highly reactive groups in the hydrophobic region.

The second major whey protein, α-lactalbumin, accounts for approximately 25% of whey protein. A total of three genetic variants (A, B and C) are known, of which the B variant predominates in the Western breeds of cattle. α-Lactalbumin B variant consists of 123 amino acids residues with a calculated molecular weight of 14,174 (Brew *et al.*, 1970). It has four disulphide bonds linking residues 6 to 22, 28 to 111, 61 to 77, and 73 to 91. α-Lactalbumin binds calcium which may stabilize the molecule against irreversible denaturation (Hiraoka and Sugai, 1984). Removal of bound Ca^{2+} with EDTA renders α-lactalbumin more susceptible to denaturation by heat or guanidine hydrochloride (Hiraoka and Sugai, 1984).

Other whey proteins, such as bovine serum albumin and the immunoglobulins occur in milk to a limited extent and may be present in conjunction with various enzymes (Shahani *et al.*, 1973). Serum albumin consists of a single polypeptide chain of 582 amino acids residues. Immunoglobulins are antibodies synthesised in response to stimulation by macromolecular antigens foreign to animal. They consist of two kinds of polypeptide chains, light (L) of MW 22,400, and heavy (H). The heavy chains are of

several types, including γ (MW 52,000), α (MW 52,000-56,000), and μ (MW 69,000). Bovine milk is also a source of two iron-binding proteins: transferrin and lactoferrin.

2.3 *Effects of heat on milk*

A number of changes occur in milk systems during heating; these changes have been extensively reviewed (Singh and Creamer, 1992; Singh, 1995). Significant changes occuring above 60 °C include whey protein denaturation, interaction between denatured whey protein and casein micelles, aggregation and dissociation of casein micelles and the conversion of soluble calcium, magnesium and phosphates to the colloidal state.

Heating of milk at temperatures above 60 °C causes denaturation of the heat labile whey proteins. Protein denaturation has been defined by some authors (de Wit, 1981; Mulvihill and Donovan, 1987) as the unfolding of the native globular form into a less ordered structure. The unfolded proteins then proceed by an entirely separate step to an aggregated form. Once denatured, the whey proteins may bind to the casein micelles or simply associate with themselves to form polymeric products.

Heating of milk above 90 °C results in whey protein denaturation and association with κ-casein on the surface of the casein micelles, giving appearance under an electron microscope of threadlike appendages, protruding from the micelles (Creamer and Matheson, 1980; Mohammad and Fox, 1987). The type of association between whey proteins and casein micelles depends on the severity of the heat treatment and involves both disulphide and hydrophobic interactions (Singh and Fox, 1987b).

Not all the denatured whey proteins complex with the casein micelles. Some remain in the serum where they may form aggregates with other whey proteins or with serum κ-casein. The extent of association of denatured whey protein with casein micelles is markedly dependent on the pH of the milk prior to heating. Heating at pH values less than 6.7 results in a greater quantity of denatured whey proteins associating with casein micelles, whereas, at higher pH values, whey proteins\κ-casein complexes dissociate from the micelle surface, apparently due to dissociation of κ-casein (Singh and Fox, 1985, 1986).

Generally, casein micelles are very stable at high temperatures due to their lack of secondary or tertiary structure and the presence of a rather complex quaternary

structure. The most pronounced effect of high temperatures on the casein micelles is increase in size (Caroll *et al.* 1971; Creamer and Matheson, 1980) which is probably due to the deposition of denatured whey proteins on the micellar surfaces and precipitation of calcium phosphate.

The hydrolytic cleavage of peptide and phosphate bonds in casein may occur on heating at the elevated temperatures. Dalgleish *et al.* (1987) reported that approximately 65% of the casein phosphate was released after heating milk for 30 min at 130 °C. Dephosphorylation of casein on heating skim milk at any temperature proceeds more slowly than that of sodium caseinate (Pyne and McHenry, 1955; Davis and White, 1959) Two-fold concentration of skim milk increased the rate of dephosphorylation; preheating unconcentrated skim milk caused little change in the rate of dephosphorylation while preheating concentrated milk reduced the rate of dephosphorylation.

Heat treatments and/or exposures to alkaline pH can lead to change in the covalent structure of proteins, resulting in modification of amino acids residues and the formation of intra- and inter-molecular covalent cross-links. Heat treatment, in the presence of reducing sugars, favours Maillard reaction between the carbonyl group of sugar and the ε-amino group of lysine. Cysteine or phosphoserine residues may undergo β-elimination, yielding dehydroalanine residues which may then react with lysine to form lysinoalanine cross-links, or with the thiol groups of cysteine residues to form lanthionine cross-links when protein solutions are heated at moderately high pH (de Groot and Slump, 1969).

2.4 Concentration of milk by membrane processes

2.4.1 Introduction

Membrane filtration was originally developed for desalination of salt or brackish water, some 35 years ago. The membranes were made of cellulose acetate. Over the years, membranes have become tougher and more durable as the type of material which can be used increases and the range of applications expands. Cleaning techniques, too, have been improved; many membranes nowadays are able to accept harsher cleaning chemicals and higher temperatures, so steam sterilization becomes feasible.

In general, certain processes can be performed that were not previously possible. Bacteria, yeast and moulds etc., can be removed without damaging the product. Yields can be increased either by reducing losses or by upgrading low quality materials; they can be used to concentrate, clarify or separate at low temperatures so there is no change in the phase and the materials are protected from damage or changes in product characteristics. Another attribute is the consistency of the permeate quality which can be obtained, even from widely varying feedstock.

The current membrane processes are based on four main groupings:

Reverse osmosis (RO) uses the tighest membrane and smallest pore size. These membranes allow only water to pass through and retain most chemical species. RO operates typically at pressures in the range 15 to 60 bar.

Nanofiltration (NF) or "loose RO" retains most chemical species but allows through a proportion of the low molecular weight materials and water. Operating pressures range from 10 to 30 bar.

Ultrafiltration (UF) retains suspended particles, bacteria and larger molecular weight materials (such as proteins) but allows through salts, sugars and materials with molecular weight less than the pore size. UF operates within a range of 2 to 10 bar.

Microfiltration (MF) utilises a very open membrane so retains all particles in the range of 0.05 to 8 microns; this includes bacteria but allows through salts, sugars and smaller proteins. Operating pressures are very low, typically from 0.3 to 5 bar depending on the application.

2.4.2 The RO process and its application in dairy processing

In normal osmosis solvent passes from the solvent side of the membrane through to the concentrate side thus diluting the solution and at the same time setting up a pressure. If an external pressure greater than the osmotic presure is applied on the concentrate side, i.e. at A in Fig. 2.4, the process may be reversed, and the solvent will be pushed out of the concentrate back into the solvent side. This is the principle of RO process. RO can be used for concentrating or for purifying solvents by collecting either the concentrate (retentate) or the filtrate (permeate) according to the requirements. A schematic representation of RO is shown in Fig. 2.4a with a practical arrangement of a membrane in tubular form Fig. 2.4b.

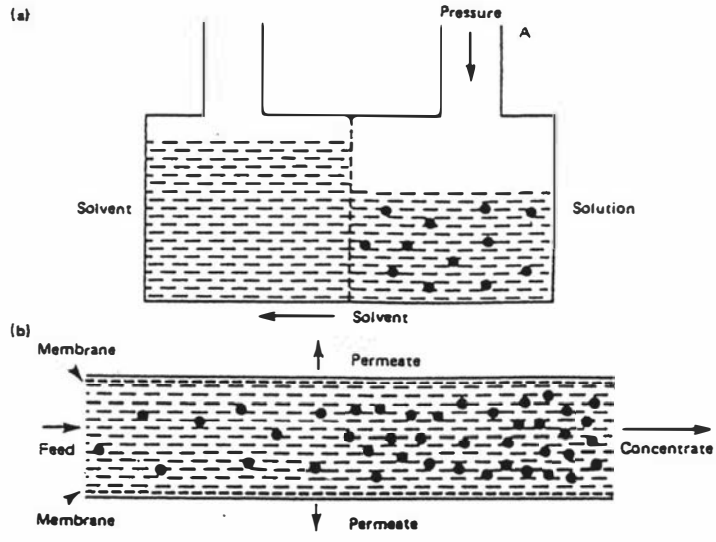

Fig. 2.4 Reverse osmosis (a) the principle (b) the practice
Adapted from Glover (1985)

The key to effective separation using RO is the structure of the membrane. The active layer of modern RO membranes is believed to be very thin, approximately 5-30 nm, with a pore size of about 5-20 A°. These "asymmetric" membranes are supported on a porous backing, for example, fibreglass, filter paper, metal (SS), ceramics, sintered glass, etc. and are assembled in a suitable physical configuration.

The actual basis of separation is still not completely understood. The "preferential sorption-capillary flow" mechanism proposed by Sourirajan and Matsuura (1985) is the most logical and probable. It is based on the Gibbs adsorption isotherm model. In this model, solutions containing salts whose surface tension increases with concentration, such as inorganic salts, will have a "negative excess" of the solute absorbed on the membrane surface i.e. there will be a layer of water molecules on the membranes surface. If the membrane contains pores of the appropriate size (twice the thickness of the water layer) and a pressure sufficient to overcome the osmotic pressure or chemical potential difference is applied, the adsorbed solvent (water) layer will flow through these pores. Thus the control of the pore size and providing an appropriate membrane surface is critical to the success of RO.

RO membranes are usually described in terms of their rejection or retention coefficients, that is the fraction of any particular dissolved substance they retain.

Modern membranes have retention coefficients of 0.999 for sugars and large molecules, 0.90-0.98 for smaller ions such as sodium, and between 0.1 and 0.9 for various low molecular weight organic compounds.

Cellulose acetate formed the first successful RO membrane. It was the first and surviving material of a very large number of trials on RO membranes for more than 10 years. In 1983, non-cellulosic membranes appeared. Paterson Candy International Ltd. (PCI) have their ZF99, which is superior in characteristics like-permeability, retention, pH and temperature tolerance. Its water flux at 50 bar pressure and at 15 °C is 75% greater than that from cellulose acetate under similar conditions. These membranes can be operated safely over a pH range of 3 to 11 and up to a temperature of 80 °C, which greatly facilitates cleaning. The cleaning for cellulose acetate is restricted to expensive neutral enzymes and 35 °C. The De Danske Sukkerfabrikker (DDS) company manufactured a new RO membrane designated as HR and described as a thin film composite membrane consisting of a UF membrane coated with a very thin polymer layer. The polymer is the effective RO membrane, laid on a polysulphone layer which in turn is supported on a poly-propylene backing. It has properties very similar to the PCI new membrane. Cleaning can be done with approved agents since the membrane is susceptible to damage by chlorine and hydrogen peroxide (Glover, 1985).

A major application of RO in dairy industry is for the concentration of whey and for the concentration of the permeate obtained during the UF of whey. It is possible to concentrate milk by RO to between 24 and 30% total solids (Coton *et al.*, 1970; Glover, 1971). Concentration of milk by RO gives a product with a minimum level of cooked flavour (McKenna, 1970) which can be used in ice cream manufacture, instead of milk powder, to give a better texture and remove the powdery taste (Bundgaard *et al.*, 1972). Other uses include the manufacture of yoghurt (Bundgaard *et al.*, 1972; Dixon, 1985) and ymer (Nielsen *et al.*, 1972). Most of these applications use skim milk concentrated to between 10 and 15% total solids and in some cases like ymer, the yield of the product is increased by 50% (Bundgaard *et al.*, 1972; Nielsen *et al.*, 1972).

Whole milk and skim milk concentrated to about 30% total solids by RO has been used as an alternative to concentrate by evaporation prior to spray drying and for the direct production of evaporated milk (Abbot *et al.*, 1979).

Agbevavi *et al.* (1983) made cheddar cheese from whole milk concentrated two-fold by RO. They reported using 50% less starter culture and 60% less rennet as compared to the cheese made from unconcentrated milk. The composition of cheese made from RO concentrated milk was close to that of cheese made from regular milk.

In another study cheddar cheese was made in a commercial factory from whole milk concentrated by RO at 5, 10, 15, and 20% reductions in volume (Bynum and Barbano, 1984). Proteolysis was similar for control cheese and cheese made from RO milks, during the first 3 months of ripening. Cheese made from milk reduced in volume by 20% using RO has a significantly greater lactose content than the control cheese, and gave good quality aged cheddar cheese. Composition of the cheese made from milk concentrated by RO was comparable to that of control cheese. There was an increased retention of whey solids and improved milk fat recovery which resulted in increased yield of cheese by 2 to 3%, when the milk was reduced in volume by 20%.

Glover (1985) reported that the main application of reverse osmosis in dairying is concentration of whey to facilitate its handling, transport, storage, and to serve as a first step in drying. Reverse osmosis for preconcentration has been suggested for the following products: (a) whey powder, (b) whey protein powder, (c) demineralized whey powder, and (d) whey based animal feed.

The use of RO concentrated skim milk (14% T.S.) and diluted RO concentrated skim milk (from initial 23% T.S. diluted to 14% T.S.) have been tried in the manufacture of yoghurt (Dixon, 1985). The RO process yoghurts were preferred over the control yoghurt (made from skim milk fortified with SMP). Although, there was no difference in the flavour of the products, the physical properties, measured in terms of viscosity and syneresis, RO yoghurts were superior to the control yoghurt. The apparent viscosity increased and syneresis decreased in RO yoghurts.

Davis *et al.* (1977) compared yoghurt made from milk concentrated by RO to 15% total solids with that made from milk adjusted to comparable concentrations of solids by addition of skim milk powder. The quality of yoghurt prepared from RO concentrates was at least equal to that of yoghurt prepared from the milk plus milk powder.

Kocak (1985) studied the possibility of using RO concentrates (26% TS) and diluted RO concentrate (13% TS) for UHT processing. The results from these studies

indicate that UHT processing of RO concentrates either as such or as reconstituted milk is quite feasible.

Dixon (1985) studied the implications of the use of RO to concentrate milk in production areas for the quality of subsequent milk and allied liquid products and concluded that RO technology could be introduced with no apparent effect on milk quality. No particular problem was noticed while making butter from churning of blends of RO cream with normal cream (70-80% of RO cream) (Dixon, 1985). However, butter granules drained quickly and the fat losses in the butter milk were higher than expected. The curd content of the butter increased perhaps due to presence of higher SNF content in the RO concentrated milk from which the cream was derived. This resulted in an increase in the butter yield (Dixon, 1985). Also no problems were encountered during the manufacture of powder from RO skim milk and thus the use of RO can be freely interchanged with conventional evaporation (Dixon, 1985).

Gupta and Pal (1993) explored the feasibility of transporting raw milk from rural chilling centres in concentrated form to city dairies. Reverse osmosis (RO) was used for concentration of buffalo milk. The organoleptic quality of fresh RO concentrated milk and reconstituted RO milk was identical to that of normal buffalo milk. The flavour of the stored RO milk did not change up to 72 h at 5°C despite slight increase in free fatty acid (FFA), titrable acidity and microbial count. The energy requirements for concentration of buffalo milk to 1.5 fold and 2 fold using RO process were calculated to be 369.7 and 470.9 KJ per kg of water removed, respectively. About 50% reduction in volume of buffalo milk employing RO at a chilling centre handling 10, 000 l milk/day and located at a distance of 300 Km from the main processing plant can offer 25% savings in costs of transportation with a payback period of slightly over a year.

However, the RO process has some limitations. There is a decrease in the flux during the concentration process which may be due to various reasons such as build up of material at the membrane surface (concentration polarization), the reduction of the driving force with the increasing feed concentration and membrane compaction at high operating pressures (Marshall *et al.*, 1968: Skudder *et al.*, 1977). The second problem that relates to the quality of milk is the damage to fat globules. This damage is caused probably by the shearing action in high pressure pumps and high operating pressures during concentration process. The fat globules are homogenized during this process.

Also product suffered with an increase in bacterial numbers when cellulose acetate (CA) membranes (operating temperatures below 45 °C) were used. But with continued improvement of membrane material and design of RO plants has minimised this problem to a considerable extent (Marshall, 1985). With the use of non-CA membranes, bacterial growth can be prevented since a higher operating temperature (50-55 °C) can be used.

2.5 Rheological properties of milk concentrates

2.5.1 Introduction

Rheology is the study of the deformation of materials, subjected to applied forces. A distinction is usually made between fluids and solids; fluids will flow under the influence of forces, whereas solids will stretch, buckle or break (Lewis, 1993). The viscosity of a fluid is defined as the internal friction within the fluid. When a fluid is subjected to a shearing force over a surface area it will undergo a deformation, known as flow. All fluids and many semi-fluid materials have a measurable viscosity. The science of rheology is mainly concerned with measuring the viscosity and other properties of materials under different conditions in order to understand the factors that influence their deformability (O'Connor *et al.*, 1995). A basic understanding of the rheological behaviour of foods is essential for process design and evaluation (in designing pumping and piping systems, and in heat and mass transfer operations), quality control (to check the consistency of raw materials, and in-process and final products) and finally consumer acceptability (e.g. mouth feel) (Holdsworth, 1971; Hermansson, 1975).

2.5.2 Classification of the rheological behaviour of fluids

The viscosity of a fluid food depends on its temperature and composition and may also depend on the shear rate or shear stress, the duration of shear, and the previous shear history. Fluids can be broadly classified into Newtonian and non-Newtonian fluids. The rheological properties of Newtonian fluids are independent of the shear rate and previous shear histories, and are dependent only on temperature and composition (Rao, 1977). Water, milk, whey and vegetable oils are common examples of Newtonian fluids.

Newtonian fluids represent the simplest type of fluid flow behaviour and obey the following law:

$$\tau = \eta \dot{\gamma} \quad \text{(Pa)} \tag{2.1}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (per s) and η is a constant called the coefficient of viscosity (Pa s). The shear stress is defined as force/area and the rate of deformation, termed the shear rate, is determined by the velocity gradient. The two main units used for viscosity measurement are the poise (P) (cgs) and the pascal second (Pa s)(SI). The cgs unit poise (P) is smaller than the pascal second (Pa s) by factor of 10. For example the viscosity of water at 20.2 °C is 1 mPa s or 1 cP (Lewis, 1993; O'Connor *et al.*, 1995).

Many of the fluids that are found in food processes display more complex flow behaviour than Newtonian fluids and these fluids are referred to as non-Newtonian. They can be classified according to their dependency on the shear rate, or shear rate and duration of shear. For time-independent fluids, at a constant temperature, the viscosity depends only on the magnitude of the shear stress or shear rate. If the viscosity decreases when the shear rate is increased, the fluid is shear-thinning and is known as a pseudoplastic fluid. Concentrated milk and many emulsions are shear-thinning fluids. Shear-thinning fluids commonly obey the power law:

$$\tau = k \dot{\gamma}^n \tag{2.2}$$

where k (Pa s^n) and n (dimensionless) are constants called the consistency index and flow behaviour index respectively and may have different values over different ranges of shear rates (O' Connor *et al.*, 1995).

If the viscosity increases as the shear rate is increased, the fluid is a shear-thickening (dilatant) fluid. Dilatant flow is rare in foods. High concentrations of raw starch in water and melted chocolate exhibit this kind of behaviour. For Newtonian fluids $n = 1$, pseudoplastic fluids $n < 1$ and for dilatant fluids $n > 1$. The viscosity is also referred to as the apparent viscosity, since it is not a constant at a given temperature but is dependent on the shear rate (Holdsworth, 1971):

$$\eta_{app} = \tau / \dot{\gamma} = k \dot{\gamma}^{n-1} \quad \text{(Pa s)} \tag{2.3}$$

Non-newtonian fluids with time dependent properties are subdivided into two categories. At a constant temperature and shear rate, if viscosity decreases as a function of time, then the fluid is thixotropic and if the viscosity increases with time, the fluid is rheopectic.

Some fluids possess both viscous and elastic properties and hence are called viscoelastic (Holdsworth, 1971).

Plastic flow is characterized by a yield stress (τ_0) below which there is no flow and above which shear rate varies linearly or non-linearly with shear stress. This type of behaviour is common in foods like ketchup, whipped cream, butter and margarine, which often obey Bingham's equation

$$\tau = k\dot{\gamma}^n + \tau_0 \qquad (2.4)$$

where τ_0 is the yield stress.

2.5.3 Rheology of milk

2.5.3.1 Rheology of whole milk

Normal milk is a fat-in-water dispersion containing 10-12% suspended particulate, dissolved or colloidal matter which comprises fat, proteins, lactose and minerals (Rao, 1977). In spite of this complex nature, normal milk behaves as a Newtonian liquid (except at low shear rates or at low temperatures, where it is non-Newtonian). In normal milk the viscosity is affected by temperature, fat content, total solids content, and the solid to liquid fat ratio. Fat contents ≤ 10% have no influence on the viscous character of milk (Randhahn, 1974) especially when the temperatures are high (> 40 °C). Raw milk has characteristic rheological properties in the temperature range of ≤ 40 °C owing to its fat content. Liquid to solid fat ratio changes on decreasing temperature (Randhahn, 1974). At low temperatures, raw or low-pasteurized milk and cream deviate from Newtonian behaviour because of the cold agglutination of the fat globules (Mulder and Walstra, 1974). The lower the temperature, the stronger are the attractive forces between the globules, and the higher the shear rate needed for Newtonian behaviour and the higher the apparent viscosity at lower shear rates.

Several workers have measured the viscosity of milk and have obtained as many values. Cox (1952) attributed most of the discrepancies to differences in experimental conditions and to the fact that milk from widely varying sources and with different

compositions might be expected to have different properties. Caffyn (1951) used homogenized milk in order to avoid complications from the effect of creaming. He established that the viscosity of milk was about twice (2 mPa s) that of water at 20 °C, but decreased rather more rapidly with rising temperature, falling to about 0.6 mPa s at 80 °C. There was a slight discontinuity in the viscosity-temperature relationship at about 40 °C and Caffyn suggested that the melting of fat was the cause, since the milk was stored at 0 °C before the test; the fat was probably solid and took appreciable time to melt even at temperatures above its melting point. The alternative cause might possibly have been a slight alteration in the milk proteins (denaturation at temperatures in excess of 40 °C) (Prentice, 1972). Caffyn (1951) also demonstrated that milk was not quite Newtonian in its behaviour, since the measured viscosity decreased slightly on repeated shearing at temperatures below 60 °C and rose slightly at temperatures above 60 °C, behaviour he ascribed to the changes in the protein structure of milk.

Walstra and Jenness (1984) have discussed Newtonian and non-Newtonian behaviour of liquid milk products. For Newtonian liquids the viscosity is well described by the Eilers' relation:

$$\eta = \eta_{ref} \left(1 + \frac{1.25 \sum (\phi_i)}{1 - \frac{\sum (\phi_i)}{\phi_{max}}} \right)^2 \tag{2.5}$$

which reduces to the well-known Einstein equation

$$\eta = \eta_{ref} \ (1 + 2.5 \ \phi_i \ ) \tag{2.6}$$

for dilute dispersions where $\sum (\phi_i) \rightarrow 0$. ϕ_i is the volume fraction of the dissolved particles. Thus for milk ϕ_i includes lactose molecules, protein and fat globules. The hydrodynamic volume includes the hydration water, "hairy" layers, and cavities in the particles, and for milk

$$\sum (\phi_i) = \phi_{fat} + \phi_{cas} + \phi_{wp} + \phi_{lac} \tag{2.7}$$

where ϕ_{fat} , ϕ_{cas} , ϕ_{wp} , and ϕ_{lac} are the volume fractions of fat globules, casein micelles, whey proteins and lactose.

$$\phi_i = C_{vi} \ V_i \tag{2.8}$$

where $C_{v\ i}$ is the volume concentration of the component in the product (kg/m^3 of product), and V_i is the voluminosity of the component i (m^3/kg of dry component). ϕ_{max} is the assumed value of $\sum (\phi_i)$ for maximum packing of all dispersed particles (0.9 for fluid milk products). However, Snoeren *et al.* (1982, 1983) used an experimentally determined value of 0.79 for skim and whole milk concentrates. η_{ref} is the viscosity of the liquid in which the particles are suspended (water and salts). For milk, η_{ref} is about 1.02 times the viscosity of water. Snoeren *et al.* (1982, 1983) in their studies have included lactose in η_{ref} rather than including it in $\sum (\phi_i)$ (van Vliet and Walstra, 1980; Walstra and Jenness, 1984).

Einstein's equation (Eq. 2.6) assumes no particle-particle interaction whereas Eilers' equation (Eq. 2.5) accounts both for the presence of the dispersed phase and for hydrodynamic interaction between particles during flow. The volume fraction of the casein (ϕ_{cas}) in the milk is the main determinant of $\sum (\phi_i)$, and thus of viscosity. Large differences in viscosity between different milks are almost certainly attributable to differences in ϕ_{cas}, which in turn depends on factors such as the concentration of colloidal calcium phosphate, calcium ion activity and pH (Walstra and Jenness, 1984).

Eilers' equation fairly well predicts the viscosity of skim milk, milk, cream, concentrated milk and milk ultrafiltrates up to $\phi = 0.6$. Beyond this value, the viscosity increases steeply with ϕ and flow deviates significantly from Newtonian behaviour. The deviation may result from interaction between particles and from their deformability (Walstra and Jenness, 1984). Shear thinning behaviour is observed. Apparent viscosities are always higher than the viscosities predicted by Eilers' equation. The main reason is the aggregation of particles; the aggregates contain interstitial liquid and have irregular shapes, and consequently the effective ϕ is increased. The aggregates are held together by interaction forces between the particles. The shearing action of the flow exerts a stress on the aggregates, which may disrupt them into smaller and more rounded ones. In this way effective ϕ, and thus apparent viscosity decreases with increasing shear rate. If the force acting on the aggregates is larger than the interaction forces, the latter are negligible, and the liquid shows Newtonian behaviour. Consequently, the higher the shear rate, the smaller is the effect of shear rate on viscosity (Walstra and Jenness, 1984).

Van Vliet and Walstra (1980) have discussed the relationship between the viscosity and fat content of milk and cream using the Eilers' equation (Eq.2.5). They observed that a comparison of the published data on the flow behaviour of milk and cream at higher shear rates revealed conflicting results which can be explained by differences in experimental conditions, cold agglutination and churning effects. In the absence of these effects and at not very low shear rates milk behaves as a Newtonian liquid. Excellent agreement was obtained between measured viscosity (η) as a function of fat content (ϕ_{fat}) and theoretical relations between η and the volume fraction of dispersed particles, but only if the volume occupied by the protein particles and lactose molecules in the milk is taken into account (Eq. 2.7).

Taking η_{ref} as the viscosity of skim milk, van Vliet and Walstra (1980) showed that the results of Phipps (1969) cannot be made to agree with Eilers' relation for any reasonable value of ϕ_{max}. ϕ_{fat} was calculated from the w/w fraction of fat as given by Phipps (1969). By taking $\sum (\phi_i) = \phi_{fat}$ one wrongly neglects the contribution to the hydrodynamic interaction of particles other than fat globules. Generally all particles or molecules with a radius of ≥ 0.5 nm contribute to the hydrodynamic interaction. Thus the protein particles (pro) and lactose molecules (lac) have to be included in the calculation of $\sum (\phi_i)$.

$$\sum (\phi_i) = \phi_{fat} + \phi_{pro} + \phi_{lac}$$

where $\phi_{pro} \sim 0.10 - 0.13$ and $\phi_{lac} \sim 0.045 - 0.050$. The continuous phase is then roughly a 1% solution of small molecules, mainly salts, with a viscosity 1.02 times that of water. The value of $\phi_{pro} + \phi_{lac}$ is ~ 0.16. So in Eilers' equation $\sum (\phi_i) = \phi_{fat} + 0.16$ should be used, and for η_{ref} the viscosity of the milk salt solution, in order to have the results of Phipps (1969) in excellent agreement with Eilers' equation taking $\phi_{max} = 0.88$ (Van Vliet and Walstra, 1980).

A number of workers (Fernandez-Martin, 1972 (low shear rates); Bakshi and Smith, 1984 (shear rate 0-1353 s^{-1}); Wayne and Shoemaker, 1988 (shear rate 121-485 s^{-1})) have found that unconcentrated whole milk behaves as a Newtonian liquid whereas Rohm *et al.* (1996) observed that raw milk followed non-Newtonian behaviour at low shear rates (1-1500 s^{-1}).

2.5.3.2 Rheology of Skim milk

Like whole milk, skim milk also behaves as a Newtonian liquid (Fernandez-Martin, 1972; Bakshi and Smith, 1984; Wayne and Shoemaker, 1988; Stepp and Smith, 1991; Horne, 1993). Skim milk has a viscosity approaching that of whole milk. At 20 °C the viscosity of whole milk is 2 mPa s, of skim milk is 1.6 mPa s, of rennet whey is 1.3 mPa s and of a 5% lactose solution is 1.2 mPa s. This indicates that the casein is the main contributor to the viscosity of milk (Glover, 1985). Any factors affecting the stability of casein such as acidity, salt balance and heat treatment will affect the viscosity (Jenness and Patton, 1959; Glover, 1985). The viscosity of skim milk decreases as the temperature rises.

2.5.3.3 Rheology of concentrated milks

The viscosity of a dispersion or solution increases in a non-linear fashion as the concentration increases. At higher concentrations, small additional changes in the concentration will lead to large changes in viscosity. In processing, this could result in reduced flow rates, high pressure drops, decreased turbulence, and in heating operations, severe fouling. In concentration processes such as evaporation, reverse osmosis and ultrafiltration, the extent of concentration attainable will be limited by viscosity considerations. There is often a transition from Newtonian to non-Newtonian behaviour as concentration proceeds (Lewis, 1993).

2.5.3.3.1 Concentrates prepared by heat evaporation

A number of workers have studied the viscosity of concentrated milks prepared by heat evaporation (Rao *et al.* 1964; Fernandez-Martin, 1972; Buckingham, 1978; Bloore and Boag, 1981; Horne, 1993). The viscosity of concentrated milks depends largely upon the composition, pretreatment, temperature, total solids content and holding time in the evaporator. The viscosity of milk concentrates restricts the maximum concentration that can be achieved without adversely affecting the properties of the resultant milk powder (Bloore and Boag, 1981). The viscosity of freshly concentrated milk has been found to decrease with increasing shear due to compaction of casein micelles (Rao *et al.*, 1964). Fernandez-Martin (1972) observed that concentrated milks were shear thinning and followed power law behaviour. Shear thinning effects increased with decreasing

temperature and increasing total solids. The values of the flow behaviour index (n) ranged from 0.9 and 1 (Fernandez-Martin, 1972). The viscosity of milk concentrates increases with total solids content according to a polynomial function which appears to be independent of the fat/SNF ratio (Fernandez-Martin, 1972). The protein to lactose ratio has a marked influence on the concentrate viscosity. When the protein/lactose ratio is high, the viscosity is high (Wood, 1996). Bloore and Boag (1981) reported that the viscosity of concentrated skim milk increases linearly with increasing total solids (from 43.9% to 51.6%) and protein content. Horne (1993) observed increasing shear thinning behaviour in skim milk concentrates as the concentration increased. Buckingham (1978) compared the kinematic viscosity of freshly prepared New Zealand skim milk concentrates to reconstituted New Zealand skim milk powders and found that below 40% (w/v) total solids, the behaviour of the two was similar, whereas above 40% (w/v) total solids, age thickening and seasonal variations in the viscosity of freshly prepared concentrates became significant.

Snoeren *et al.* (1982, 1983, 1984) used the hydrodynamical approach similar to van Vliet and Walstra (1980) and Walstra and Jenness (1984) to study the viscosity of whole milk concentrates obtained by heat evaporation. As mentioned earlier (Walstra and Jenness, 1984), the viscosity of skim milk concentrate depends on the volume fractions of components of the milk and on the viscosity of the milk serum. The relationship between viscosity and volume fraction is given by Eilers' equation (Eq.2.5). The volume fraction occupied by the protein depends on the protein content, protein composition, heat treatment of the milk, and on the degree of concentration. During storage the viscosity of skim milk concentrate increases regardless of the heat treatment of the milk. This phenomenon is called age-thickening. A so-called structural viscosity is formed, which can be disrupted by agitation (Snoeren *et al.*, 1982). In a later study Snoeren *et al.* (1983) found that the viscosity of whole milk concentrate depends on the volume fractions of the protein material and of the fat present in milk, and the viscosity of the continuous medium. The interdependence of these factors is given by Eilers' equation. Viscosity and age-thickening characteristics of the concentrate were given in a viscosity-time-shear diagram from which the apparent viscosity at a given rate of shear could be inferred, as well as the increase of the viscosity with time. Age thickening in highly concentrated systems may be due to the loosening of casein micelles. As a consequence of

concentration, the pH drops and the ionic strength increases, both of which favour the solubility of β-casein, which results in an increased voluminosity of the casein (Snoeren *et al.* 1984).

2.5.3.3.2 Concentrates prepared by membrane processes

With recent advances in membrane processing in the dairy industry, a number of workers have studied the viscosity of milks concentrated by membrane processes (Culioli *et al.*, 1974; Randhahn, 1976; Setti and Peri, 1976; Jelen, 1979; de Boer and Nooy, 1980; Kristensen *et al.*, 1981; Kocak, 1985; Versteeg, 1985; Hallstorm and Dejmek, 1988a, b; Rao and Renner, 1988; Stepp and Smith, 1991; Sierzant and Smith, 1993; Gupta and Pal, 1993). In membrane processes, viscosity affects the mass transfer coefficient through its effect on diffusivity and on the fluid flow properties of the system (Jelen, 1979). An essential factor affecting membrane processes is the change in flow properties with increasing total solids content, since these properties determine the flow conditions in ultrafiltration plants (Randhahn, 1976). Like heat evaporation concentrates, the viscosity of concentrates prepared by membrane processes increases significantly with concentration due to the predominant effect of the proteins, especially the casein.

Culioli *et al.* (1974) found non-Newtonian behaviour in UF concentrated milks; the viscosity reaches a low and constant value at shear rates above 300 s^{-1}. With flow velocities over membranes usually in the region of 2 m s^{-1} shear rates will be above this value in channel assemblies, though not always in wide tubes. This aspect of milk rheology (non-Newtonian behaviour in channel assemblies) is of little consequence, particularly at temperatures of around 50 °C at which most membrane processing is carried out, but the realization of this behaviour enabled De Danske Sukkerfabrikker (DDS) to improve the design of channels in their flat plate UF system (Kristensen *et al.*, 1981). More serious is the increase in the viscosity as the concentration factor increases.

Randhahn (1976) studied the flow properties of skim milk concentrates obtained by ultrafiltration (UF) up to a solids content of 27% at temperatures ranging from 5 to 60 °C. The skim milk was concentrated at 20 °C. The time-independent pseudoplastic properties were well described by the power law. He demonstrated a clear dependence of both the rheological parameters (k and n) on solids content and on temperature. The value

of k increased rapidly at a temperature of 20 °C as the solids content exceeded 20%. At lower temperatures such an increase occured at lower solids contents. Between the parameters k and n, there existed a relationship which itself was independent of temperature (Randhahn, 1976).

de Boer and Nooy (1980) showed that ultrafiltered skim milk concentrates behaved as non-Newtonian liquids as the protein concentration increased. Setti and Peri (1976) found that the viscosity of UF skim milk concentrates increased with increasing protein content and decreasing temperature. Hallstorm and Dejmek (1988a) reported that the non-Newtonian behaviour of skim milk concentrates obtained by ultrafiltration could be completely ascribed to the protein concentration and the hydration of caseins.

Stepp and Smith (1991) studied the effects of concentration and temperature on the viscosity of skim milk retentates obtained by UF. Determinations were made at 20, 30, 40 and 50 °C and over a protein range of 3-19.21%. The power law equation was used to determine values of n and k for the samples. The n values decreased and k values increased with increasing protein concentration. Skim milk retentates became more pseudoplastic with increasing concentration.

Sierzant and Smith (1993) studied the flow properties of whole milk retentates obtained by UF as affected by temperature. Whole milk was ultrafiltered to various volume concentration ratios of 2X, 3X, and 4X. Viscosity measurements at three temperatures (5, 50, 75 °C), followed by regression analysis of selected points from the flow curves, gave n and k values. These values indicated the samples became more shear-thinning with decreasing temperature and increasing concentration.

It is interesting to note that milks concentrated by UF are more viscous than those concentrated to the same total solids by evaporation since the latter have lower protein and fat contents (Glover, 1985); lactose is lost in the permeate during UF concentration.

The concentration of milk by reverse osmosis (RO) is basically a concentration process where mainly water molecules are removed, whereas in the concentration of milk by UF, lactose and minerals along with water molecules are removed. So far little work has been published on the rheological properties of RO concentrates and there is thus a need to identify if and how RO concentrates differ from UF concentrates.

Versteeg (1985) found that the viscosity of whole milk (measured by rotational viscometery) and its reverse osmosis concentrates decreased with increasing temperature in the range 5-30 °C.

Gupta and Pal (1993) studied viscosity changes in buffalo milk concentrated by reverse osmosis to 1.5X and 2.0X using a falling ball viscometer. They observed that there was no appreciable increase in the viscosity of the concentrates when stored at 5 °C after 24 and 48 h. The samples during storage were however occasionally shaken to simulate conditions during transportation, which may have been responsible for breaking any structure that had developed over the storage period.

2.5.4 Further discussion of factors affecting the rheology of milk

Rheology of dairy fluids is affected by a number of factors. The most important factors include temperature, thermal history, composition, concentrations of fat and protein, shear rate, processing treatments (separation, homogenization and heating) and storage.

2.5.4.1 Effect of temperature

The viscosity of a fluid is a function of the inter-molecular forces that restrict molecular motion. These forces depend upon the inter-molecular spacings, which determine the free volume and which are affected by changes in both temperature and pressure (Holdsworth, 1971). Since the temperature and the macroscopic components of the liquid system have a very marked effect on the rheology of dairy products, it is necessary to measure rheological properties under constant temperature conditions and also over a range of temperatures which are likely to be encountered in practice (Holdsworth, 1971). The relationship between temperature and viscosity of pure fluids is well described by the Arrhenius equation:

$$\eta = A\, e^{\Delta E / RT} \qquad (2.9)$$

where A is a constant and ΔE is the activation energy for flow obtained from the slope of a logarithmic plot of $\ln \eta$ *versus* $1/T$.

$$\ln \eta = \ln A + (\Delta E / R)\,(1/T) \qquad (2.10)$$

In general, the higher the activation energy the greater is the effect of temperature on viscosity. Tang *et al.* (1993) found values of E in the range 16.9-26.0 kJ/mol and values

of the factor A in the range 0.69-2.71 μPas^n for whey protein concentrate solutions containing 2-30% total solids. Eq. 2.10 can be rewritten to include the effect of concentration (c) on apparent viscosity as

$$\eta_{app} = A\, c^x\, e^{\Delta E / RT} \tag{2.11}$$

where c = total solids %, x and A are constants.

In general, the Arrhenius equation is applicable to simple liquids and for dispersions of long chain molecules and polymeric substances. It is considered that long chain molecules do not flow as a whole but in segments and clusters, and that the viscosity depends on the chain length. Eyring (1936) proposed a general theory with regard to non-Newtonian shear thinning flow and the effect of temperature on the flow properties as:

$$\eta = A\, f\, e^{(a-bf)/T} \tag{2.12}$$

where A, a and b are functions of inter-molecular distances, partition and other thermodynamic properties of the system, and f is the shearing force. From this equation it can be seen that the viscosity decreases with increasing shear force since (a - bf) is an exponential term. When a liquid is heated at constant pressure, two basic factors combine to reduce its viscosity. The first is that the thermal energy of the molecules increases and the second is that the intermolecular distances increase, i.e. thermal expansion occurs. For many fluids the second factor predominates.

Fernandez-Martin (1972) showed that the apparent viscosity of skim milks (fat = 0.11%), half and half milks (fat = 1.56%) and whole milks (fat = 3.05%) decreased with increasing temperature from 0 to 80 °C. The effect of temperature on the viscosity could be described by the relation:

$$\log \eta = a_0 + a_1 \theta + a_2 \theta^2 \tag{2.13}$$

where a_0, a_1 and a_2 are constants and θ is temperature in °C.

For creams containing up to 50% fat, Phipps (1969) reported that viscosity decreased linearly with increase in temperature from 40 to 80 °C, and all the creams showed Newtonian behaviour (since the fat was in the molten state in the temperature range studied). A regression equation relating the fat content, and the viscosity of the continuous medium, to the viscosity of cream was developed. The slope of the line was linearly related to temperature and the intercept (the viscosity of the continuous medium) was inversely related to the temperature.

Temperature has a definite effect on the values of n and k of milk concentrates. Randhahn (1976) observed an increase in n from 0.72 to 0.925 and a decrease in k from 353.1 to 7.64 (mPa s^n) as temperature increased from 4.5 to 58.3 °C for skim milk concentrates containing 23.66% total solids.

Buckingham (1978) observed a decrease in the kinematic viscosity of the reconstituted skim milk at various solids concentrations as the temperature increased from 15 to 70 °C. Bloore and Boag (1981) found that the viscosity of skim milk concentrates fell to a minimum at 60 °C and then increased as the temperature was raised from 60 to 80 °C. These results are similar to those found by de Boer and Nooy (1980) and Snoeren *et al.* (1982) for skim milk concentrates. The decrease in the viscosity up to 60 °C is mainly due to the decrease in the viscosity of serum and the increase in viscosity beyond 70 °C is due to the denaturation of whey proteins.

Bertsch and Cerf (1983) found that the viscosity of a variety of milk products containing 0.03 to 15.5% fat and 9.03 to 22.83% T.S, respectively, decreased as the temperature was increased from 70 to 135 °C. These results are in contrast to those found by Buckingham (1978) and Snoeren *et al.* (1982). These differences may be attributed to the lower temperatures ranges used by Buckingham (1978) (15-70 °C) and Snoeren *et al.* (1982) (50-70 °C) and also the fact that the availability of the whey proteins for interaction with caseins is greater in concentrated systems, resulting in more whey protein-casein interactions than in unconcentrated systems.

Bakshi and Smith (1984) observed that the effect of fat content on the viscosity is much greater at low temperatures. They reported the viscosity of whole milk at 10 °C as 2.5 mPa s, and this decreased to 1.75 mPa s at 20 °C. At 30 °C, the viscosity of skim milk, 1% fat milk, 2% fat milk and whole milk were the same, i.e. 1.25 mPa s. The viscosities of skim milk, 1 and 2% fat milks, whole milk, half and half (whole milk: whipping cream 50:50) and whipping cream (30% fat) were measured in a temperature range 0-30 °C; the viscosity varied exponentially with temperature and linearly with the fat content of the milk. A relationship between viscosity (η), fat content (f) and temperature (θ) of the form:

$$\ln \eta = -8.9 + 0.1f + 2721.5 / \theta \qquad (2.14)$$

was developed, which was used to calculate the pumping requirements of milk with various fat contents at various temperatures.

Versteeg (1985) found that the viscosity of whole milk and its reverse osmosis concentrates decreased with increasing temperature in the range 5-30 °C. Rao and Renner (1988) studied the effect of heating, at 65-80 °C for 5 min, of whole milk UF concentrates (35% total solids) on the viscosity (measured at 19 °C) and heat denaturation of whey proteins, and found that viscosity and denaturation increased linearly with increase in temperature from 65-80 °C; a significant change in whey protein denaturation and hence viscosity was observed in the temperature range of 75-80 °C.

Stepp and Smith (1991) and Sierzant and Smith (1993) used the power law equation to characterize the flow behaviour of ultrafiltered skim milk concentrates in the temperature range 20-50 °C and of whole milk concentrates in the temperature range of 5-75 °C, respectively. Stepp and Smith (1991) found a decrease in the viscosity of skim milk concentrates, corresponding to a decrease in k values and increase in n values, as the temperature increased from 20 to 50 °C, whereas Sierzant and Smith (1993) reported that at a low temperature (5 °C), the samples exhibited increasing shear thinning (decreasing n values) with increase in concentration. The increasing concentration of protein in samples and temperatures near denaturation temperatures of some whey proteins could explain the shear thinning behaviour (Sierzant and Smith 1993). The samples with 34-36% total solids exhibited shear thinning behaviour at 5 °C, approached Newtonian behaviour at 50 °C but then again showed shear thinning behaviour at 75 °C (Sierzant and Smith 1993). These results are similar to those found by Bloore and Boag (1981) and Snoeren *et al.* (1982) as described earlier.

*2.5.4.2 **Effect of thermal history***

Heat treatment of milk results in temporary changes in milk structure, and therefore also in viscosity. Thus the viscosity of milk after heat treatment is not only a function of temperature but also depends on the thermal history of the sample (Janal and Blahovec, 1974). Marked thermal hysteresis of viscosity has been observed by Janal and Blahovec (1974). They demonstrated different patterns of thermal hysteresis. In one pattern the viscosity values during cooling from 85 to 15 °C were lower than the viscosity values during prior heating from 15 to 85 °C. This was probably caused by temporary reversible processes of melting and crystallization of fats. In other patterns the viscosity values

during cooling were higher than the viscosity values during heating. They related this to the increased rate of coagulation and denaturation of proteins at temperatures above 40 °C.

2.5.4.3 Effect of concentration and composition

This section deals with the effect of changing the concentration of milk. Milk shows a Newtonian behaviour up to 10% total solids. As the total solids concentration increases, the behaviour shifts towards non-Newtonian, resulting in pseudoplastic behaviour. The values of n and k vary with the concentration of total solids. As concentration increases the value of n decreases and the value of k increases, at a constant temperature and shear rate.

At a given temperature, milk viscosity increases either with increasing fat content when solids-not-fat (SNF) content is kept constant, or with increasing SNF content for constant fat percentage; there is general agreement that milk viscosity is a non-linear function of total solids content (Fernandez-Martin, 1972). Bloore and Boag (1981) reported that the viscosity of skim milk concentrates rose exponentially with increasing total solids content. They also observed a marked increase in concentrate viscosity towards the end of each New Zealand dairying season (March) which largely is attributable to the increased protein content of the milk (which in turn is due to combined effects of the lactational cycle of cows and of late summer weather on pasture growth).

When milk is concentrated by removal of water, $\sum (\phi_i)$ increases because of the concentration effect *per se* and because particle-particle (especially micelle-micelle) interactions increase owing to smaller interparticle distances. Increased interaction results in increased aggregate formation, the effects of which are essentially the same as those of concentrating only fat globules, as in cream, i.e. apparent viscosity increases, shear thinning becomes more pronounced and deviation from Newtonian behaviour persists to higher shear rates (Walstra and Jenness, 1984).

The viscosity of liquid milk products increases with increase in fat content. Structurally, cream differs from milk only in that it contains a much higher concentration of fat globules and from concentrated milk in that its serum is not concentrated. It has essentially the same composition as the original milk, except for a higher fat content and a very small quantity of serum lost during the separation process (Prentice, 1972).

Milk and cream show non-Newtonian behaviour under the conditions that favour cold agglutination of fat globules (temperature < 40 °C and low shear rate) (Randhahn, 1974; Randhahn and Reuter, 1978, Walstra and Jenness, 1984). At low shear rates, the aggregates of fat globules present in milk and cream contain trapped interstitial plasma, and this results in a higher effective volume fraction. At high shear rates, the shearing forces cause the aggregates to become more regular in shape or break down, and the volume fraction, and thus the apparent viscosity (defined as $(\eta_{app})_{\dot{\gamma}} = \tau / \dot{\gamma}$), then decrease. Successive increases in shear rate has smaller and smaller effects on viscosity as shearing forces become large compared with the attractive forces holding fat globules together.

At high fat contents, non-Newtonian behaviour is more pronounced and persists to higher shear rates. For example, a 40% fat cream at a temperature > 40 °C might be Newtonian at $> 10\ s^{-1}$, while a 49% fat cream might be Newtonian only at $> 100\ s^{-1}$.

Lower temperatures enhance cold agglutination, resulting in stronger fat globule aggregates; this increases both apparent viscosity and deviation from Newtonian behaviour, the latter persisting to higher shear rates (Walstra and Jenness, 1984). Separation of milk at temperatures ≥ 40 °C gives cream in which cold agglutination is greatly reduced owing to loss of agglutinin in the skim milk. Conversely, cold separation enhances cold agglutination (Mulder and Walstra, 1974). Thus, separation conditions influence the rheology of cream.

In a comprehensive study, Phipps (1969) determined the dynamic viscosity coefficients of cream. The fat content and temperature ranges were 0-50% and 40-80 °C, respectively. Over the shear rates 7-100 s^{-1}, all cream samples at all temperatures exhibited Newtonian behaviour (measurement temperature 40 °C). This contrasts with the results of Prentice (1967), who showed non-Newtonian behaviour in cream of about 50% fat at measurement temperature 20 °C. This probably reflects the effect of solidification of fat at lower temperatures.

Randhahn and Reuter (1978) have reported that raw cream of up to 40% fat shows pseudoplastic behaviour which appears to be due to the temperature dependent orientation of the whey protein euglobulin (immunoglobulin) and the formation of fat globule clusters particularly at low temperatures. Fat contents of > 40% further affect the flow properties

of cream, since the fat globules tend to become orientated in the flow direction. They suggested the Cross equation to describe the exact pseudoplastic behaviour of raw cream.

Snoeren *et al.* (1982, 1984) carried out detailed investigations of the rheological properties of skim milk concentrates. They found that skim milk concentrates of TS ≥ 43% were shear thinning; the apparent viscosity, at a specified shear rate, of concentrates was poorly related to T.S., but closely related to the total volume fraction of protein, ϕ_{pro}, calculated as:

$$\phi_{pro} = \phi_{cas} + \phi_{nwp} + \phi_{dwp} \qquad (2.15)$$

where 'nwp' is native whey proteins and 'dwp' is denatured whey proteins.

Snoeren *et al.* (1984) proposed the following explanation of age thickening. Concentration of milk causes an increase in ionic strength and a reduction of pH. These changes reduce the amount of calcium bound to protein, especially to β-casein. This in turn leads to increased solubility of β-casein, the consequence of which is a loosening of the casein micelle structure, increasing ϕ_{cas} and thus ϕ_{pro}. Snoeren *et al.* (1984) pointed out that only a very small increase in casein voluminosity is required to cause a substantial increase in viscosity.

Kristensen *et al.* (1981), Bakshi and Smith (1984), Snoeren *et al.* (1983), Sierzant and Smith (1993) and Rohm *et al.* (1996) observed that the viscosity of whole milk and its concentrates increases linearly with increase in fat content at a constant temperature.

*2.5.4.4 **Effect of shear rate***

In general, the viscosity of milk decreases with increasing shear rate indicating pseudoplastic behaviour, only if temperature and shear rate are low. As stated earlier in section 2.3.3.1, the shearing action of flow exerts a stress on the aggregates of particles which are held together by interaction forces in concentrated milks resulting in disruption into smaller aggregates. Thus apparent viscosity decreases with increase in shear rate. If the shear force acting on the aggregates is larger than the interaction forces, the latter are negligible, resulting in Newtonian behaviour. Consequently, the higher the shear rate, the smaller is the effect of shear rate on viscosity (Walstra and Jenness, 1984).

Rohm *et al.* (1996) observed non-Newtonian behaviour in raw milk (which was due to the cold agglutination of fat globules) in the temperature range 10 to 40 °C and the

shear rate range 1-1500 s^{-1}. The viscosity diminished heavily with increasing shear rate, at low shear rates (< 250 s^{-1}) but remained stable at higher shear rates (> 800 s^{-1}). de Boer and Nooy (1980) observed that heat evaporated skim milk concentrates (28 to 31% protein) had high viscosity at shear rates < 500 s^{-1} whereas there was a small decrease in the viscosity above this shear rate at measurement temperature of 55 °C. For heat evaporated skim milk concentrates, Horne (1993) measured the viscosity in the shear rate range 1.33-291 s^{-1} and found that all flow curves showed a decreasing viscosity with increasing shear rate. All curves tended to a limiting shear stress value at low shear rates and at high shear rates the stress rose linearly with the rate of shear.

Creams generally show shear thinning behaviour, but at high shear rates may show shear thickening due to the churning of fat. Because of this fact, creams are rheologically characterised at low shear rates to avoid the churning of fat (Phipps, 1969; Prentice and Chapman, 1969; Mulder and Walstra, 1974; Langley, 1984). Phipps (1969) showed that all cream samples at temperatures between 40 to 80 °C (where the fat is in molten state) showed Newtonian behaviour in the shear rate range of 7-100 s^{-1}. Homogenized cream shows shear thinning behaviour at low temperatures as shear rate increases. For a cream of 17% fat and a viscosity of 10 mPa s, homogenized at 40 °C and 210 atm., the viscosity measured at room temperatue was found to be 640 mPa s at low shear rate of 25 s^{-1} which decreased to 120 mPa s at higher shear rate of 200 s^{-1} (Mulder and Walstra, 1974). Langley (1984) measured viscosity in the shear rate range of 0.5 - 15 s^{-1} for creams containing 20, 40 and 50% fat. They all showed shear thinning. Higher fat creams were more susceptible to churning at high shear rates.

2.5.4.5 Effect of processing operations

2.5.4.5.1 Separation of milk

Creams separated at lower temperatures (< 35 °C) have higher viscosities than creams separated at higher temperatures (> 45 °C). This is attributed to the fact that at lower temperatures of separation, the agglutinin is still attached to the fat globules and results in a higher cream viscosity because of a greater extent of agglutination (Mulder and Walstra, 1974; Scurlock, 1986). Prentice and Chapman (1969) found no significant difference in

the viscosities of fresh cream separated at temperatures of 35 °C and 45 °C, which suggests that it is extremes in temperature that are important.

2.5.4.5.2 Homogenization of milk

Homogenization increases the viscosity of whole milk (Trout, 1950; Jenness and Patton, 1959; Bertsch and Cerf, 1983). As the homogenization pressure increases, the viscosity increases in a linear manner (Whitnah *et al.*, 1956b). The increase in viscosity caused by homogenization is attributed to the increased fat surface area, the increased amounts of protein bound to the fat globule surface and to changes in fat globule clustering. The level of fat in the homogenized milk also has an influence on the viscosity. Homogenizing milks of relatively high fat content (> 5-6%) results in very high viscosities.

Prentice and Chapman (1969) reported that homogenization had the greatest influence on the properties of cream; the average viscosity of homogenized samples was almost 15 times that of non-homogenized samples. Homogenized cream has much higher viscosity owing to clusters of fat globules immobilizing part of the plasma and thereby considerably increasing the effective fat volume fraction (Mulder and Walstra, 1974).

2.5.4.5.3 Heat treatment of milk

Heating of milk has a marked effect on its viscosity because of denaturation of whey proteins at temperatures > 60 °C. The denatured whey proteins associate with the casein micelles resulting in an increased micelle size and a change in micelle-micelle interaction, which increase the viscosity (Walstra and Jenness, 1984). Langley and Temple (1985) reported an exponential increase in the viscosity of skim milk as the temperature of heating increased from 80 to 140 °C for a heating time of 15 min. An Arrhenius plot of ln k against 1/T showed two distinct regions, one above and one below a value of 1/T of 0.00255 (120 °C). They suggested that this could be due to structural changes in β-lactoglobulin alone or in association with other milk proteins. They observed a large change in the voluminosity of casein as a result of whey protein deposition and hence viscosity between 120 and 140 °C which they explained on the basis of the unfolding of β-lactoglobulin that takes place at around 130 °C.

Jeurnink and Kruif (1993) studied the effect of heating on the viscosity of skim milk. Skim milk was heated at 85 °C for different holding times. As a result of such heating, whey proteins, in particular β-lactoglobulin, denatured and associated with casein micelles resulting in an increase of the volume of the casein micelles but also a different interaction between them. A quantitative model for the viscosity of dispersions of so-called hard spheres was developed and used to describe the changes caused by heat treatment of skim milk. It was observed that after heating, the casein micelles became larger and acquired a mutual attraction. The unfolding of the whey proteins and their subsequent association with the casein micelles appeared to be responsible for these changes, resulting in the increase in viscosity.

Hydration of milk proteins can be influenced by preheating the milk. Snoeren *et al.* (1982) observed an increase in the viscosity of skim milk concentrates in proportion to the intensity of preheat treatment (95 °C for 5 min > 85 °C for 1 min > 70 °C for 10 s) which was due to the increased protein voluminosity as a result of whey protein denaturation. Bloore and Boag (1981) reported that a high temperature-short time preheat treatment (113 °C for 10 s) of concentrated skim milk gave a lower viscosity than a low temperature long time preheat treatment (80 °C for 120 s) although both these treatments gave a similar whey protein nitrogen index.

Prentice and Chapman (1969) studied the effect of heat treatment on cream at different temperatures. Creams heat treated at 66 °C for 15 s after separation were almost twice as viscous as those heated to 74 °C for 15 s. Any heating results in a reduced viscosity of the cream when it emerges from the plant, but this may to some extent be overshadowed by a subsequent thickening during storage.

2.5.4.6 Effect of storage

The viscosity of milk increases upon storage. Whitnah *et al.* (1956a) reported an increase in the viscosity, measured at temperatures in the range 4-44 °C, of both non-homogenized and homogenized pasteurized milk during storage at 4 °C; the increase was found to be approximately linear with storage time. Kocak (1985) studied the changes in apparent viscosity of UHT sterilized RO whole milk concentrates (26% total solids) during subsequent storage at 2, 10, 20, 30 and 40 °C and observed that the apparent viscosity of

the samples stored at 2 or 10 °C increased linearly reaching 33 and > 50 mPa s after 102 days storage respectively. In contrast, the samples stored at higher temperatures displayed viscosity changes that were more characteristic of age gelation (small changes in viscosity followed by a sudden rise). Similar changes were observed by Harwalkar and Vreeman (1978a) in UHT sterilized evaporated skim milk (23% total solids) stored at 28 °C. Langley (1984) studied the changes in the viscosity of processed cream (20, 40 and 50% fat) during storage at 5 °C and observed that the viscosity of the heat-treated creams increased with time. The rate of increase was dependent on the homogenization pressure, fat content and heating temperatures. Viscosity increased with increase in fat content and with increase in homogenization pressure, and decreased with increase in heat treatment temperature. Upon storage all the creams became more viscous (Langley, 1984).

Phipps (1982) found that the apparent relative viscosity of stored homogenized UHT "coffee" cream increased with time at a rate which was a function of mean fat globule diameter and fat volume fraction; the rate was also influenced by the addition of stabilizers (sodium carbonate, 0.1% w/v and sodium citrate, 0.1% w/v) to cream before processing. Viscosity increases were attributed to the progressive flocculation of fat globules and to the strengthening of the structure formed by gradual accumulation of casein micelles on to globule surfaces and their points of contacts (bridging).

Snoeren *et al.* (1982, 1983, 1984) observed that during storage, the viscosity of skim and whole milk concentrates increased regardless of the heat treatment of the milk. Structural viscosity developed during storage which could be disrupted by agitation.

Gupta and Pal (1993) studied viscosity changes in buffalo milk concentrated by reverse osmosis to 1.5X and 2.0X. They observed that there was no appreciable increase in the viscosity of the concentrates when stored at 5 °C after 24 and 48 h which is in contrast to the results from other studies (Whitnah *et al.*, 1956a; Kocak, 1985; Harwalkar and Vreeman, 1978a; Snoeren *et al.*, 1982, 1983, 1984). The samples during storage were however occasionally shaken to simulate conditions during transportation, which may have been responsible for breaking any structure that had developed over the storage period.

2.5.5 Summary

2.5.5.1 Newtonian behaviour in milk systems

Fresh milk systems (skim milk, whole milk and cream) are Newtonian liquids under the following conditions: fat content < 40% w/w, temperature > 40 °C (milk fat present in molten state, no cold agglutination of fat globules and moderate shear rates (Mulder and Walstra, 1974). Rheological behaviour of such systems is defined by Newton's law of viscosity (Eq. 2.2), which determines the coefficient of viscosity.

At temperatures < 40 °C, whole milk shows behaviour close to Newtonian if cold agglutination is absent. Rheology of milk and cream depends on the state and concentration of fat and casein and also on the factors which affect these. In milk, lactose and whey proteins influence the viscosity to a relatively small extent. Fat content has a relatively large influence, and the casein content has by far the greatest influence.

The viscosity of whole milk, skim milk, cream and some milk concentrates (if Newtonian) can be predicted by Eilers' equation, which relates the viscosity of a system to the viscosity of the continuous medium and the volume fraction of the dispersed components. Volume fraction pertains to hydrodynamic volume, and accounts for the particle shape and water of hydration as well as volume *per se*. Concentration increases the volume fraction of the dispersed components and hence results in an increase in viscosity. The volume fraction of fat and casein mainly account for the total volume fraction of a milk system. The volume fraction depends on the voluminosity and volume concentration of the dispersed components. Differences in ϕ_{cas} , which depends on the concentration of CCP, Ca^{2+} activity and pH, between milks results in large differences in their viscosity values.

The main factors affecting Newtonian behaviour of milk and cream are composition, concentration, temperature, thermal history and processing conditions. The effects of composition and concentration of milks and creams can be predicted by Eilers' equation. Viscosity increases with increase in total solids, but for given total solids, is inversely related to % fat content, because of the lower voluminosity of fat compared to casein in particular. When ϕ_{max} for a milk system exceeds 0.6, viscosity increases steeply with ϕ, and the rheological behaviour changes to non-Newtonian.

Viscosity is inversely related to the temperature *per se*. Cooling of milk results in an increase in viscosity because at lower temperatures the viscosity of the continuous medium increases and also the voluminosity of casein increases and thus ϕ_{cas} . Also β-casein dissociates from the micelles at low temperatures. This increases the viscosity because dissociated protein molecules have higher hydrodynamic volumes. Thus the rheological behaviour changes to non-Newtonian.

Warming milk to temperatures above ambient causes the viscosity to decrease, because the viscosity of continuous medium decreases and because ϕ_{cas} decreases moderately. The viscosity of whole milk depends on both temperature and temperature history. Janal and Blahovec (1974) found that the hysteretic pattern for fresh milk usually showed higher viscosities during heating than during cooling. This effect may be due to the reversible melting and crystallization behaviour of the milk glycerides. Mild heat treatment like pasteurization results in no noticeable changes in the rheological properties whereas heating to 85 °C causes an increase in the casein micelle size, and also mutual attraction between the casein micelles. These changes are attributed to the association of whey proteins with casein micelles, and result in viscosity increases.

2.5.5.2 Non-Newtonian behaviour in milk systems

Raw milk and cream show non-Newtonian behaviour under conditions that favour cold agglutination of fat globules (temperature < 40 °C and low shear rate) (Randhahn, 1974; Walstra and Jenness, 1984). Shear thinning is the predominant rheological behaviour. At low shear rates, the aggregates of fat globules contain trapped interstitial plasma, and thus have high effective volume fraction. On increasing shear rate, the shear forces cause aggregates to become regular in shape or break down, resulting in a decrease in the apparent viscosity. At sufficiently high shear rates, behaviour becomes Newtonian.

In milk systems with high fat contents, the non-Newtonian behaviour is more pronounced and persists to higher shear rates, e.g. 40% fat cream at temperature > 40 °C might be Newtonian at $\dot{\gamma} > 10\ s^{-1}$, while a 49% fat cream might be Newtonian only at $\dot{\gamma} > 100\ s^{-1}$. Low temperatures enhance cold agglutination, resulting in stronger fat

globule aggregates. This increases the apparent viscosity and also deviation from Newtonian behaviour, the latter persisting to higher shear rates.

Homogenization of milk and cream results in an increase in the viscosity at high shear rates. The increase is inversely related to fat globule size. Raw cream containing cold agglutinated fat globules, and homogenized cream containing homogenization clusters can exhibit time dependent shear thinning (as distinct from time-independent pseudo-plasticity). High fat cream shows shear thickening. Shearing induces partial coalescence of fat globules, thus increasing effective ϕ_{fat} by entrapment of plasma. Partial coalescence appears to explain the rebodying of cream resulting from temperature fluctuations. Total coalescence causes little change in viscosity because ϕ_{fat} remains almost constant.

On concentration of milk the volume fractions of the dispersed components increase . Also there is increased micelle-micelle interaction as the distance between the micelles is smaller. In concentrated milks, the apparent viscosity increases as shear thinning becomes more pronounced, and deviation from Newtonian behaviour persists even at high shear rates (Walstra and Jenness, 1984).

The volume fraction of protein depends on the volume fraction of casein and whey proteins. Heating of milk or concentrated milk results in whey protein denaturation and therefore results in an increase in the volume fraction of the whey proteins; the voluminosity of denatured whey proteins is three times higher than that of native state. Also the hydrodynamic volume of the casein micelles increases, as a result of precipitation of calcium phosphate. These changes in the state of the proteins cause the viscosity of the system to increase.

Concentration of milk causes an increase in ionic strength and a reduction in pH. The amount of calcium bound to protein, especially β-casein is reduced. β-casein as a result becomes soluble which causes loosening of casein micelle structure, thus increasing the volume fraction of the casein. A small increase in the volume fraction of casein to causes substantial increase in viscosity of concentrated milks.

2.6 The effects of homogenization and heat treatment on milkfat globules

2.6.1 The nature of fat globules in normal milk

Bovine milk contains approximately 3-5% fat which is in the form of globules, ranging from 0.1 to 10 μm diameter, with the bulk of the fat globules having diameters between 2 and 5 μm (McPherson and Kitchen, 1983). The fat globules in the milk are generally considered to be truly spherical and are surrounded by a thin membrane called the milk fat globule membrane (MFGM). The MFGM (approximately 10 nm in thickness) is derived mainly from the apical plasma membrane of the mammary secretory cells at the time of milk fat secretion, and contains proteins, glycoproteins, enzymes, phospholipids, triglycerides, cholesterol, glycolipids and other minor components (McPherson and Kitchen, 1983). The protein in the MFGM represents about 1% of the total milk protein, and helps to maintain the integrity of the membrane. MFGM lipid comprises 1-2% of the total lipids (McPherson and Kitchen, 1983). The MFGM in normal milk is mainly composed of proteins (about 48%) and phospholipids (about 33%), cerebrosides (about 4%), cholesterol (2%), gangliosides (1%), water (about 11%) and enzymes and minerals (about 1%) (Walstra and Jenness, 1984).

The MFGM acts as a natural emulsifying agent enabling the fat to remain dispersed throughout the aqueous phase of milk (McPherson and Kitchen, 1983). The MFGM prevents the fat globules from flocculating and coalescing, and protects the fat against enzyme action. The MFGM is also closely involved with natural processes in milk (creaming and agglutination) and is affected by various processing treatments such as cooling, heating and homogenization of milk products. The responses of the various MFGM components to these processes determines the stability and acceptability of the final products (Mulder and Walstra, 1974).

2.6.2 The effects of heat on fat globules in normal milk

Membrane proteins may denature on heating milk above 70 °C, resulting in exposure of reactive thiol groups. Therefore, thiol-disulphide interchange reactions may occur between membrane proteins, and whey proteins may also participate in these reactions. Dalgleish and Banks (1991) and Houlihan *et al.* (1992a) found that whey proteins,

especially β-lactoglobulin, became associated with the membranes of natural milkfat globules at temperatures > 70 °C. Houlihan *et al.* (1992a) found that κ-casein also became incorporated into the membrane with increase in heating time from 2.5 to 20 min at 80 °C. However, no κ-casein and only a small amount of β-casein were detected by Dalgleish and Banks (1991) using temperatures between 65 and 90 °C and holding time 0-20 min; the presence of β-casein was not caused by heating. It was concluded that whey proteins alone, rather than whey protein/κ-casein complexes, bind to the fat globules during heating (Dalgleish and Banks, 1991). Dalgleish and Banks (1991) suggested that a layer of whey proteins, especially β-lactoglobulin, binds to the membrane proteins on heating through intermolecular disulfide bonds. Houlihan *et al.* (1992a) found losses of some original MFGM proteins (polypeptides) in milk on heating at 80 °C for 2.5 to 20 min and suggested whey proteins may have displaced these proteins. However, Kim and Jimenez-Flores (1995) claimed that β-lactoglobulin and α-lactalbumin were involved in heat-induced interactions with MFGM components; direct disulfide binding between whey proteins and MFGM proteins could not explain their results, suggesting that interaction between these two groups of proteins are more complex than direct disulfide bond formation.

Rather than direct disulfide bond formation, whey proteins (particularly β-lactoglobulin) may possibly deposit on the MFGM with the displacement from the membrane of polypeptides such as the cysteine-containing protein (49 kDa protein) of the MFGM (Kim and Jimenez-Flores (1995)). Corredig and Dalgleish (1996b) found that both α-lactalbumin and β-lactoglobulin bind via intermolecular disulfide bridges to the surface of the milk fat globule on heating milk from 65-85 °C. Whey proteins in whole milk have more affinity for the MFGM than for the casein micelle surface (Corredig and Dalgleish, 1996b).

Membrane proteins and lactose may participate in the Maillard reaction when milk is heated above 100 °C, but the recent work of Berg and van Boekel (1994) has shown that the Maillard reaction is not very important in milk from a quantitative point of view; therefore its importance for the milk fat globule membrane is difficult to estimate. More important may be the formation of dehydroalanine (an intermediate product in the

Maillard reaction) from cysteine residues present in the MFGM proteins (Walstra and Jenness, 1984); dehydroalanine is quite reactive and lysinoalanine and lanthionine residues may be formed which cause inter- and intra-molecular crosslinks between membrane proteins which can be expected only when the heat treatment is severe.

Phospholipids migrate from the fat globules to the aqueous phase during heating, to some extent (Houlihan *et al.* 1992a). The zeta-potential (or electrophoretic mobility) of fat globules changes as a result of heating (Fink and Kessler, 1985a; Dalgleish and Banks, 1991), indicating that changes occur on the surfaces of the globules, probably owing to the binding of whey proteins.

The changes in the membrane composition and structure, as a result of heating, would be expected to affect the stability of the fat globules. Owing to the absence of cold agglutination, creaming would be slower, and the cream layer formed would be more closely packed, which could result in partial coalescence on cooling when fat crystallization starts (Walstra, 1983). van Boekel and Folkerts (1991) found that batch heating of 30% fat cream for up to 40 min at 130 °C caused no change in the size distribution of the natural milkfat globules, suggesting that the changes in membrane composition discussed above do not impair coalescence stability. Also, indirect UHT heating at up to 150 °C for 30 s of milk with 4% fat or cream containing 20 or 30% fat caused no changes in globule size distribution (Streuper and van Hooydonk, 1986; van Boekel and Folkerts, 1991). Hence, even the turbulence that occurs in combination with heating during indirect UHT treatment does not appear to cause coalescence (or disruption).

These results are in contrast to those of Fink and Kessler (1985b) who observed a small increase in fat globule size in the temperature range 90-125 °C for 0.9 to 63 s (from 4.25 μm to 4.55 μm), followed by a decrease in the temperature range 125-150 °C (from 4.55 μm back to 4.25 μm); these changes in particle size were accompanied by similar changes in "free fat" (actually extracted fat) and electrophoretic mobility. Fat globule coalescence and disruption, and aggregation were also studied by van Boekel and Folkerts (1991), but with natural milkfat globules no aggregation was observed after either indirect UHT or batch heating.

Fink and Kessler (1985a) studied UHT heating of non-homogenized milk and cream. They reported that the fat globule membrane becomes progressively more "permeable" to fat on heating milk between 105 and 125 °C (because of loss of membrane forming constituents, in particular phospholipids and lipoproteins which are responsible for the stabilisation of the fat globule membrane), while the membrane becomes increasingly dense at temperatures above 125 °C (because of the heat induced deposition and polymerization of whey proteins on the membrane similar to the deposition of whey proteins on the casein micelles). Their finding that coalescence does occur to some extent on heating milk between 105 and 125 °C has been refuted by later work (van Boekel and Walstra, 1989, Streuper and van Hooydonk, 1986, Melsen and Walstra, 1989; van Boekel and Folkerts, 1991, Dalgleish and Banks, 1991). van Boekel and Folkerts (1991) stated that fat as such cannot permeate through a membrane, and that it will never be free as milk has abundant surface active compounds that will cover denuded fat instantaneously. The explanation of Fink and Kessler (1985a) that polymerization of whey proteins causes a more dense membrane at temperatures > 125 °C is at variance with the fact that the effect does not depend at all on heating time (van Boekel and Folkerts, 1991). Dalgleish and Banks (1991) found that heating at 85 °C for 4 min has a maximum effect on the association of whey proteins with fat globules. A possible explanation for the discrepancy between the findings of Fink and Kessler (1985a) and those of other workers mentioned above may be due to a possible difference in the equipment used by Fink and Kessler which might have resulted in the induction of partial coalescence at some processing stage.

With direct UHT heating, disruption of fat globules has been observed (Zadow, 1969; Ramsey and Swartel, 1984; van Boekel and Folkerts, 1991; Corredig and Dalgleish, 1996a), probably because steam injection causes severe turbulence and flash cooling causes cavitation. The disruption in such cases is, for a milk containing 4% fat, more or less comparable with homogenization at 100 bar. Incidentally, this effect is not limited to natural milkfat globules, but also occurs with recombined milkfat globules (Melsen and Walstra, 1989).

Heating of milk is always accompanied by some agitation, which is of importance for physical stability as it may lead to changes in globule size as a result of coalescence or disruption (Mulder and Walstra, 1974). Consequently, changes in the surface layers of the

MFGM may occur. Coalescence causes desorption of membrane material whereas disruption results in the adsorption of surface active material from the milk plasma. When foaming occurs, fat globules may come in contact with air bubbles still uncovered by plasma proteins; hence spreading of membrane material over bubbles may occur, and when such bubbles disappear this material is released into the plasma; meanwhile the plasma proteins adsorb on to the partly denuded fat globules (Mulder and Walstra, 1974). These changes are thus not the result of heating *per se*, but of the agitation during heating. Whether and to what extent coalescence or disruption occurs depends on the type of apparatus used. In the case of direct UHT heating by steam injection, for example, disruption is very pronounced and has nearly the same effect as homogenization (Zadow, 1969; Ramsey and Swartzel, 1984; van Boekel and Folkerts, 1991; Correidg and Dalgleish, 1996a).

2.6.3 The effects of homogenization on milk fat globules

Homogenization is the process having the most significant effect on the MFGM. It markedly reduces fat globule size, with a consequent 4 to 6 fold increase in surface area. Since the amount of available original membrane material is insufficient to cover this area, plasma proteins adsorb on to the fat globule surface; the new membranes in homogenized milk have been shown to consist of MFGM material, caseins, casein micelles and whey proteins (Anderson *et al.* 1977; Darling and Butcher, 1978). It is this adsorbed material which accounts for some of the differences between non-homogenized and homogenized milks. Homogenization retards creaming because fat globules are smaller and because the new membrane structure does not favour cluster formation (Brunner, 1974).

Intense turbulence, in homogenizers, causes casein micelles to become preferentially adsorbed over the serum proteins, and large casein micelles adsorb preferentially, particularly on small globules (Walstra and Oortwijn, 1982). The adsorbed casein micelles are partly spread on the fat surface, posssibly as submicelles. As a result, most of the newly formed membrane consists of casein (sub) micelles, while serum proteins make up a much smaller part, about 5%, but covering about 25% of the surface area (Walstra and Oortwijn, 1982); some differences between the compositions of the new membranes of small and large fat globules also exist.

If milk fat is homogenized into skim milk (i.e. if making recombined milk), the resulting fat globules have a surface layer that consists almost exclusively of plasma proteins. Sharma *et al.* (1996) found that although the whey proteins in recombined milks make up a much smaller proportion (about 10%) of the total protein at the fat globule surface, they appear to cover a disproportionately greater area (about 33%) of the fat globule surface.

The relative content of original membrane material in the new membrane will depend on the increase in surface area, and thus on the intensity of homogenization. Darling and Butcher (1978) claimed that desorption of natural membrane components occurs during homogenization and that these membrane proteins are not readsorbed to the same extent as in the original state. McPherson *et al.* (1984) claimed that during homogenization phospholipids were lost from the fat globules, but their conclusion was refuted by Walstra (1985).

After homogenization, clusters of fat globules may be found in which casein micelles are shared by globules (Ogden *et al.*, 1976). Also, submicelles and even molecular proteins were reported to link globules together (Darling and Butcher, 1978). Whether homogenization clusters are formed or not depends primarily on the fat/protein ratio, on homogenization pressure and factors that determine the protein load. As a rule of thumb, homogenization clusters will not be formed in cream if the fat content is less than 8%, whereas formation can never be prevented if the fat content is more than 18% (Walstra, 1983). Two stage homogenization may (partly) prevent the occurrence of homogenization clusters (Walstra, 1983).

The membranes of homogenized miik fat globules may impart less stability against coalescence than do natural membranes (unlike the membranes of recombined fat globules, which result in greater stability) (Mulder and Walstra, 1974; Melsen and Walstra, 1989). However, the reduced sizes of the globules makes them more stable, particularly to creaming (unless homogenization clusters are present) and coalescence (Walstra, 1983). The homogenized fat globules act as large casein micelles and will participate in any reaction of caseins, such as renneting, acid precipitation and heat coagulation (van Boekel and Walstra, 1989).

2.6.4 The effects of heat treatment on homogenized milk fat globules

When milk fat globules are homogenized before heating, the globules behave differently during and after heating as compared to natural milk fat globules. The membranes of the homogenized globules have a different composition and the size of the globules is smaller.

The subsequent heating of homogenized milk causes complex formation between whey proteins and the casein adsorbed on the fat globule surface. Homogenized fat globules act as large casein micelles because of the casein in their new membranes and thus participate in any reaction of the caseins as stated above (van Boekel and Walstra, 1989). van Boekel and Walstra (1989) stated that, because of this, homogenization is expected to greatly speed up aggregation of casein particles. The heat stability of homogenized, unconcentrated milk is indeed lower than that of non-homogenized, unconcentrated milk (or skim milk), the more so when the homogenization pressure is high (Sweetsur and Muir, 1983a). Problems of heat stability are encountered in the manufacture of sterilized cream, due to the high fat content and to the fact that sterilized cream is always homogenized to prevent creaming during storage. Severe heat coagulation of homogenized cream occured during indirect UHT heating at temperatures above 135 °C for 10 to 27 s (van Boekel and Folkerts, 1991); the same was observed for recombined cream (Melsen and Walstra, 1989). There was no significant effect of holding times greater than 10 s. Melsen and Walstra (1989) reasoned that rapid heat coagulation of casein-covered globules occurs but that the prevailing turbulence in the heat exchanger disrupts large aggregates again, so that aggregates will not become larger than a certain size; hence there should be no further effect of holding time after a certain maximum time. However, Fink and Kessler (1985b) did find increasing aggregation with increasing holding time (0.9-63 s at 90-145 °C).

Homogenization of concentrated milk has a detrimental effect on heat stability and may cause problems in practice, owing to the fact that homogenized fat globules act as large casein micelles. In a complex system such as concentrated milk, coagulation is possible between casein micelles and casein-covered fat globules, between casein-covered fat-globules and between casein micelles. There are several, as yet unexplained, phenomena. Some of these are described below.

Homogenizing milk before concentration decreases the heat stability of the concentrate much more than does homogenization after concentration (Sweetsur and Muir, 1982). Forewarming has, in general, a positive influence on the heat stability of concentrated milk but the effect is more pronounced when the milk is homogenized. Preheating (at 80-100 °C for 5-30 min) of concentrated milk before homogenization gives a better heat stability than preheating after homogenization, and the higher the preheating temperature, the better the stability (Sweetsur and Muir, 1982). Preheating at a higher pH gives a better heat stability (Sweetsur and Muir, 1982). Blocking sulfydryl groups before homogenization of un-preheated concentrated milk prevents the destabilizing effects of homogenization (Sweetsur and Muir, 1983b). Homogenized concentrated milk was more heat stable at 110 °C than the non-homogenized control, whereas the opposite was true at 120 °C (Sweetsur and Muir, 1983b).

All in all, it seems that homogenized concentrated milk is more sensitive to stabilizing treatments (preheating, stabilizing salts) than non-homogenized concentrated milk, and stabilizing treatments have a larger effect if applied before homogenization (Sweetsur and Muir, 1982). It is also clear that interaction between whey proteins and caseins plays a crucial part in the heat coagulation of homogenized fat globules in concentrated milk.

After heating, homogenized fat globules are normally stable against coalescence during storage (Melsen and Walstra, 1989). If homogenization clusters are present, creaming may be rapid. Darling and Butcher (1978) reported that whey proteins were more strongly attached to the MFGM after pasteurization of homogenized cream than before; apparently, the membrane develops into a more cohesive network after a heat treatment and on storage.

2.6.5 The effects of homogenization on heat-treated milkfat globules

When whole milk or cream is heated (before homogenization), the whey proteins are denatured (if the temperature is > 70 °C) and interact with both the κ-casein of casein micelles (at the natural pH) and the native fat globule membrane (Dalgleish and Banks, 1991). During subsequent homogenization, the micellar complex of casein and whey

protein will adsorb on to the newly created fat surfaces (Sharma and Dalgleish, 1994). Competition between the caseins and whey proteins for the fat surface will be small or even absent. On the other hand, if milk is homogenized before it is heated, the caseins, either as semi-intact micelles or as micellar fragments, cover the newly formed fat globule surface (Walstra and Oortwijn, 1982) and no whey proteins are present on the membrane (Sharma and Dalgleish, 1993). The subsequent heating of this milk causes complex formation between whey proteins and the casein adsorbed on the fat globule surface. These two treatments, homogenization and heat treatment, cause major variations in the quality of processed dairy products such as evaporated and sweetened condensed milk (van Boekel and Walstra, 1989).

It is not known whether the heat-induced casein-whey protein complexes are desorbed or remain adsorbed during homogenization. All in all, homogenization after heating gives fat globules with thicker adsorbed layers (Oortwijn and Walstra, 1979). This in turn favours the formation of homogenization clusters (Walstra, 1983).

An advantage of homogenizing after heating is, of course, that heat coagulation is no longer possible and this is important for the production of concentrated milk (Muir, 1984), which is very sensitive to heat coagulation. Homogenization after sterilization must be aseptic.

Direct UHT heating by steam injection causes disruption of fat globules to much the same degree as do homogenizers (Zadow, 1969; Ramsey and Swartzel, 1984; van Boekel and Folkerts, 1991, Corredig and Dalgleish, 1996a). However, high-pressure homogenization after direct heating is still necessary to disrupt protein aggregates that may have been formed by the heating process; otherwise sediment will form during storage (Ramsey and Swartzel, 1984).

Although the adsorbed layers on fat globules homogenized after heating will, on average, be thicker, favouring homogenization clusters, than those of fat globules that have been homogenized before heating, appreciable differences in stability are not to be expected. Formation of homogenization clusters is favoured by thick adsorbed layers, but two-stage homogenization can diminish the extent of clustering again (Walstra, 1983). The globules will be stable against coalescence because of the thick adsorbed layers, but flocculation is possible as a result of reactions of caseins adsorbed on fat globules. In the

case of thick adsorbed layers, creaming is reduced, especially in concentrated milk; some sedimentation can even occur.

2.7 Storage-induced changes in UHT treated milks

Heat treatment of milk is used to increase its shelf life, since raw milk is a highly perishable product and also is unsafe for consumption without further heat treatment. In industrialised countries, especially those producing milk far in excess of their requirements, there is an increasing trend to process milk in a limited number of centralised dairy factories. The distribution of milk to wider and remote areas, particularly where refrigeration is not available, necessitates the prolongation of the shelf life of the milk beyond the limits afforded by pasteurization. Several methods, for example freezing of milk, addition of sucrose to concentrated milk (SCM) and sterilization are effective in preserving milk for long periods (Harwalkar, 1992).

Shelf life of a product may be defined as the period over which a product retains acceptable bacteriological, chemical and physical characteristics. A product may be considered stable during storage for as long as it remains a more or less homogenous liquid. Two types of physical instability occur during storage of heat treated milk products; a) sedimentation and/or creaming, and b) gelation.

Sedimentation involves settling or precipitation of colloidal particles. Generally, a layer of proteinaceous material is formed at the bottom of the storage container. Sedimentation and creaming are related to the size and density of the particles present in milk immediately after manufacture, and could be enhanced by aggregation of particles during storage. The factors which affect the aggregation of particles during storage and thus enhance sedimentation and/or creaming, include voluminosity of the resulting aggregates, initial volume fraction of particles and rate of aggregation (Nieuwenhuijse, 1995).

Gelation is characterised by loss of fluidity of the product as a result of changes during storage (Harwalkar, 1992). Gelation has been described by various terms, such as coagulation (Samulesson and Holm, 1966; Snoeren *et al.*, 1979), thixotropic gel formation (Swanson *et al.*, 1965); age thickening (Morr, 1969, Hostettler, 1972;

Seehafer, 1972; Schmidt, 1980), partial gelation (Samuelsson and Holm, 1966; Hostettler, 1972) or lumpiness (Tarassuk and Tamasma, 1956).

Gelation of milk or concentrated milk is strictly a storage defect. It should not be confused with the thickening during sterilization nor with coagulation observed during incubation of certain types of evaporated milks. The product shows a curd-like consistency when gelation occurs, and is irreversible after this stage. On prolonged storage of gelled products, syneresis does occur, though it may be absent during initial gelation period (Harwalkar and Vreeman, 1978a, b).

Gelation of heat treated milk during storage has been the subject of many reviews over the years (Levinton and Pallansch, 1961a, b, 1962; Seehafer *et al.*, 1962; Levinton *et al*, 1963; Ellerston and Pearce, 1964; Samuelsson and Holm, 1966; Hostettler, 1972; Harwalkar and Vreeman, 1978a, b, Snoeren *et al.*, 1979; Mehta, 1980; Schmidt, 1980; de Koning *et al.* 1985; Manji *et al.*, 1986; Harwalkar, 1992, Nieuwenhuijse, 1995; McMahon, 1996).

2.7.1 Factors affecting gelation

The various factors that affect the rate and extent of gelation of milk products include heat treatment, homogenization and sequence of processing, total solids, composition and quality of milk, additives and temperature of storage (see reviews by Harwalkar, 1992; Nieuwenhuijse, 1995; McMahon, 1996).

2.7.1.1 Heat treatment

Milk sterilized by different processes vary considerably in stability during storage. It is now well recognised that retort sterilized milk (115-120 °C for 15-20 min) remains resistant to gelation for long periods of storage, whereas UHT sterilized milk (135-140 °C for 4-5s) gels during storage (Harwalkar, 1992). Onset of gelation varies with heat treatment given to milk before or during sterilization and with the extent of whey protein denaturation. Samuelsson and Holm (1966) observed that by increasing sterilization temperatures from 142 °C to 152 °C and increasing heating time from 6s to 12s, milks could be held longer without gelation. Similarly, Zadow and Chituta (1975) observed increase in gelation time when sterilization temperature was increased from

135 °C to 140 °C and holding time increased from 3 s to 5 s. Manji and Kakuda (1988) correlated the protection against gelation to the extent of whey protein denaturation. Greater heat treatment (percent whey protein denaturation) of milk or concentrated milk retarded gelation.

Sterilization of concentrated milk at higher temperatures and longer holding times also retard gelation. However, at equivalent sterilizing effectiveness, higher sterilization temperatures with shorter exposure times result in reduced resistance to gelation (Ellerston and Pearce, 1964; Swanson *et al.*, 1965). Heat treatments in excess of those required for sterilization are needed to achieve greater stability against gelation (Harwalkar, 1992).

UHT sterilizing method also influences the gelation (Corradini, 1967; Corradini, 1971; Blanc *et al.*, 1980). Sterilization by direct heating methods (e.g. direct steam injection) resulted in less protection against gelation during storage than sterilization by indirect methods (Harwalkar, 1992). The wide variation in the length of the gelation time, ranging from less than 3 months to over 12 months, as observed by many researchers (Hostettler, 1972; Andrews, 1975; Corradini, 1975; Zadow and Chituta, 1975; Harwalkar and Vreeman, 1978a; Farah, 1979a; Snoeren *et al.*, 1979) could be attributed, at least in part, to the different methods of sterilization.

Forewarming, a beneficial step in improving stability of concentrates during retort sterilization methods, plays an important role in the gelation of UHT sterilized concentrated milk. Zadow and Chituta (1975) observed a moderate delay of gelation time by forewarming at 72 °C for 30 s (~ 13 days) or at 80 °C for 30 min (~ 21 days) as compared to control (no forewarming) for unconcentrated UHT milk.

Leviton *et al.* (1963) studied the effect of forewarming treatments on the retardation of gelation in HTST sterilized milk concentrates (37 % T.S.). Forewarming of milk at 280 °F for 15 s, compared with forewarming at 295 °F for 0.6 s, resulted in the increase in the viscosity of the concentrates. On storage for 102 days at 70 °F, the viscosity of the milk concentrate forewarmed at 280 °F for 15 s increased significantly, whereas the viscosity of the milk concentrate forewarmed at 295 °F for 0.6 s increased only slightly. These results showed that variations in the forewarming regime may be expected to bring about variations in the stability and viscosity of the concentrates and consequently in the degree of gel formation.

2.7.1.2 ***Homogenization***

In sterilized concentrated milk, homogenization reduces the gelation time but the effect is dependent on the position of the homogenization step in the manufacturing process. Placing the homogenization step before concentration and sterilization gives a product with reduced stability against gelation, whereas concentration before sterilization and then followed by homogenization renders a product more stable towards gelation (Leviton *et al.*, 1963).

Omission of homogenization in the manufacture of sterilized concentrated milk would benefit the product both in terms of heat stability and age-gelation but can not be avoided if creaming is to be limited during subsequent storage. No studies have been carried out to establish the influence of severity of homogenization or two stage multipass homogenization on the gelation of UHT sterilized concentrated milks.

Pouliot *et al.* (1990) observed that application of high pressure homogenization prior to in-can sterilization promoted gelation in an infant formula. As a result of increased adsorption of protein on fat surfaces due to the high pressure homogenization, the modified surface is believed to make it more susceptible to aggregation and gelation.

2.7.1.3 ***Total solids content***

Gelation of concentrated milk, sterilized by HTST or UHT methods, is hastened by increasing the total solids content (Leviton *et al.*, 1963; Ellerston and Pearce, 1964). Increased severity of heat treatment has less influence on retarding the gelation of concentrates containing higher total solids than of less concentrated milks. Stewart *et al.* (1959) observed that heat treatment given to concentrated milk (32-45% T.S.), followed by dilution to 26% T.S., imparts to the diluted milk a greater stability against gelation.

Gelation time decreases by increasing the total solids content in the UHT sterilized milk which is mainly due to the SNF portion, and fat is not directly involved in gelation (Tarassuk and Tamasma, 1956).

2.7.1.4 Composition of milk

Factors, which affect the composition of milk, may indirectly affect the gelation behaviour of sterilized milk. Summer milk gives more stable products than winter milk (Zadow and Chituta, 1975). UHT-treated mastitic milk is more susceptible to gelation than normal milk (Swartling, 1968). Early lactation milk is more susceptible to storage gelation of UHT products (Zadow and Chituta, 1975). Evaporated milk from Jersey cows (higher protein/water ratio) is more susceptible to gelation than evaporated milk from other breeds.

Addition of dialysed acid whey to milk to increase whey protein content by 10% and subsequently processing the milk into evaporated milk (26% T.S.) sterilized by HTST method, markedly hastens the onset of gelation (Ellerston and Pearce, 1964). Schmidt (1969) found that addition of α_s-, β- and κ-caseins to milk influenced the characteristics of the casein micelles but did not affect the gelation time of UHT sterilized concentrated milk.

2.7.1.5 Quality of milk

The bacteriological quality of raw milk is also important in gelation. Sterilized milk prepared from poor quality milk is very susceptible to gelation during storage (Swartling, 1968; Zadow and Chituta, 1975; Snoeren *et al.*, 1979). The types of microorganism or spores present in poor quality milk are important (Zadow and Chituta, 1975; Snoeren *et al.*, 1979). Organisms that produce heat-stable enzymes e.g. proteinases, cause most serious gelation problems.

In order to suppress the growth of psychrotrophs that produce heat-stable proteinases, which could affect storage stability, a thermization treatment of milk (63-65 °C for 12-15 s) before storage in bulk tanks has been recommended (Ashton, 1979). This treatment is effective against psychrotrophs but not against their proteolytic enzymes.

2.7.1.6 Additives

In order to develop suitable additives for controlling age gelation in sterilized milk or concentrated milk during storage, lot of research has been done in the past three decades

(see reviews by Harwalkar, 1992, Nieuwenhuijse, 1995). Addition of 0.5 g orthophosphate/l reduced gelation time of direct (142 °C for 4 s) UHT sterilized milk from 13 to 12 weeks (Snoeren *et al.*, 1979). Addition of 1 g sodium citrate/l followed by adjustment of the pH to 6.75 resulted in a similar reduction of gelation time whereas 3 g/kg had a slightly smaller effect (Kocak and Zadow, 1985b). Addition of 2 g sodium citrate/l, with no pH adjustment, to a slightly more intensely sterilized milk (142 °C for 6 or 12 s), however, reduced gelation time from 21 (no addition) to 14 weeks and from more than 39 (no addition) to 27 weeks, respectively (Samuelsson and Holm, 1966). Addition of sodium phosphate and sodium citrate hasten, while polyphosphates delay, the gelation process in both UHT sterilized milk (Samuelsson and Holm, 1966; Snoeren *et al.*, 1979) and concentrated milk (Leviton and Pallansch, 1962; Leviton *et al.*, 1962, 1963; Schmidt, 1968; Schmidt and Buchheim, 1968; Harwalkar and Vreeman, 1978a). The extent of protection against gelation by polyphosphates increases with the chain length and concentration. Polyphosphates with an average of 4.8 phosphorous atoms per chain are effective against gelation (Leviton and Pallansch, 1962; Leviton *et al.*, 1962, 1963). Cyclic condensed phosphates are more effective against gelation than linear polyphosphates because the former are less susceptible to hydrolysis than the latter (Leviton *et al.*, 1962; Leviton and Pallansch, 1962). A mixture of monophosphate and polyphosphate augments gelation (Leviton *et al.*, 1962; Leviton and Pallansch, 1962).

Addition of sugars (lactose, sucrose, dextrose and sorbitol) and phosphatides delay the gelation of sterilized concentrated milk (Leviton and Pallansch, 1962). Harwalkar and Vreeman (1978b) observed a decrease in the gelation time on addition of hydrogen peroxide to UHT sterilized skim milk concentrate, whereas the addition of sodium hydroxide (Board and Mullet, 1970) or sodium carbonate (Seehafer, 1972) to adjust the pH of 3-fold sterile concentrate to near neutrality increases the gelation time and adjusting the pH of the unconcentrated milk prior to UHT sterilization had no effect on gelation (Zadow and Chituta, 1975).

2.7.1.7 Storage temperature

The occurrence of gelation in UHT sterilized milk is markedly influenced by storage temperature. However, there is no unanimous agreement between different investigators regarding the effect of storage temperature on gelation. Samel *et al.* (1971) observed that the gelation time (13 month) was independent of storage temperature between 4 and 30 °C, but at 37 °C, gelation was not detected even after 2 years. Other researchers (Hostettler *et al.*, 1957; Andrews, 1975; Zadow and Chituta, 1975; Andrews *et al.*, 1977; Farah, 1979a) have also reported greater resistance to gelation in UHT milk stored at 35 °C and above. Zadow and Chituta (1975) observed minimum gelation-free storage life at 25-35 °C but the storage life was extended considerably at 2 °C or at 40 °C. Andrews *et al.* (1977) reported that the samples of UHT milk stored at 4 °C gelled in 19 months whereas samples stored at 30 to 35 °C did not gel even after 28 months. Hostettler *et al.* (1957) observed that UHT sterilized milk, stored in glass containers, gelled in 10 months at room temperature but remained liquid at 35 °C. The samples in glass containers at 35 °C turned brown but remained white when stored in tinned containers at 35 °C and gelled at about the same time as the sample stored at room temperature. A study by Manji *et al.* (1986) showed similar temperature dependence. Milk samples sterilized by the direct heating system were more resistant to gelation when stored at 37 and 4 °C compared with samples stored at 22 to 25 °C.

Studies on sterilized evaporated milk showed better agreement among researchers regarding the effect of storage temperature on gelation. In general, the higher the storage temperature, the faster the HTST sterilized evaporated milk gelled. (Ellerston and Pearce, 1964; Levinton *et al.*, 1963).

2.7.2 Physico-chemical changes during storage

*2.7.2.1 **pH decrease***

Many researchers have observed that pH decreases during storage of UHT milk (Andrews and Cheeseman, 1971; Andrews and Cheeseman, 1972; Andrews, 1975; Zadow and Chituta, 1975; Kocak and Zadow, 1985b; Manji *et al.*, 1986). The decrease in pH is greater in samples stored at high temperatures (Zadow and Chituta, 1975;

Andrews *et al.*, 1977; Kocak and Zadow, 1985b; Manji *et al.*, 1986). Andrews *et al.* (1977) observed during storage of UHT milk that the higher the storage temperature, the greater was the decrease in pH from the value of 6.7 which was obtained for the milks immediately after processing; the pH decreased from 6.7 to 6.5 after storage at 4 °C, and to 5.6 after storage at 37 °C for 34 months. This is compatible with the loss of positive charge on protein molecules caused by a loss of free ε-NH_2 groups of lysine in a Maillard type of reactions (Andrews and Cheeseman, 1971, 1972; Andrews, 1975). However, Venkatachalam *et al.* (1993) observed that the pH of the UHT treated samples containing added sucrose also decreased when stored at 35 °C, suggesting that the Maillard reaction may not be the only cause, as these samples had insignificant browning.

Method of sterilization (direct or indirect) has no definite effect on pH decrease. Manji *et al.* (1986) studied the effect of storage time and temperature on the pH of direct and indirect UHT processed milks. Both types of milk showed little change in pH during storage at 4 °C. Samples stored at 22 to 25 °C showed slightly decreased pH whereas both direct and indirect samples stored at 37 °C showed significant decrease in the pH after 60 days storage. Andrews *et al.* (1977) reported similar effects and concluded that the level and extent of pH decrease was not related to age gelation.

Kocak and Zadow (1985a) studied the effect of storage time and storage temperature on the pH of UHT milk. In general, for samples stored at 20 °C or below there was a little change in pH during storage. Samples stored at 20 °C showed a general decrease in pH. In samples stored at or above 30 °C, the decrease in pH was most pronounced after about 100 days of storage. The extent of pH drop as related to storage temperature was in the following order: 50 > 40 >30 > 25 > 20 > 15 > 10 > 2 °C. Again, as reported by Andrew *et al.* (1977), the onset of age gelation could not be related to the extent of decrease in pH.

Samples with additive show a slightly different pattern in pH decrease during storage of UHT milk. Kocak and Zadow (1985b) noted the pH of additive-treated UHT samples increased immediately after UHT processing with the magnitude of the change decreasing in the following order: 0.05% $CaCl_2$ > 0.3% sodium citrate > 0.1% sodium citrate > 0.1% sodium hexametaphosphate (SHMP) > 0.3% EDTA > 0.1% EDTA. The pH of the control samples was reduced on UHT processing. They also observed that the

pH of all samples showed a general decrease on storage at 25 °C. In samples containing additives there was initially a higher rate of decrease in pH during storage. Samples containing $CaCl_2$ had the most severe drop in pH. After 7 days of storage, the pH of these samples was well below that of the control. On further storage upto 200 days, the rate of decrease in pH of these samples was comparable to the control and other additive containing samples. Again, there appeared to be no relation between the extent or rate of pH decrease and the onset of age gelation.

Venkatachalam *et al.* (1993) while studying the storage of ultrafiltered UHT concentrated skim milk observed that the pH of all samples (control, control + 3 or 6% lactose, control + 3 or 6% sucrose) stored at 4, 20 and 35 °C for 22 weeks decreased during storage. The greatest decrease occurred in samples with 3 or 6% lactose. The rate and extent of pH decrease during storage were highest at 35 °C followed by those of samples at 20 °C. Minimal changes occurred at 4 °C. The pH of gelled samples ranged from 6.54-6.68 and the pH of non-gelled samples ranged from 6.08-6.41. The change in pH was considered to be due to gradual precipitation of calcium phosphate during storage, shifting the calcium-phosphate equilibrium toward formation of Ca^{2+} and PO_4^{3-} from other forms of ionic species and releasing protons that decrease pH. Dephosphorylation of proteins, occurring during storage, would liberate free H^+, resulting in a decrease in pH. Alkaline milk phosphatase can cause this and there are reports in literature that reactivation of alkaline milk phosphatase during storage of milk occurs. However, Harwalkar and Vreeman (1978a) found no increase in inorganic P during storage and suggested no dephosphorylation had occurred.

*2.7.2.2 **Proteolysis***

Proteolysis occurs during storage of milk, if the proteolytic enzymes present in milk are not completely inactivated by heat treatment. The proteolytic activity during storage of UHT milk can be due to extracellular proteinases from the growth of psychrophilic bacteria (Law *et al.*, 1977; Renner, 1988; Driessen, 1989) or native milk proteinase (plasmin) (Snoeren *et al.*, 1979; Burton, 1984; de Koning *et al.*, 1985) that survive UHT processing. Native milk proteinase (plasmin) acts mainly on β- and α_{s2}-caseins (Snoeren *et al.*, 1979; Snoeren and van Riel, 1979; Snoeren and Both, 1981;

de Koning and Kaper, 1985; de Koning *et al.*, 1985) and not on κ-casein (Visser, 1981; Manji and Kakuda, 1988; Kohlmann *et al.*, 1988). Plasmin hydrolyses β-casein to yield three large C-terminal fragments, γ_1, γ_2 and γ_3- caseins (Corradini and Pecis, 1979; Visser, 1981). In contrast, extracellular proteinases of microbial origin predominantly attack κ-casein with the formation of material similar to para-κ-casein (Law *et al.* 1977; Snoeren and Van Riel, 1979) followed by extended nonspecific hydrolysis (Law *et al.*, 1977; Driessen, 1989).

Driessen (1983) as cited by Nieuwenhuijse (1995) based on determination of the TCA-soluble free amino groups found that plasmin gives an increase in non-casein nitrogen (NCN) but hardly any in non-protein nitrogen (NPN), whereas both NCN and NPN increase following proteolysis by bacterial proteinases. Samuelsson and Holm (1966) observed an inverse relationship between the increased level of NPN and the time of onset of gelation.

Numerous workers have observed protein breakdown during storage of both unconcentrated (Samel *et al.*, 1971; Andrews, 1975; Corradini, 1975) and concentrated milk (Hostettler *et al.*, 1968; Hostettler, 1972; Harwalkar and Vreeman, 1978b; Snoeren *et al.*, 1979) sterilized by UHT treatment. In general, during storage, there is a progressive decrease in the amount of casein nitrogen and a corresponding increase in NCN and NPN. UHT treatment by a direct heating method results in a higher degree of proteolysis during storage than in milk sterilized by indirect methods or retort sterilization (Samuelsson and Holm, 1966; Samel *et al.*, 1971; Farah. 1979b; Blanc *et al.*, 1980).

Snoeren *et al.* (1979) and de Koning *et al.* (1985) reported proteolysis by plasmin only. Snoeren *et al.* (1979), for direct UHT-sterilized (142 °C for 2 s) milk, reported that complete breakdown of β- and α_{s2}- caseins occurred in about 60 days at 28 °C, and of α_{s1}- casein in about 100 days; about 50% of κ-casein was, however, still intact after 100 days. de Koning *et al.* (1985) reported similar rates of proteolysis except for κ-casein which hardly hydrolysed. In contrast, proteolysis in indirect UHT sterilized (142 °C for 2 s) milk could not be detected and NPN increased only very slightly (Snoeren and Both, 1981). de Koning and Kaper (1985) observed similar results (i.e. proteolysis was absent) in UHT sterilized concentrated milk, made from

milk preheated at 120 °C for 3 min. Manji *et al.* (1986) found that the difference in the extent of proteolysis between direct and indirect UHT sterilized milk correlated well with the difference in the plasmin activity upon measuring proteolysis as 6% TCA-soluble amino groups.

Rate of proteolysis in UHT milk is also affected by the storage temperature. Increased breakdown of protein at a higher temperature of storage was observed by Samel *et al.* (1971), but no correlation was observed between the extent of proteolysis and the time of onset of gelation. They found that the samples stored at 4, 20, or 30 °C gelled at about the same time (13 months) in spite of varying levels of protein breakdown. Samples stored at 37 °C showed extensive proteolysis but did not gel. Slower proteolysis was observed during storage of UHT sterilized concentrated milk than in UHT sterilized unconcentrated milk (Harwalkar and Vreeman, 1978b). Harwalkar and Vreeman (1978a) observed a slow rate of protein breakdown as indicated by the decrease in casein nitrogen and increase in NCN and NPN. Also, they noted that the rates of protein breakdown were unaffected by the addition of orthophosphate or SHMP. Similar observations were noted by Snoeren *et al.* (1979) regarding the effect of added phosphates on the changes during storage of UHT sterilized unconcentrated milk.

2.7.2.3 Dissociation, association and microstructure of casein micelles

Casein particles undergo a number of changes during storage. An increase in the amount of non-sedimentable casein has been observed, which results from a partial dissociation of the casein micelles (Wilson *et al.*, 1963; Morr, 1969) and is seen by electron microscopy as fine particles or subunits of casein (Wilson *et al.*, 1963; Schmidt, 1968; Harwalkar and Vreeman, 1978b). Large casein micelles dissociate more rapidly at 4 °C than at 20 or 37 °C. Shifts in the mineral balance during storage may be the probable cause for this dissociation. Aoki and Imamura (1974a) found α_s - casein (total) to be the principal protein that dissociated, whereas Schmidt (1968) and Harwalkar and Vreeman (1978b) indicated that κ-casein and β-lactoglobulin may dissociate as well, since the spikes on the micelles in heated milk presumably consist largely of these proteins (Singh and Creamer, 1992). Dissociation of α_s- (and β-) casein would imply that organic P in the supernatant should increase during storage.

Also, there is association of proteins due to increased formation of protein polymers during storage (Aoki and Imamura, 1974a, b; Andrews, 1975). These polymers result from covalent binding or cross-linking (other than S-S bridges) of casein polypeptides chains through carbonyl intermediates resulting from Maillard-type reactions (Andrews and Cheeseman, 1971, 1972; Andrews; 1975). The polypeptide chains may also be linked through S-S bridges. The extent of polymerisation is dependent upon time and temperature of storage. The amount of protein in S-S linked polymers decreased during storage of UHT-milk (Andrews, 1975). After six months of storage, the proportion of milk proteins existing as covalently-bound polymers was 50% at 37 °C, 40% at 30 °C, 26% at 20 °C and 21% at 4 °C (Andrews, 1975). The extent of polymer formation does not appear to be related to gelation time since UHT milk stored at 4 °C gelled sooner than milk stored at 30 or 37 °C, although the milk stored at 4 °C contained the lowest amount of polymers (Andrews, 1975).

The above mentioned changes progress independently of changes in viscosity, and there are changes in the structure of casein micelles which coincide with the observed changes in the viscosity or gelation. Electron microscopy shows that during storage of UHT sterilized milk, the casein micelles associate increasingly during the period in which a rapid increase in viscosity and gelation occur (Harwalkar and Vreeman, 1978b; Farah, 1979a). The changes in the microstructure of the casein micelles during storage of UHT sterilized milk are gradual.

Harwalkar and Vreeman (1978b) have given a good description of the changes occurring in the UHT sterilized concentrated skim milk. During initial period, there is no change in viscosity, the micelles remain spherical and well separated from each other, although the surface appearance changes and the filamentous appendages or hairiness on the micelle surface become prominent. After 10 weeks of storage, when viscosity starts to rise, the casein micelles show a slight distortion and thread-like tails appear on the micelle surfaces. Similar observations were made by Andrews *et al.* (1977) and Blanc *et al.* (1980). After 13 weeks of storage, when milk had become viscous, pairs or triplets of casein micelles were observed. The micelles were attached either by fusion between micelles or by thin, fibre-like material, analogous to the bridging material described by Caroll *et al.* (1971). The micelles still retained their identity and several unattached micelles were also present. After 17 weeks of storage,

when the samples had gelled, there was severe distortion and aggregation of casein micelles into chains that were connected through fibre-like material to form a continuous network. Other workers have also observed network formation by linkages between casein micelles in gelled concentrated milks (Wilson *et al.*, 1963; Hostettler *et al.*, 1968; Schmidt, 1968; Schmidt and Buchheim, 1968; Carroll *et al.*, 1971; de Koning *et al.*, 1985) and unconcentrated milks (Andrews *et al.*, 1977; Farah, 1979a; Blanc *et al.*, 1980; de Koning *et al.*, 1985). Therefore, gelation appears to result from gradual change in the casein micelles rather than from an abrupt coalescence of micelles shortly before gelation (Schmidt, 1968; Schmidt and Buchheim, 1968).

2.7.3 Proposed mechanisms of gelation

Gelation has been attributed to various changes in UHT milk during storage and to the various conditions that alter the gelation time. In general, gelation occurs when casein micelles lose colloidal stability and form a three dimensional network (Harwalkar, 1992; Singh *et al.*, 1989). The interaction between micelles is preceded by changes at the surface of the micelles, as a result of which they become more reactive. However, the forces that lead to the modification of the micelle surface are not fully understood. Several models have been hypothesised to explain the mechanism of age gelation. The two possible changes which enhance the interaction between the micelles can be categorised as changes that arise from proteinase activity or from non-enzymic (physico-chemical) reactions (Harwalkar, 1992).

2.7.3.1 ***Proteinases hypothesis***

Proteolytic enzymes that survive UHT treatment or that reactivate during storage may cause gelation of UHT milk during storage (Samuelsson and Holm, 1966; Bengtsson *et al.*, 1973; Adams *et al.*, 1975; Zadow and Chituta, 1975; Law *et al.*, 1977; Corradini and Pecis, 1979; Snoeren and van Reil, 1979; Snoeren *et al.*, 1979; Renner, 1988). During gelation of UHT treated milks, hydrolysis of κ-casein occurs, similar to hydrolysis by rennet, but the aggregation of the particles is different from that of renneted casein micelles. Paynes (1978) considers that the phenomenon of initial age thinning followed by an explosive growth of the average particle weight, results from the action of a

clotting proteinase. Coagulation kinetics studies can explain certain forms of gelation (where proteolysis is observed) in sterilized milk but is not applicable to some other types of gelation, for example: samples of sterilized milk where gelation was observed without accompanying proteolysis (Paynes, 1978; de Koning and Kaper, 1981; Harwalkar *et al.*, 1983; de Koning *et al.*, 1985); samples where gelation could not be correlated with the rate and extent of proteolysis, as influenced by storage temperature (Wilson *et al.*, 1961; Samel *et al.*, 1971; Manji and Kakuda, 1988). Also, observed gelation of evaporated milk that was cold-stored before retort sterilization (Leviton *et al.*, 1963; Heintzberger *et al.*, 1972). de Koning and Kaper (1981, 1985), while studying the physicochemical changes during storage of UHT-sterilized concentrated casein micelles dispersion isolated from aseptically drawn milk, reported typical age-thinning followed by gelation without accompanying proteolysis. Proteinase inhibitors were used to control proteolysis. The initial decrease in the viscosity (age-thinning) has been linked to heat treatment rather than proteolysis (de Koning *et al.*, 1985).

Two separate mechanisms for the age gelation of unconcentrated and concentrated UHT sterilized skim milk have been suggested by de Koning *et al.* (1985). Age gelation of unconcentrated skim milk is preceded by proteolysis whereas gelation of concentrated skim milk occurs even when the proteolysis is prevented. Similar observations were reported by Manji and Kakuda (1988).

There is a wide variation in the nature and extent of proteolysis and the occurrence of gelation could not be related to any specific degree of protein breakdown (Harwalkar, 1992). This may be due to wide variation in the specificity of the proteinases both indigenous or of bacterial origin. Some of these proteinases lack the highly specific proteolytic action of rennet, which attacks Phe-Met bond between residues 105 and 106 of κ-casein to yield a macropeptide and para-κ-casein. Usually very slow rate of hydrolysis of κ-casein during age-gelation, as compared to that during renneting, plays a part, allowing slow aggregation of partly denuded casein particles, not only by colloidal interactions, but also by bond formation between reactive groups on the κ-casein hairs in a no-longer dense hairy layer, as suggested by Walstra (1990). In rennet treated milk, 80-90% of κ-casein is degraded before an increase in viscosity (Green *et al.*, 1978; Green and Morant, 1981) whereas in sterilized milks (or

concentrated milks) only 5-10% of κ-casein is degraded as evidenced by the low levels of macropeptide at the time of gelation (Samel *et al.*, 1971; Harwalkar and Vreeman, 1978a).

2.7.3.2 Non-enzymatic basis of gelation

The absence of a quantitative relationship between gelation time and proteolytic activity has prompted some authors (de Koning and Kaper, 1981; de Koning *et al*, 1985; Manji and Kakuda, 1988; Samel *et al.*, 1971) to attribute gelation to physicochemical processes, including those involving whey proteins, chemical modification of casein micelles by Maillard reaction, milk salts, modification of κ-casein during storage, sulphydryl-disulfide reactions, changes in casein micelle surface potential and casein micelle dissociation.

Extensive proteolysis accompanies gelation of unconcentrated milks whereas no proteolysis takes place during gelation of concentrated milks (Hostettler *et al.*, 1968; de Koning *et al.*, 1985). Gelation of concentrated milks may be affected by the nature and extent of heat-induced complex formation between whey proteins and casein (Hostettler *et al.*, 1968). In a severe heat treatment, like retort sterilized milk, a complete and irreversible interaction occurs between whey protein and casein and resultant complex protects the micelles from further changes and interactions during storage, whereas in milder heat treatment, such as UHT treatment by direct steam injection, an incomplete and reversible complex is formed that does not protect the micelles from further changes and interaction during storage (Harwalkar, 1992).

Andrews and Cheeseman (1971, 1972) and Andrews (1975) suggested that gelation is caused by polymerisation of casein and whey proteins by Maillard type reactions that are promoted by higher storage temperature. However, failure to observe gelation when sterilized milk is stored at temperatures exceeding 35 °C is not consistent with this hypothesis (Samel *et al.*, 1971). Turner *et al.* (1978) suggested that the reaction between lactose and amino groups (predominantly ε-NH_2 groups of lysine) involves Schiff's base formation and Amadori rearrangement to yield keto and enol structures. Maillard reaction during storage could crosslink protein chains into very large complexes, because lactose, but not its degradation products, react with proteins.

This reaction occurs more frequently with casein fractions (and more frequently with κ-casein than α_s- or β-casein) than with whey proteins (Turner *et al.*, 1978). Reactions between lactose and κ-casein may affect casein micelles stability during storage. However, Maillard browning of UHT-concentrated milk is similar regardless of the rate of gelation (Harwalkar and Vreeman, 1978a). Some workers (de Koning and Kaper, 1981, 1985) could not correlate Maillard reaction with age gelation because UHT treated casein micelle dispersions with lactose (reducing sugar) or sorbitol (nonreducing sugar) gelled at about the same time. However, shelf life increases when sugars (both reducing and nonreducing) are added to milk prior to UHT sterilization (Nakai *et al.*, 1964). Changes on the surface of the casein micelles detected by electron microscopy are not explained by Maillard reactions occurring during storage (Andrews *et al.*, 1977).

An alternative hypothesis is that casein micelles lose their ability to aggregate in the presence of rennet when lysine groups in κ-casein are blocked (Samel *et al.*, 1971). Apart from Ca^{2+} bridging and hydrophobic interactions, ionic interactions between casein micelles through oppositively charged regions of suitable configuration may also be involved in their aggregation. Lysine residues may contribute to the configuration of such regions (Samel *et al.*, 1971) and could delay the gelation of sterile milk because the blockage of their ε-NH_2 groups by interaction with lactose could prevent casein micelles from interacting and gelling.

Venkatachalm *et al.* (1993) suggested that age gelation is not promoted by occurrence of the Maillard reaction, based on their comparison of stability of UHT-sterilized skim milk concentrates containing lactose or sucrose. Intramicellar protein-protein reactions occurring at 35 °C provided protection against age gelation rather than carbohydrate-protein Maillard reactions. The streaking observed in SDS-PAGE of UHT milk stored at 35 °C was due to protein modifications other than Maillard browning by use of non-reducing sugars in the samples. They proposed that age gelation involves dissociation of proteins from the casein micelles and the formation on the micelle surface as protuberances and tendrils. Aggregation of the protein particles occurs through these appendages, not through the original micelle surface as in rennet or acid coagulation of milk.

McMahon (1996) has proposed the following model for the age gelation of UHT milk. UHT processing of milk denatures β-lactoglobulin which then covalently bonds with κ-casein to form large polymeric β-lactoglobulin/κ-casein complexes. Such bonding weakens the ionic bonds that anchor κ-casein (via α_{s1} - casein) to the micelles. During storage of UHT milk, the β-lactoglobulin/κ-casein complex is gradually released from the micelle as multiple anchor sites of κ-casein are broken. The β-lactoglobulin/κ-casein complex accumulates in the serum phase as large protein aggregates that have been completely released from the micelles or as long tendrils that are still partially attached to micelles. When a critical volume concentration of β-lactoglobulin/κ-casein complex is attained, a gel network of cross linked β-lactoglobulin/κ-casein complex is formed with any attached casein micelles being incorporated into the network. Crosslinking of the β-lactoglobulin/κ-casein complex continues, and other proteins are incorporated into the network, until a semi-rigid gel is produced.

3. MATERIALS AND METHODS

3.1 Raw milk supply

Raw whole milk from Friesian cows was obtained from the Massey University Dairy Farm, and processed immediately.

3.2 Processing treatments

All milk processing was carried out in the pilot plant at the Department of Food Technology, Massey University, Palmerston North.

3.2.1 Separation

Whole milk was separated at 50 °C using a disc bowl separator (Model 103 AE, Alfa-Laval, Sweden).

3.2.2 Homogenization

Whole milk was homogenized at 50 °C using a two-stage homogenizer (Rannie Homogenizer, Model LAB 100, Rannie a/s, Roholmsvej 8 DK - 2620, Albertslund, Denmark), operating at 150 bar for the first stage and 50 bar for the second stage.

3.2.3 Reverse osmosis

Whole milk, skim milk or homogenized milk was concentrated to various volume concentration factors (i.e. 1.5X, 2.0X, 2.5X and 3.0X) by reverse osmosis (RO) using a DDS LAB-20 plate and frame membrane processing unit (De Danske Sukkerfabrikker, Nakskov, Denmark) equipped with DDS membrane Type HR 95 PP. Total membrane area was 0.324 m^2. The membranes had a NaCl permeability of < 5%. RO concentration was carried out at a temperature of 50 °C, an initial retentate flow rate of 0.2 kgs^{-1}, and retentate inlet and outlet pressures of 40 and 38 bars gauge respectively. Single stage recirculation of the retentate was carried out until the required concentration had been reached.

3.2.4 Pasteurization and UHT sterilization

Pasteurization (72 °C for 15 s) and UHT sterilization (140 °C for 4 s) were both carried out in a pilot-scale continuous indirect UHT plant incorporating shell and tube and "spiraflow" heat exchangers (Alfa-Laval, Australia). Flow rates of 1.00 l/min and 1.26 l/min, which corresponded to 15 s and 4 s holding time, were used for pasteurization and sterilization treatments respectively. The UHT plant was sterilized at 131 °C for 30 min using water as heating medium prior to actual UHT-treatment. After UHT-treatment the milk stream was cooled to 20 °C in the regeneration section.

Homogenization of UHT treated milks was carried out at 55 °C and 137.9 bar gauge using a single-stage adjustable homogenizing valve built into the cooling section of the UHT plant. For homogenization carried out separately from UHT sterilization, the heating sections of the UHT plant were used merely to preheat the product stream to 55 °C prior to its passage through the homogenizing valve. When homogenization was not required, the valve was adjusted to zero homogenizing pressure.

3.3 Aseptic filling

For storage studies of RO-UHT milk samples, the samples were aseptically filled in screw capped glass bottles (4.2 cm diameter, 11.2 cm height, capacity 100 ml) inside a stainless steel chamber (45 cm width, 40 cm length and 80 cm height), fabricated in the Food Technology Department workshop, maintained under a laminar flow by means of compressed air fed at 60 psi. The compressed air was passed first through a filter (Main line filter, model AFF 8B, flow rate 1500 l/min, Japan), followed by a separator (Micro mist separator, model AMD 350, flow rate 1000 l/min, Japan) and then fed onto the top of the chamber. The chamber was sprayed with chlorine solution (1000 ppm) and the glass bottles which had been autoclaved, were placed into the chamber prior to switching on the compressed air. The outlet pipe of the UHT plant was made to pass through the chamber. Once a sterile environment was created inside the chamber, the bottles were filled and immersed in sodium hypochlorite solution (1.5% v/v) for 5 min outside the chamber. The sample bottles were labelled, wrapped in aluminium foil and then transferred into constant temperatures rooms, maintained at 5, 20 or 37 °C.

3.4 Compositional analysis

Fat content of milks was determined using the Gerber method (British Standards Institution, 1969). Total solids were determined using the A.O.A.C. (1984) method. Protein was determined as total nitrogen (N) using the Kjeldahl method, using Kjeltec 1007 Digestor and Kjeltec 1026 Distilling unit (Tecator, Sweden). Non-casein nitrogen (NCN) was determined by precipitating the casein fraction with acetic acid and determining the N content of the filtrate. The non-protein nitrogen (NPN) was measured as N soluble in 12 % trichloroacetic acid (IDF, 29:1964). The concentration of whey protein N was calculated by subtracting the NPN values from the NCN values. A factor of 6.38 was used to convert nitrogen to protein. The analyses were carried out in duplicate.

$$Percent\ whey\ protein\ denaturation = \frac{Whey\ protein N_{unheated} - Whey\ protein\ N_{heated}}{Whey\ protein\ N_{unheated}} \times 100$$

3.5 Measurement of viscosity

Viscosity of milks and their corresponding concentrates were measured at 5, 15, 25, 40 and 60 °C, using a Bohlin VOR Rheometer (Bohlin Rheologi AB, Lund, Sweden) operating in its steady shear mode (Bohlin *et al.*, 1984). The C25 concentric cylinders measuring system, consisting of a 25 mm diameter fixed bob and a 27.5 mm diameter rotating cup, was used in all experiments. Temperatures were maintained by inbuilt cooling/heating water bath of the Bohlin VOR Rheometer System. The sample (13 ml) was carefully loaded into the cup and the bob was then lowered slowly into the cup until the milk solution just reached the top surface of the bob. In order to prevent the evaporation of water and consequent surface drying, the sample surface was covered with liquid paraffin in all the tests. The samples were initially equilibrated for 1 min and the viscosity was determined in the shear rate range 73-921 s^{-1} , unless specified otherwise. Flow curves (shear stress, τ, *versus* shear rate, $\dot{\gamma}$) were obtained from the Bohlin's software. Shear stress and viscosity were reproducible for both increasing and decreasing shear-rate sweeps, with no discernible thixotrophic behaviour.

3.6 Particle size distribution analysis

Particle size distributions (psd) of milk samples were measured at room temperature with a Malvern MasterSizer/E (Model MS 20, Malvern Instruments Limited, Malvern, Worcestershire, United Kingdom) at the New Zealand Dairy Research Institute (NZDRI), Palmerston North. From the distributions, the volume to surface mean diameter d_{32} (also known as the Sauter mean diameter or d_{vs}), the volume moment mean diameter, d_{43} and the specific surface area were calculated by the MasterSizer's software.

The MasterSizer was set up to estimate particle size in 0.1 to 80 μm range. The optical model was set to a polydisperse model, the presentation parameter to 2NAD (N corresponds to the refractive index of the fat particles relative to that of the surrounding medium 1.095 (1.456 for fat:1.330 for dispersant), A corresponds to the fat particle absorption, 0.00, and D corresponds to the dispersant refractive index, 1.330). The optical properties brought together in the presentation parameter are a requirement of the Mie theory, absorption being the imaginary component for the refractive index (Kerker, 1969). This technique measures the angular distribution of the scattered light energy at 633 nm over 32 concentric photodetector elements using 'Reverse Fourier Optics'. A lower limit of 0.1 μm is readily achievable with this technique, this limit being set by the upper limit of the scattering angle. The measured angular scattering data are analysed using Mie theory of light scattering to recover the particle size distribution in the sample.

Measurements were made on samples after dilution with milli Q water and after dilution with a protein dissociating medium (0.05M EDTA and 2% w/v SDS in milli Q water) (1 ml sample was diluted with 3 ml dissociating medium), to disperse the protein aggregates. This procedure allows the determination of the so-called apparent and 'real' mean particle diameters of fat globules. It was verified that the use of the dissociating medium on unflocculated/unaggregated particles did not significantly affect the particle size distribution (Tomas *et al.*, 1994; Gelin *et al.*, 1994).

3.7 pH measurement

The pH of the samples was measured by Orion 720 A pH meter (Orion research, Boston, MA 02129 USA). The pH meter was first calibrated at pH 4 and then at pH 7 using standard solutions at 20 °C. The pH of the stored samples was measured at 5, 20 or 37 °C by setting the temperature knob of the pH meter to these temperatures.

3.8 Penetration test

The penetration test was performed using an Instron Universal Testing Machine (Table Model 4502, Instron Corporation, USA). The bottle containing the samples was placed on the compression plate of a load cell (compression type-10 N) mounted at the base of the instrument. A 14 mm cylindrical probe was mounted underneath the crosshead. The crosshead was positioned in such a way that the probe was within 0.5 mm of the surface of the milk sample. The crosshead was lowered at the rate of 10 mm/min into the milk sample. The force exerted on the probe was measured by the load cell and recorded continuously by a computer data recorder. Gel strength (failure force) was defined as the force either at the first peak on the force-deformation curve (Dunkerley and Hayes, 1980) or at the point where the slope of the curve changed suddenly. The ratio of penetration force to displacement was taken as an index of gel strength.

3.9 Microbiological analysis

Standard plate count method was used for estimating the bacterial population in the aseptically filled samples initially and after storage for 25 weeks.

3.10 Electrophoresis

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

3.10.1 Preparation of reagents and buffer solutions

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

Acrylamide (29.2 g) and N, N-bis acrylamide (0.8 g) were dissolved in deionised water (Milli Q reagent water system, Millipore Corporation, Bedford, MA, USA), to a final volume of 100 ml, filtered and stored at 4°C in a dark bottle.

3.10.1.2 1.5 M TRIS-HCl buffer, pH 8.8

TRIS (tris hydroxymethyl aminoethane, 18.15 g) (United States Biochemicals Corporation, Cleveland, Ohio, USA), was dissolved in 60 ml of deionised water, the pH adjusted to 8.8 with 1 M HCl and volume made to 100 ml with deionised water and stored at 4 °C.

3.10.1.3 0.5 M TRIS-HCl buffer, pH 6.8

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

3.10.1.4 10% SDS

SDS (10 g) was dissolved with gentle stirring in deionised water and the volume made to 100 ml, and stored at room temperature.

3.10.1.5 SDS-reducing buffer (sample buffer), 100 ml

The following solutions were added to 50 ml of deionised water: 0.5 M TRIS-HCl buffer (12.5 ml), glycerol (10 ml), 10% (w/v) SDS (20 ml), 2-mercaptoethanol (0.25 ml) and 0.05% (w/v) bromophenol blue (2.5 ml). The sample buffer was made fresh each day.

3.10.1.6 5X Electrode (running) buffer, pH 8.3

Electrode buffer (5X) was made by dissolving TRIS (9 g), glycine (43.2 g) and SDS (3 g) with 600 ml deionised water. The pH was adjusted to 8.3 and then stored at 4°C. For each electrophoresis run, 60 ml of 5X stock buffer was diluted with 240 ml of deionised water.

3.10.1.7 Preparation of resolving gel (16% , w/v, 0.375 M TRIS, pH 8.8), 10 ml

The following solutions were mixed: deionised water (2.02 ml), 1.5 M TRIS-HCl buffer (2.50 ml), 10% (w/v) SDS solution (100 µl) and acrylamide/bis mixture (5.3 ml). The mixture was degassed for 15 minutes, followed by the addition of 10% (w/v) ammonium persulphate (50 µl), prepared fresh, and TEMED (tetramethylethylenediamine) (5 µl). After gentle mixing, the contents were poured between electrophoresis casting plates (Bio-Rad Mini Protean, Bio-Rad, Richmond, CA, USA). A small quantity of deionised water was added to form an upper layer and the acrylamide solutions were allowed to polymerise at room temperature for 40 min. The water layer was then removed with filter paper before pouring the stacking gel.

3.10.1.8 Preparation of stacking gel (4.0%, w/v, 0.125 M TRIS, pH 6.8), 5 ml

The following solutions were mixed: deionised water (3.05 ml), 0.5 M TRIS-HCl buffer (1.25 ml), 10% (w/v) SDS solution (50 µl) and acrylamide/bis mixture (0.65 ml). The mixture was degassed for 15 min, followed by the addition of 10% (w/v) ammonium persulphate (25 µl) and TEMED (5 µl). The stacking gel mixture was carefully poured on top of the resolving gel. The slot former (plastic comb) was immediately placed in the gel sandwich. Polymerization was carried out at room temperature for 35-40 min. The comb was removed by pulling it straight up slowly and gently, and the formed slots were rinsed with deionised water. Water was removed using filter paper. The gel plates were placed in the electrode chamber and samples were loaded on the slots.

3.10.2 Sample preparation and running gels

3.10.2.1 Sample preparation

Samples were dispersed in measured aliquots of SDS sample buffer and heated in a water bath at 95 °C for 5 min and cooled to room temperature. The sample (10 µl) were applied to the slots of the SDS gel.

3.10.2.2 Running gels

The gels were run on 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 disappeared from the gel. The approximate running time was 50 min after which gels were removed from the plates and transferred gently to staining solution.

3.10.3 Staining/destaining

The gels were put into plastic boxes and 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 plastic boxes containing gels were mounted on a rocking table in order to stain the gels uniformly with the staining solution. The staining solution was drained carefully after 1 hour and replaced with destaining solution (100 ml of isopropyl alcohol and 100 ml of acetic acid diluted to 1 l). The destaining solution was again changed after 1 hour with fresh destaining solution. The destaining was continued for about 19 hours. After that the destaining solution was replaced with distilled water.

3.11 Transmission electron microscopy

3.11.1 Sample fixation and staining

Milk samples for transmission electron microscopy (TEM) were prepared as follows. Milk (1.8 ml) was fixed by adding 25% glutaraldehyde solution (0.2 ml), blending the mixture on a vortex mixer for 2-3 s, and allowing to stand for 20 min at room

temperature. Fixed sample (1 ml) was then mixed with an equal quantity of hot 3% agar solution, and the mixture blended on a vortex mixer for 2-3 s. The mixture was spread on a microscopic slide, allowed to solidify, and cut into 1 mm^3 blocks.

The sample blocks were transferred into the Bijous bottles and washed in a 0.2M sodium cacodylate-HCl buffer (pH 7.2) for 30 min. The buffer was changed after 60 min, and then again after 60 min. The samples were then post-fixed in 1% (w/v) osmium tetraoxide in cacodylate-HCl buffer for 2 h, washed with distilled water, stained with 1% (w/v) aqueous uranyl acetate solution for 30 min and finally washed again with distilled water.

3.11.2 Sample dehydration and embedding

The stained blocks were dehydrated using a graded series of (50, 70, 90%) ethanol-water mixtures followed by absolute ethanol, and were then embedded in epoxy resin (Araldite CY212, Taab Laboratories, United Kingdom). The embedding procedure involved mixing of incomplete resin mixture (10 ml dodecenyl succinic anhydride (DDSA) + 10 ml epoxy resin + 1 ml butyl phthalate) with the dehydrated blocks and placing them on rollers (speed 14 rpm) for 6 hours, then replacing the incomplete resin mixture with a complete resin mixture (incomplete resin mixture + 0.4 ml benzyldimethylamine (BDMA)) and continued on rollers for another 6 hours in order to complete the infiltration of the resin into the blocks.

3.11.3 Sample moulding and hardening

The infiltrated sample blocks were carefully placed in the plastic moulds and labelled. Freshly prepared complete resin mixture was then poured into the moulds. The moulds were placed in an oven, maintained at 60 °C for 24 h, for hardening.

3.11.4 Trimming and thin sectioning of sample blocks

The hardened blocks, after cooling to room temperature, were trimmed with a block trimmer (Reichert-Jung TM 60, C. Reichert Optische Werke AG, Wien, Austria), carefully sectioned (70-90 nm thickness) with a diamond knife (DDK, Delaware Diamond Knives, Inc., Delaware, USA), using a ultramicrotome (Reichert-Jung Ultracut

E, C. Reichert Optische Werke AG, Wien, Austria). The thin sections were then mounted on 300 mesh copper grids (Probing and structure, Queensland, Australia) previously dipped in chloroform-cellotape mixture, stained with lead citrate for 2 min, washed with distilled water and dried on filter paper.

3.11.5 Viewing and development of electron micrographs

The sections were viewed under a transmission electron microscope (Philips EM 201C, The Netherlands) at an accelerating voltage of 60 kV at the Crown Research Institute (CRI) , Palmerston North. The negatives were enlarged three times and the electron micrographs were developed at the CRI.

4. RHEOLOGICAL PROPERTIES OF MILK CONCENTRATES

4.1 Flow Properties of milk concentrates

4.1.1 Introduction

The viscosity of concentrated milks obtained by membrane separation processes, ultrafiltration (UF) and in particular reverse osmosis (RO), has received relatively little attention. The rapid increase in the use of membrane processes in the dairy industry makes it necessary to study the properties of the resulting fluids. Various workers (Culioli *et al.*, 1974; Randhahn, 1976; Jelen, 1979; de Boer and Nooy, 1980; Kristensen *et al.* 1981; Hallstrom and Dejmek, 1988a, b; Rao and Renner, 1988; Stepp and Smith, 1991; Sierzant and Smith, 1993) have reported the viscosity of milk concentrates obtained by UF whereas other workers (Kocak, 1985; Versteeg, 1985; Gupta and Pal, 1993) have reported the viscosity of milk concentrates obtained by RO. The viscosity of concentrates increases with increase in the protein and total solids contents. Therefore the viscosity of concentrates partially determine the concentration factor (C.F.) that can be reached during membrane processing and the behaviour of the concentrates during processing. It is also important to understand how factors such as heating temperature and storage time may alter the flow behaviour of these concentrates. The objectives of this investigation were:

- to determine the rheological properties of milk concentrates obtained by reverse osmosis;
- to determine if and how these properties depended on the temperature of measurement, concentration, and storage time and temperature;
- finally to determine if and how UHT treatment altered the rheological properties of the concentrates.

4.1.2 Experimental protocol

Bulk raw whole milk from a Friesian herd was obtained from Massey University No. 1 Dairy Farm. Experimental protocols for Trials I and II, showing how samples for rheological measurements were produced, are given in Figs. 4.1 and 4.2. Trial I was carried out in September and October, 1993, on whole milk (A), homogenized milk, skim milk and the corresponding concentrates. Trial II was carried in March, 1995, on whole milk (B) and concentrates of it.

The processing, reverse osmosis concentration and UHT treatment used in Trial I and II are described in detail in Chapter 3.

In Trial I, for each milk type, duplicate sets of experiments (Fig. 4.1) were performed on different days. The samples were collected at various concentration factors in each set of experiments, as and when the particular C.F. was reached. Data obtained from these duplicate sets were more or less similar, hence data from only one set was used.

In Trial II, since the samples at each C.F. had to be subsequently UHT treated (Fig. 4.2), the experiments were planned in such a way that each C.F. experiment was performed on a different day. For each C.F. run (unheated or UHT treated), the feed (1.0X) was taken as a control. Hence there were four sets of data for the feed (1.0X), both unheated and UHT treated. The four sets of data showed similar trends in values, hence only one set of data was used.

4.1.3 Results and Discussion

4.1.3.1 Compositions of milks and their concentrates

The compositions of various milks and their concentrates from Trials I and II are shown in Tables 4.1 and 4.2 respectively. Unconcentrated milk, i.e. feed (1.0X), was considered a reference. In all milks there was an increase in the concentration of fat, protein and total solids in proportion to the concentration factor obtained. There was little difference between the initial compositions of whole milk (A) and the homogenized milk used for Trial I. The whole milk (B) used for trial II had a higher fat content than whole milk (A). Skim milk, when concentrated to the same concentration factor as that of whole milk, contained less total solids owing to the absence of fat.

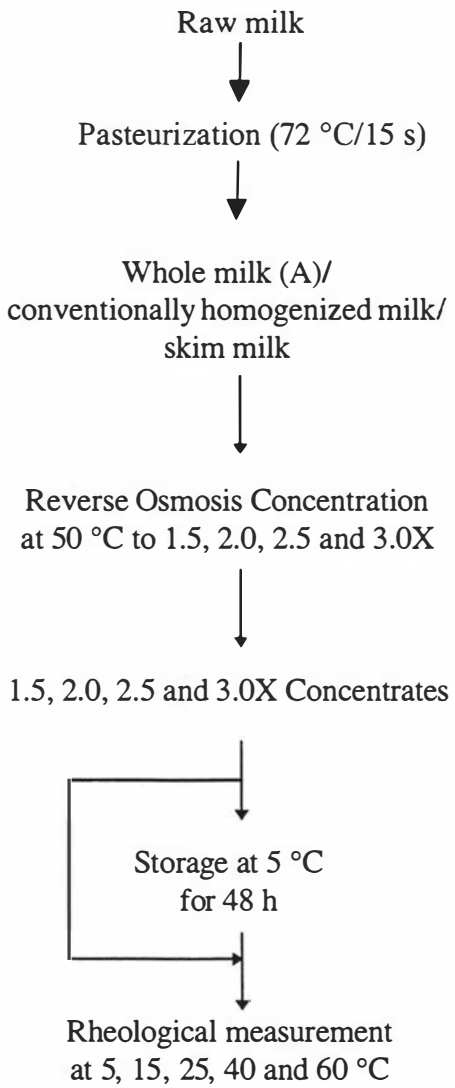

Fig. 4.1 Experimental protocol for Trial I.

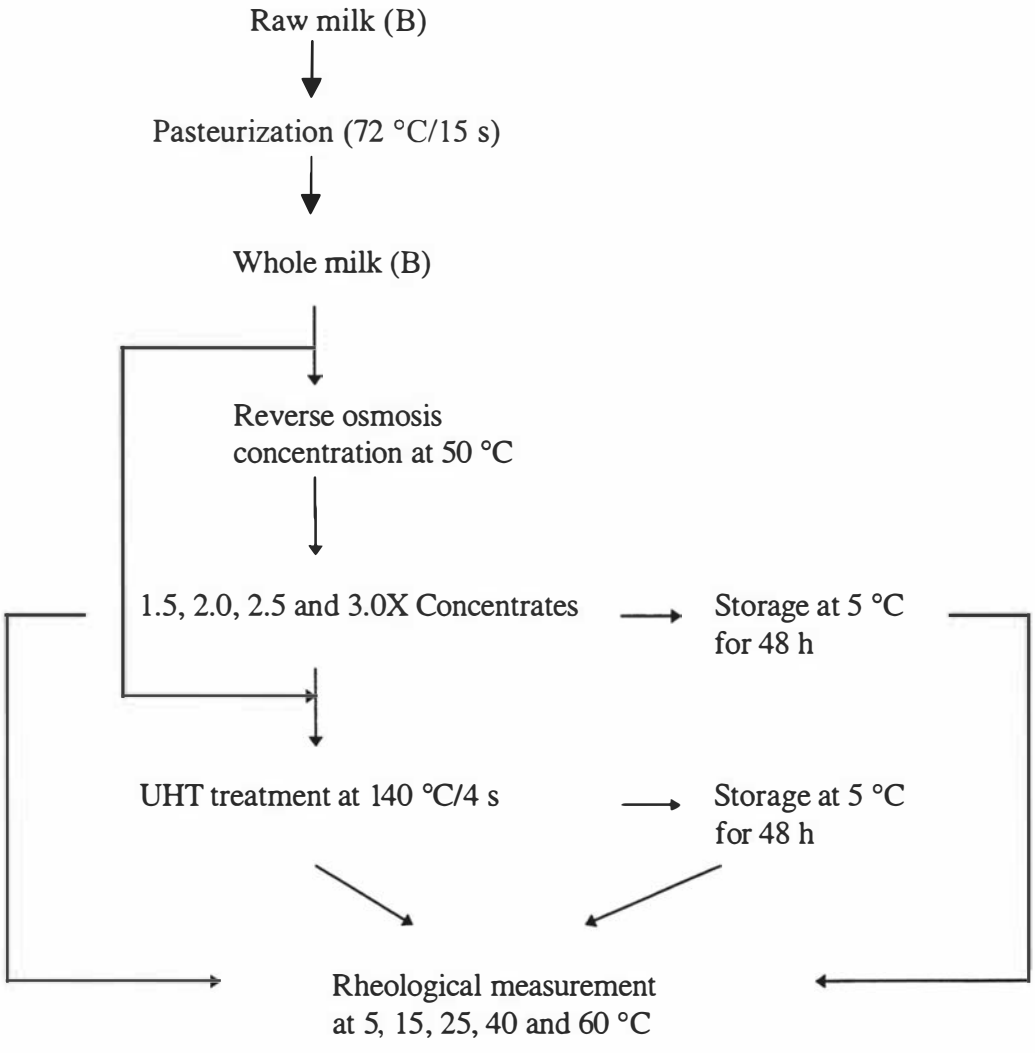

Fig. 4.2 Experimental protocol for Trial II.

At a given concentration factor, differences in total solids between whole milk (A) and homogenized milk may have been due to the fact that the nominal concentration factor may not have been reached at the time a sample was collected. There may also have been errors in total solids measurement. At a given concentration factor, differences in total solids between whole milk (A) and whole milk (B) were probably mainly due to the fact that they were different milks with different initial total solids contents.

The exact concentration factors were difficult to achieve from batch to batch. Based on % total solids measurement, the actual concentration factors of samples 1.5X, 2.0X, 2.5X and 3.0X were on average, 1.43, 1.90, 2.40, and 2.92 for whole milk (A); 1.46, 1.93, 2.43, and 2.99 for homogenized milk; 1.42, 1.83, 2.29, and 2.80 for skim milk; and 1.51, 2.00, 2.58, and 3.02 for whole milk (B) and UHT treated whole milk. It was assumed that UHT treatment did not alter the total solids content.

Non-casein nitrogen (NCN) values for Trial II are shown in Table 4.2. NCN increased with increase in concentration for both whole milk (B) and UHT treated whole milk. Also at each concentration, a decrease in the NCN values was observed as a result of UHT treatment. This possibly may be attributed to the denaturation of whey proteins.

Table 4.1 Compositions of whole milk (A), homogenized milk, skim milk and their concentrates (Trial I).

Milks	C.F.	FAT %	Protein %	Casein %	T.S. %
Whole milk (A)	1.0X	4.2	3.50	3.17	13.36
	1.5X	6.1	5.00	4.56	19.08
	2.0X	8.2	6.73	6.12	25.26
	2.5X	10.6	8.49	7.62	32.00
	3.0X	12.9	10.45	9.53	39.00
Homogn. milk	1.0X	4.2	3.57	3.22	13.36
	1.5X	6.6	5.20	4.68	19.56
	2.0X	8.4	6.94	6.26	25.84
	2.5X	10.8	8.70	7.86	32.40
	3.0X	13.2	10.62	9.75	40.00
Skim milk	1.0X	-	3.43	3.08	9.30
	1.5X	-	4.86	4.31	13.25
	2.0X	-	6.34	5.63	17.06
	2.5X	-	7.87	6.97	21.34
	3.0X	-	9.82	8.62	26.05

Table 4.2 Compositions of whole milk (B) and its concentrates, UHT treated whole milk (B), and UHT treated concentrates (Trial II).

C.F.	Fat %	Protein %	Casein %	Non-casein nitrogen %		T.S. %
				Whole milk (B)	UHT treated whole milk (B)	
1.0X	4.60	3.42	3.09	0.051	0.022	13.25
1.5X	7.40	5.02	4.54	0.075	0.029	19.96
2.0X	9.40	6.80	6.25	0.084	0.030	26.57
2.5X	12.20	8.65	7.94	0.112	0.048	34.24
3.0X	13.50	11.04	10.23	0.126	0.060	40.08

4.1.3.2 Characterization of Rheological Behaviour

The experimental shear stress-shear rate data obtained with the Bohlin rheometer (section 3.5, Chapter 3) for the various milks and concentrates were fitted with the power law equation:

$$\tau = k\,\dot{\gamma}^{n} \qquad \text{(Pa)} \qquad (4.1)$$

where τ = shear stress (Pa)

$\dot{\gamma}$ = shear rate (s^{-1})

k = consistency index ($Pa.s^{n}$)

n = flow behaviour index (dimensionless)

$$\log \tau = \log k + n \log \dot{\gamma} \qquad (4.2)$$

The slope of a plot of log τ *versus* log $\dot{\gamma}$ gives the value of the flow behaviour index (n), and the intercept at log $\dot{\gamma} = 0$ (i.e. $\dot{\gamma} = 1$) is the value of log k.

Figs. 4.3a-e show typical flow curves obtained for whole milk (A), homogenized milk, whole milk (B), UHT treated whole milk (B), skim milk and their corresponding 3.0X concentrates before storage, at various temperatures in the range 5-60 °C. On plotting similar data obtained after storage for 48 h at 5 °C, there was a slight upwards shift in all the flow curves (results not shown). At all temperatures, there was a linear relationship between τ and $\dot{\gamma}$ and the values of τ decreased with increasing temperature at a given $\dot{\gamma}$ The values of k and n obtained at various temperatures for all the milk types are given in Tables 4.3-4.7.

In all samples, the value of n was little affected by either temperature or total solids concentration either before or after storage at 5 °C for 48 h. Milks at the normal concentration (1.0 X) can be considered Newtonian, ($n \approx 1$). Whole milk (B) (Table 4.5) was the only milk that exhibited significant shear thinning, and then only at 3.0X concentration. Apart from this, there were no obvious trends with concentration or temperature.

These results are in agreement with Fernandez-Martin (1972) who found that unconcentrated milks were Newtonian liquids, but that concentrated milks (up to about 30% total solids) showed a weak dependence on shear, with the n value ranging between 0.9 and 1.0. Similar results were obtained by Randhahn (1976) and Stepp and Smith

(1991) for skim milk UF concentrates, and Sierzant and Smith (1993) for whole milk UF concentrates. Randhahn (1976) reported a decrease in n from 0.98 for skim milk (9% total solids) to 0.675 for skim milk concentrate (26.7% total solids) and an increase in k from 0.020 Pa s^n for skim milk (9% total solids) to 4.284 Pa s^n for skim milk concentrates (26.7% total solids) measured at 20 °C. Sierzant and Smith (1993) reported a decrease in n from 1.03 for whole milk (12.30% total solids) to 0.806 for UF whole milk concentrate (34.93% total solids) and an increase in k from 0.0034 Pa s^n for whole milk (12.30% total solids) to 0.0087 Pa s^n for UF whole milk concentrate (34.93% total solids). In these results, since the n values are different, k values are not strictly comparable.

The average values of n determined in the present work are 0.98 (± 0.032) for whole milk (A); 0.98 (± 0.035) for homogenized milk, 0.93 (± 0.105) for whole milk (B), 0.98 (± 0.045) for UHT treated whole milk and 0.97 (± 0.038) for skim milk. When the samples were stored at 5 °C for 48 h, the average n values remained almost unaffected. In the literature, n values for RO whole milk concentrates have not been reported so far. However, the values reported here are in the same range as for UF concentrates.

The k values reported here showed definite trends with respect to concentration and temperature. The k values in general increased with increase in concentration and decreased with increase in temperature in all the unheated samples with some exceptions. In UHT treated whole milk the values of k increased as concentration increased but a peculiar trend was observed with samples having 19.96% total solids, which may be due to either an error in the measurement or some unknown reasons. In all samples, the values of k were generally higher when the samples were stored at 5 °C for 48 h and measurement made at 5 °C. No such trend was observed at 60 °C where k values before and after storage were generally similar.

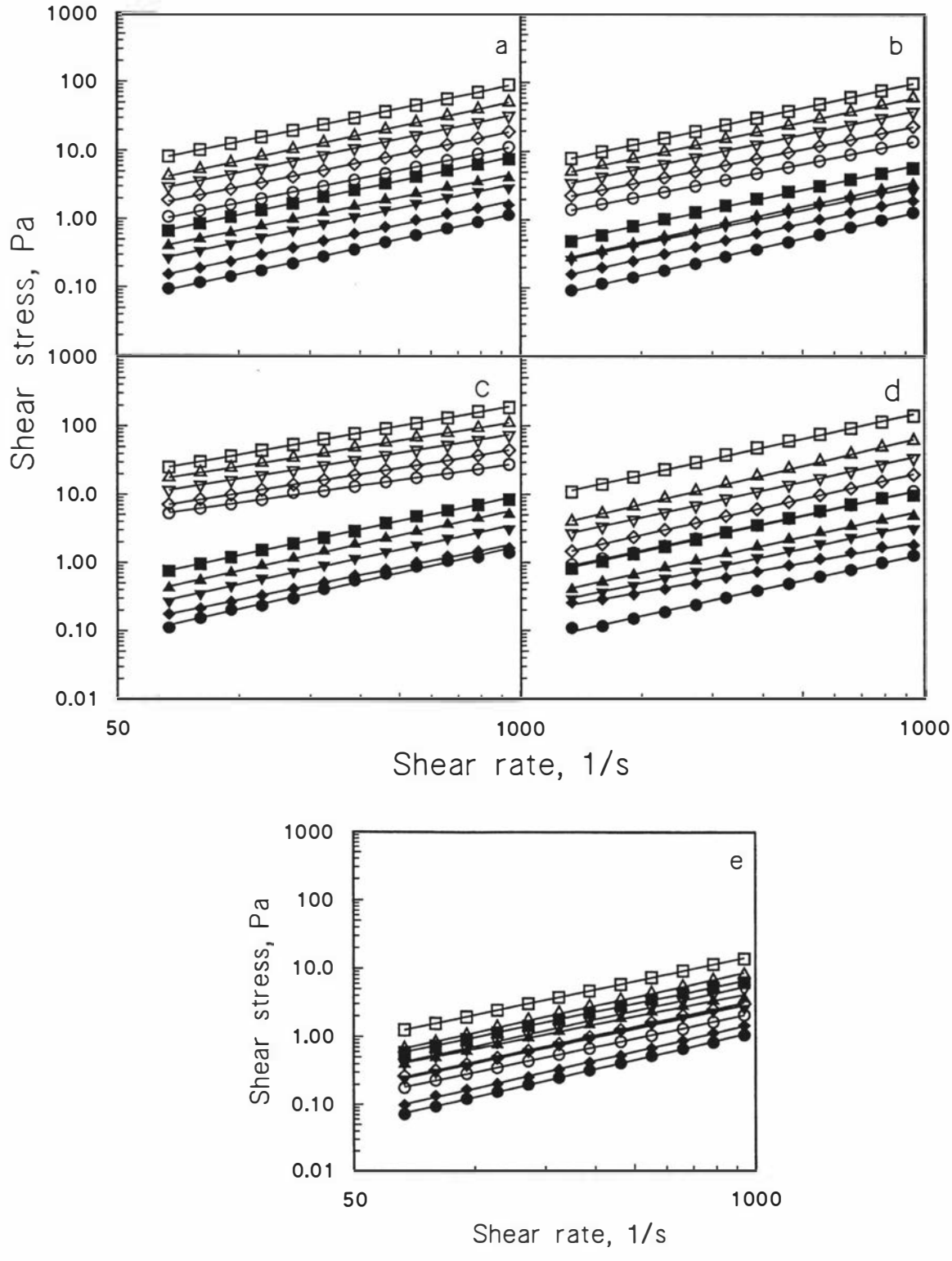

Fig. 4.3 Flow curves for (a) whole milk (A) (1.0X) and its concentrate (3.0X), (b) homogenized milk (1.0X) and its concentrate (3.0X), (c) whole milk (B) (1.0X) and its concentrate (3.0X), (d) UHT treated whole milk (B) (1.0X) and its concentrate (3.0X), (e) skim milk (1.0X) and its concentrate (3.0X) at various temperatures; 1.0X, ■ 5, ▲ 15, ▼ 25, ♦ 40, or ● 60 °C and 3.0X, □ 5, Δ 15, ∇ 25, ◊ 40, or ○ 60 °C.

Table 4.3 Flow behaviour constants for whole milk (A) at different temperatures and total solids content

C.F.	Total Solids %	Parameter		Temperature (°C)				
				5	15	25	40	60
1.0X	13.36	k	a	.0102	.0072	.0042	.0023	.0011
			b	.0140	.0058	.0044	.0022	.0010
		n	a	0.98	0.94	0.97	0.98	1.01
			b	0.91	0.97	0.93	1.00	1.02
1.5X	19.08	k	a	.0065	.0073	.0041	.0031	.0016
			b	.0146	.0074	.0056	.0031	.0017
		n	a	1.06	1.00	1.00	0.98	1.01
			b	0.99	0.99	0.98	0.99	1.00
2.0X	25.26	k	a	.0165	.0106	.0090	.0057	.0037
			b	.0379	.0122	.0079	.0053	.0033
		n	a	1.04	0.98	0.96	1.00	0.94
			b	0.90	0.99	0.99	0.96	0.96
2.5X	32.00	k	a	.0363	.0199	.0164	.0094	.0060
			b	.0330	.0236	.0168	.0122	.0069
		n	a	0.98	1.00	0.96	0.97	0.96
			b	1.05	1.00	0.98	0.95	0.95
3.0X	39.00	k	a	.1402	.0602	.0469	.0327	.0176
			b	.1056	.0999	.0559	.0370	.0270
		n	a	0.95	0.99	0.96	0.93	0.95
			b	1.05	0.98	0.97	0.93	0.89

(a) before (b) after 48 h storage at 5 °C
$r^2 > 0.972$ for (a) }
$r^2 > 0.964$ for (b) } Significant at $p < 0.002$
Units of k : Pa s^n

Table 4.4 Flow behaviour constants for homogenized milk at different temperatures and total solids content

C.F.	Total Solids %	Parameter		Temperature (°C)				
				5	15	25	40	60
1.0X	13.36	k	a	.0072	.0039	.0043	.0021	.0009
			b	.0118	.0053	.0042	.0027	.0011
		n	a	0.99	1.00	0.96	1.00	1.05
			b	0.98	0.99	0.97	0.96	1.02
1.5X	19.56	k	a	.0223	.0063	.0049	.0029	.0016
			b	.0234	.0062	.0062	.0032	.0017
		n	a	0.92	0.99	0.99	1.00	1.02
			b	0.95	1.00	0.97	0.98	0.98
2.0X	25.84	k	a	.0134	.0105	.0079	.0056	.0035
			b	.0143	.0171	.0094	.0050	.0036
		n	a	1.06	1.00	1.00	0.98	0.98
			b	1.05	0.96	0.98	0.99	0.99
2.5X	32.40	k	a	.0451	.0226	.0176	.0101	.0077
			b	.0656	.0317	.0197	.0142	.0100
		n	a	0.96	0.99	0.97	0.98	0.96
			b	0.95	0.98	0.98	0.94	0.93
3.0X	40.00	k	a	.1136	.0779	.0595	.0431	.0276
			b	.2750	.1113	.0772	.0538	.0337
		n	a	0.99	0.98	0.95	0.92	0.91
			b	0.92	0.96	0.94	0.90	0.89

(a) before (b) after 48 h storage at 5 °C

$r^2 > 0.977$ for (a) }

$r^2 > 0.954$ for (b) } Significant at $p < 0.004$

Units of k : Pa s^n

Table 4.5 Flow behaviour constants for whole milk (B) at different temperatures and total solids content

C.F.	Total Solids %	Parameter		Temperature (°C)				
				5	15	25	40	60
1.0X	13.25	k	a	.0113	.0057	.0039	.0032	.0014
			b	.0032	.0065	.0053	.0026	.0016
		n	a	0.98	1.02	0.99	0.93	1.03
			b	1.15	0.99	0.95	0.96	0.98
1.5X	19.96	k	a	.0084	.0069	.0068	.0043	.0030
			b	.0112	.0096	.0060	.0038	.0021
		n	a	1.09	1.02	0.97	0.95	0.94
			b	1.04	0.96	0.99	0.97	0.99
2.0X	26.57	k	a	.0213	.0180	.0102	.0056	.0035
			b	.0219	.0129	.0126	.0071	.0044
		n	a	0.99	0.94	0.96	0.97	0.96
			b	0.99	0.99	0.94	0.94	0.93
2.5X	34.24	k	a	.0530	.0325	.0221	.0148	.0084
			b	.0897	.0404	.0215	.0166	.0092
		n	a	0.98	0.97	0.97	0.96	0.97
			b	0.96	0.98	1.01	0.95	0.96
3.0X	40.08	k	a	.7948	.7429	.4722	.2991	.3165
			b	1.2563	.9038	1.0183	.4844	.3921
		n	a	0.80	0.74	0.75	0.74	0.66
			b	0.78	0.73	0.65	0.67	0.61

(a) before (b) after 48 h storage at 5 °C
$r^2 > 0.971$ for (a) }
$r^2 > 0.957$ for (b) } Significant at $p < 0.008$
Units of k : Pa s^n

Table 4.6 Flow behaviour constants for UHT treated whole milk (B) at different temperatures and total solids content

C.F.	Total Solids %	Parameter		Temperature (°C)				
				5	15	25	40	60
1.0X	13.25	k	a	.0108	.0054	.0046	.0068	.0012
			b	.0075	.0063	.0043	.0023	.0013
		n	a	1.01	1.01	0.97	0.83	1.00
			b	1.06	1.01	0.98	0.99	1.01
1.5X	19.96	k	a	.3371	.1228	.0932	.0275	.0121
			b	.4283	.2328	.1078	.0212	.0098
		n	a	0.73	0.80	0.78	0.86	0.90
			b	0.70	0.71	0.76	0.89	0.92
2.0X	26.57	k	a	.1058	.0327	.0139	.0101	.0054
			b	.2821	.0770	.0109	.0090	.0057
		n	a	0.96	1.00	1.04	1.00	1.00
			b	0.86	0.94	1.18	1.04	1.02
2.5X	34.24	k	a	.0489	.0394	.0272	.0153	.0091
			b	1.4200	.7342	.0478	.0233	.0122
		n	a	1.18	1.07	1.05	1.04	1.03
			b	0.76	0.73	1.10	1.00	1.01
3.0X	40.08	k	a	.1488	.0390	.0348	.0179	.0126
			b	.3466	.3772	.0909	.0296	.0209
		n	a	1.01	1.08	1.01	1.02	0.99
			b	0.96	0.82	0.94	0.97	0.95

(a) before (b) after 48 h storage at 5 °C

$r^2 > 0.995$ for (a) }

$r^2 > 0.923$ for (b) } Significant at $p < 0.014$

Units of k : Pa s^n

Table 4.7 Flow behaviour constants for skim milk at different temperatures and total solids content

C.F.	Total Solids %	Parameter		Temperature (°C)				
				5	15	25	40	60
1.0X	9.30	k	a	.0104	.0084	.0038	.0012	.0008
			b	.0152	.0102	.0043	.0018	.0010
		n	a	0.94	0.90	0.97	1.04	1.05
			b	0.93	0.89	0.95	1.03	1.03
1.5X	13.25	k	a	.0140	.0058	.0039	.0019	.0011
			b	.0148	.0067	.0039	.0021	.0007
		n	a	0.89	0.96	0.98	1.00	1.02
			b	0.93	1.00	0.98	0.99	1.09
2.0X	17.06	k	a	.0087	.0044	.0048	.0029	.0016
			b	.0134	.0074	.0046	.0024	.0014
		n	a	0.99	1.02	0.97	0.95	0.97
			b	0.98	0.97	0.98	0.99	1.02
2.5X	21.34	k	a	.0172	.0077	.0043	.0032	.0019
			b	.0151	.0093	.0050	.0030	.0021
		n	a	0.93	0.97	1.00	0.97	0.97
			b	1.07	0.99	0.99	1.00	0.99
3.0X	26.05	k	a	.0206	.0089	.0059	.0037	.0031
			b	.0271	.0094	.0068	.0043	.0024
		n	a	0.95	1.00	0.99	0.98	0.95
			b	0.96	1.00	0.99	0.99	1.01

(a) before (b) after 48 h storage at 5 °C
$r^2 > 0.901$ for (a) }
$r^2 > 0.948$ for (b) } Significant at $p < 0.013$
Units of k : Pa s^n

4.1.3.3 Effect of concentration

For Newtonian fluids, Newton's law of viscosity is:

$$\eta = \tau / \dot{\gamma} \tag{4.3}$$

where τ is the shear stress (Pa) , η is the coefficient of viscosity (Pa s) and $\dot{\gamma}$ (s^{-1}) is the shear rate.

For all the samples the measured viscosity decreased with increasing shear rate. Such behaviour is called shear thinning and implies that the liquid has not a true but an apparent viscosity. By analogy with Eq. (4.3), Eq. (4.1) can be used to define a shear rate dependent apparent viscosity:

$$\eta_{app} = \tau / \dot{\gamma} \qquad \text{(Pa.s)}$$

$$= k \cdot \dot{\gamma}^{n-1} \tag{4.4}$$

Fig. 4.4 illustrates the apparent viscosity calculated in this way as a function of total solids at a fixed shear rate of 367 s^{-1} for all milks at the various measurement temperatures before and after 48 h storage. The apparent viscosity increased with increase in total solids concentration at a given temperature and decreased with increase in temperature at a given total solids concentration. The higher viscosities at lower temperatures were probably the result of higher milk plasma viscosity and higher casein micelle hydrodynamic volume (Walstra and Jenness, 1984). Homogenized milk was more viscous than unhomogenised whole milk (A) at the same temperature and fat content. This was due to the increase in the volume of the fat fraction as a consequence of a larger number of smaller fat globules, and thus a greater fat surface area; the MFGM is counted as part of the fat fraction (Mulder and Walstra, 1974). Whole milk (B) was found to be more viscous than whole milk (A) at a given total solids content, more so at a higher total solids level, probably because of the fact the two milks have different initial composition and also due to the fact that the nominal concentration factor may not have been reached at the time a sample was collected. UHT treated whole milk (B) was more viscous in 20-35% total solids range but was less viscous at higher concentration (40% total solids) (Fig. 4.4d) than the unheated milk. When the samples were stored at 5 °C for 48 h, the apparent viscosity at a given temperature and total solids concentration was similar to or greater than the value measured at the same temperature before storage (Fig. 4.4). In the case of milk containing

fat, the viscosity was higher after storage at 5 °C for 48 h particularly at a measurement temperature of 5 °C. The temperature plays an important part because of the change in the ratio of the solid to liquid fat with change in the temperature. At lower temperatures, the fat will be present in solid or crystalline form and hence results in an increase in the viscosity, where as at temperatures over 40 °C, most of the fat will be present in liquid or molten state and therefore results in a decrease in the viscosity of the milk and its concentrates. In UHT treated whole milk (B) there was an increase in the apparent viscosity especially above 25% total solids after storage when measured at 5 °C. There was only a slight increase in the viscosity of skim milk under similar conditions.

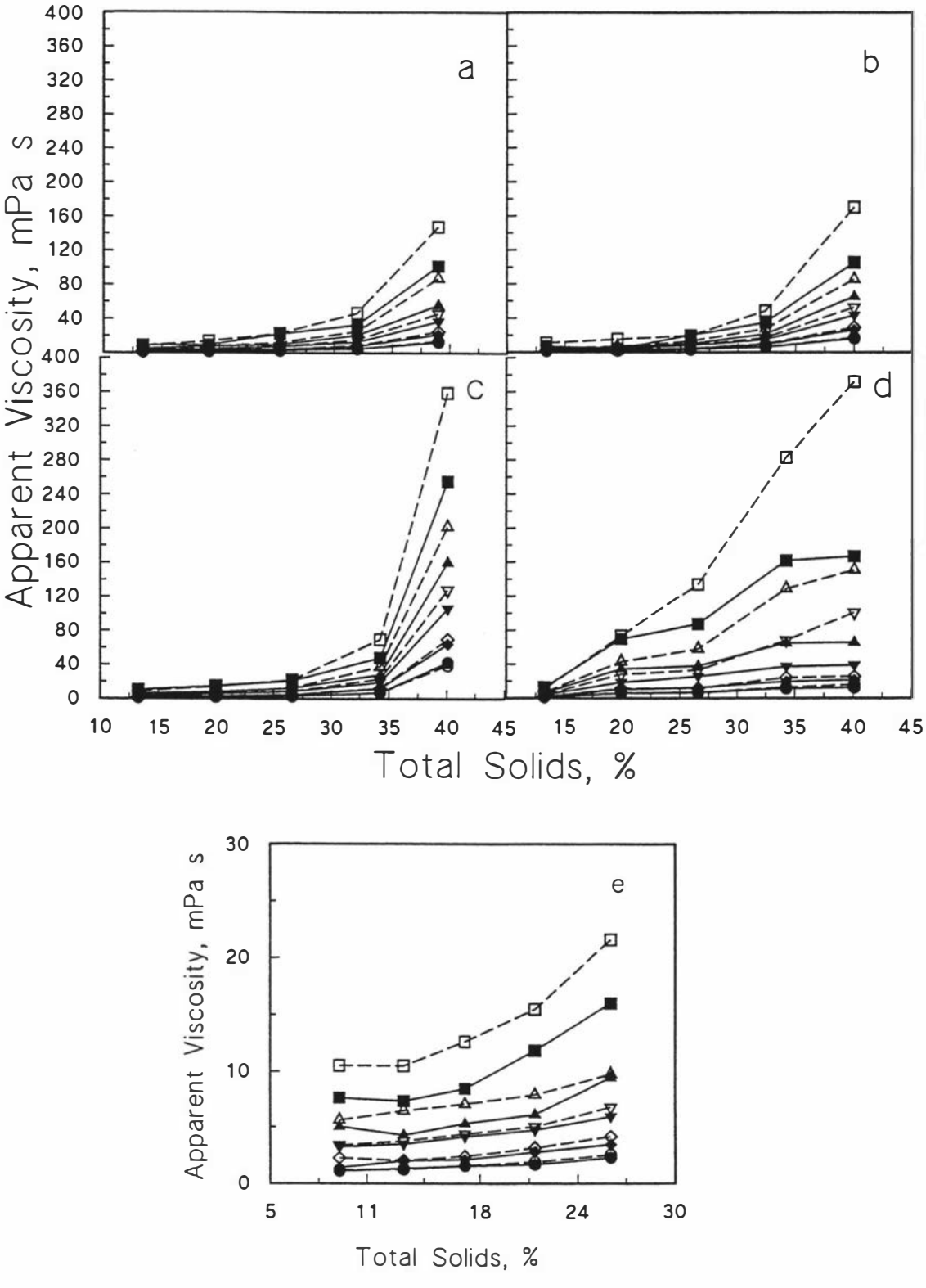

Fig. 4.4 Apparent viscosity at a shear rate 367 s^{-1} as a function of total solids for (a) whole milk (A) and its concentrates, (b) homogenized milk and its concentrates, (c) whole milk (B) and its concentrates, (d) UHT treated whole milk (B) and its concentrates, (e) skim milk and its concentrates at various temperatures; ■, □ 5, ▲, Δ 15, ▼, ∇ 25, ♦, ◊ 40, or ●, ○ 60 °C. Dotted lines with open symbols represent milks and concentrates that had been stored for 48 h at 5 °C.

Table 4.8 Percentage increase in viscosity (measured at 5 °C and at a shear rate 367 s^{-1}) of various samples

Milks	Total solids %	% increase in Viscosity after storage for 48 h at 5 °C
Whole milk (A)	25.26	3.13
	39.00	45.00
Homogenized milk	25.84	2.75
	40.00	45.00
Whole milk (B)	26.57	4.80
	40.00	41.00
UHT treated Whole Milk (B)	26.57	53.00
	40.00	123.00
Skim milk	26.05	35.00

On storage for 48 h at 5 °C, the percentage increase in the viscosity for various milks are given in Table 4.8. The data in the table suggests that milk fat makes a significant contribution towards increase in viscosity after storage, which may be due to the time-dependent solidification of fat at low temperatures. At higher measurement temperatures, i.e. 40 and 60 °C, no pronounced increase in viscosity was observed on storage which may be due to the fact that at these temperatures all the fat would have been in the liquid state (Caffyn, 1951). Also as result of UHT treatment there was significant increase in the viscosity of whole milk samples. This may be due to the denaturation of the whey proteins and their reaction with κ-casein. Also as a result of subsequent UHT treatment of whole milk (B), at 26.50% T.S., the percentage increase in the viscosity values was 320.00% for un-stored milks, whereas the combined effects of UHT treatment and storage for 48 h at 5 °C of the same milk, at similar total solids resulted in an increase of viscosity to 544.00%. This increase may be due to the fact that upon UHT treatment and storage, there is swelling of the micelles which results in the increase in the hydrodynamic volume of casein micelles.

4.1.3.4 Effect of temperature

As the flow behaviour index, n, remains essentially constant with temperature, and the consistency index, k, decreases with increasing temperature, an Arrhenius equation was used to quantify the effect of temperature on k:

$$k = A\, e^{E/RT} \qquad (\mu Pa\ s^n) \tag{4.5}$$

where E is the activation energy (kJ/mol), R is the universal gas constant, A is the frequency factor ($\mu Pa\ s^{-1}$) and T is the absolute temperature ($^\circ K$). The units of A are questionable because the units of k depend on n. Fig.4.5a-e shows the Arrhenius type relationship between consistency index and temperature. By plotting log k against 1/T, the parameters E/R and A were obtained.

$$\log k = (E / 2.303\ R)\ (1/T) + \log A \tag{4.6}$$

The values of E and A for whole milk (A), homogenized milk, skim milk and their concentrates are given in Table 4.9; and for whole milk (B), UHT treated whole milk (B) and their concentrates in Table 4.10. For each milk type, the values of E and A vary rather haphazardly with increase in concentration. This could be due to the difficulties in measuring very low viscosities especially at higher temperatures. The ranges of the values of the activation energy and the frequency factor are given in Table 4.11.

The values of activation energy generally increased for all milks after storage, whereas the values of frequency factor decreased after storage. There are no published values for E and A for concentrated milks in the literature with which the current results can be compared. However, Tang *et al.* (1993) determined values of E in the range 16.9-26.0 kJ/mol and values of the frequency factor A from 0.69-2.71 $\mu Pa\ s^n$ for whey protein concentrate solutions containing 2-30% total solids.

The Arrhenius plots shown in Fig. 4.5 were found to be linear for unheated samples, which suggests that the state of the fat and therefore the deformability of fat globules (in the case of whole and homogenized milks) seemed not to matter. There is about a 50% increase in solid fat to be expected when the temperature is decreased from 60 to 5 °C. In the case of UHT treated whole milk (B) the Arrhenius plots showed no definite pattern, which could have been due to the formation of aggregates as a result of heating.

The rheological properties of concentrated milks are very complex and depend mainly on temperature and concentration and on the physical states of their dispersed phases, milk fat and proteins.

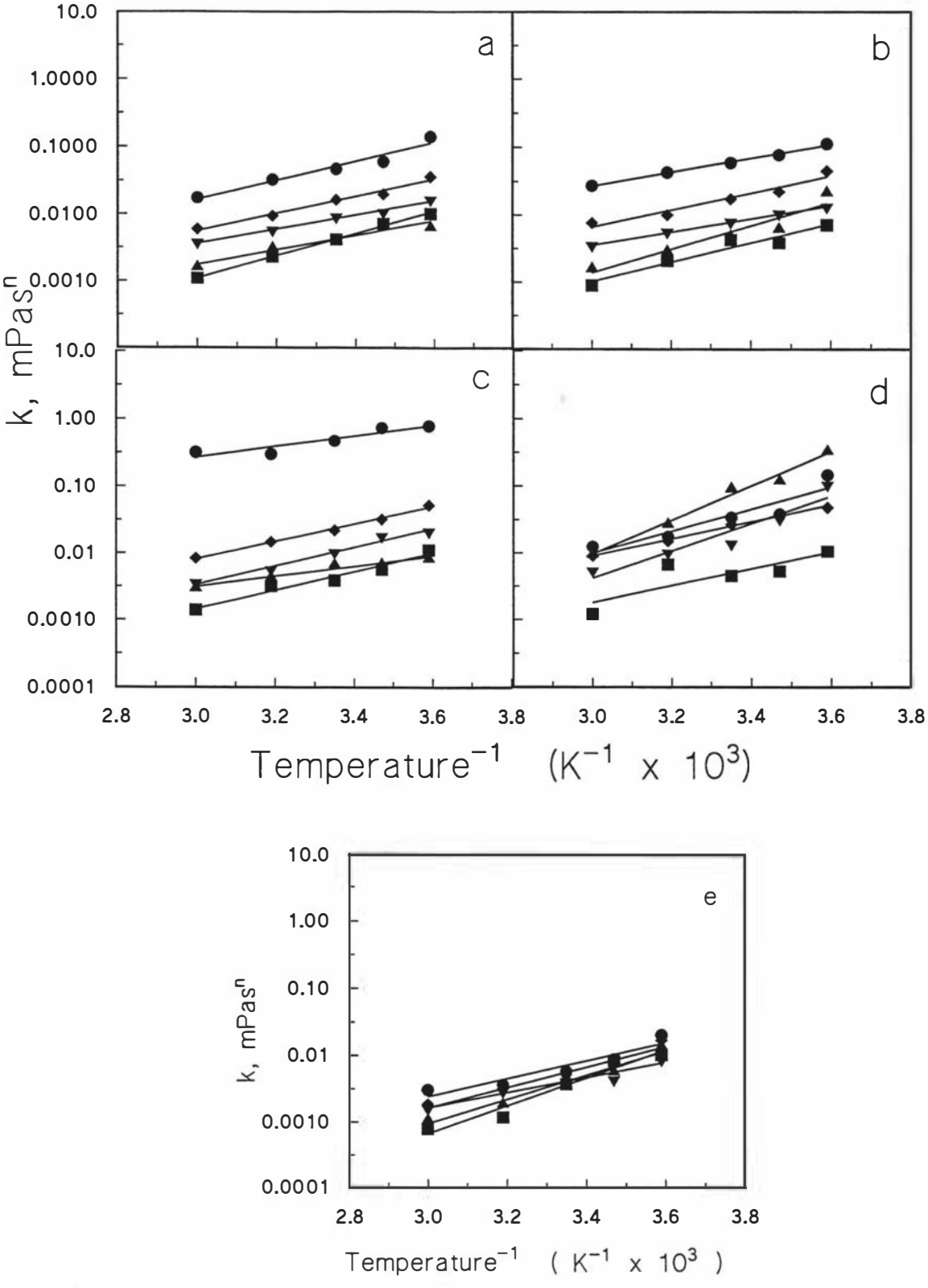

Fig. 4.5 $\log_{10}$ of consistencey index, k, as a function of the reciprocal of absolute temperature for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk at various concentration factors (total solids) (C.F.); ■ 1.0X, ▲ 1.5X, ▼ 2.0X, ♦ 2.5X, or ● 3.0X before storage.

Table 4.9 Values of activation energy of flow E and parameter A in the Arrhenius equation for whole milk (A), homogenized milk, skim milk and their concentrates.

C.F.		Whole milk (A)			Homogenized milk			Skim milk		
		E	A	r^2	E	A	r^2	E	A	r^2
1.0X	a	31.9138	0.0110	0.997	27.6994	0.0466	0.941	40.0862	0.0003	0.957
	b	35.4141	0.0027	0.982	30.8550	0.0169	0.970	40.5170	0.0003	0.980
1.5X	a	21.1527	0.8497	0.928	33.6793	0.0072	0.910	34.8339	0.0033	0.972
	b	29.2714	0.0422	0.986	33.1182	0.0098	0.900	36.2470	0.0019	0.978
2.0X	a	20.5208	2.2192	0.989	18.9223	3.8618	0.998	21.7769	0.6462	0.934
	b	31.6209	0.0300	0.900	22.8435	0.9176	0.909	31.8296	0.0131	0.990
2.5X	a	24.5955	0.7952	0.980	24.2336	1.0641	0.947	29.3710	0.0412	0.937
	b	21.5854	2.9234	0.996	25.2906	0.9289	0.927	28.3695	0.0646	0.966
3.0X	a	26.7535	1.0782	0.948	19.3911	24.9516	0.994	25.6831	0.2321	0.892
	b	21.0340	12.7908	0.957	27.3873	1.5055	0.926	31.4236	0.0252	0.932

(a) before (b) after storage for 48 h at 5 °C.
p = 0.000014 - 0.015
Units: E = kJ/mol, A = μPa s^n

Table 4.10 Values of activation energy of flow E and parameter A in the Arrhenius equation for whole milk (B), UHT treated whole milk (B) and their concentrates.

C.F.		Whole milk (B)			UHT treated whole milk (B)		
		E	A	r^2	E	A	r^2
1.0X	a	26.9105	0.0879	0.958	24.4404	0.2692	0.686
	b	14.2802	11.3841	0.504	25.9052	0.1162	0.986
1.5X	a	14.6832	15.6819	0.955	46.3016	0.0006	0.981
	b	24.4672	0.3163	0.988	56.5726	0.000012	0.979
2.0X	a	27.1632	0.1850	0.982	39.3586	0.0028	0.907
	b	21.7539	1.7266	0.969	54.1561	0.000010	0.827
2.5X	a	25.290	0.8910	0.993	24.6510	1.2540	0.992
	b	30.2997	0.1450	0.940	71.5807	0.00000004	0.883
3.0X	a	15.2719	1087.42	0.863	31.6630	0.1100	0.869
	b	17.0021	832.530	0.896	45.9416	0.0010	0.910

(a) before (b) after storage for 48 h at 5 °C.
p = 0.00024 - 0.17
Units: E = kJ/mol, A = μPa s^n

Table 4.11 The ranges of the values of activation energy, E (kJ/mol) and frequency factor, A (μ Pas ^{n}) for various milks.

Milks		E (kJ/mol)	A (μ Pas ^{n})
Whole milk (A)	a	20.52-31.91	0.01-2.21
	b	21.03-35.41	0.0027-12.79
Homogenized milk	a	18.92-33.68	0.007-24.95
	b	22.84-33.11	0.009-1.50
Whole milk (B)	a	14.68-27.16	0.087-1087.42
	b	14.28-30.29	0.140-832.50
UHT treated whole milk (B)	a	24.44-46.30	0.0006-1.25
	b	25.90-71.58	4.0×10^{-8} -0.1162
Skim milk	a	21.77-40.08	0.0003-0.646
	b	28.37-40.52	0.0003-0.064

(a) before (b) after storage for 48 h at 5°C

4.1.3.5 Combined effects of temperature and concentration

From the above results, it can be summarised that the viscosity of RO concentrated milks increased with increase in the concentration, and that the viscosity of milk and its concentrates decreased with increase in temperature. In this section mathematical modelling of the combined effects of temperature and concentration on the viscosity of milk was performed using shift factor and Fernandez-Martin (1972) approaches.

4.1.3.5.1 Using a shift factor

To obtain equations for predicting k in terms of temperature and total solids content, a shift factor was used to collapse the set of log k *versus* 1/T curves for each type of milk on to one curve. The most satisfactory shift factor identified was the value of log k at 1/T = 3.3 (K^{-1} x 10^3). This value of log k was termed log k^*. Each log k^* was predicted using the appropriate Arrhenius equation. When all log k values were divided by the appropriate log k^* shift factors, reasonably satisfactory coincidence of the curves in each set was obtained (Fig. 4.6).

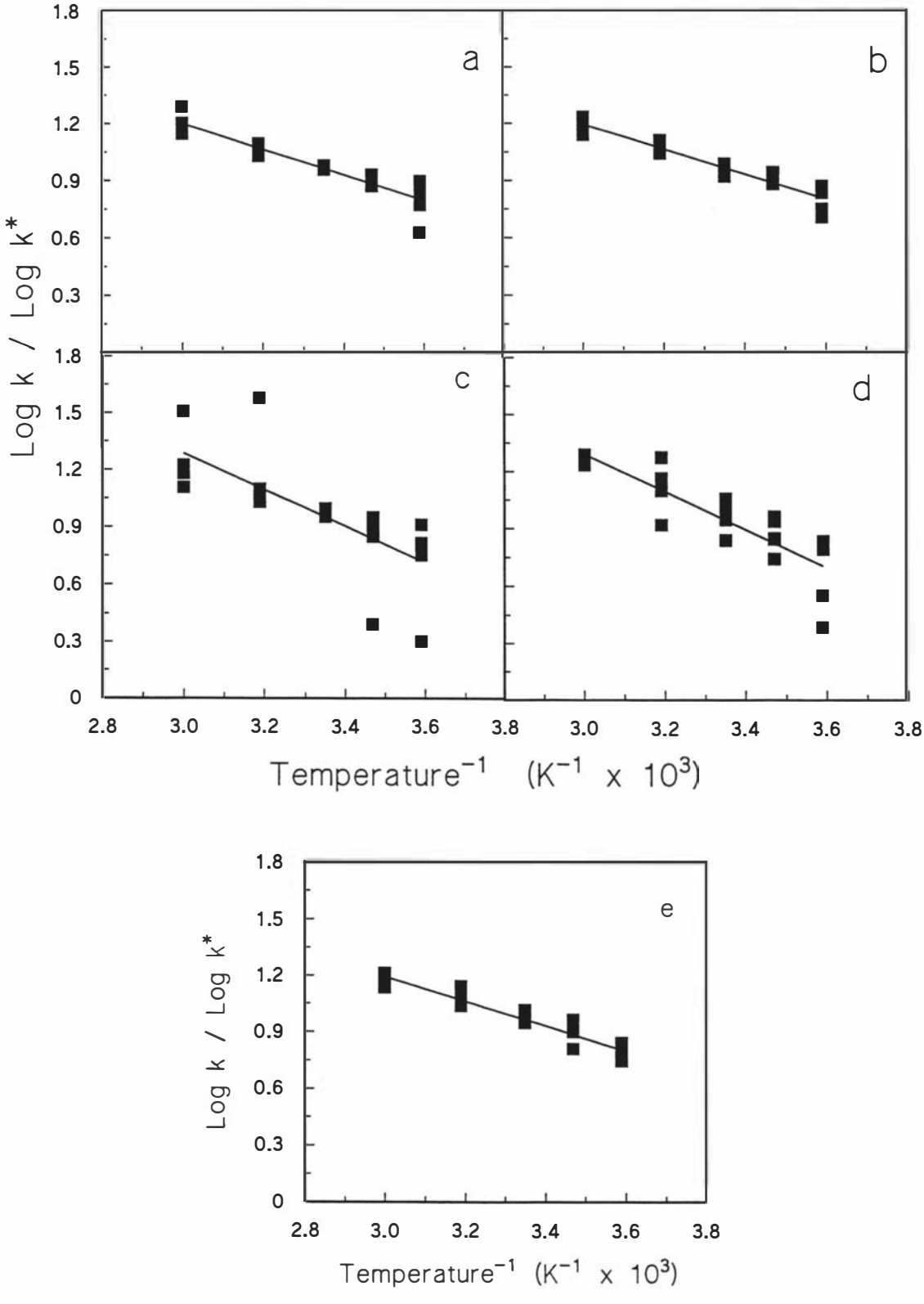

Fig. 4.6 log k / log k* as a function of the reciprocal of absolute temperature for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk.

For each milk, log k/log k^* was linearly regressed against 1/T to give equations of the following form:

$$\log k/\log k^* = - A\,(1000/T) + B \qquad (4.7)$$

The shift factors were related to the total solids concentration as shown in Fig. 4.7.

log k^* was quadratically regressed against total solids concentration to give equations of the form:

$$\log k^* = a_0 + a_1\,(T.S.) + a_2\,(T.S.)^2 \qquad (4.8)$$

A, B, a_0, a_1 and a_2 are constants (whose values are given in Table 4.12), 1/T = reciprocal of absolute temperature and T.S.= total solids concentration (% w/w).

Combining equations 4.7 and 4.8 into one equation by substituting for log k^* in Eq.(4.7) gives:

$$\log k = [\, - A\,(1000/T) + B\,] \,.\, [\, a_0 + a_1(T.S) + a_2(T.S)^2\,] \qquad (4.9)$$

This equation, together with the n values in Table 4.12, provides a means of predicting the flow properties of a reverse osmosis concentrate at any temperature between 5 and 60 °C and any total solids content in the range 13.36-39.00% for whole milk (A), 13.36-40.00% for homogenized milk and 9.30-26.05% for skim milk, and 13.25-40.08% for whole milk (B) and UHT treated whole milk (B).

4.1.3.5.2 Using the Fernandez-Martin (1972) approach

Another possible way of fitting the data is the approach used by Fernandez-Martin (1972), to establish a general expression relating the viscosity of milks to temperature and total solids content. Since flow behaviour index, n, was more or less constant, the consistency index, k ($mPas^n$), was used for the regressions.

For every milk type, a quadratic equation relating temperature (θ, °C) to log k was obtained:

$$\log k = a_0 + a_1\,\theta + a_2\,\theta^2 \qquad (4.10)$$

The values of a_0, a_1 and a_2 for milks and their concentrates over the temperature range 5-60 °C are given in Tables 4.13a and 4.13b for Trials I and II respectively. With increase in concentration, the value of constant a_0 increases, a_1 shows no definite trend and a_2 decreases for all unheated milks.

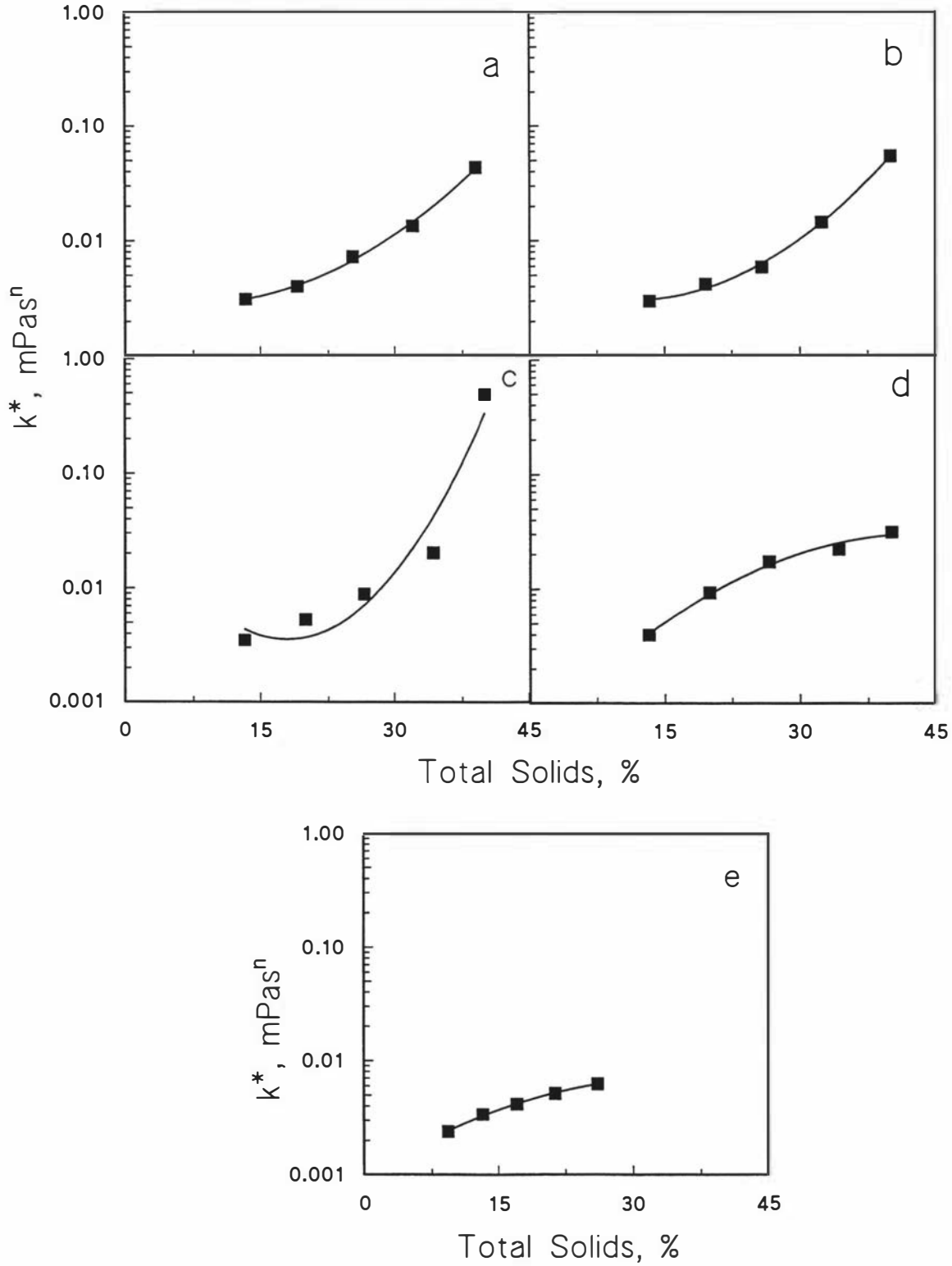

Fig. 4.7 log k* as a function of total solids before storage for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk.

Table 4.12 Values of A, B, a_0 , a_1 , a_2 in Eq. 4.9 for various milks.

Milk		A	B	r^2	a_0	a_1	a_2	r^2	Average value of flow behaviour index (n)	Coefficient.of variation of flow behaviour index (n) (%)
Whole milk (A)	a	0.672	3.21	0.885	-2.3603	-0.0264	0.001334	0.991	0.981	3.26
	b	0.738	3.44	0.943	-2.4083	-0.0198	0.001245	0.994	0.975	4.10
Homogenized milk	a	0.656	3.16	0.919	-2.6363	-0.0073	0.001037	0.996	0.981	3.56
	b	0.812	3.68	0.842	-2.3580	-0.0263	0.001446	0.998	0.967	3.82
Whole milk (B)	a	0.958	4.16	0.554	-1.0643	-0.1497	0.004095	0.945	0.934	10.7
	b	1.158	4.86	0.559	-1.2695	-0.141331	0.004118	0.946	0.928	4.10
UHT treated whole milk	a	0.992	4.27	0.754	-3.1963	0.0750	-0.000828	0.991	0.976	10.2
	b	1.921	7.48	0.692	-3.0779	-0.1043	-0.001338	0.964	0.932	12.3
Skim milk	a	0.658	3.16	0.913	-2.6054	0.0010	0.000548	0.993	0.975	3.89
	b	0.766	3.54	0.958	-2.3140	-0.0242	0.001126	0.984	0.993	3.82

(a) before (b) after 48 h storage at 5°C. For all regressions : $p < 0.009$
Coefficient of variation of n = (standard deviation / mean) × 100

Table 4.13a Quadratic relationship between consistency index (k) and temperature (θ) showing the polynomial parameters obtained by the least squares method for whole milk (A), homogenized milk and skim milk before storage over the temeprature range 5-60 °C in the Eq. 4.10.

Milk	C.F.	Total Solids %	a_0	a_1	a_2	r^2
Whole milk (A)	1.0X	13.36	-1.8752	-0.020516	0.00004005	0.997
	1.5X	19.08	-2.1080	-0.007356	-0.00006297	.0946
	2.0X	25.26	-1.7206	-0.015500	0.00006119	.0992
	2.5X	32.00	-1.3560	-0.021438	0.0001174	0.988
	3.0X	39.00	-0.7819	-0.025786	0.0001657	0.965
Homogenized milk	1.0X	13.36	-2.1309	-0.010292	-0.00008145	0.962
	1.5X	19.56	-1.5405	-0.039330	0.0003144	0.955
	2.0X	25.84	-1.8130	-0.011718	0.00001685	0.999
	2.5X	32.40	-1.2312	-0.027727	0.0002175	0.989
	3.0X	40.00	-0.881182	-0.015135	0.00006541	0.996
Skim milk	1.0X	9.30	-1.7392	-0.032983	0.0001617	0.959
	1.5X	13.25	-1.7095	-0.035025	0.0002382	0.993
	2.0X	17.06	-2.0434	-0.013736	0.00002292	0.937
	2.5X	21.34	-1.6309	-0.034300	0.0002744	0.982
	3.0X	26.05	-1.5593	-0.033820	0.0003044	0.983

Table 4.13b Quadratic relationship between consistency index (k) and temperature (θ) showing the polynomial parameters obtained by the least squares method for whole milk (B) and UHT treated whole milk (B) before storage over the temeprature range 5-60 °C in the Eq. 4.10.

Milk	C.F.	Total Solids %	a_0	a_1	a_2	r^2
Whole milk (B)	1.0X	13.25	-1.8942	-0.020674	0.00008562	0.961
	1.5X	19.96	-2.0473	-0.005541	-0.00004177	0.974
	2.0X	26.57	-1.5356	-0.019669	0.00006822	0.981
	2.5X	34.24	-1.1874	-0.020594	0.00009849	0.996
	3.0X	40.08	0.04118	-0.019122	0.0001627	0.917
UHT treated whole milk	1.0X	13.25	-2.0955	0.002032	-0.0002431	0.769
	1.5X	19.96	-0.316308	-0.036478	0.0001612	0.985
	2.0X	26.57	-0.779296	-0.050912	0.0004440	0.976
	2.5X	34.24	-1.2163	-0.014787	0.00001459	0.993
	3.0X	40.08	-0.702404	-0.042094	0.0003743	0.945

At a given temperature, the total solids concentration (T.S.) dependence of log k for each milk type was expressed by a single relation of the form:

$$\log k = b_0 + b_1 (T.S) + b_2 (T.S)^2 \qquad (4.11)$$

The values for b_0, b_1 and b_2 at each measurement temperature for the different milks are given in Tables 4.14a and 4.14b for Trials I and II respectively. With increase in temperature, the value of the constant b_0 decreases, b_1 shows no definite trend and b_2 decreases for all unheated milks. The values of these constants for heated whole milk do not show a definite trend; the r^2 values are generally very much similar to those for unheated milks.

A general expression was derived for each milk by separately regressing b_0, b_1 and b_2 against temperature (θ, °C) to enable k to be calculated at any temperature (5-60 °C) and any total solids concentration (9.3-40.08%). The expression took the form:

$$\log k = (A_0 + A_1 \theta + A_2\theta^2) + (B_0 + B_1 \theta + B_2 \theta^2)\ (T.S.) + (C_0 + C_1 \theta + C_2 \theta^2)\ (T.S)^2 \qquad (4.12)$$

where $(A_0 + A_1 \theta + A_2\theta^2) = b_0$

$(B_0 + B_1 \theta + B_2 \theta^2) = b_1$

$(C_0 + C_1 \theta + C_2 \theta^2) = b_2$

The values of A_0, A_1, A_2, B_0, B_1, B_2, C_0, C_1 and C_2 are given in Table 4.15 for all the milk types.

Eq. 4.12, together with the n values in Table 4.12, provides a further means of predicting the flow properties of a reverse osmosis concentrate at any temperature between 5 and 60 °C and any total solids content in the range 13.36-39.00% for whole milk (A), 13.36-40.00% for homogenized milk and 9.30-26.05% for skim milk and 13.25-40.08% for whole milk (B) and UHT treated whole milk (B).

Table 4.14a Quadratic relationship between consistency index (k) and total solids (T.S.) showing the polynomial parameters obtained by the least squares method for whole milk (A), homogenized milk and skim milk before storage over the different total solids range in the Eq. 4.11.

Milk	Temperature. (°C)	b_0	b_1	b_2	r^2
Whole milk (A)	5	-1.4883	-0.073119	-0.002308	0.970
	15	-1.7594	-0.051018	0.001661	0.999
	25	-2.3319	-0.022203	0.001233	0.985
	40	-2.6174	-0.016077	0.001141	0.991
	60	-3.3017	0.017928	0.0005472	0.990
Homogenized milk	5	-2.2062	0.001437	0.0007438	0.875
	15	-2.4992	-0.005763	0.001008	0.998
	25	-2.1430	-0.037487	0.001514	0.995
	40	-2.6164	-0.021473	0.001307	0.994
	60	-3.4905	0.025893	0.0005518	0.996
Skim milk	5	-1.7551	-0.034941	0.001463	0.620
	15	-1.3751	-0.106243	0.003134	0.719
	25	-2.4091	-0.004787	0.0004258	0.789
	40	-3.6436	0.095313	-0.001885	0.989
	60	-3.3503	0.025903	0.0002303	0.993

Table 4.14b Quadratic relationship between consistency index (k) and total solids (T.S.) showing the polynomial parameters obtained by the least squares method for whole milk (B) and UHT treated whole milk (B) before storage over the different total solids range in the Eq. 4.11.

Milk	Temperature (°C)	b_0	b_1	b_2	r^2
Whole milk (B)	5	-0.560772	-0.158828	0.004197	0.973
	15	-1.0980	-0.132190	0.003807	0.942
	25	-1.2229	-0.130642	0.003716	0.934
	40	-0.989539	-0.162725	0.004263	0.948
	60	-1.3236	-0.162884	0.004448	0.912
UHT treated whole milk	5	-3.6440	0.190972	-0.003189	0.369
	15	-4.0924	0.199770	-0.003401	0.487
	25	-3.4449	0.130848	-0.002112	0.287
	40	-2.5617	0.049299	-0.0007610	0.230
	60	-4.1320	0.128470	-0.001876	0.644

Table 4.15 General expression relating consistency index (k) to temperature (θ) and total solids (T.S.) concentration; the regression values of its parameters in the Eq. 4.12.

Milk	i	0	1	2	r^2
Whole milk (A)	A_i	-1.2801	-0.039185	0.00009606	0.984
	B_i	-0.082662	0.002352	-0.00001188	0.966
	C_i	-0.002666	0.0002434	-0.00000324	0.682
Homogenized milk	A_i	-2.3906	0.018964	-0.0006176	0.913
	B_i	0.021699	-0.003490	0.00005928	0.897
	C_i	0.0003923	0.00006627	-0.000001061	0.936
Whole milk (B)	A_i	-0.569802	-0.027504	0.0002703	0.610
	B_i	-0.154837	0.001126	-0.00002245	0.437
	C_i	0.004187	-0.00002446	0.0000005013	0.667
UHT treated whole milk	A_i	-4.4024	0.076521	-0.001145	0.402
	B_i	0.255311	-0.007799	0.00009114	0.700
	C_i	-0.004271	0.0001284	-0.000001415	0.726
Skim milk	A_i	-0.995669	-0.075118	0.0005493	0.775
	B_i	-0.108810	0.005930	-0.00005606	0.495
	C_i	0.003427	-0.0001732	0.000001867	0.481

4.1.3.5.3 Goodness of shift factor based and Fernandez-Martin type approaches

Linear plots of experimental versus predicted values of k are shown in Figs. 4.8 and 4.9. It can be seen that for whole milk (A), homogenized milk and skim milk both approaches give reasonably accurate predictions, with the Fernandez-Martin approach being somewhat better than the shift factor approach. For whole milk (B) the Fernandez-Martin approach is again better, but neither is particularly good. For UHT treated whole milk (B), both approaches give poor predictions; however the Fernandez-Martin approach appears to be more valid as the plotted points are, at least, scattered about the line of perfect prediction.

On comparing the predicted and experimental k values of the milk samples after storage, it can be seen that of all unheated milks, homogenized milk gave better predictions using the shift factor approach. UHT treated whole milk (B) did not improve the predictions up on storage. Skim milk samples after storage gave similar predictions to un-stored milks.

Using the Fernandez-Martin approach, all the milks on storage showed better predictions on storage than un-stored milks except for UHT treated whole milk (B) which showed anomalous behaviour. As the Fernandez-Martin approach is essentially a simple curve fitting one, it is not possible to give sound reasons for this.

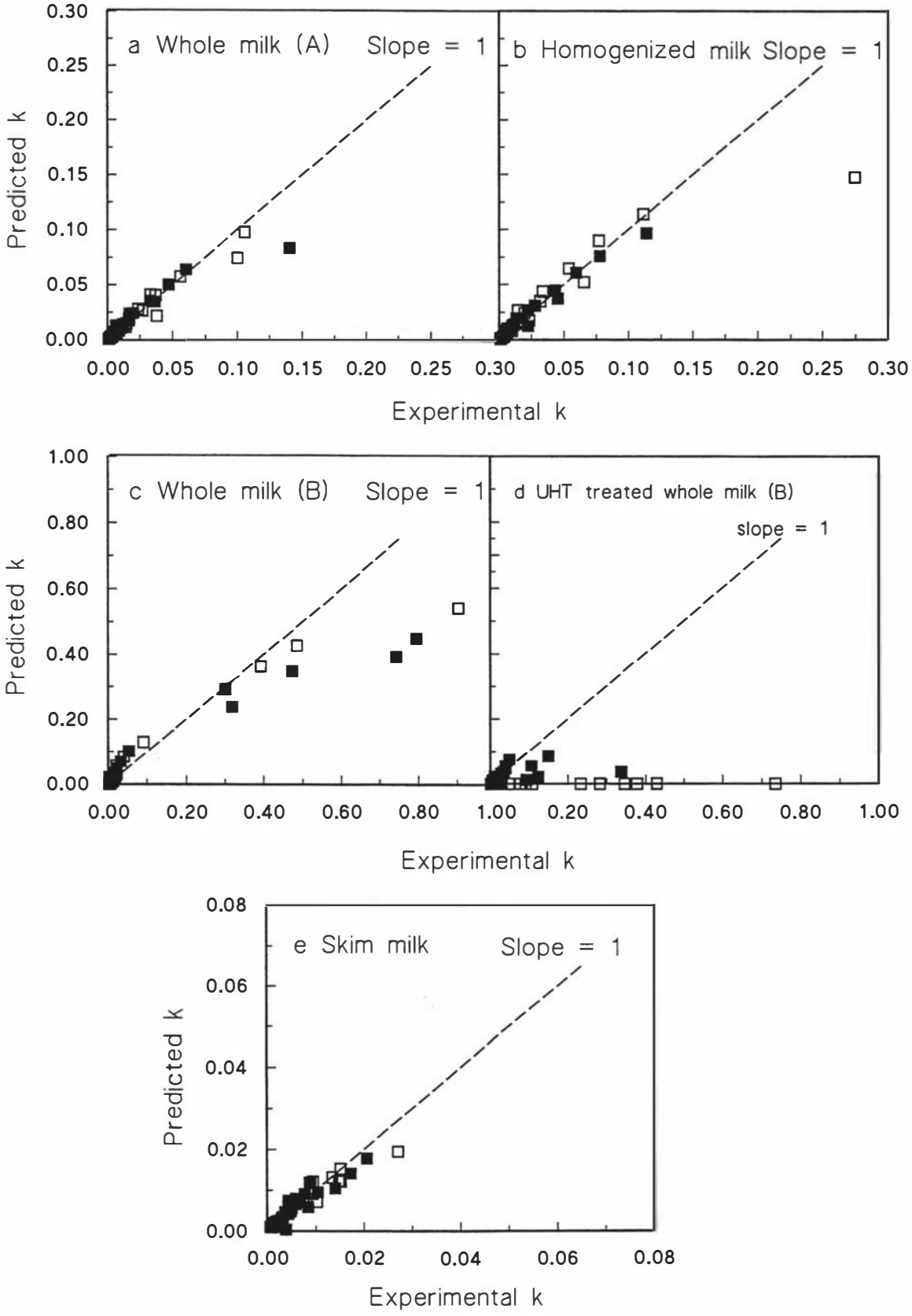

Fig. 4.8 Comparision between predicted and experimental k values using the shift factor approach for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk ■ before and □ after storage.

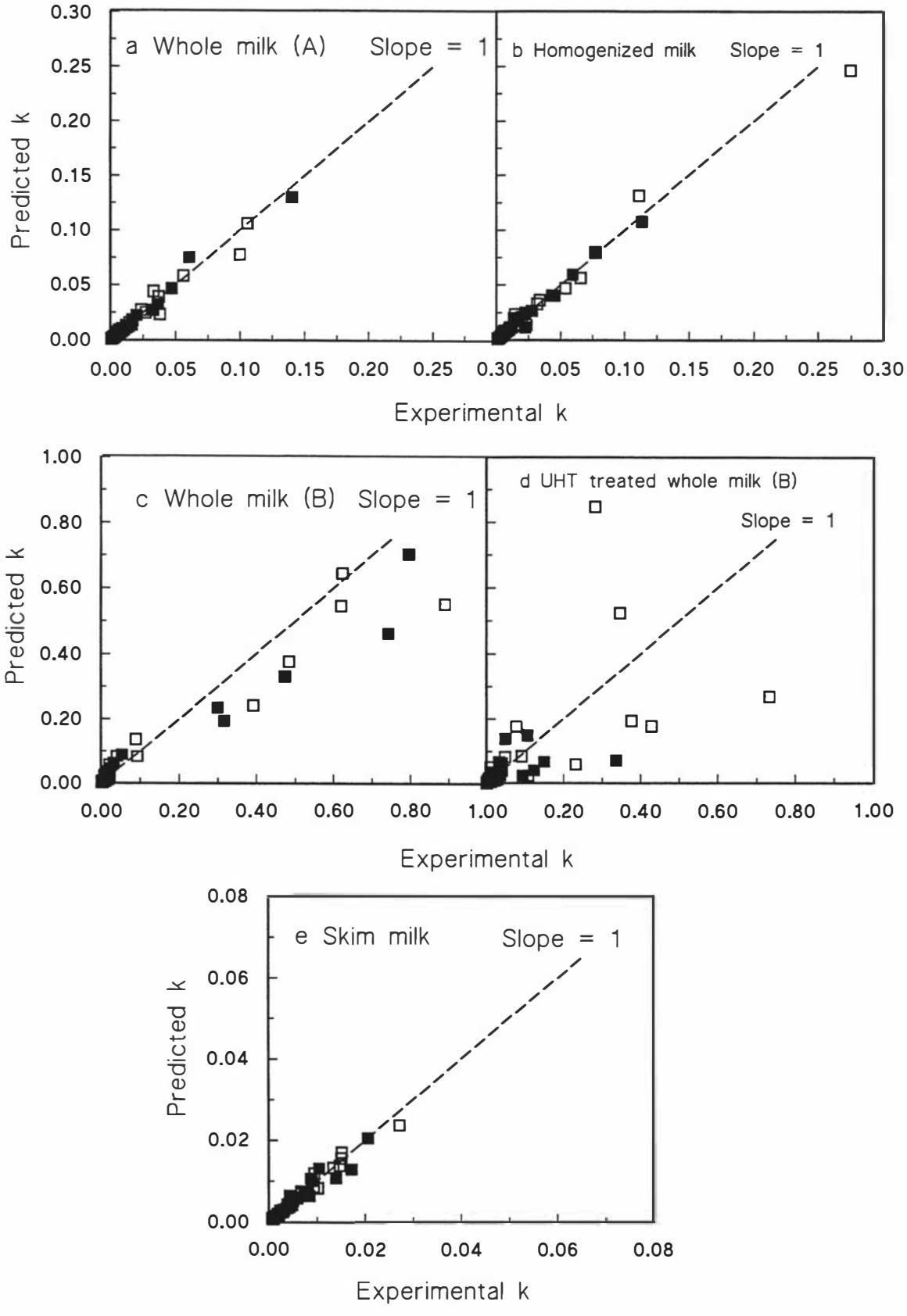

Fig. 4.9 Comparision between predicted and experimental k values using the Fernandez-Martin (1972) approach for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk ■ before and □ after storage.

4.2 Hydrodynamic approach to the viscosity of milk concentrates

4.2.1 Introduction

In this section, the data from Section 4.1 has been analysed using a hydrodynamic approach to explore how the viscosity at 25 °C of concentrated milks changes, as a function of concentration, UHT treatment and storage for 48 h at 5 °C.

The viscosity η of a dilute dispersion of solid compact spheres is given by Einstein's equation (Eq. 2.6). However, this relation holds only for very dilute systems and not for concentrated systems, where hydrodynamic interactions take place between the dispersed particles. The viscosity of concentrated systems can be well described by Eilers' equation (Eq. 2.5). The value of ϕ_{max} depends on the shapes of the dispersed particles, the size distributions of these particles and the definition of the continuous medium. To represent the continuous medium in concentrated milks, Snoeren *et al.* (1982) used a solution of the milk salts and 5% lactose, whereas for milk van Vliet and Walstra (1980) chose a 1% solution of small molecules, mainly salts for milk products in general. Hence, an attempt has been made to analyse the data in both ways using the Snoeren *et al.* (1982) approach (Scheme A) and the van Vliet and Walstra (1980) approach (Scheme B) to observe the effects of including lactose in the continuous medium (Scheme A) or considering the lactose as a dispersed component (Scheme B).

4.2.2 Calculations

4.2.2.1 4.2.2.1 Calculation of the volume fractions (ϕ), and the viscosity of the continuous medium (η_{ref}), for Scheme A

Calculation of the volume fractions (ϕ) for scheme A

In skim milk the total volume fraction of the dispersed particles is given by the sum of the volume fractions of casein (ϕ_{cas}) the native whey protein ((ϕ_{nwp}) and the denatured whey protein ((ϕ_{dwp}) as follows:

$$\begin{aligned}\phi_{\text{skim milk}} &= \phi_{cas} + \phi_{nwp} + \phi_{dwp} \\ &= C_{cas}V_{cas} + C_{nwp}V_{nwp} + C_{dwp}V_{dwp}\end{aligned} \qquad (4.13)$$

where C_{cas}, C_{nwp}, and C_{dwp} are the volume concentrations of casein, native whey protein and denatured whey protein, respectively (kg / m^3) and V_{cas}, V_{nwp} and V_{dwp} are the voluminosities of casein, native whey protein and denatured whey protein, respectively (m^3 / kg). For whole milks, the volume fraction is calculated in a similar way to that of the skim milk except that contribution of fat to volume fraction ($C_{fat}V_{fat}$) is taken into account. Therefore Eq. 4.13 can be rewritten for whole milk as:

$$\phi_{\text{whole milk}} = C_{cas}V_{cas} + C_{nwp}V_{nwp} + C_{dwp}V_{dwp} + C_{fat}V_{fat} \qquad (4.14)$$

The above stated equations are for scheme A where lactose has not been included in ϕ.

The values for V_{cas}, V_{nwp}, V_{dwp}, V_{fat} were 3.57×10^{-3} , 1.07×10^{-3} , 3.09×10^{-3} and 1.075×10^{-3} (m^3 / kg) (Snoeren *et al.*, 1982, 1983). The values of C_{cas}, C_{nwp}, C_{dwp}, C_{fat} were calculated from their mass concentration given in Table 4.1 for whole milk (A), homogenized milk and skim milk (Trial I), and in Table 4.2 for whole milk (B) and UHT treated whole milk (B) (Trial II) and were further converted to volume concentrations for individual component (kg / m^3) as follows:

Assuming the concentration of the individual component in the milk to be W% by weight; then volume concentration is:

$$C_{v,i} = \frac{W \times \rho_{MILK}}{100 \times 1000} \qquad (kg / l) \qquad (4.15)$$

The densities of milks and concentrates were calculated from the equation:

$$\rho = \left(\frac{1}{\frac{x_{SNF}}{1635} + \frac{x_{water}}{1000} + \frac{x_{fat}}{930}} \right) \qquad (kg / m^3) \qquad (4.16)$$

where x_{SNF}, x_{water} and x_{fat} are the mass fractions of SNF, water and fat contents, respectively and the values for the densities of SNF, water and fat were taken from Jenness and Patton (1959).

For unheated milks, the volume fraction of protein was taken as the sum of the volume fractions of casein micelles and whey proteins i.e. $\phi_{pro} = \phi_{cas} + \phi_{wp}$ and for UHT treated whole milk, the volume fraction of the protein was taken as the sum of the volume fractions of casein micelles, native whey proteins and denatured whey proteins, i.e. $\phi_{pro} = \phi_{cas} + \phi_{nwp} + \phi_{dwp}$. For UHT treated whole milk (B), the concentration of denatured whey

proteins was taken as the difference in non-casein nitrogen (Table 4.2) (converted to protein using the factor 6.38) between heated and unheated milk.

The total volume fraction of concentrates, $\phi_{conc.}$, were calculated from volume fractions of the unconcentrated milks, ϕ_{milk} , using the following equation:

$$\phi_{conc} = \phi_{milk} \left(\frac{TS_{conc}}{TS_{milk}} \right) \left(\frac{\rho_{conc}}{\rho_{milk}} \right) \tag{4.17}$$

where ρ is the density (kg/m^3).

Calculation of viscosity of continuous medium (η_{ref}) for scheme A:

The viscosity of the continuous medium was taken as a linear function of the salts and lactose content and was calculated using the following equation at 25 °C

$$\eta_{ref} = \eta_{water} + \left(\Delta\eta_s + \Delta\eta_{lac} \right) \left(\frac{TS_{conc}}{TS_{milk}} \right) \qquad (mPas) \tag{4.18}$$

where $\Delta\eta_s = 0.02\ \eta_{water} = (0.02)\ (0.894) = 0.01788$ mPa s

and $\Delta\eta_{lac} = \eta_{5\%\ lactose} - \eta_{water} = 1.03 - 0.894 = 0.136$ mPa s. The values of η_{water} (0.894 mPa s), $\eta_{5\%\ lactose}$ (1.03 mPa s) at 25 °C were obtained from Jenness and Patton (1959). By substituting these values in the Eq. 4.18, η_{ref} was calculated for each milk and concentrate. Eq. 4.18 was used for scheme A where lactose was included in η_{ref}. For scheme B lactose was included in the total volume fraction, hence η_{ref} for scheme B does not include a contribution from lactose molecules (see Eq. 4.21 below). Consequently, the values of η_{ref} were higher and the values of volume fraction ϕ were lower in scheme A than in scheme B.

4.2.2.2 Calculation of the volume fractions (ϕ), and viscosity of continuous medium (η_{ref}) for scheme B

Calculation of the volume fractions (ϕ) for scheme B:

For scheme B the equations (Eqs. 4.13 and 4.14) remained the same except for the addition terms for lactose. Thus for skim milk Eq. 4.13 becomes:

$$\phi_{skim\ milk} = C_{cas}V_{cas} + C_{nwp}V_{nwp} + C_{dwp}V_{dwp} + C_{lac}\ V_{lac} \tag{4.19}$$

Similarly, the Eq. 4.14 for whole milk becomes:

$$\phi_{\text{whole milk}} = C_{cas}V_{cas} + C_{nwp}V_{nwp} + C_{dwp}V_{dwp} + C_{fat}V_{fat} + C_{lac}V_{lac} \qquad (4.20)$$

C_{lac} was claculated in the same way as C for other components. V_{lac} was taken as 1.00×10^{-3} (m^3/kg) (Walstra and Jenness, 1984).

Calculation of viscosity of continuous medium (η_{ref}) for scheme B:

In scheme B the viscosity of the continuous medium was taken as a linear function of the salts and water only and not the lactose content (since lactose was included in the total volume fraction, ϕ). Therefore η_{ref} and was calculated at 25 °C using the following equation:

$$\eta_{ref} = \eta_{water} + (\Delta\eta_s)\left(\frac{TS_{conc}}{TS_{milk}}\right) \qquad (mPas) \qquad (4.21)$$

4.2.2.3 Calculation of the basic viscosity as predicted by Eilers' equation

The basic viscosity of a milk or concentrate (i.e. the viscosity owing to the continuous phase and the total volume fraction, but excluding structural viscosity) was predicted using Eilers' equation (Eq. 2.5), using appropriate values of η_{ref} and ϕ for schemes A and B. ϕ_{max} was taken as 0.79. In order to plot smooth curves of basic viscosity against volume fraction, values of ϕ and η_{ref} were calculated at a number of concentrations, using the ϕ of the unconcentrated milks calculated using Eq. 4.13 for skim milk and Eq. 4.14 for whole milks. ϕ at various concentrations was calculated using Eq.4.17 and η_{ref} at various concentrations was calculated using Eq. 4.18 and Eq. 4.21.

4.2.2.4 Calculations of the experimental values of basic viscosity

For each milk type, the experimentally measured basic viscosity was obtained by plotting the values of η_{app} (calculated as τ / γ) at different shear rates (73-921 s^{-1}) (measured at 25 °C) as a function of the reciprocal of shear rate and extrapolating linearly to $(\text{shear rate})^{-1} = 0$ (i.e. shear rate = ∞), as shown in Fig. 4.10. From the linear regression equation obtained from the data, the basic viscosity was calculated as in the following example for whole milk (B).

$$\log \eta_{app,\infty} = -2.69\ (\text{shear rate})^{-1} + 0.613 \qquad (4.22)$$

Substituting $(\text{shear rate})^{-1} = 0$ into Eq. 4.22 gives a basic viscosity of 4.1 mPa s.

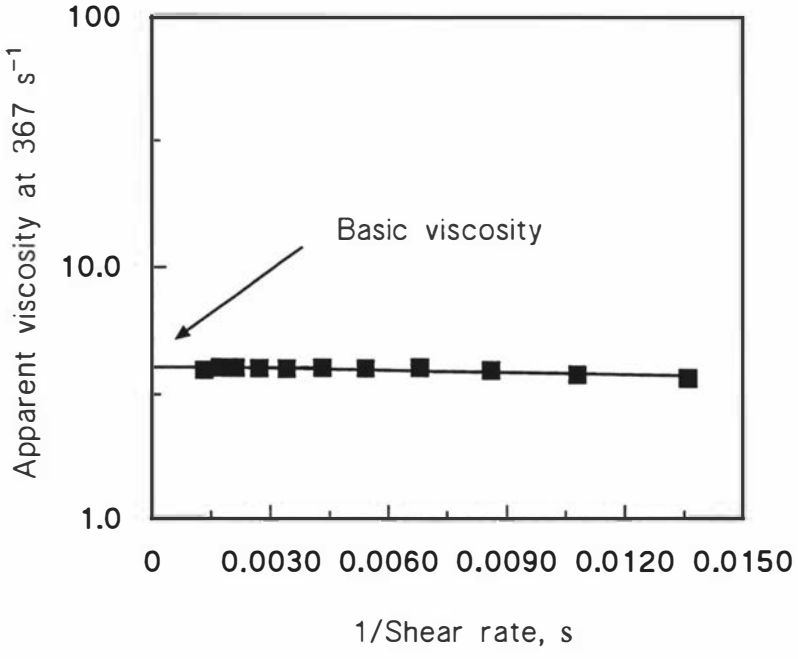

Fig. 4.10 Example showing how basic viscosity was found by extrapolation for whole milk (B) at a measurement temperature of 25 °C.

4.2.3 Results and Discussion

Fig. 4.11 shows comparisons between experimental values of the basic viscosity of various milk types and prediction based on scheme A. The experimental values were higher than the values predicted by Eilers' equation. There was a marked difference between the experimental and predicted values in the case of whole milks at higher ϕ (Fig. 4.11a-d); but not much difference between these two values for skim milk (Fig. 4.11e). The basic experimental viscosity increased with increase in volume fraction for all unheated milks (Fig. 4.11a, b, c, e), and thus exhibited the trend expected from Eilers' equation. In the case of UHT treated whole milk (B) (Fig. 4.11d), there was an increase in experimental basic viscosity up to a volume fraction of 0.35, and then the viscosity remained more or less constant with further increase in volume fraction. On storage for 48 h at 5 °C, there was an increase in the experimental basic viscosity for all whole milks. The values of experimental basic viscosity for skim milk were more or less similar before and after storage. After storage the basic viscosity of UHT treated whole milk (B) increased considerably with increase in volume fraction (Fig. 4.11d). For whole milk (A) the increase in experimental basic viscosity after storage was 21.28% at ϕ = 0.58; for homogenized milk 22.0% at ϕ = 0.58; for whole milk (B) 17.60% at ϕ = 0.58; for UHT treated whole milk 127.28% at ϕ = 0.58 and for skim milk 14.52% at ϕ = 0.36. An appreciable difference in the viscosity of the two whole milks (whole milk (A) and whole milk (B)) (Fig. 4.11a and Fig. 4.11c) was observed which possibly may have been due to differences in the initial compositions of the two milks.

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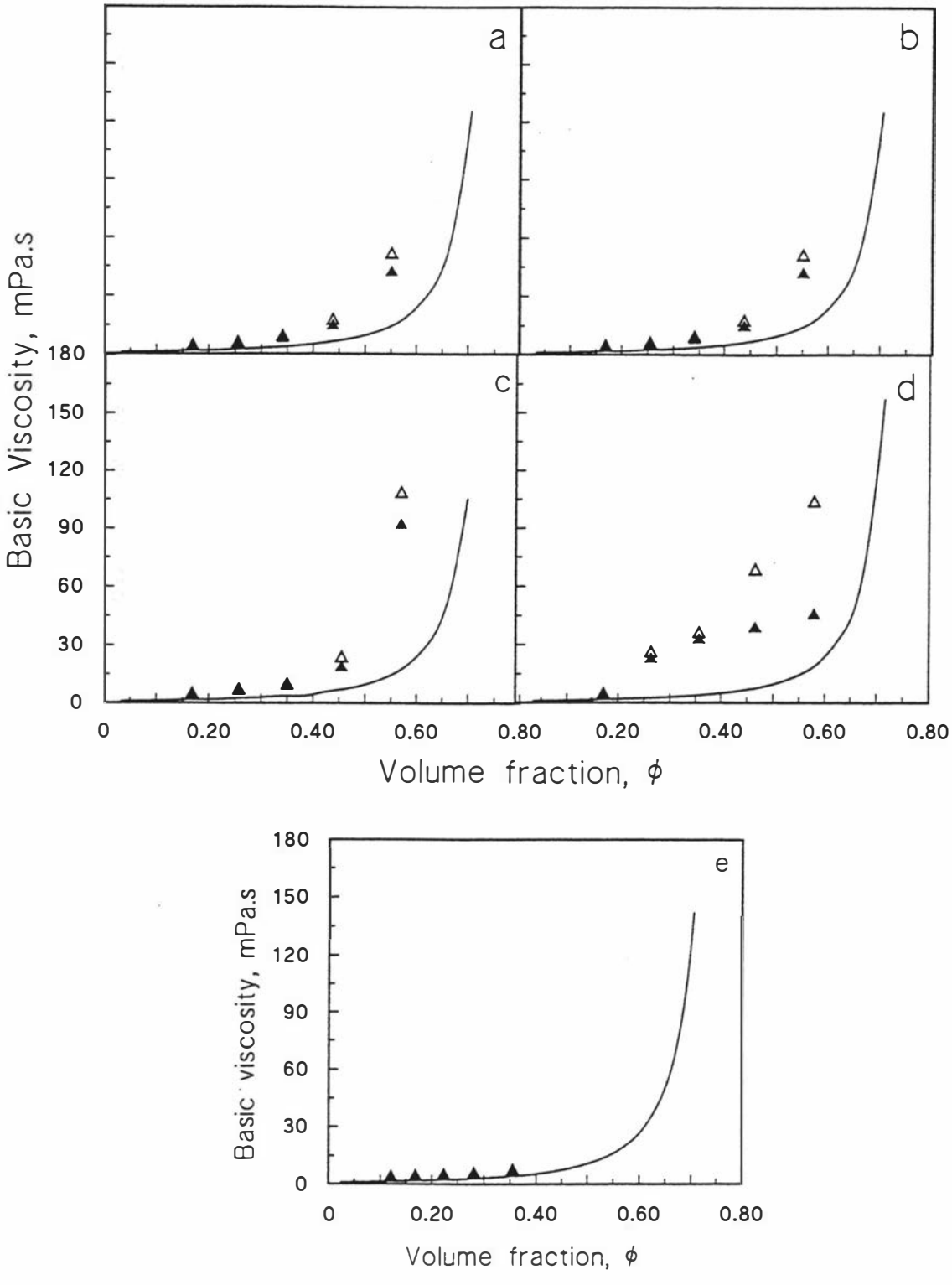


Fig. 4.11 Basic viscosity ($\eta_{app,\infty}$) of concentrates obtained at 25 °C for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk as a function of volume fraction (ϕ). Experimental points are (▲) before and (Δ) after 48 h storage. The solid curve represents values predicted by Eilers' equation using Scheme A.

The increasing deviation between experimental and predicted basic viscosities at higher ϕ would not have been the result of increasing inaccuracy in viscosity measurement, since viscosities were higher at higher ϕ, and thus more accurately measurable.

The experimental values for the basic viscosity were higher than the values predicted by Eilers' relation (Fig.4.11) for whole milk. The main reason was probably the aggregation or weak flocculation of fat globules (Walstra and Jenness, 1984; Horne, 1993), also observed from electron micrography of RO concentrated milks in the present study (to be discussed in Chapter 5). The aggregates possibly contained interstitial liquid and had irregular shapes and consequently had volume fractions greater than Eilers' equation allowed for. The other possible reason may be that the structural effect of aggregation on the viscosity of milks might not be removed when extrapolating the basic viscosity to shear rate = ∞.

After storage for 48 hours at 5 °C, there was an increase in the experimental basic viscosity possibly because the hydrodynamic volume of the casein micelles increased, which was more predominant at higher volume fractions and especially in the systems containing fat. Further, UHT treatment of whole milk (B) caused higher viscosities over much of the volume fraction range (Figs. 4.11c and 4.11d). As a result of heat treatment the viscosity of the milk concentrates increases significantly owing to the increased voluminosity of protein particles specially casein. Also whey proteins on heating get denatured and the voluminosity of denatured whey protein is three times higher than the native whey protein, hence the increase in the viscosity. Non-stored UHT treated whole milk showed a peculiar trend i.e. viscosity increased at an ever decreasing rate with increase in volume fraction but after storage the viscosity increased almost linearly with increase in volume fraction suggesting that structures formed during heating were reversible and during storage period such aggregates/structures became irreversible.

Fig. 4.12 shows comparisons between experimental basic viscosities and those predicted by Eilers' equation using Scheme A (lactose included in η_{ref}) and Scheme B (lactose included in volume fraction, ϕ), for milks and concentrates before storage. Similar trends were observed for the increase in basic viscosity with increase in volume fraction for the two schemes. It can be observed that the plot of Eilers' equation for scheme B is shifted to the right relative to the plot for scheme A because of the inclusion of the volume fraction of lactose in the total volume fraction.

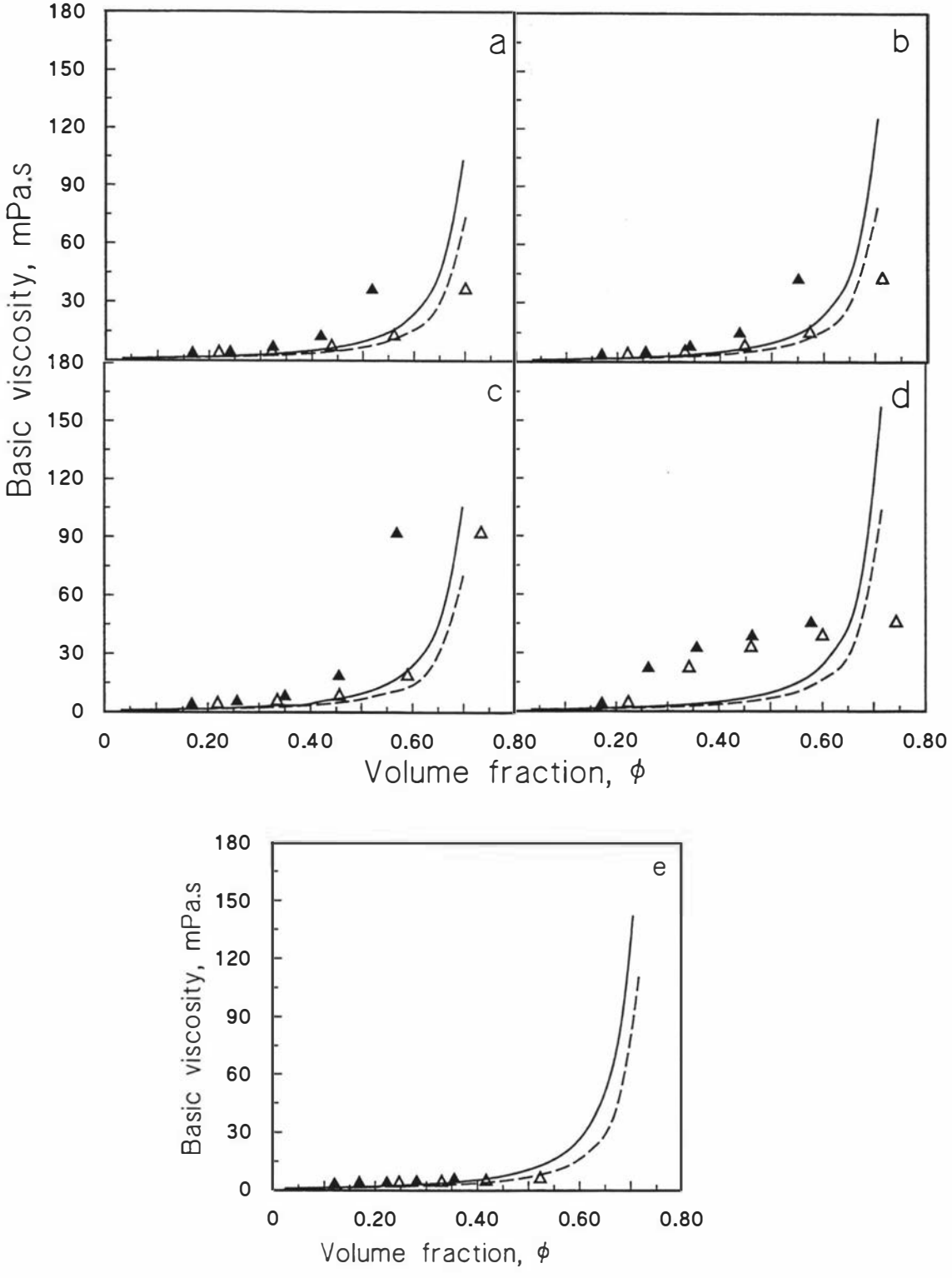


Fig. 4.12 Basic viscosity ($\eta_{app,\infty}$) of concentrates obtained at 25 °C for (a) whole milk (A), (b) homogenized milk, (c) whole milk (B), (d) UHT treated whole milk (B), (e) skim milk as a function of volume fraction (ϕ) before storage. ▲ and the solid curve represent the experimental points and Eilers' equation respectively for scheme A (lactose included in η_{ref}). Δ and dotted curve represent the experimental points and Eilers' equation respectively for scheme B (lactose included in volume fraction, ϕ).

A closer relation exists between the experimental and predicted values of basic viscosity using scheme B where lactose was included in volume fraction (Fig. 4.12), for all unheated milks than when using scheme A. UHT treated whole milk (B) showed similar trends in both the schemes. The extent of deviation of experimental values from the prediction values for the two schemes were as follows: Using Scheme A for whole milk (A) at $\phi = 0.54$ the deviation from the prediction was 64.81%; for homogenized milk at $\phi = 0.54$ the deviation was 68%; for whole milk (B) at $\phi = 0.57$ the deviation was 79.80%; for UHT treated whole milk the deviation at $\phi = 0.57$ was 53.27% and for skim milk, the deviation from predicted value at $\phi = 0.33$ was 42.56%. Similarly, on using Scheme B for whole milk (A) at $\phi = 0.55$ the deviation from the prediction was 20.11%; for homogenized milk at $\phi = 0.54$ the deviation was 12.15%; for whole milk (B) at $\phi = 0.59$ the deviation was 27.02%; for UHT treated whole milk the deviation at $\phi = 0.71$ was -143.49% and for skim milk, the deviation from predicted value at $\phi = 0.31$ was 35.96%. In other words, the Scheme B gave less deviation of experimental values from predicted values, as compared to Scheme A. Therefore, based on these results, it is recommended to include lactose in volume fraction rather than in viscosity of continuous medium. This is in line with the recommendation of van Vliet and Walstra (1980).

4.3 Conclusions

Whole milk, homogenized milk, skim milk and heated whole milk and the concentrates prepared from them by reverse osmosis, are slightly pseudoplastic liquids whose flow properties can be characterised by the power law. For each milk type, n was independent of temperature and total solids concentration, while the dependence of k on these factors was modelled mathematically by means of total solids concentration-dependent shift factors and the Fernandez-Martin (1972) approach using appropriate linear and quadratic regressions.

The measured values of n, and the equations for k, provide a means of predicting rheological behaviour for process engineering design purposes.

A hydrodynamic approach using Eilers' equation was used to explore how the viscosity of concentrated milks changes, as a function of concentration, UHT treatment and storage for 48 hours at 5 °C. Inclusion of lactose in the total volume fraction provides

better agreement between the experimental and predicted values of the basic viscosity. Also the experimental values of basic viscosity for milk concentrates at various volume fractions are higher than the values predicted by the Eilers' equation. The reason for this difference may be that the Eilers' equation does not account for intersititial liquid trapped between the fat and protein particles in aggregates in the concentrated milks. If these aggregates existed, then they must have been shear resistant, since the concentrates were all close to Newtonian in behaviour (rather than being shear thinning). Because of the Newtonian behaviour, extrapolating the plots of apparent viscosity versus $(\text{shear rate})^{-1}$ to $(\text{shear rate})^{-1} = 0$ (shear rate = ∞), did not "remove" structural viscosity caused by aggregation. This could explain the fact that experimental basic viscosities of the whole milks were significantly higher than those predicted by Eilers' equation, especially at higher volume fraction. A further (part-) explanation might be an increase in casein micelle voluminosity, not accounted for by the value used with Eilers' equation, owing to the precipitation of colloidal calcium phosphate as calcium phosphate on to the micelles.

5. THE EFFECTS OF HOMOGENIZATION AND UHT STERILIZATION ON MILKS CONCENTRATED BY REVERSE OSMOSIS

5.1 INTRODUCTION

Sterilized concentrated whole milk has been an established dairy product for many years (Muir, 1984). Concentration, coupled with shelf stability at ambient temperatures, makes the product a convenience one, and eases the complexity and costs of storage and distribution. These advantages make sterile concentrated milk an attractive product in the context of increasing the availability and consumption of milk in developing countries in particular.

In-container thermal processing (canning) is the traditional means of sterilizing milk concentrates. However, because this method involves heating at a relatively low temperature for a relatively long time (e.g., 115 °C for 30 min), significant undesirable nutritional and organoleptic changes can occur in milk. Such changes can be reduced greatly in extent (for the same bacteriological sterilizing effect) by using ultra-high temperature-short time (UHT) continuous sterilization (Muir, 1984; Kessler, 1981).

Homogenization is a vital operation in the production of sterilized whole milk concentrate; it is needed to ensure that creaming does not take place during storage. However, homogenization greatly reduces the heat stability of concentrated milk, so it is preferable to homogenize concentrates (aseptically) after sterilization rather than before (Muir, 1984; van Boekel and Walstra, 1995). This option is commonly used in the manufacture of heat-evaporated concentrates.

In the case of the reverse osmosis (RO) concentration process, however, the types of pressure relief valve normally used in full-scale RO plants to maintain the trans-membrane pressure gradient can result in significant homogenization of whole milk (de Boer and Nooy, 1980). Since aseptic RO concentration is not currently feasible, the heat stability of the milk is inevitably lowered, prior to sterilization, by the RO concentration step. de Boer and Nooy (1980) discussed possible ways in which this problem can be circumvented, but these do not appear to have been applied commercially.

No previous work has been reported on the effects of simultaneous RO concentration and homogenization on the stability of whole milk to subsequent UHT sterilization. The objective of the work reported in this chapter was to investigate any such effects by examining the following:

- the effects of concentration of milk by RO on the state of fat globules;
- the microstructure of RO concentrated milks;
- the effects of homogenization and UHT treatment on fat globule-protein interactions in RO milk concentrates;
- the effect of pH adjustment of, and the addition of disodium phosphate to, RO concentrated milk on fat globule-protein interactions.

5.2 MATERIALS AND METHODS

5.2.1 Raw material

Raw whole milk from Friesian cows was obtained from a Massey University dairy farm, and processed immediately.

5.2.2 Experimental protocol

Raw milk was processed as shown in Fig. 5.1a to investigate the effect of heat treatment on the particle size distribution of the fat globules in unconcentrated and RO concentrated milks. The milks were heated in an indirect UHT mini-pilot plant, held for 4 s at 80, 90, 100, 120, 130 or 140 °C, and cooled to ~ 30 °C . Particle size distributions with or without prior treatment with protein dissociating medium were determined by the light scattering method described in Chapter 3.

In order to investigate the effects of homogenization and UHT treatment on fat globules, three types of milks were produced for experimental purposes (Fig 5.1b): unconcentrated whole milk, RO concentrated whole milk, and reblended concentrated whole milk. Raw whole milk was pasteurized and then concentrated by reverse osmosis to obtain RO concentrated whole milk.

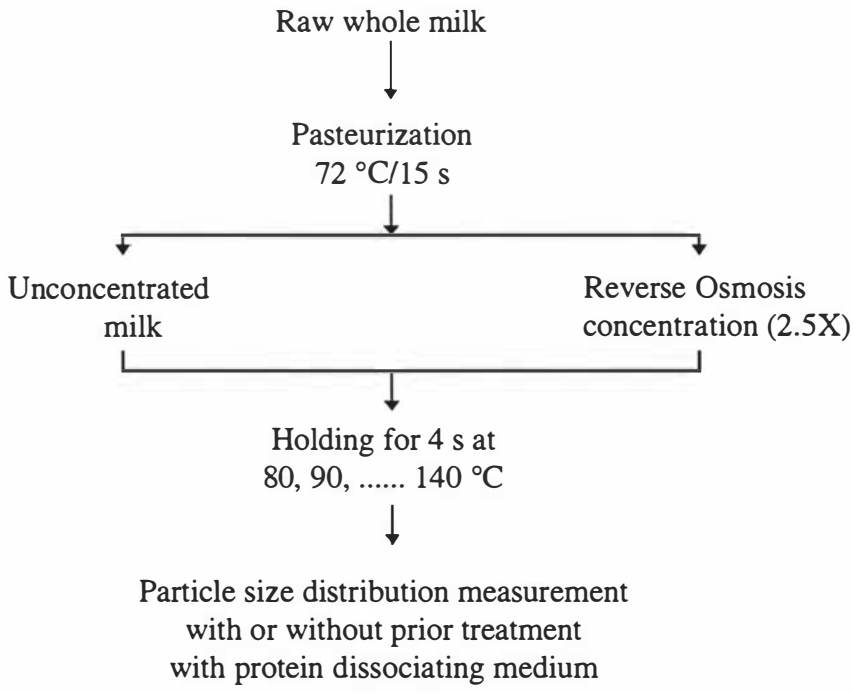


Fig. 5.1a Experimental scheme used to produce and analyse UHT treated unconcentrated and RO concentrated milk systems.

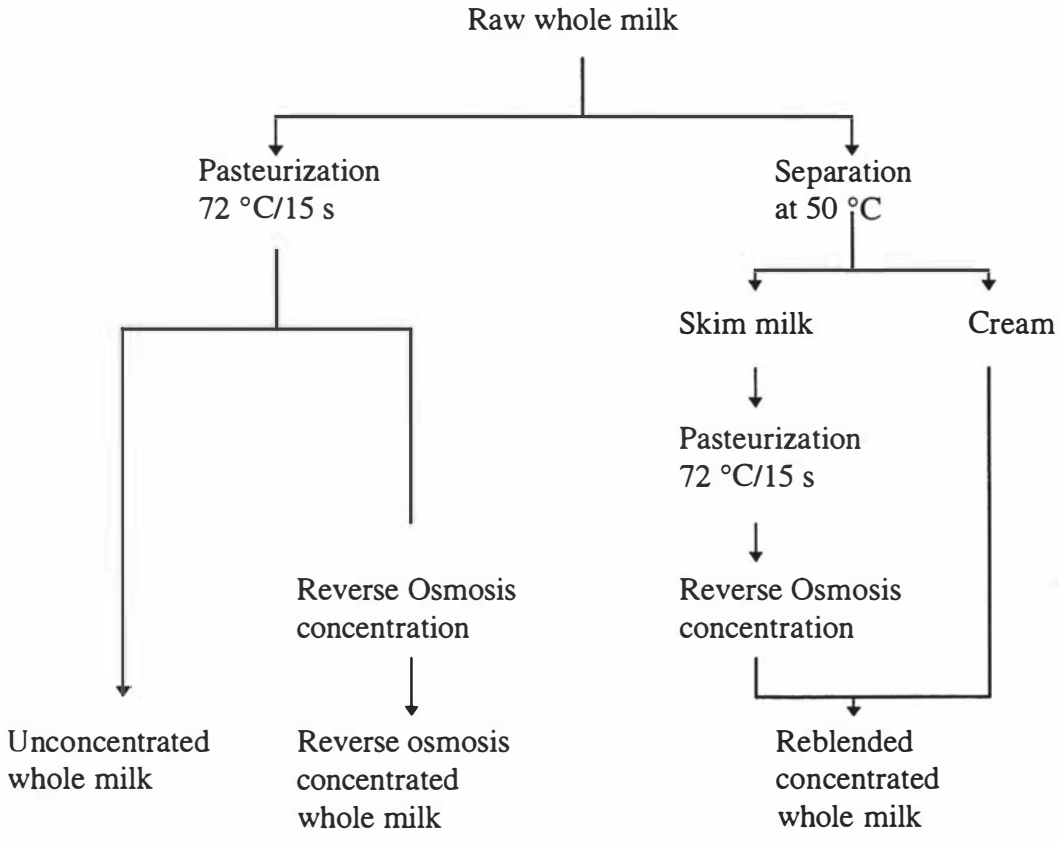


Fig. 5.1b Experimental scheme used in the processing of raw milk to produce three types of milk for experimental purposes.

To prepare reblended milk, raw whole milk was first separated at 50 °C. The resulting skim milk was pasteurized, concentrated by reverse osmosis, and remixed (reblended) with the cream. Each of the three milks was subjected to various processing treatments as shown in Fig. 5.2. These treatments included homogenization, UHT treatment and combinations of the two.

Processing treatments (separation, pasteurization, sterilization and RO concentration) and analyses (particle size distribution measurements and transmission electron microscopy) were carried out as described in Chapter 3.

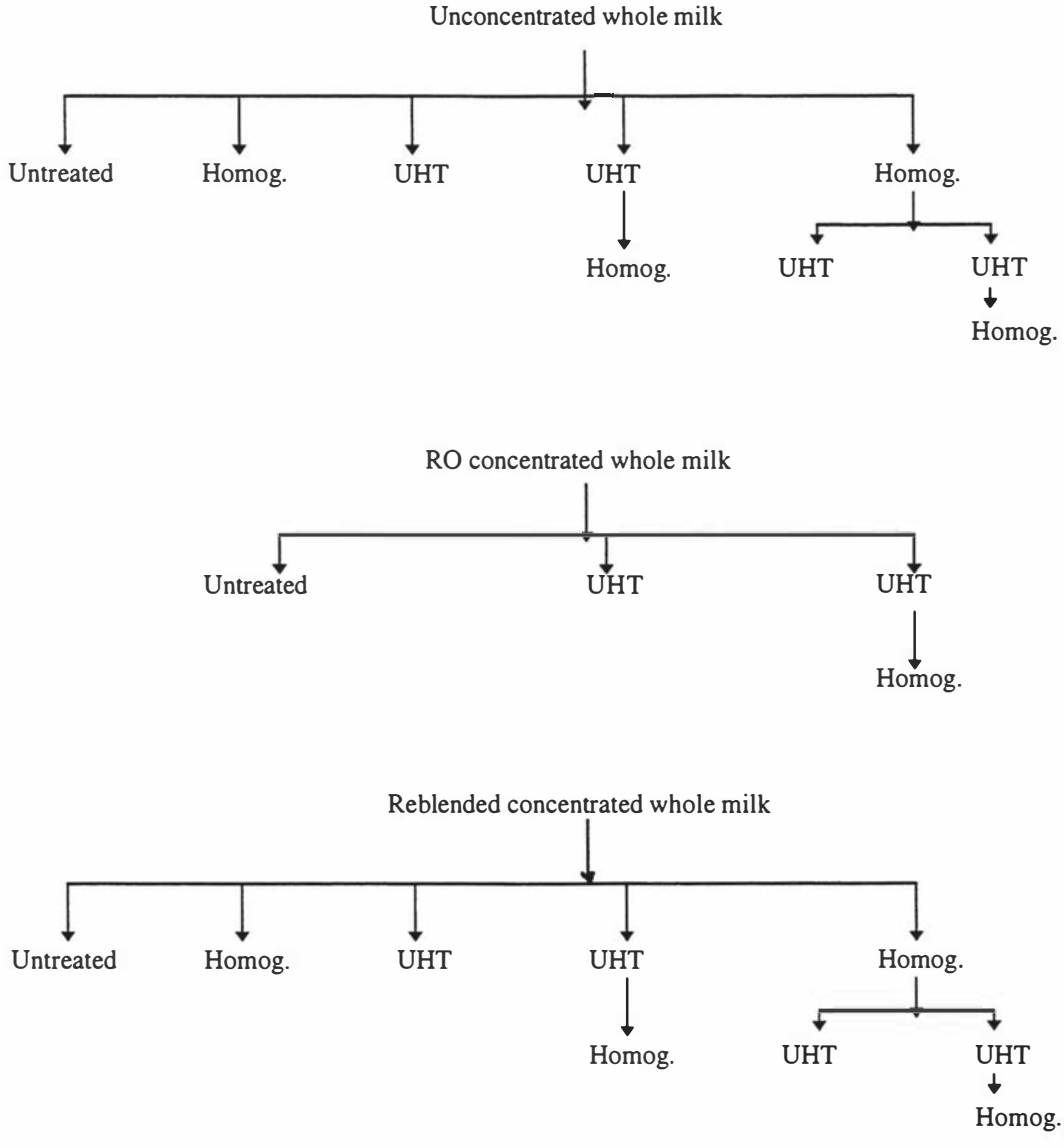


Fig. 5.2 Experimental processing treatments applied to the three types of milk investigated (Homog. = single-stage homogenization; UHT = ultra-high temperature sterilization; RO = reverse osmosis).

5.3 RESULTS AND DISCUSSION

5.3.1 Effects of RO concentration of whole milk on the state of the fat globules

5.3.1.1 Particle size distributions

Whole milk was concentrated to various volume concentration factors (1.19X, 1.56X and 2.36X), and particle size distributions (volume percentage versus diameter) were determined using light-scattering. As the light-scattering technique used was unable to distinguish whether a scattering particle was a casein micelle or a fat globule, the volume distribution derived from the light scattering data was of all particles present in the system. However, because of the greater scattering power of fat globules, heavily weighted by their size, it is likely that the measured distributions were predominantly those of fat globules. Any contribution by casein micelles was likely to have influenced only the low diameter side of the distributions (McCrae and Lepoetre, 1996).

Another measurement was made after a sample of the same milk had been treated with protein dissociating medium (0.05 M EDTA and 2% w/v SDS) to disperse the casein micelles. EDTA complexes with calcium and magnesium, causing dissociation of casein micelles, and SDS acts as a dispersing agent. Hence in this system, the distribution measured by light-scattering can be considered to have been due to individual fat globules only.

Fig 5.3a shows the changes in the particle size distribution of whole milk as it was being concentrated by the RO process. Normal whole milk showed a bimodal size distribution with the particle size ranging from 0.1 to 10 μm. As the milk was concentrated, the particle size distribution shifted gradually from a bimodal to a monomodal distribution with the particle size ranging from 0.1 to 2 μm, thus clearly demonstrating the homogenizing effect of the RO concentration process. The particle size distribution remained unchanged in each case when the milks were treated with protein dissociating medium. This suggests that the presence of casein micelles in the system had only a minor effect on particle size distribution. In addition it also indicates that fat globule clusters were not present in these milks (Fig. 5.3b).

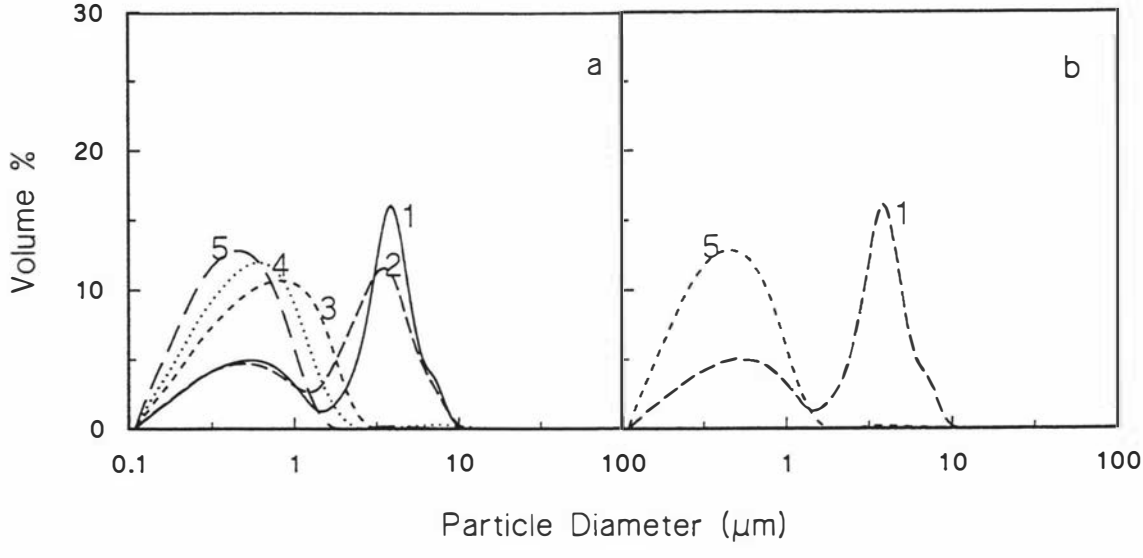


Fig. 5.3 Effects of RO concentration of whole milk on the particle size distribution. a) 1, normal whole milk; 2, immediately after the start of concentration (~ 1.0X, 1 min); 3, after ~ 1.19X (25 min); 4, after ~ 1.56X (70 min); 5, after ~ 2.36X (150 min); b) after treatment with protein dissociating medium; 1, normal whole milk; 5, RO concentrated milk (2.36X).

Fig. 5.4 shows the change in the weight-average mean particle diameter (d_{43}) with increase in concentration factor. (The time required to achieve a particular concentration factor is also indicated). There was a sudden decrease in d_{43} from 4.76 μm (0 min) to 2.43 μm (1 min), although the concentration factor remained essentially unchanged. The d_{43} decreased from 0.77 μm to 0.52 μm as the concentration factor was increased from 1.19 to 2.36X. The times required to achieve the concentration factors of 1.19, 1.56 and 2.36 were 25, 70, and 150 min (Fig 5.4). These observations suggest that the fat globules were disrupted mainly at the beginning of the RO concentration process, and that continuous recycling of the milk to achieve higher concentration factors caused further slight disruption of fat globules. Thus it appears that the RO process, in addition to concentrating the milk solids, had a homogenizing effect on the fat globules. In order to check this, a sample of whole milk was homogenized using a conventional two-stage valve homogenizer; the resulting change in particle size distribution was found to be similar to that obtained by RO concentration (Fig. 5.5).

The milk, during recycling in the RO concentration process, passed continuously through the pressure relief valve, and this caused the disruption of fat globules and consequently reduced the fat globule size. de Boer and Nooy (1980) also noted the homogenizing effect on concentration of milk by RO. They suggested that dead end RO, or RO at low temperature (<10 °C) might prevent damage to milk fat globules.

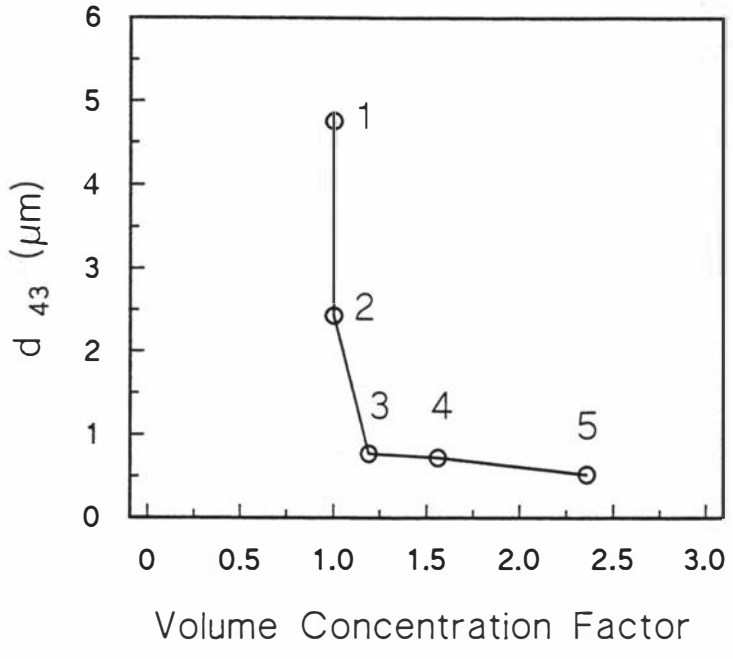


Fig 5.4 Effect of RO concentration of whole milk on the weight-average mean particle diameter, d_{43}. 1, normal whole milk; 2, immediately after the start of concentration (~ 1.0X, 1 min); 3, after ~ 1.19X (25 min); 4, after ~ 1.56X (70 min); 5, after ~ 2.36X (150 min).

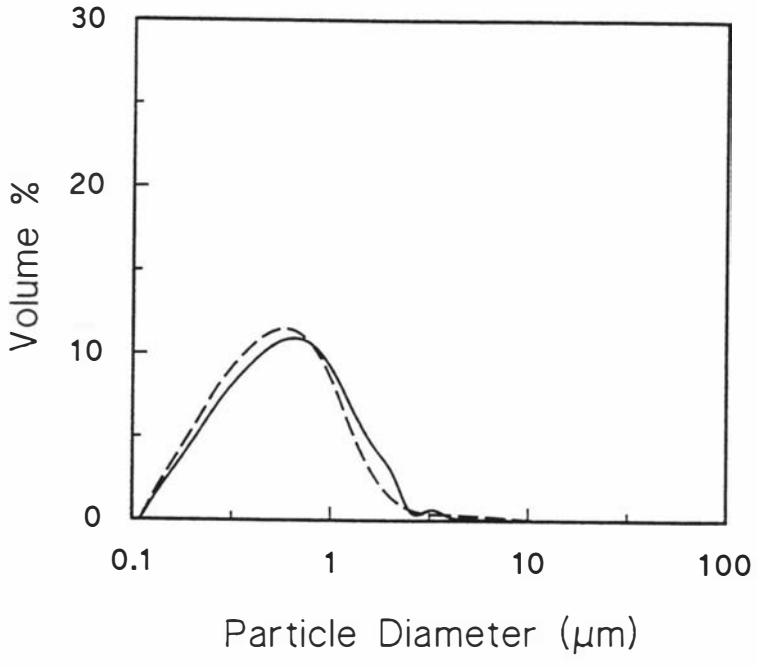


Fig. 5.5 Particle size distribution of unconcentrated whole milk homogenized by a two-stage conventional homogenizer with (——) and without (----) protein dissociating medium.

5.3.1.2 Electron microscopic examination of RO concentrated milks

Typical electron micrographs obtained for RO concentrated milks (2.5X) are shown in Fig. 5.6a-d. The casein particles are stained black on account of adsorbed osmium, whereas the fat globules are colourless.

This appearance is due to the saturated character of milk fat, which is not fixed by osmium tetraoxide and is washed out by the dehydration steps during sample preparation. A few remnants of the membranes of fat globules are still visible. Few distinct casein micelles were observed in any of the preparations. Many very small fat globules, with diameters in the range 0.02-0.3 μm (average = 0.04 μm), were present (Fig. 5.6a). These fat globules were not completely separated from one another, but in many cases were grouped together, and apparently held together by quantities of protein. Although each fat globule had a distinctly defined membrane, there were in addition extra bodies of protein, presumably derived from casein micelles, which held together clumps of the smaller fat globules, and which were also attached to parts of the larger fat globules (Fig. 5.6b-d). No isolated intact casein micelles could be observed; all were attached to the fat globules. The most remarkable particles that were observed in RO concentrated milks seemed to have been derived from casein micelles, since they were approximately spherical in shape, were stained heavily and contained small fat globules embedded within their structures. These particles appeared to be unique to RO concentrated milks since they were not observed in conventionally homogenized unconcentrated whole milk (Fig. 5.7).

As can be seen from Fig.5.7(a-c), the fat globules in the conventionally homogenized unconcentrated milk were partly surrounded by smooth and fairly thin membranes of protein, and at some points casein micelles or micellar fragments were attached. This is typical of what has been observed in previous studies of homogenized milks (Henstra and Smith, 1970; Dalgleish *et al.*, 1996). Not only were there casein micelles on the surfaces of the fat globules, they were also found "free" in the serum. The homogenization process had not broken the micelles, as most of the micelles in the serum appeared to be intact. The fat globules had a diameter in the range 0.02-1.00 μm, with an average of 0.09 μm.

Although the particle size distribution of RO concentrated milk as measured by light-scattering experiments was similar to that of conventionally homogenized whole milks (Figs. 5.3a and 5.5), the electron microscopy showed marked differences in the make-up of protein and fat particles (Figs. 5.6c and 5.7c). It is worth noting that unconcentrated homogenized milk is being compared here with the RO concentrated milk. Reblended milk (skim milk concentrated by RO and blended with cream) that had been homogenized using a conventional valve homogenizer (Fig. 5.18a) exhibited protein and fat particle structures similar to those of unconcentrated homogenized milk. (Reblended milks are discussed in section 5.3.2.2).

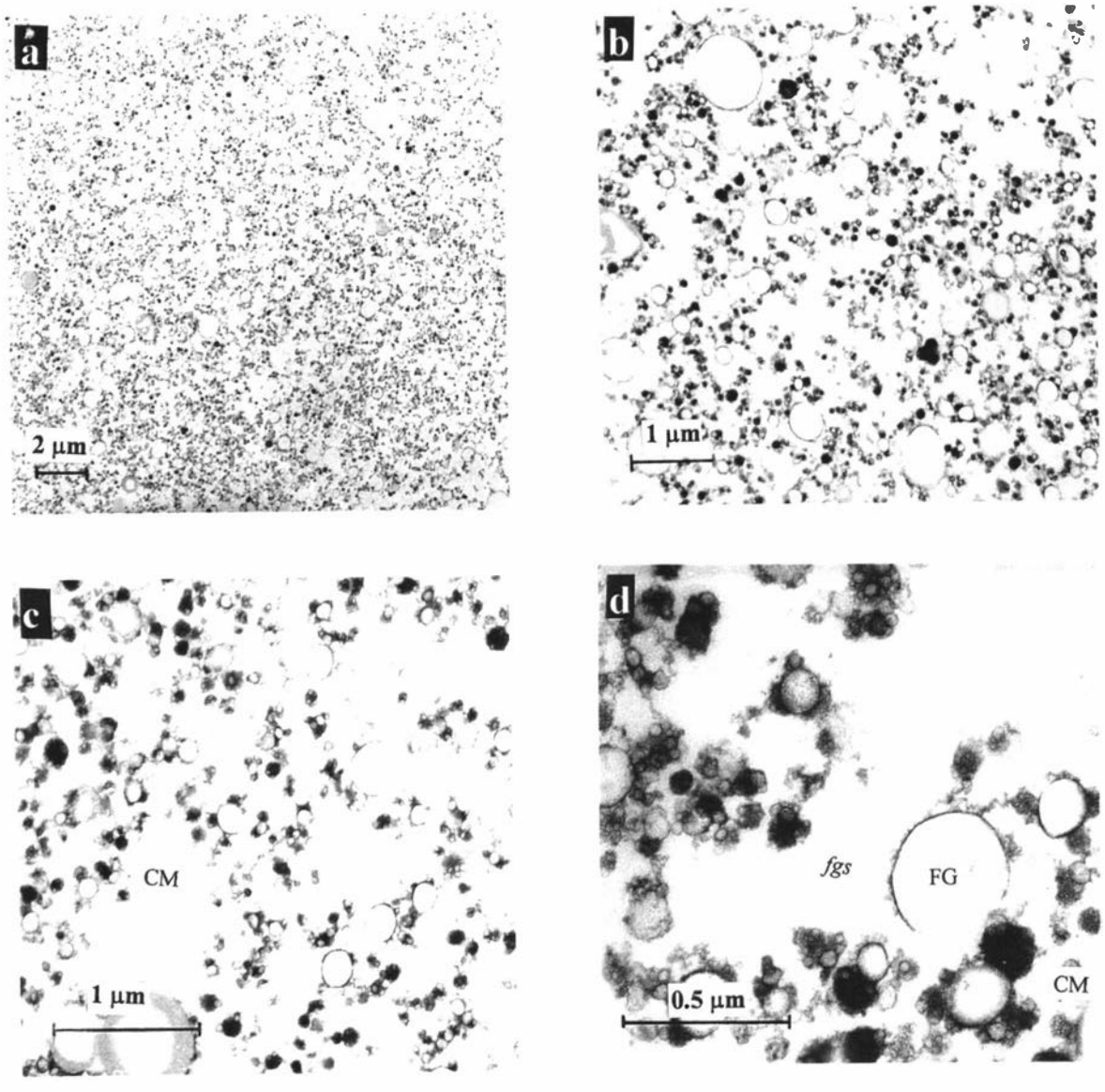


Fig. 5.6 Transmission electron micrographs of RO concentrated (2.5X) whole milk showing fat globules (FG), casein micelles (CM), fat globule surface (*fgs*). Magnification a) 3400X, b) 11200X, c) 21200X, d) 48600X.

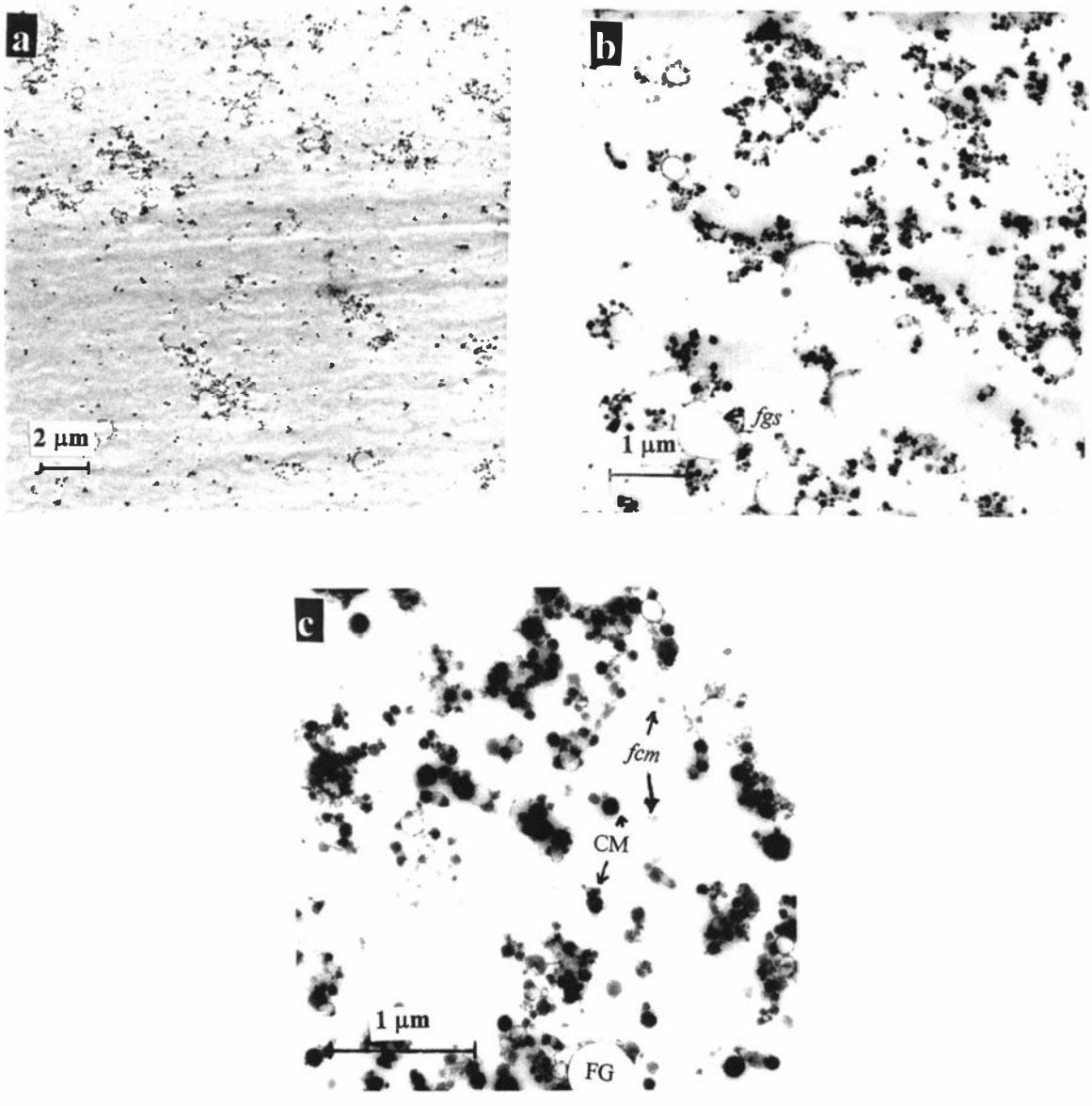


Fig. 5.7 Transmission electron micrographs of conventionally homogenized unconcentrated whole milk showing fat globules (FG), casein micelles (CM), fat globule surface (*fgs*), fragmentation of casein micelles (*fcm*). Magnification a) 3400X, b) 11200X, c) 21200X.

5.3.1.3 Particle size measurements from electron microscopy

The sizes of particles in RO concentrated milk, unconcentrated homogenized milk and homogenized reblended milk were determined from electron micrographs by measuring the diameters of the individual fat globules. Number of globules is plotted against globule diameter in Fig. 5.8. RO concentrated milk had a narrow size distribution compared to the other two milk systems. The average diameter of the fat globules was found to be 0.042 μm for RO concentrated milk as compared to 0.16 μm for conventionally homogenized unconcentrated milk and 0.12 μm for conventionally homogenized reblended milks.

The d_{43} of the fat globules, determined from light-scattering, was found to be only slightly greater for the conventionally homogenized milks (0.76 μm) as compared with RO concentrated milk (0.58 μm) (Table 5.2). This may reflect the inability of the light-scattering equipment to measure the large number of small fat globules, especially those below 0.1 μm, present in RO concentrated milks.

No previous work has been reported on the nature of fat and casein particles in RO concentrated milks. However, it is interesting to note that the microfluidization of whole milk can produce a population of very small fat globules with diameters of the order of 100 nm or less as measured by integrated light scattering (Strawbridge *et al.* 1995) and electron microscopy (Dalgleish *et al.* 1996). The principle of operation of the microfluidizer is different from that of the conventional homogenizer; the liquid is divided into two or more microstreams that are projected against one another at an angle of 180° (Robin *et al.*, 1992). These microstreams have very high velocities and undergo a sudden pressure drop when they collide. Using electron microscopy, Dalgleish *et al.* (1996) observed that in microfluidised milk, small fat globules, in many cases, were embedded in the casein micelles, and often several of these small globules were found embedded in a single casein micelle. (Since these "micelles" were spherical and made of protein, it was assumed that they were casein micelles, whether native micelles or reformed casein particles). The formation of the fat-protein particles was presumed to result from the "firing" of the small fat globules at the micelles with sufficient energy that they were capable of penetrating the micellar structure, in a way in which a large fat globule would

be unable to do. Dalgleish *et al.* (1996) described the microfluidised milks as micro-emulsions of milk fat and protein.

Dalgleish *et al.* (1996) suggested two possible mechanisms for the uniquely structured microfluidized milk particles. Firstly, the casein micelles are broken up due to the severe conditions present in the equipment. The broken up casein micelle would act more effectively as an emulsifier, because of the increased number of casein particles in the solution which could cover a larger area of the interface. Alternatively, the micelles may not be broken *per se*, but they may be thrown against fat globules with such force as to disrupt these and allow them to spread. The presence of embedded fat globules within the micelles supports this supposition. The mechanism of formation of fat and protein structures observed in RO concentrated milk (Fig.5.6) is not clear. RO is much less severe, operating typically at a pressure of 40 bar. As the milk is being concentrated, it suffers repeated homogenization during repeated passes through the pressure relief valve in its path; the valve coincidentally acts also as a homogenizing valve.

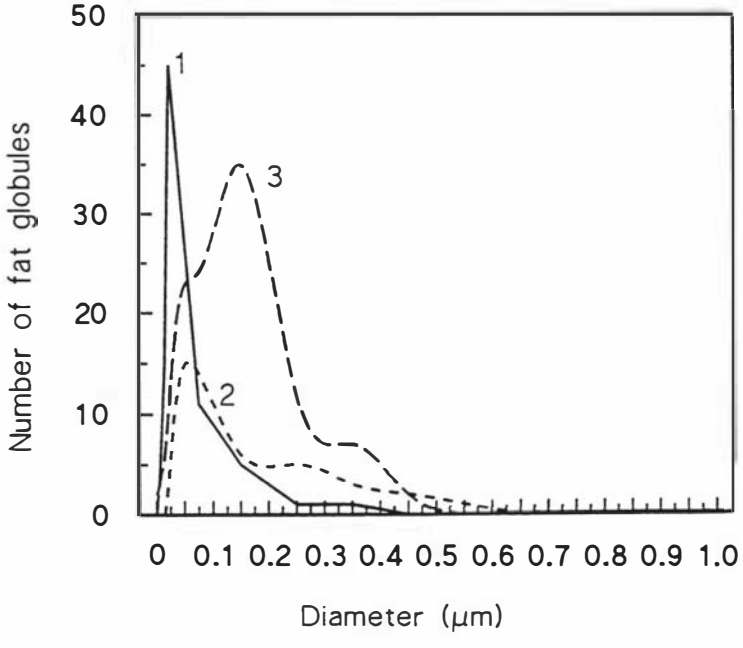


Fig. 5.8 Fat globule size distributions in 1, RO concentrated milk; 2, unconcentrated homogenized milk 3, homogenized reblended milk; size distributions were estimated from several electron micrographs by counting globules with different diameters.

5.3.2 Effects of UHT sterilization on milks concentrated by reverse osmosis

5.3.2.1 Effect of holding temperature on particle size distribution

In preliminary experiments, unconcentrated homogenized whole milk (homogenized in a conventional valve homogenizer), and RO concentrated whole milk, were heated to temperatures in the range 80 to 140 °C and held for 4 s in the UHT plant. The effects of this heat treatment on the particle size distributions of of these two milks are shown in Figs. 5.9 and 5.10, respectively. Particle size distribution measurement was used to characterize aggregate formation.

When unconcentrated homogenized milk was heated there was no change in particle size at holding temperatures of up to 125 °C, but at 130 °C some large particles were formed (Fig. 5.9 b). Further increase in temperature resulted in an increase in the proportion of large particles in the system. In the case of RO concentrated milk, the trend was somewhat similar (Fig. 5.10a, b) but the formation of large particles started at a somewhat lower holding temperature (125 °C).

In unconcentrated homogenized milk, there was not much change in the weight-average particle diameter (d_{43}) at the lower end of the temperature range used but the d_{43} increased markedly with increase in temperature from 120 °C to 130 °C (Table 5.1). In the RO concentrated milks there was a continuous change in the d_{43} over the entire range of the temperatures used (Table 5.1). However, the particles formed in the unconcentrated system were larger than the particles formed in the RO concentrated system at the higher end of the temperature range investigated.

Fig. 5.11 shows the extent of whey protein denaturation in unconcentrated homogenized milk and RO concentrated milk after being held at various temperatures for 4 s. The extent of whey protein denaturation at a given temperature was slightly greater in the RO concentrated milks than in the unconcentrated whole milks. On plotting d_{43} versus the extent of whey protein denaturation (Fig. 5.12), it was found that in both types of milk, approximately 63-65 % of whey protein denaturation was required for the formation of large particles or aggregates. Whey protein denaturation of up to 60% did not result in the formation of aggregates.

In both systems, treatment with protein dissociating medium restored the particle size distributions to the form resulting from homogenization alone, indicating the absence of either coalescence or disruption of fat globules (Table 5.1). The formation of large particles thus appears to have resulted from aggregation or flocculation caused by heat-induced changes in casein micelles and whey proteins.

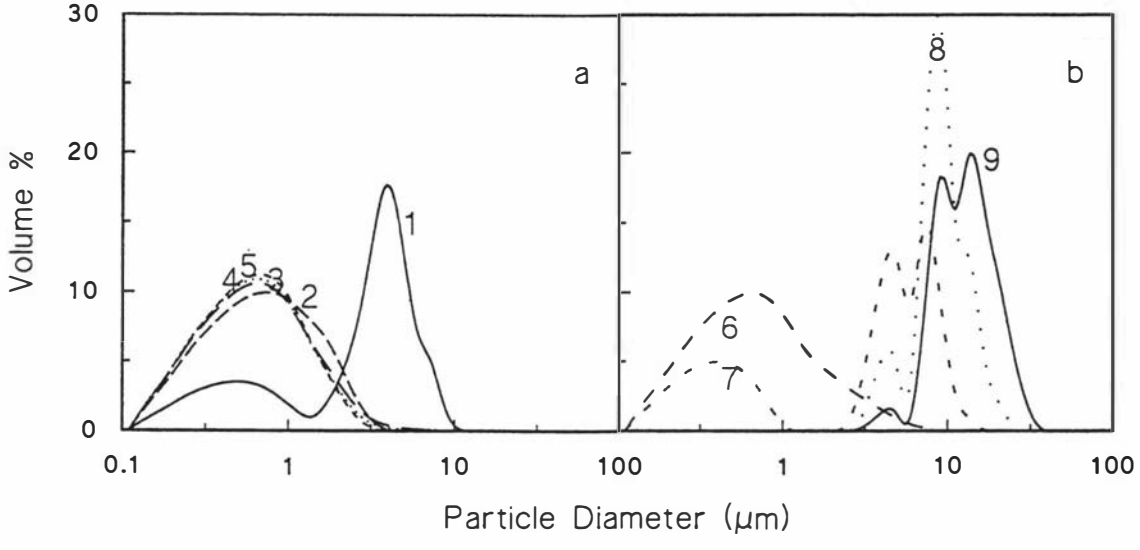


Fig. 5.9 Effect of holding temperature (holding time = 4 s) on particle size distribution in unconcentrated milk a) 1, unheated whole milk; 2, unheated homogenized milk; 3, homogenized milk heated at 80 °C; 4, 100 °C; 5; 120 °C b) 6, 125 °C; 7, 130 °C; 8, 135 °C or 9, 140 °C.

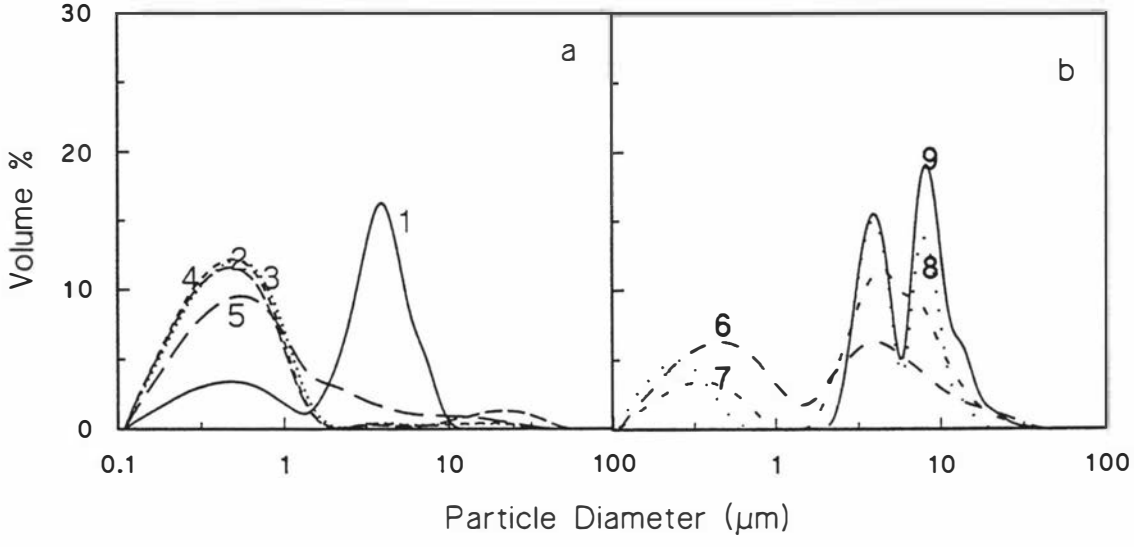


Fig. 5.10 Effect of holding temperature (holding time = 4 s) on particle size distribution in a) 1, unheated whole milk; 2, unheated RO concentrated milk; 3, RO concentrated milk heated at 80 °C; 4, 100 °C; 5, 120 °C b) 6, 125 °C; 7, 130 °C; 8, 135 °C or 9, 140 °C.

Table 5.1 Volume to surface mean diameter (d_{32}) and weight average mean diameter (d_{43}) of unconcentrated homogenized milk and RO concentrated whole milk held at different temperatures for 4 s.

System	Heating Temperature (°C)	Without protein dissociating medium		With protein dissociating medium	
		d_{32} (μm)	d_{43} (μm)	d_{32} (μm)	d_{43} (μm)
Unconcentrated homogenized milk	Nil	0.53	0.99	0.42	0.60
	80	0.46	0.73	0.41	0.60
	90	0.47	0.77	0.42	0.65
	100	0.46	0.77	0.43	0.68
	110	0.47	0.78	0.43	0.68
	120	0.47	0.81	0.43	0.69
	125	0.50	0.97	0.42	0.70
	130	0.94	5.86	0.43	0.69
	135	8.13	9.46	0.41	0.75
	140	11.88	14.33	0.43	0.69
RO concentrated milk	Nil	0.40	0.53	0.38	0.55
	80	0.38	0.68	0.38	0.55
	90	0.38	0.71	0.38	0.54
	100	0.40	0.93	0.37	0.54
	110	0.39	0.95	0.38	0.55
	120	0.49	1.68	0.38	0.54
	125	0.67	3.55	0.38	0.55
	130	1.09	5.04	0.38	0.55
	135	0.82	6.60	0.38	0.57
	140	5.55	7.29	0.37	0.55

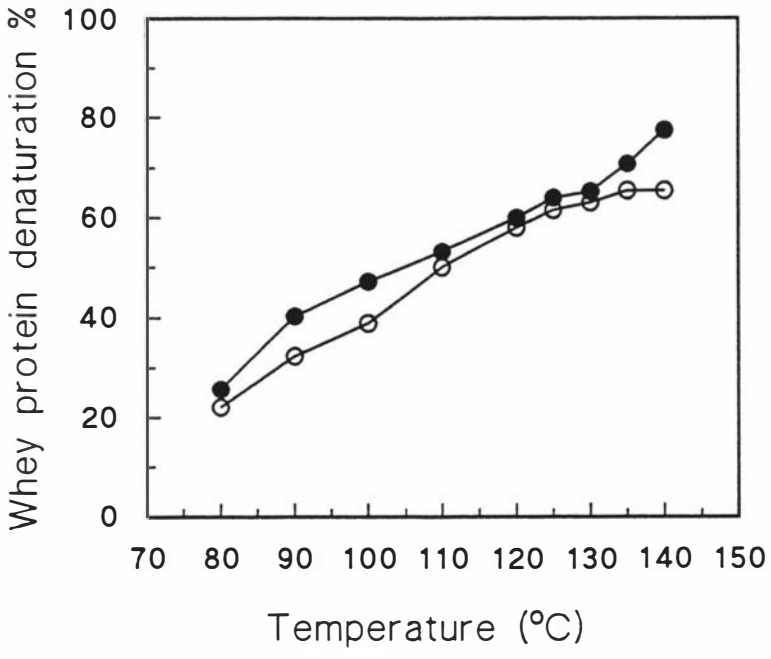


Fig. 5.11 Effect of holding temperature (holding time = 4 s) on the extent of whey protein denaturation in (○) unconcentrated homogenized milk and (●) RO concentrated milk.

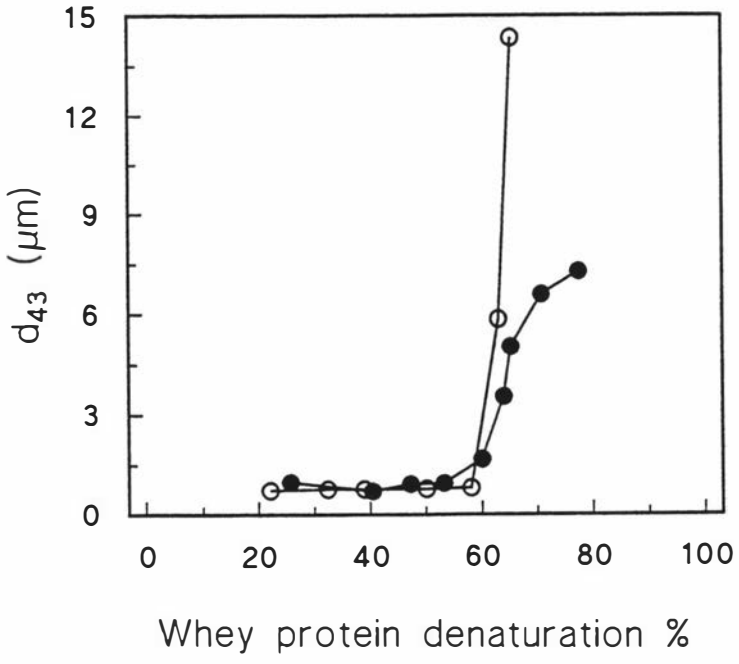


Fig. 5.12 Relationship between the extent of whey protein denaturation and the average particle size (d_{43}) in (○) unconcentrated homogenized milk and (●) RO concentrated milk.

5.3.2.2 Effects of combined homogenization and UHT treatments

(i) Unconcentrated systems

Fig. 5.13 shows particle size distributions for unconcentrated milk treated in various ways. Particle size distributions of milk UHT sterilized, and milk UHT sterilized and subsequently homogenized (Figs. 5.13a, b), were essentially identical to those of whole milk and homogenized milk respectively (Figs. 5.3a and 5.5). Again, treatment with protein dissociating medium did not change the particle size distributions.

When conventional homogenization preceded UHT sterilization, the latter resulted in the formation of large particles in the milk (Fig. 5.13c); d_{43} increased by a factor of 3.5 and d_{32} increased by a factor of 9.4 when compared to non-homogenized, unheated milk (Table 5.2). The average size (d_{43}) of the aggregates was 9-11 μm. Electron microscopy of these milks (Fig. 5.14a, b, c) showed that after UHT treatment the micelles had lost their spherical structures, and they appeared to have fused together with the fat globules embedded among them to form large particles. Fat globules retained their spherical structures after UHT treatment. Some particles smaller than casein micelles had been formed; presumably these were casein micelle fragments or denatured whey proteins. Some of the casein micelles were in the serum phase whereas some were attached to the fat globule surfaces. In some instances, one micelle was shared between two or more fat globules. Micelles in the serum developed small protuberances, possibly consisting of denatured whey proteins. Some casein micelles adsorbed on the fat globule surfaces had lost their spherical structure, possibly owing to spreading on the surfaces. Also, the micelles increased in size as result of UHT treatment.

There is no direct reference available in the literature to which the above results can be compared. However, Singh *et al.* (1996) published electron micrographs of heat treated recombined milk. The microstructures of protein and fat particles observed after heat treatment (130 °C for 4 min) showed the aggregation behaviour of these particles. The casein micelles (both "free" and attached to the fat globules) in milks heated at pH 6.3 or 6.7 had filamentous appendages projecting irregularly from their surfaces. The appendages were larger and more clearly distinguishable at pH 6.3 than at pH 6.7. Mohammad and Fox (1987) identified these appendages formed on heating skim milk as denatured whey proteins complexed with micellar κ-casein. On heating milk at

Table 5.2. Volume to surface mean diameter (d_{32}), weight average mean diameter (d_{43}) and specific surface area (S.S.A) of whole milk systems processed in various ways, measured with and without treatment with protein dissociating medium. (H = homogenized, UHT = UHT sterilized, RO = RO concentrated, S + C = reblended milk (skim milk plus cream)).

System	Experimental Processing Treatment	Without protein dissociating medium			With protein dissociating medium		
		d_{32} (μm)	d_{43} (μm)	S.S.A ($m^2 g^{-1}$)	d_{32} (μm)	d_{43} (μm)	S.S.A ($m^2 g^{-1}$)
Unconcentrated pasteurized whole milk	None	1.02	3.13	5.86	1.22	3.43	4.90
	H	0.46	0.76	12.94	0.43	0.70	14.00
	UHT	1.07	3.43	5.59	1.35	4.25	4.42
	UHT-H	0.35	0.48	17.27	0.33	0.45	18.22
	H-UHT	9.63	11.09	0.62	0.43	0.73	14.05
	H-UHT-H	1.01	7.20	5.93	0.40	0.63	15.03
RO concentrated pasteurized whole milk (RO)	RO	0.40	0.58	15.09	0.40	0.56	15.09
	RO-UHT	5.53	7.46	1.08	0.41	0.55	14.76
	RO-UHT-H	0.83	4.70	7.24	0.37	0.51	16.09
Reblended concentrated whole milk (S + C)	S+C	1.10	3.18	5.48	1.22	5.71	4.93
	S+C-UHT	2.00	13.51	2.99	0.88	3.07	6.81
	S+C-UHT-H	0.70	5.60	8.54	0.40	1.59	14.93
	S+C-H	0.47	0.75	12.85	0.43	0.67	14.08
	S+C-H-UHT	5.92	7.45	1.01	0.45	0.93	13.25
	S+C-H-UHT-H	0.83	4.26	7.26	0.35	0.49	17.12

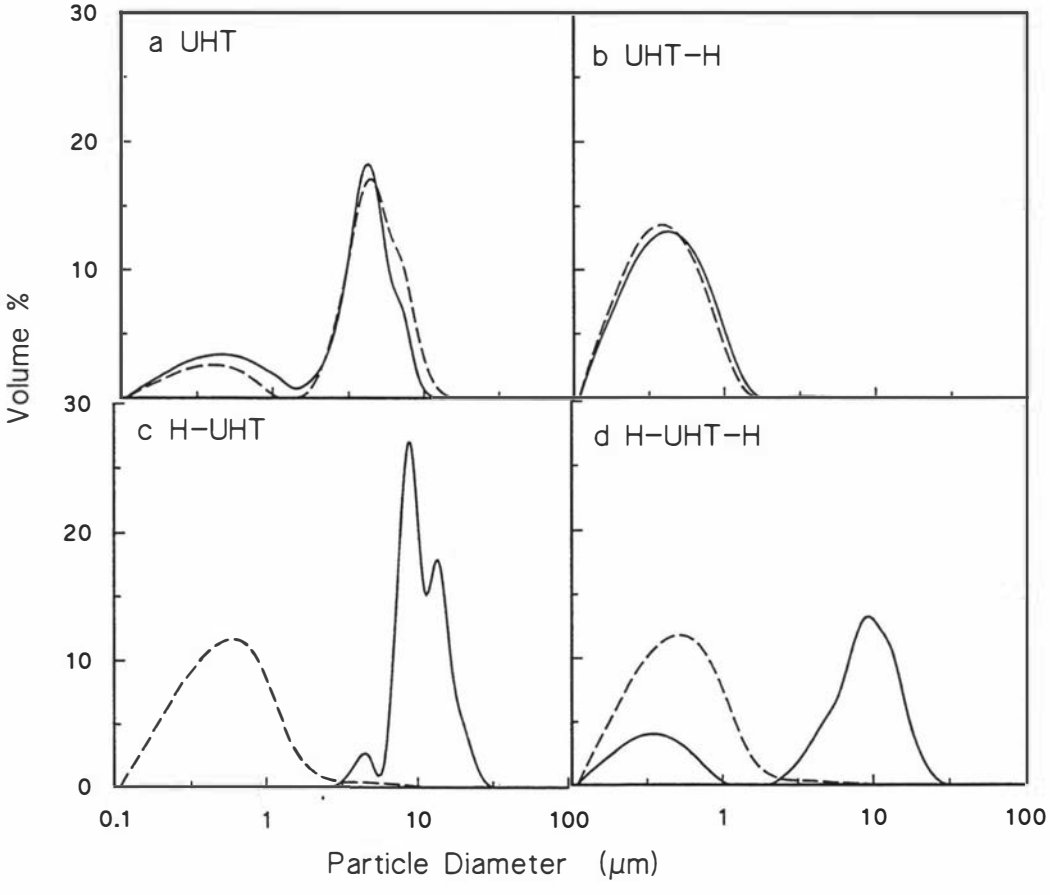


Fig. 5.13 Particle size distributions of unconcentrated whole milk (a) UHT sterilized, (b) UHT sterilized and subsequently homogenized (UHT-H), (c) homogenized and subsequently UHT sterilized (H-UHT), (d) homogenized, UHT sterilized and then homogenized again (H-UHT-H), without (——) and with (----) protein dissociating medium.

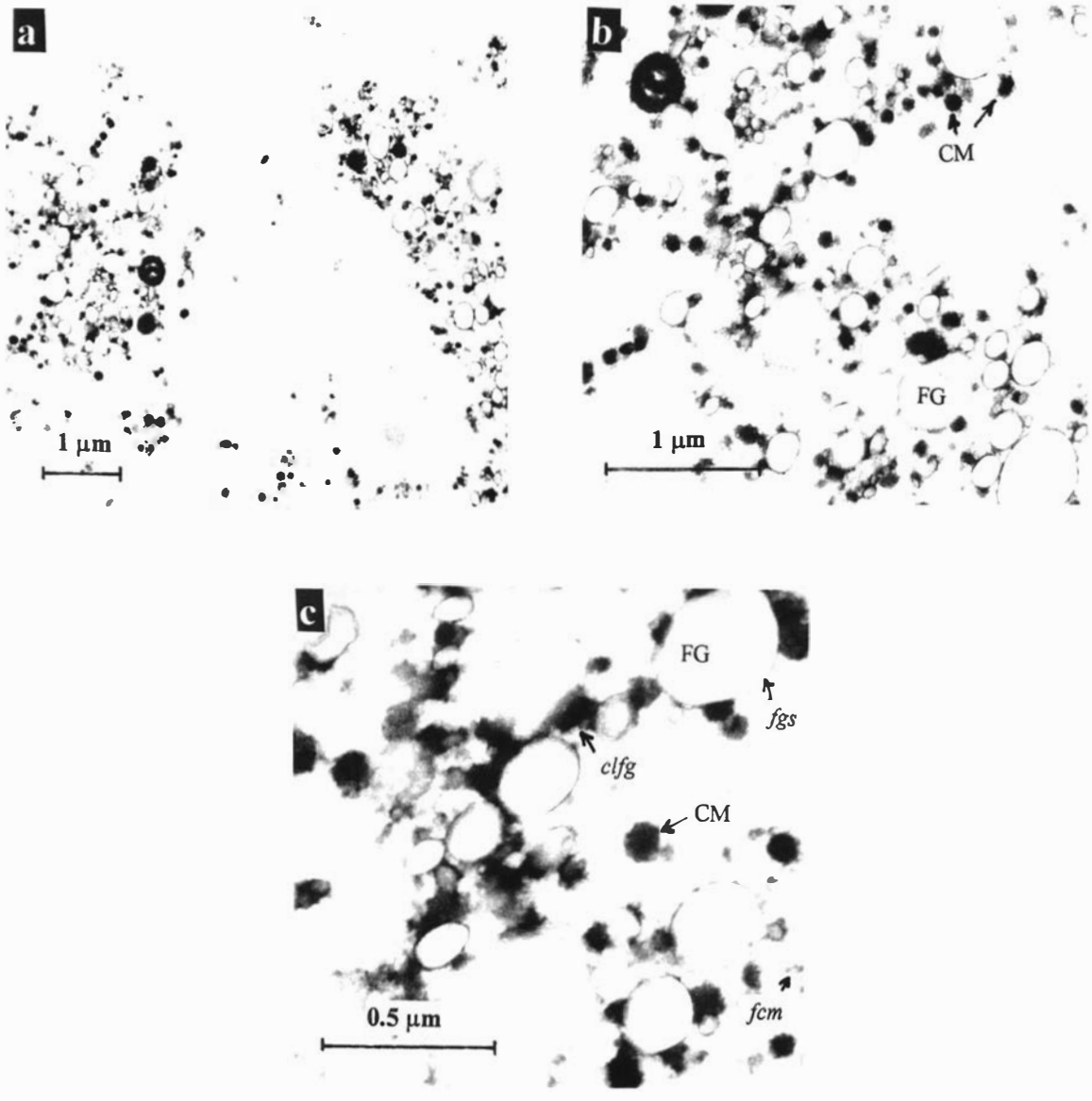


Fig. 5.14 Transmission electron micrographs of unconcentrated whole milk that had been homogenized and then UHT sterilized showing fat globules (FG), casein micelles (CM), fat globule surface (*fgs*), fragmentation of casein micelles (*fcm*), casein linking fat globules (*clfg*). Magnification a) 11200X, b) 21200X, c) 48600X.

higher pH (pH 7.1), the casein micelles (both "free" and attached to fat globules) were no longer spherical and the surfaces of many of the micelles appeared to have been dissolved. The appendages observed at the micelle surface at pH 6.3 or 6.7 were absent. These results suggest that on subjecting milk to heat treatment, aggregation of casein micelles is observed which indirectly support the results in this study (i.e. aggregation leads to an increase in the particle size).

A second homogenization, applied after UHT sterilization, caused partial breakdown of the large particles formed by the UHT treatment (Fig. 5.13d). In the cases of both homogenized and subsequently UHT sterilized milk (H-UHT) , and homogenized UHT sterilized and then homogenized again milk (H-UHT-H), treatment with protein dissociating medium restored the particle size distributions to the form resulting from homogenization alone, indicating the absence of either coalescence or disruption of fat globules (Table 5.2).

Previous work (Muir, 1984; van Boekel and Walstra, 1989; Streuper and van Hooydonk, 1986; Melsen and Walstra, 1989; van Boekel and Folkerts, 1991; Corredig and Dalgleish, 1996a) has shown that natural (non-homogenized) fat globules are quite stable in indirect UHT sterilization, both to the inevitable mechanical agitation involved and to the heat treatment itself. The fat globules in fact become more stable to coalescence as a result of whey protein deposition on their surfaces (van Boekel and Folkerts, 1991; Dalgleish and Banks, 1991). Since the denatured whey protein is unreactive (Dalgleish and Banks, 1991), and because casein in non-homogenized unconcentrated milk is very heat stable (Singh, 1993), no aggregation or coagulation occurs. Fink and Kessler (1985a) reported some loss of coalescence stability at UHT temperatures in the range 105-135 °C for a range of holding times, but this finding has been refuted by later work (van Boekel and Walstra, 1989, Streuper and van Hooydonk, 1986, Melsen and Walstra, 1989; van Boekel and Folkerts, 1991, Dalgleish and Banks, 1991). The possible explanation of the discrepancy between the findings of Fink and Kessler (1985a) and those of other workers may be due to features of the equipment used by Fink and Kessler which might have resulted in the induction of partial coalescence at some processing stage.

Lack of applied back pressure during indirect UHT heating and during subsequent cooling can result in globule coalescence (if air bubbles form in the milk) or disruption (if cavitation (boiling) occurs) (Streuper and van Hooydonk, 1986).

Homogenization *per se* of whole milk can be expected to have much the same effect on the particle size distribution whether it is carried out before or after a heat treatment (Muir, 1984). When homogenization is carried out after a relatively intense heat treatment, casein and whey protein complexes adsorb on to the newly created fat surface (van Boekel and Walstra, 1989). Sharma and Dalgleish (1994) reported that the adsorbed layers are thicker (~ 34 nm) when homogenization is carried out before the heat treatment (because of the deposition of whey proteins (~0.64 mg m^{-2}) and casein on the fat surface) than when homogenization is carried out after heat treatment (~20 nm adsorbed layer thickness and ~ 0.48 mg m^{-2} of whey protein deposition). "Pre-homogenization" favours the formation of homogenization clusters if the fat content is greater than 8%. Differences in stability to coalescence are not to be expected (van Boekel and Walstra, 1989).

Homogenization of milk that has not been subjected to high temperature heating results in the adsorbed layers on the fat globules containing reactive casein (and some whey proteins) (Fink and Kessler, 1985b). The behaviour of the globules is governed mainly by the adsorbed casein (van Boekel and Walstra, 1989, Melsen and Walstra, 1989, Fink and Kessler, 1985b), which is less heat stable than native (i.e. unadsorbed) casein micelles and can undergo heat coagulation and aggregation during subsequent UHT sterilization (Muir, 1984; Melsen and Walstra, 1989; Fink and Kessler, 1985a; Dalgleish and Sharma, 1993). The result is that indirect UHT sterilization of even unconcentrated homogenized milk results in the formation of fat globule-protein aggregates (Melsen and Walstra, 1989; van Boekel and Folkerts, 1991; Fink and Kessler, 1985b). Melsen and Walstra (1989) and van Boekel and Folkerts (1991) found, by carrying out fat globule size measurements after treatment of samples with protein dissociating medium, that no globule coalescence occurred during heat aggregation (van Boekel and Walstra, 1989; van Boekel and Folkerts, 1991). They refute the findings of Fink and Kessler (1985b) that coalescence does occur to some extent.

Aggregates formed by indirectly UHT sterilizing recombined milk, in which the globule surface layers are similar to those in homogenized milk, are stronger than

homogenization clusters, but can be disrupted by turbulence. Turbulence in UHT plants thus limits aggregate size (Melsen and Walstra, 1989).

The experimental results shown in Figs. 5.3a and b, 5.5, 5.9 and 5.13 are in good agreement with the results of previous work just discussed. UHT sterilization had no effect on particle size distribution, causing neither globule coalescence nor disruption, and thus confirming the findings of van Boekel and Folkerts (1991). (The UHT plant used in the present work was pressurized by means of a back pressure device located downstream of the cooling section). Homogenization *per se* had the same effect on the particle size distribution whether or not the milk was first UHT sterilized; clustering did not occur in the homogenizing valve when homogenization followed UHT sterilization. When UHT sterilization followed homogenization, large aggregates of intact fat globules formed, and there was no evidence of coalescence. Aggregation was protein-mediated and was presumably the result of heat coagulation of adsorbed casein. The aggregates were strong, as shown by the fact that a second homogenization step, after UHT sterilization, only partially broke them down.

The aggregates formed during UHT treatment dissolved completely in the protein dissociating medium allowing the unchanged fat globule size distribution to be observed.

(ii) ***Reverse osmosis concentrated systems***

Fig. 5.15a shows the effect of UHT sterilization on the particle size distribution of RO concentrate; large particles were formed, the new particle size distribution being broadly similar to that of the analogous unconcentrated system (Fig. 5.13c). Treatment with the protein dissociating medium restored the particle size distribution to the form it had prior to UHT sterilization, indicating that none of the large particles was formed by fat globule coalescence. This was confirmed by electron microscopy; Fig. 5.16 shows that the large particles were aggregates consisting of intact globules in a protein matrix, as were the particles in the analogous unconcentrated system (Fig. 5.14). A comparison of Figs. 5.6 and 5.16 shows the dramatic change in milk concentrate structure brought about by UHT sterilization.

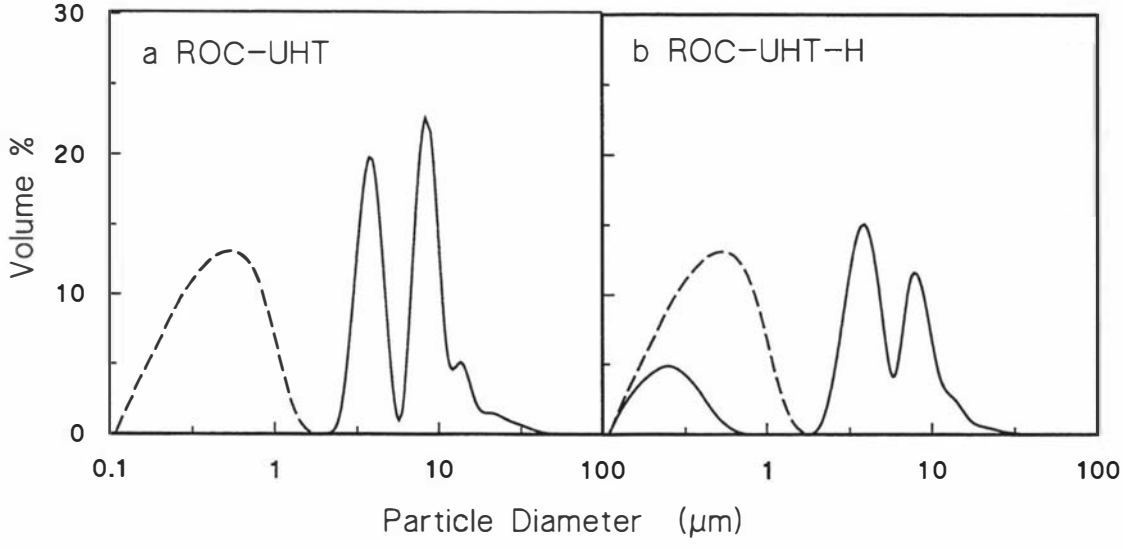


Fig. 5.15 Particle size distributions of 2.5X RO concentrated milk (a) UHT sterilized (ROC-UHT), (b) UHT sterilized and subsequently homogenized (ROC-UHT-H), without (—) and with (----) protein dissociating medium.

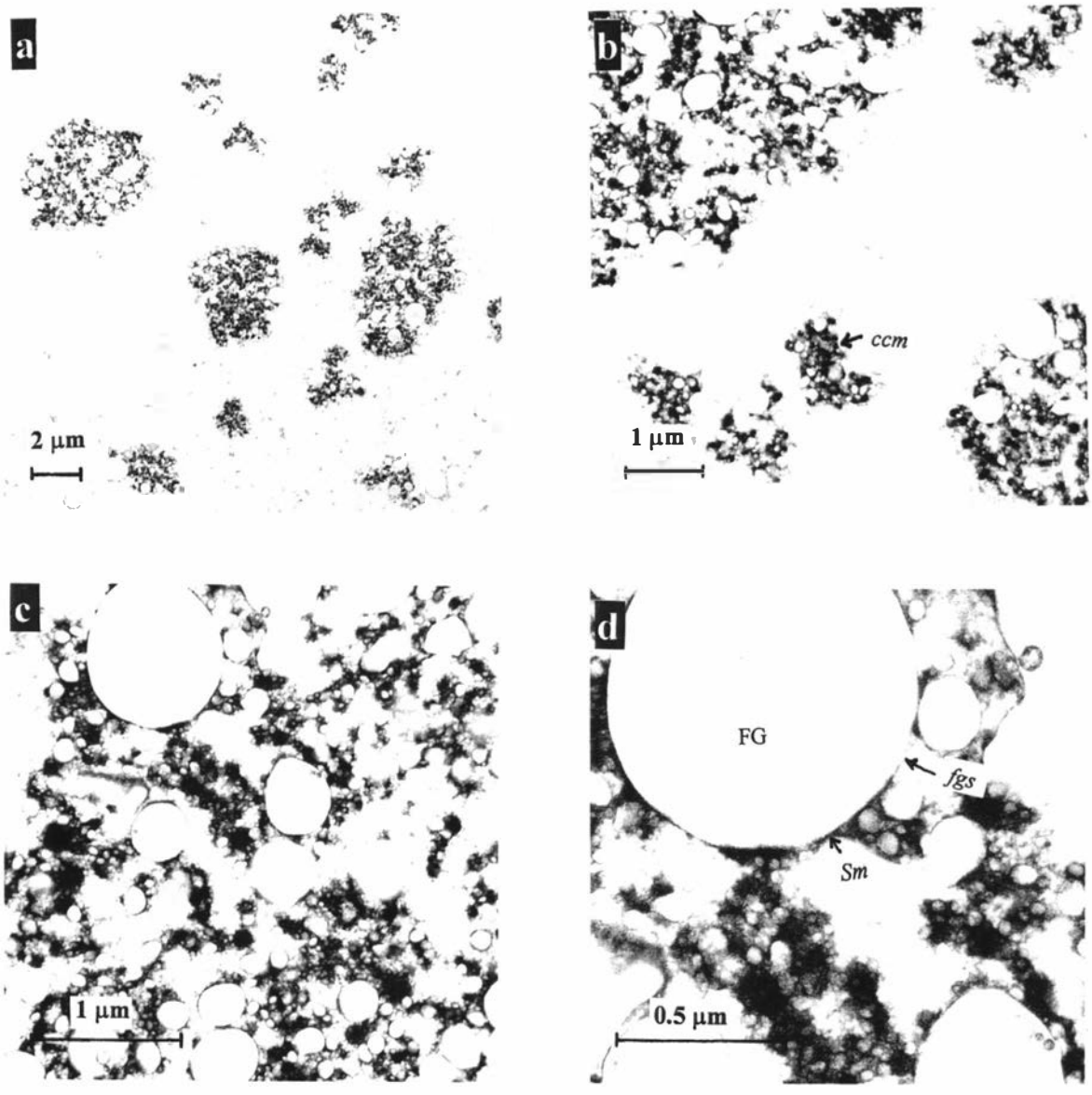


Fig. 5.16 Transmission electron micrographs of RO concentrated (2.5X) whole milk that had been UHT sterilized showing fat globules (FG), fat globule surface (*fgs*) submicellar casein (*Sm*), coalescence of casein micelles (*ccm*). Magnification a) 3400X b) 11200X, c) 21200X, d) 48600X.

The aggregates formed as a result of UHT treatment had a size range of 1.5 to 6 μm. These aggregates appeared to be more compact than those observed in unconcentrated homogenized milk (Fig. 5.14). As a result of UHT treatment, the casein micelles lost their spherical shape and were fused together, with fat micro-droplets embedded in them. No free casein micelles were observed in the serum phase; all the casein micelles were involved in aggregate formation. Some micelles appeared to have spread over the surfaces of the larger fat globules. Some of the small fat globules were in the form of bunches sticking to casein micelles. Casein micelles existed in the form of a continuous matrix, with small and large fat globules embedded in them.

Subsequent conventional homogenization of the aggregate-containing sterilized concentrate caused only partial breakdown of the aggregates (Fig. 5.15b). This behaviour was similar to that of the analogous unconcentrated system (Fig. 5.13d). However, it is interesting to note that the aggregates in the unconcentrated homogenized milk system were larger than those in the RO concentrated system after UHT treatment and subsequent conventional homogenization.

There are no reports in the literature with which these results may be compared directly. It is clear, however, that homogenization during RO concentration had a heat destabilizing effect similar to that caused by conventional homogenization of unconcentrated whole milk. It may be surmised that the basic mechanism, described above, was the same in each case.

(iii) Reblended systems

Fig. 5.17a shows the particle size distribution of untreated reblended milk, and Figs. 5.17b-f the particle size distributions of this milk after various treatments. Comparisons of Figs. 5.17a and b with Figs. 5.3a and 5.5, and with Figs. 5.13a and b, show that the particle size distributions of reblended milk (both before and after homogenization, and both before and after treatment with protein dissociating medium) were essentially identical to those of the analogous unconcentrated systems. Therefore, RO concentration of skim milk alone, followed by reblending with the previously-separated cream, had, *per se*, no effect on fat globule particle size distribution. (The apparent appearance of some large particles in the untreated reblended milk on treatment with protein dissociating medium (Fig. 5.17a) is considered to be an artefact).

Electron microscopic examination of reblended milk showed it to be similar to normal whole milk (Fig. 5.18a). The casein micelles were spherical in shape and dispersed "free" in the serum, and the natural fat globules had thin membranes. On homogenizing the reblended milk (Fig. 5.18b), the fat globules were reduced in size and the casein micelles were adsorbed on to the new fat globule surface. These micrographs are similar to those obtained for unconcentrated homogenized milk (Fig. 5.7).

Fig. 5.17c shows that when non-homogenized reblended milk was UHT sterilized, large particles formed which could be completely dispersed by protein dissociating medium to give a particle size distribution identical to that of the unheated milk (Fig. 5.17a). The large particles appeared to consist of aggregates of protein alone, with no incorporation of fat globules (Fig. 5.18c). There was little adsorption of the casein micelles on to the surfaces of the natural fat globules. The casein micelle surfaces themselves had developed tendrils or thread-like tails; possibly denatured whey proteins had attached to casein micelles via interaction between β-lactoglobulin and κ-casein. The micelles were connected to each other to form chains as well as clusters. Subsequent conventional homogenization partially broke down the aggregates (as shown by the continuous curve in Fig. 5.17d and by Fig. 5.18d), at the same time homogenizing the fat globules (as shown by the dashed curve in the Fig. 5.17d). The latter curve suggests that some coalescence or clustering of fat globules may have occurred during homogenization; some non-protein mediated clustering is evident in the electron micrograph in Fig. 5.18d.

Fig. 5.17e and Fig. 5.18e show that when reblended milk was first conventionally homogenized and then UHT sterilized, large aggregates were formed. The figures show also, when compared with Figs. 5.13c and 5.14a, and with Figs. 5.15a and 5.16a, that the particle size distribution and nature of these aggregates were very similar to those in the analogous unconcentrated and RO-concentrated whole milk systems. Furthermore, subsequent conventional homogenization, and treatment with protein dissociating medium either before or after this homogenization step (Figs. 5.17e and 5.17f), had the same effects as they did in the other whole milk systems.

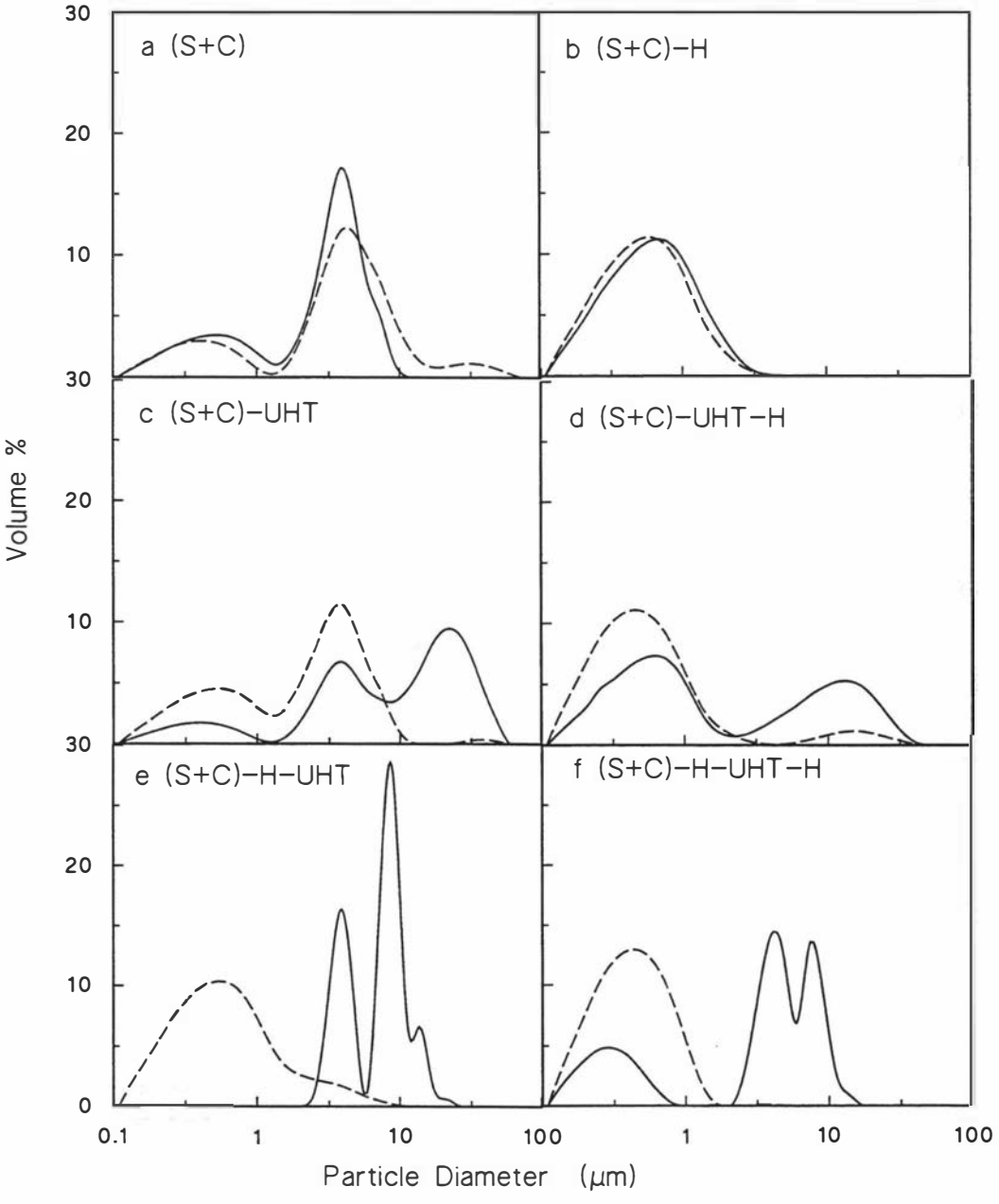


Fig. 5.17 Particle size distribution of 2.5X reblended milk (a) untreated (S+C), (b) homogenized only (S+C)-H, (c) UHT sterilized (S+C)-UHT, (d) UHT sterilized and subsequently homogenized (S+C)-UHT-H, (e) homogenized and subsequently UHT sterilized (S+C)-H-UHT, (f) homogenized, UHT sterilized and then homogenized again (S+C)-H-UHT-H, without (—) and with (----) protein dissociating medium.

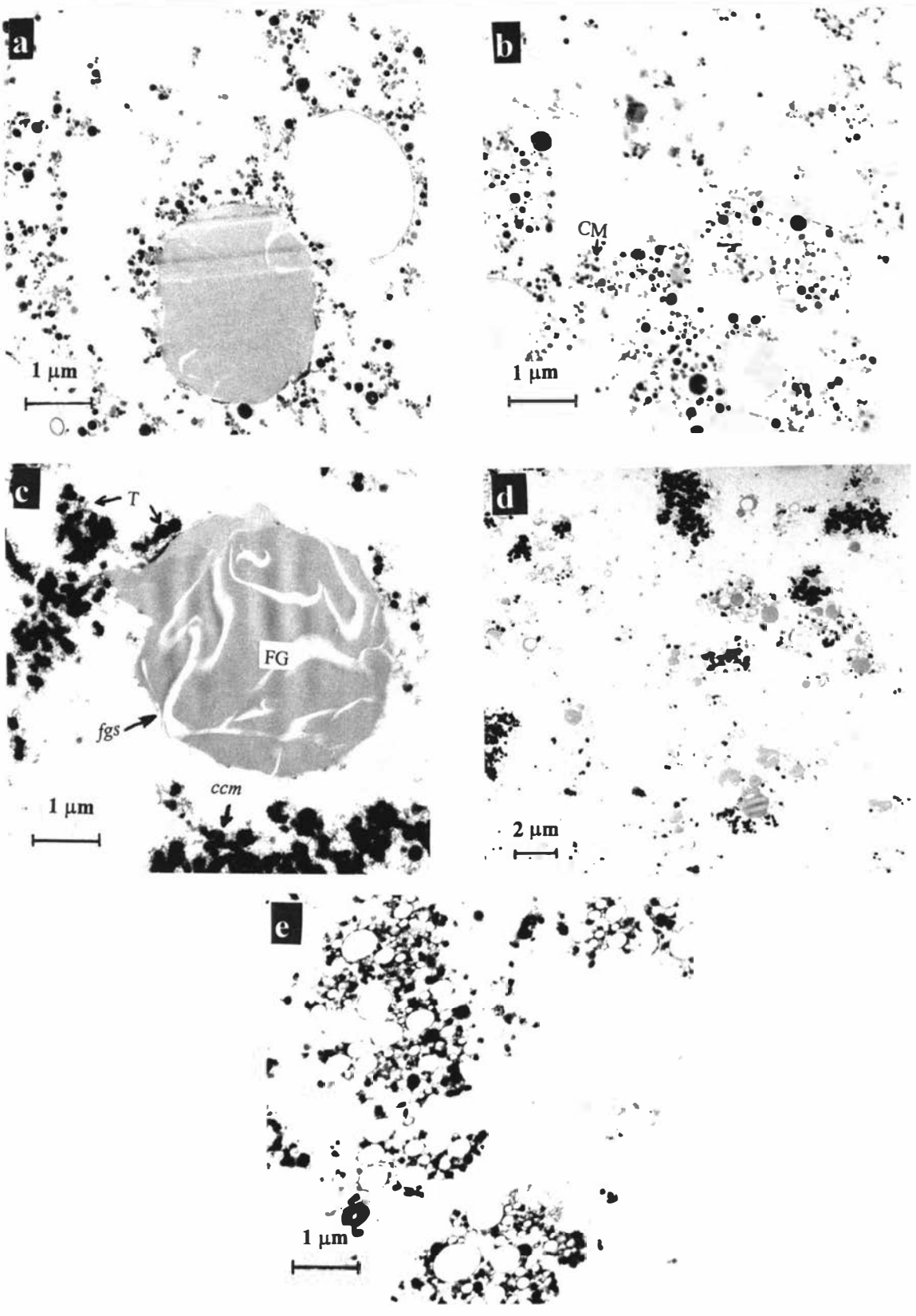


Fig. 5.18 Transmission electron micrographs of reblended milk (a) untreated (magnification 11200X); (b) homogenized only (magnification 11200X); (c) UHT sterilized (magnification 11200X); (d) UHT sterilized and subsequently homogenized (magnification 3400X); (e) homogenized and subsequently UHT sterilized (magnification 11200X) showing fat globules (FG), casein micelles (CM), fat globule surface (*fgs*), coalescence of casein micelles (*ccm*), tendril formation on casein micelles (T).

To support the results obtained when reblended milk was UHT treated (Figs. 5.17c and 5.18c), skim milk was concentrated to 2.5 fold by RO (Fig. 5.19a). UHT treatment of this skim milk concentrate caused extensive coagulation (Fig. 5.19b). Unheated casein micelles (Fig. 5.19a) had relatively smooth, uninterrupted contours compared with those of the UHT treated micelles (Fig. 5.19b) which had filamentous appendages projecting irregularly from their surfaces. These appendages joined adjacent micelles. Similar observations were reported by Mohammad and Fox (1987) for skim milk heated at 140 °C for 10 min. A comparision of Fig. 5.18c and Fig. 5.19b shows that the presence of fat in the reblended milk appeared to have provided a stabilizing effect against complete coagulation; there was only partial coagulation in the reblended milk system.

The lack of stability of RO concentrated skim milk to UHT treatment may be due to the precipitation of calcium phosphate upon heating, precipitation of denatured whey proteins on to the surfaces of the casein micelles, and heat induced aggregation of small micelles leading to the formation of larger micelles and, finally, visible clots (Mohammad and Fox, 1987). As seen in Fig. 5.19b casein micelles were aggregated into long chains presumably via whey protein-casein and/or casein-casein interactions.

Gavaric *et al.* (1989) and McMahon *et al.* (1991) published transmission electron micrographs of rennet gels made from both homogenized and non-homogenized whole milk UF retentates (which had not been UHT sterilized). It is perhaps of interest to note that their micrographs show that the structure of a rennet gel made from non-homogenized retentate is remarkably similar to the structure of the UHT sterilized non-homogenized reblended milk shown here in Fig. 5.18c. Their micrographs show also that when UF retentate was homogenized prior to rennetting, the structure of the rennet-induced gel was broadly similar to the structure of the aggregates found, during the present work, in UHT sterilized homogenized whole milk (Figs. 5.14, 5.16 and 5.18e). It would appear, therefore, that homogenization changes the stability of whole milk concentrates to rennet action in the same way that it changes their stability to UHT sterilization; van Boekel and Walstra (1989) pointed out that homogenized fat globules act as large casein micelles, and thus participate in any reaction of caseins such as rennetting, acid precipitation and heat coagulation.

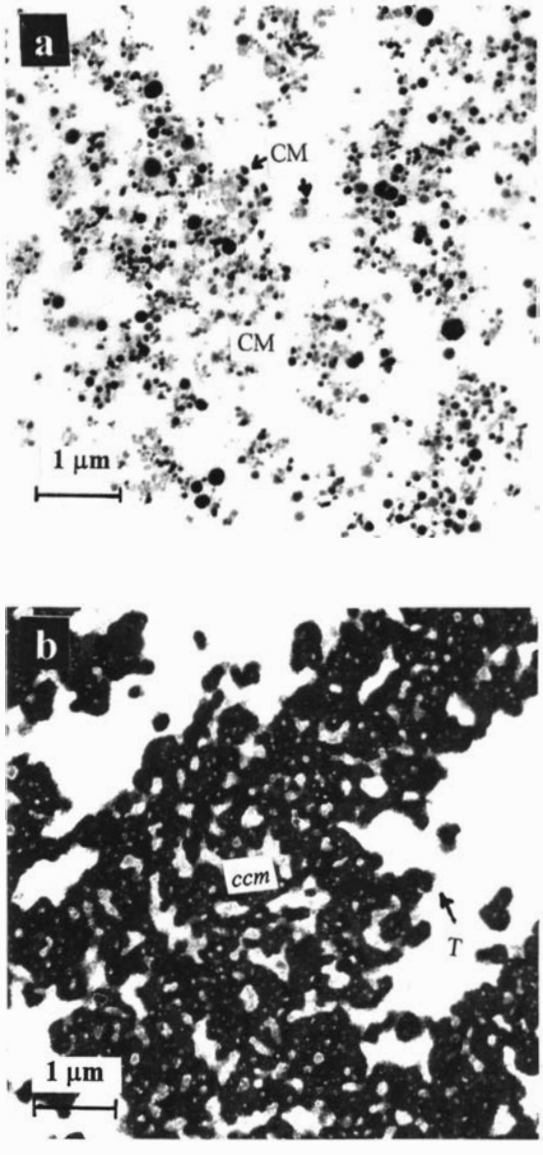


Fig. 5.19 Transmission electron micrographs of RO concentrated (2.5X) skim milk showing casein micelles (CM), coalescence of casein micelles (ccm), tendril formation on casein micelles (T). (a) untreated (magnification 11200X) (b) UHT treated (magnification 11200X).

5.3.2.3 Effect of pH adjustment and phosphate addition on particle size distribution

Effect of pH adjustment

The initial pH of the whole milk was 6.65. The pH decreased to 6.30 upon concentration by RO to 2.5X. The increased concentrations of the proteins and salts would have accounted for this decrease in pH.

UHT treatment of the RO concentrate resulted in a further decrease in pH of ~ 0.1 unit. The decrease in pH during heating may result from the following factors: precipitation of primary and secondary calcium phosphate as tertiary phosphate with the release of H^+, hydrolysis of organic phosphate ester bonds and subsequent precipitation of calcium phosphate, and the formation of organic acids, mainly formic, from thermal breakdown of lactose (Singh and Creamer, 1992).

pH adjustment of the concentrates prior to UHT treatment

The pH of RO concentrates (2.5X) was adjusted to various values in the range 6.4 to 7.1 using 1M NaOH. The concentrates were then UHT treated and their particle size distributions were measured (Fig. 5.20a-e). The particle size distribution shifted gradually towards lower diameters as pH increased; the d_{43} was 8.41 μm at pH 6.3 and 2.76 μm at pH 7.1 (Table 5.3a).

In an another experiment the pH of the concentrate was adjusted to 7.2 prior to UHT treatment. After UHT treatment, the concentrate was cooled to 20 °C and sub-samples adjusted by using 1M HCl to different values in the range 6.3 to 7.2. It is of interest that few large aggregates were found (Fig. 5.21a-e), although at pH 6.7 a d_{43} of 5.54 μm was observed (Table 5.3b). The particle size distributions were more or less the same at all pH values.

These observations revealed that the pH of the concentrate at heating had an important part to play in the formation of the aggregates.

pH adjustment of the concentrate after UHT treatment

In this case , the RO concentrate (2.5X) was UHT treated at its original pH of 6.3. A decrease in the pH (to 6.2) was observed after UHT treatment. The pH of the UHT treated concentrate was then adjusted to various values in the range 6.3 to 7.1 and particle size distribution measurements carried out. It can be seen (Fig. 5.22) that pH adjustment of the concentrates after UHT treatment (done at the original pH of 6.3) had little effect on the particle size distribution. There was a slight increase in the d_{43} with pH as the pH was adjusted from 6.3 to 6.9 at which d_{43} reached a maximum of 10.05 μm (Table 5.3b).

Table 5.3a Effect of pH adjustment prior to UHT treatment on the particle size in RO concentrates.

RO concentrate	pH adjusted to	UHT treatment	d_{43} (μm)
ROC pH 6.30	-	-	0.53
ROC pH 6.30	-	+	8.41
ROC pH 6.30	6.5	+	5.34
ROC pH 6.30	6.7	+	4.66
ROC pH 6.30	6.9	+	3.22
ROC pH 6.30	7.1	+	2.76

Table 5.3b Effect of pH adjustment after UHT treatment on the particle size in RO concentrate i) pH adjusted to 7.2 prior to UHT treatment ii) pH unadjusted prior to UHT treatment.

	RO concentrate	UHT treatment	pH adjusted to	d_{43} (μm)
i	ROC pH 7.20	+	6.30	4.68
	ROC pH 7.20	+	6.50	4.49
	ROC pH 7.20	+	6.70	5.54
	ROC pH 7.20	+	6.90	4.69
	ROC pH 7.20	+	7.10	3.79
ii	ROC pH 6.30	+	6.30	8.58
	ROC pH 6.30	+	6.50	8.91
	ROC pH 6.30	+	6.70	9.87
	ROC pH 6.30	+	6.90	10.05
	ROC pH 6.30	+	7.10	9.98

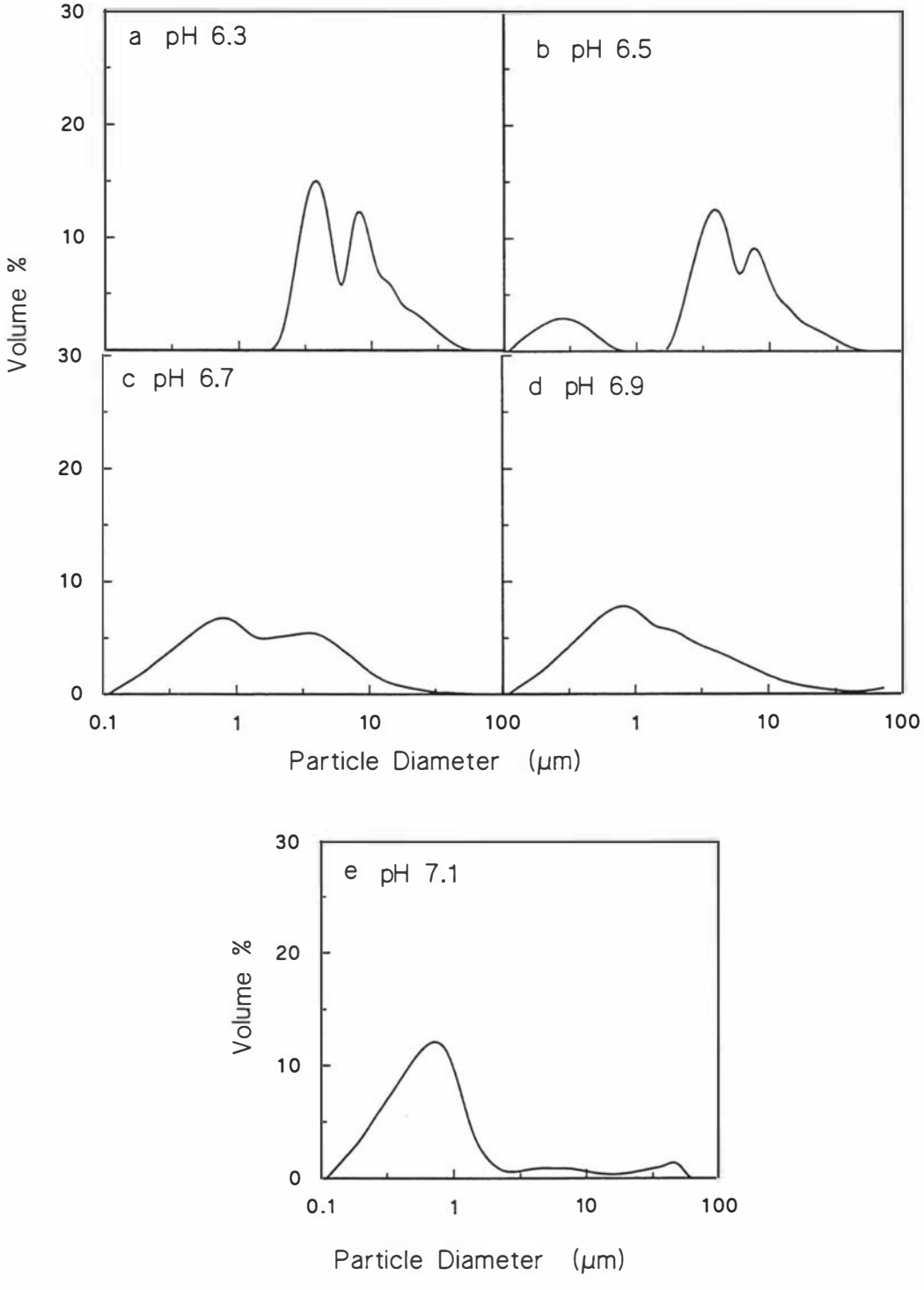


Fig. 5.20 Effect of pH adjustment prior to UHT treatment on the particle size distribution of RO concentrated milks. a) no adjustment (6.30); pH adjustment to b) 6.5, c) 6.7, d) 6.9 or e) 7.1.

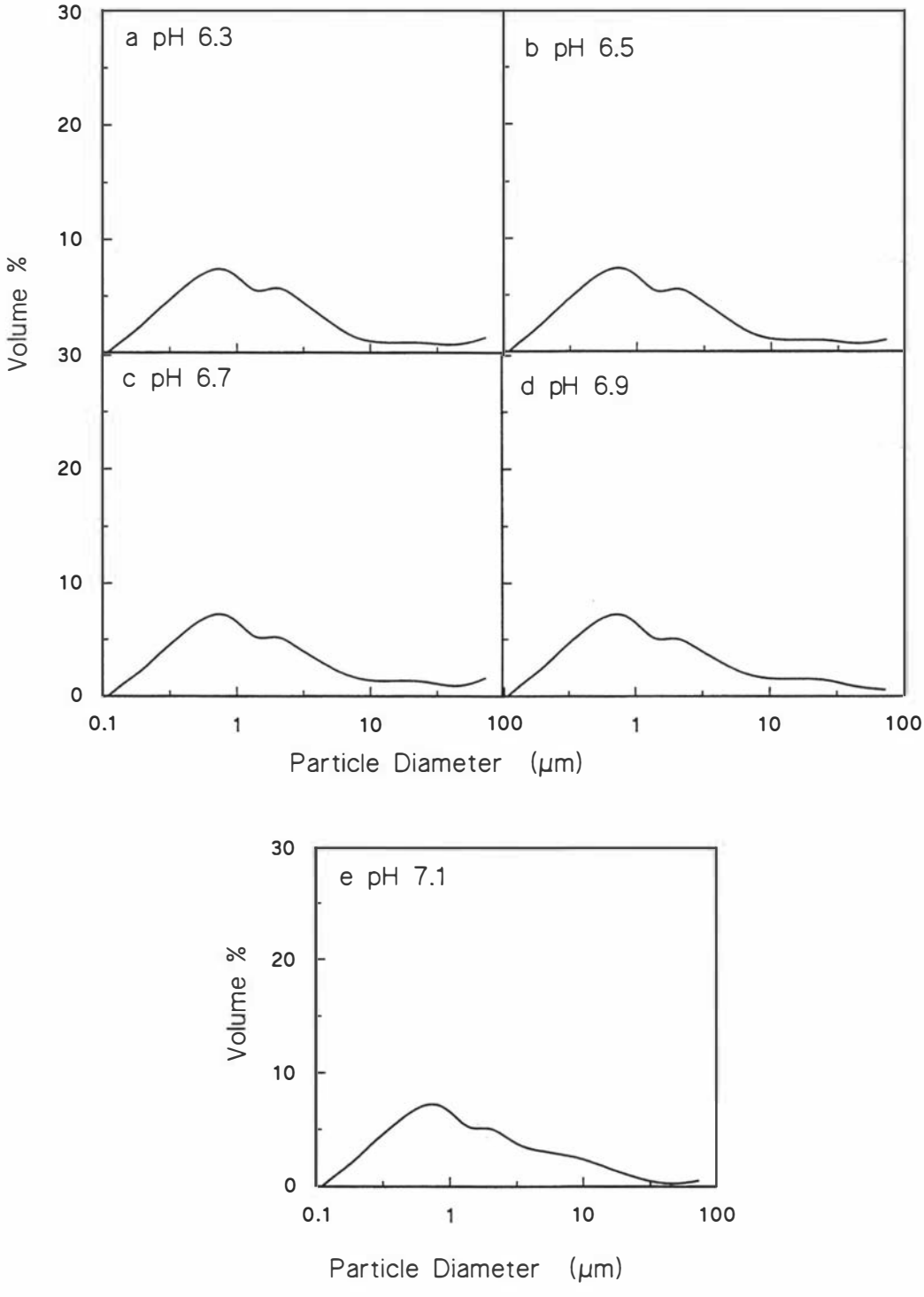


Fig. 5.21 Effect of pH adjustment after UHT treatment on the particle size distribution of 2.5X RO concentrated milks. In this case pH of the RO concentrate was first adjusted to 7.2 and then UHT treated and the pH of the resulting (heat treated) concentrate was again adjusted to pH a) 6.3, b) 6.5, c) 6.7, d) 6.9 or e) 7.1 and then subjected to particle size determination.

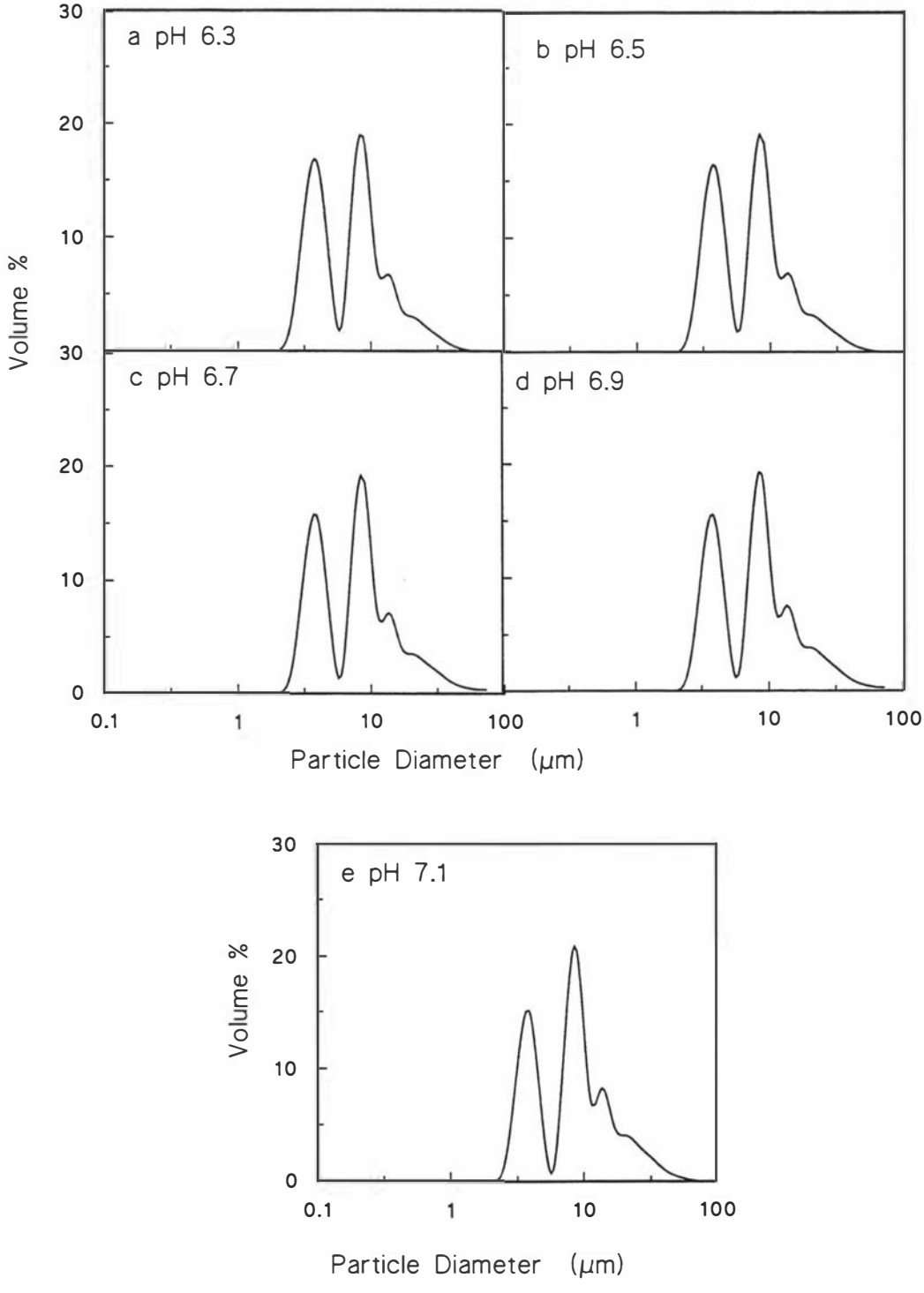


Fig. 5.22 Effect of pH adjustment after UHT treatment on the particle size distribution of RO concentrated milk. pH adjusted to a) 6.3, b) 6.5, c) 6.7, d) 6.9 or e) 7.1

From all of the above results, it can be summarised that the size of the aggregates decreased as the pH of the concentrate increased from 6.3-7.1 prior to UHT treatment and the aggregates were formed only when the concentrate was heated at a low pH (i.e. at its original pH 6.3); few aggregates were formed when pH of the concentrate was adjusted to 7.2 prior to heating. Adjustment of pH of the concentrates after UHT treatment had no effect on particle size distribution (aggregates remained as such); there was no evidence of disaggregation.

Changing the pH of milk results in a change in the charge on the protein residues and in the solubility of calcium phosphate. The negative zeta-potential of the casein micelles in milk increases by about 15% upon a pH increase from 6.2 to 7.0 (Walstra and Jenness, 1984). This results in an increase in the electrostatic repulsion between casein micelles by a factor of about 1.15^2 (= 1.32). For concentrated milk, this change is probably smaller, because of the high ionic strength in concentrated milk. Changing the pH affects the extent of formation of salt bridges. Firstly, the rate or extent of ionic bond formation becomes lower at higher pH because of lower calcium ion concentration. The calcium ion concentration in concentrated milk decreases from 1.1 mM to 0.4 mM as the pH increases from 6.2 to 7.0 (Nieuwenhuijse *et al.*, 1988). Also, a higher pH results in the supersaturation of all calcium phosphate, which may increase the rate or extent of formation of calcium phosphate bridges. The heat-induced dissociation of (especially κ-) casein from casein micelles, proceeds much faster at a high pH than at a low pH (Singh and Creamer, 1991; Nieuwenhuijse *et al.*, 1991).

Singh and Creamer (1991) investigated aggregation and dissociation of protein complexes on heating reconstituted concentrated skim milk, and found that κ-casein dissociation occured from the micelle even at normal pH, i.e. pH 6.55 for 20% T.S. milk. About 60% of the micellar κ-casein appeared to dissociate just before coagulation. They suggested that as a result of dissociation of κ-casein, the remaining micelles probably lost the protective action of κ-casein (i.e. charge and steric repulsion). Consequently these κ-casein depleted micelles aggregated to form large micelles. $Ca_2(PO_4)$ or Ca^{2+} was likely to have been involved in attaching κ-casein depleted micelles to one another.

No information is available in the literature on the heat-induced changes in RO concentrated milks. Since in the RO concentrated milks there appeared to be no

apparently intact micelles, and most of them appeared to have been adsorbed on to fat particles, it is not possible to compare the results of this study with those of Singh and Creamer (1991). Because increase in pH lowers the Ca^{2+} ion activity and also decreases the extent of aggregate formation, it is likely that Ca^{2+} ions were involved in the formation of heat-induced protein-fat aggregates.

Effect of disodium hydrogen phosphate addition

In another set of experiments (Fig. 5.23), disodium hydrogen phosphate (DSP) (Na_2HPO_4) was added to RO concentrates (2.5X) at concentrations in the range 0-0.20% (w/w). The concentrates were then subjected to UHT treatment without prior pH adjustment. The resulting particle size distributions are shown in Fig. 5.24. The particle size distribution shifted from a relatively narrow distribution (no addition, pH 6.34) to a relatively wide distribution in the lower particle size range (0.20% w/w, pH 6.52). The addition of the DSP to the concentrates (original pH 6.34) resulted in an increase in the pH of the concentrates from 6.34 (no addition) to 6.52 (0.20% w/w, DSP added) (Table 5.4a). The pH increased linearly with DSP concentration. The d_{43} decreased from 6.94 μm (no addition) to 2.22 μm (0.20% w/w DSP added) (Table 5.4a).

In further experiments (Fig. 5.23), DSP was added to the concentrate in various quantities (0-0.20%, w/w) and the pH adjusted back to that of the original (no addition) concentrate (pH 6.38), prior to UHT treatment. Similar trends were observed in this case i.e. there was a decrease in aggregate size with increasing DSP concentration (Fig. 5.25 and Table 5.4b). Aggregate size decreased as the concentration of DSP was increased from 0.05 - 0.15% (w/w).

All the above results suggest that the effect of DSP addition on aggregate formation was not a result of pH increase.

In normal heat evaporated milk concentrates containing added phosphate, the amount of calcium phosphate associated with the protein particles has been found to be larger at any pH, and the Ca^{2+} ion activity in the serum lower, than when added phosphate is absent (Nieuwenhuijse *et al.*, 1988; Augustin and Clarke, 1990). The effects of phosphate addition prior to UHT treatment on the particle size distribution of the RO concentrated milk were similar to those of a pH increase prior to UHT treatment, which

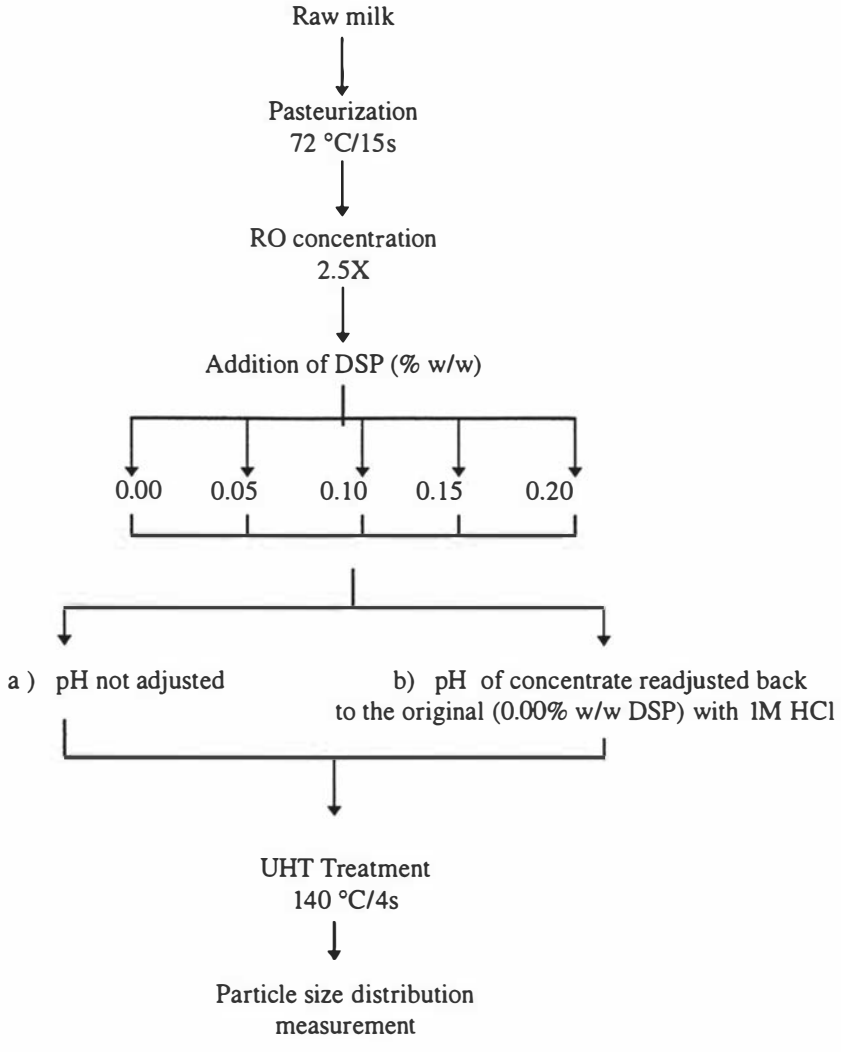


Fig. 5.23 Experimental scheme for investigating the effects of adding disodium hydrogen phosphate (DSP) to 2.5X RO concentrate a) without adjusting pH back to original value (no addition) after the addition of DSP b) with adjusting pH back to original value (no addition) after the addition of DSP.

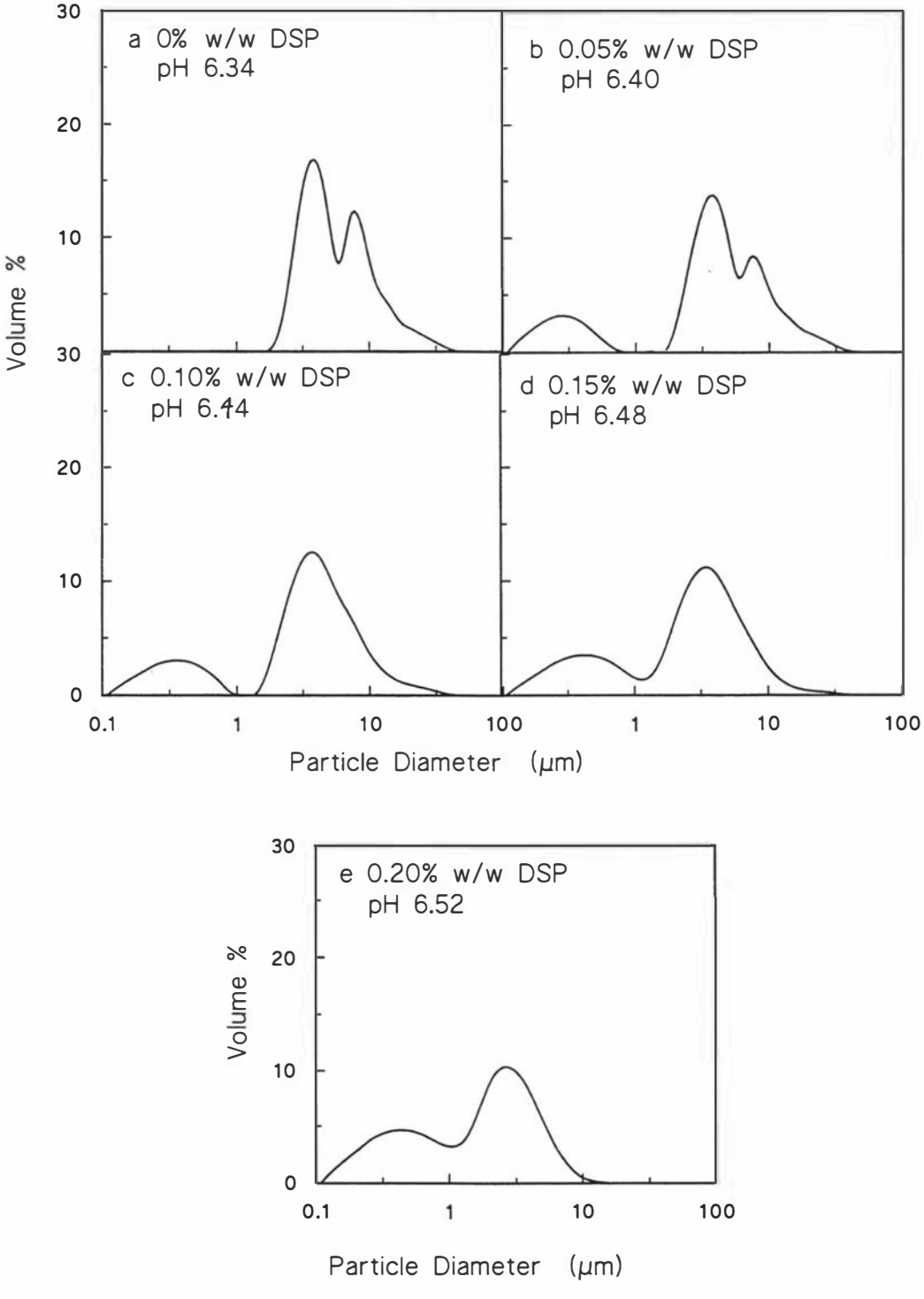


Fig. 5.24 Effect of disodium phosphate (DSP) concentration on the particle size distribution in UHT treated RO concentrated milks. DSP was added to concentrate at a) 0, b) 0.05, c) 0.10, d) 0.15 or e) 0.20% (w/w) and the concentrate then subjected to UHT treatment.

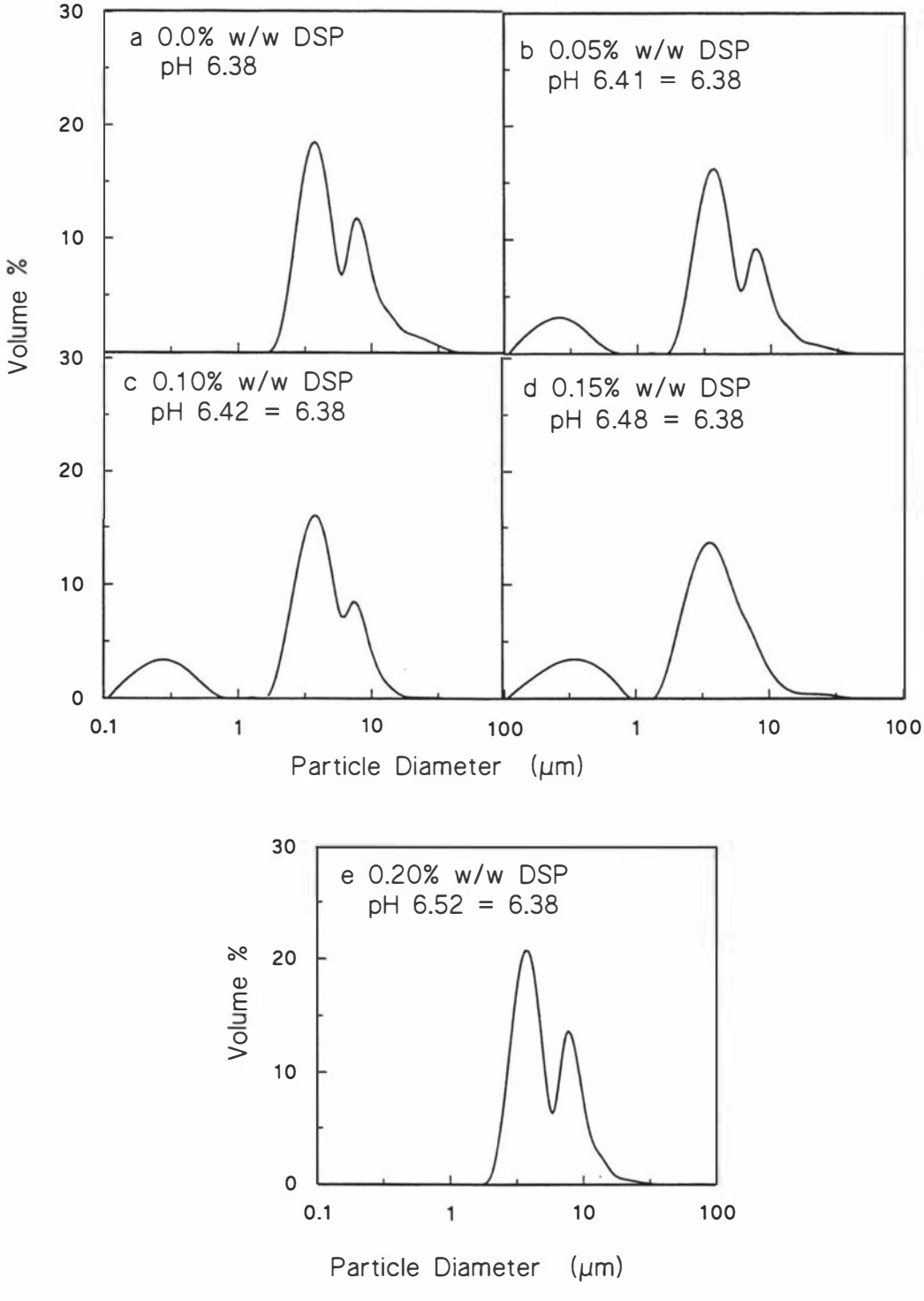


Fig. 5.25 Effect of disodium hydrogen phosphate (DSP) concentration on the particle size distribution in UHT treated RO concentrated milks. DSP was added to concentrate at a) 0, b) 0.05, c) 0.10, d) 0.15 or e) 0.20% (w/w) and pH adjusted back to that of original concentrate (no addition) and the concentrate then subjected to UHT treatment.

Table 5.4 Effect pf disodium phosphate (DSP) addition on particle size in RO concentrates (ROC) a) DSP added prior to UHT treatment, b) DSP added to RO concentrate and the pH adjusted back to that of the original concentrate prior to UHT treatment.

	RO conc	DSP (% w/w)	Resulting pH	pH adjusted to	UHT treatment	d_{43} (μm)
	ROC pH 6.34	no additon	6.34	-	-	0.55
	ROC pH 6.34	no addition	6.34	-	+	6.94
a	ROC pH 6.34	0.05	6.40	-	+	5.39
	ROC pH 6.34	0.10	6.44	-	+	4.52
	ROC pH 6.34	0.15	6.48	-	+	3.53
	ROC pH 6.34	0.20	6.52	-	+	2.22
	ROC pH 6.38	no additon	6.38	-	-	0.54
	ROC pH 6.38	no addition	6.38	-	+	6.45
b	ROC pH 6.38	0.05	6.41	6.38	+	4.92
	ROC pH 6.38	0.10	6.42	6.38	+	4.15
	ROC pH 6.38	0.15	6.48	6.38	+	3.85
	ROC pH 6.38	0.20	6.52	6.38	+	3.25

again suggest that a reduction in Ca^{2+} activity is an essential factor for protein-fat aggregation during heating.

Nieuwenhuijse *et al.*, (1988) suggested that after phosphate addition to heat concentrated milk, a new "equilibrium" between phosphate, calcium and other ions is attained very slowly at 20 °C, and which upon heating to 120 °C results in a continuing association of calcium phosphate with micelles during a certain period of holding at this temperature (10 min), while in concentrates without added phosphate this association is almost complete immediately after heating up. Thus, the phosphate addition may affect the rates of κ-casein dissociation from the micelles and of whey protein-κ-casein association.

As a result of heating milk to UHT temperatures, a number of changes to proteins, fat globules and milk salts may take place. Whey proteins denature and some of them may associate with casein micelles and / or fat globules. Denatured whey proteins may form a layer at the fat globule surface. This association is likely to involve intermolecular disulphide bond formation between β-lactoglobulin and κ-casein on the casein micelles adsorbed at the surface of the fat globules. Alternatively, the whey proteins may also react with the κ-casein of the micelles present in the serum. Also, the whey proteins may interact with other whey proteins which are already adsorbed at the surfaces of the fat globules (Singh, 1993; Singh *et al.*, 1996).

Heating of milk also causes the transfer of soluble calcium and phosphate to the colloidal state. Calcium phosphate may be deposited on to the casein micelles present in the serum or on to the micelles present at the fat globule surfaces or both. Other important reactions as a result of heat treatment of milk are the Maillard reaction, the formation of covalent cross-links and a decrease in pH. All these reactions are expected to occur simultaneously during heating.

Aggregation of protein and fat globules is obviously due to heat-induced chemical or physical changes in the salts or in protein particles ("free" as well as adsorbed at the fat globule surface themselves). Denaturation and subsequent association of whey proteins with casein micelles or fat globules, and changes in calcium phosphate are likely to be the key reactions responsible for inducing flocculation or aggregation.

In unconcentrated homogenized milks there may be possible mechanisms for the aggregation of fat globules during UHT-treatments. First, the denatured whey proteins may interact with other whey protein molecules or with κ-casein at the surfaces of more than one globule, hence inducing a kind of bridging flocculation. The second possibility is that because of spreading of the casein micelles at the fat globule surface, the fat globules may act as κ-casein depleted micelles (Singh *et al.* 1996). These globules may therefore be sensitive to Ca^{2+} ions in the serum, because of the high Ca^{2+} ion sensitivity of α-$_{s1}$ and β-caseins. Calcium bridging of these fat globules is possible. Thus, it is likely that two types of fat globules emerge during heating. In one type denatured whey proteins are deposited on these globules and are actively involved in joining them together. Another type of fat globule might be one that is sensitive to Ca^{2+} ion concentrations because of insufficient concentrations of κ-casein on its surface.

In homogenized reblended milks reactions similar to those stated above are possible. In addition, the dissociation of κ-casein from the surfaces of micelles is likely to occur. It is possible that dissociation of the κ-casein occurs from the micelles in the serum as well from the micelles adsorbed to the fat globule surfaces. There may be more of the second type of fat globule in these systems. Thus calcium bridging would be more important.

In RO concentrated milks, the electron micrographs show that no micelles were present as such, and that some casein particles of unknown structures were present. The location of κ-casein in these structures is uncertain. It is possible that the aggregation in this system is more likely to involve calcium linkages. This is supported by the results for the effect of pH increase and phosphate addition prior to UHT treatments, both of which decrease calcium ion activity.

5.4 CONCLUSIONS

Concentration of milk by RO caused a reduction in the diameters of the fat globules and induced marked changes in the microstructures of fat globules and protein particles. In milk concentrated by reverse osmosis there was a large population of smaller fat globules (< 0.1 μm). Aggregates in UHT treated RO concentrated milk formed at lower

temperature (125 °C for 4 s) than aggregates in UHT treated unconcentrated milks (130 °C for 4 s).

The large aggregates that formed in whole milk as a result of UHT sterilization following homogenization, or reverse osmosis concentration and homogenization (these operations taking place either simultaneously or in this sequence), consisted of intact fat globules held in a protein matrix. It is likely that the matrix consisted partly of casein, because of the known behaviour of casein micelles in whole milk upon homogenization and subsequent heating (van Boekel and Walstra, 1995; van Boekel and Folkerts, 1991; Henstra and Smith, 1970; van Boekel, 1993; McMahon *et al.*, 1993). Aggregate formation was independent of milk concentration.

The aggregates could be completely dispersed by the protein dissociating medium used. This suggests that aggregation must have been the result of electrostatic and hydrophobic interactions; disulphide bonds would not have been involved.

When whole milk containing natural (non-homogenized) fat globules was UHT sterilized, aggregates formed only in the concentrated system (reblended milk). The aggregates consisted only of protein; fat globules were not incorporated in them. Aggregation was probably the result of partial coagulation. Extensive coagulation occurred when RO concentrated skim milk was UHT sterilized under the same conditions (Fig. 5.19b). Coagulation in reblended milk was less marked because of the diluting effect of the cream, in which the plasma phase would have had the same composition as in unconcentrated whole milk. (Coagulation of unconcentrated skim milk at UHT temperatures occurs only after prolonged heating (Mohammed and Fox, 1987)).

The size of the aggregates decreased as the pH of the concentrate was increased from 6.3 - 7.1 prior to UHT treatment. The largest aggregates were formed only when the concentrate was heated at a low pH (i.e. at its original pH 6.3), whereas few aggregates were formed when the pH of the concentrate was adjusted to 7.2 prior to heating. Adjustment of the pH of the concentrates after UHT treatment had no effect on particle size distribution (aggregates remained as such). There was no evidence of disaggregation in the entire pH range studied. The addition of disodium hydrogen phosphate to RO concentrates had a similar effect to that of increasing the pH.

6. STORAGE-INDUCED CHANGES IN UHT STERILIZED RO CONCENTRATED MILKS: EFFECTS OF HOMOGENIZATION, FOREWARMING AND ADDED PHOSPHATES

6.1 Introduction

The main aim of heat treating milk is to extend shelf-life. The shelf life of milk can be extended by several means, such as freezing, addition of sucrose to concentrated milk, and sterilization (Harwalkar, 1992). The primary objective of sterilization is microbiological sterility but it also causes physical and chemical changes which are of great importance, as they affect the quality (flavour and nutritional) and shelf life of the product (Burton, 1984, 1988).

UHT sterilization of concentrated milk products is gaining increased acceptance, mainly because these products have a great marketing convenience and show minimal flavour changes during considerable periods of storage without refrigeration (Harwalkar, 1992). However, age thickening, followed by gelation, during storage of such products is a serious problem.

Concentration of milk by RO imparts a homogenizing effect to the fat globules (Chapter 5). However, homogenization impinges on two important aspects of long-life products. First, it reduces initial heat stability, that is, the ability of the product to withstand heat sterilization without coagulation. Second, it enhances the rate of gelation during subsequent product storage (McCrae and Muir, 1993). Gelation has been described as coagulation, sweet curd formation, thixotropic gel formation, age thickening, partial gelation, or lumpiness (Harwalkar, 1992). Gelation is always preceded by a sharp rise in viscosity (Ellerton and Pearce, 1964). During initial stages of storage the viscosity drops. This is followed by a long period of little change and then viscosity suddenly increases, resulting in gel formation. Gelation results in milk with a custard-like consistency, which is an irreversible condition (Harwalkar and Vreeman, 1978a).

To date most of the work carried out on storage-induced changes (gelation) relates to UHT treated heat evaporated concentrated milk (Tarassuk and Tamsma, 1956; Levinton and Pallansch, 1962; Ellerston and Pearce, 1964; Carroll *et al.*, 1971; Harwalkar and Vreeman, 1978a, b; de Koning and Kaper, 1985; de Koning *et al.*, 1985, de Koning *et al.*, 1992; Venkatachalam *et al* , 1993) and unconcentrated milk (Schmidt, 1968, 1969; Samel *et al.*, 1971; Andrews and Cheeseman, 1971, 1972; Zadow and Chituta, 1975; Andrews *et al.*, 1977; Snoeren *et al.*, 1979; Snoeren and Both, 1981; Kocak and Zadow, 1985a, b; Manji *et al*, 1986) and little information is available on the milks concentrated by RO and subsequently UHT treated. Kocak (1985) studied the storage stability of UHT treated RO concentrates (26% T.S.). The present work involved study of storage-induced changes in RO concentrates at high total solids (32~35%) and also aimed to explore the effects of the following variables on the storage stability:

- storage temperature,
- homogenization after UHT treatment,
- forewarming of milk before concentration and
- addition of phosphates to concentrates before UHT treatment

6.2 Material and Methods

6.2.1 Raw material

Raw whole milk from Friesian cows was obtained from a Massey University Dairy Farm, and processed either immediately or stored at 5 °C and processed the next day.

6.2.2 Experimental protocol

In order to determine the effects of homogenization after UHT treatment, and forewarming of milk prior to concentration, on the storage stability of RO concentrated milks, four experiments (Experiments. A, B, C, D; Tables 6.1a and 6.2a) were carried out as described in Figs. 6.1 and 6.2. The milks were concentrated to 2.5X in a Lab-20 RO plant and then UHT treated in an indirect UHT mini-pilot plant at 140 °C for 4 s, cooled to ~ 30 °C

aseptically into 100 ml glass bottles and stored at 5, 20, or 37 °C. (For more details, see Chapter 3).

Processing treatments (separation, pasteurization, RO concentration, UHT treatment, and homogenization) and analyses (particle size distributions, viscosity, penetration test, pH, non-protein nitrogen (NPN), electrophoresis and electron microscopy, of the various samples were carried out using the methods described in Chapter 3. Forewarming (90 °C for 2 min) of the milk was carried out in the indirect UHT mini-pilot plant using an extended holding tube.

Samples for each experiment (A-D) were produced on 4 days per week (Monday and Tuesday- Trial I; Wednesday and Thursday-Trial II) from September to November 1995. Fresh raw milk was obtained on Monday and Wednesday mornings, and divided into two lots. One lot was processed fresh and the other stored at 5 °C to be used the next day (i.e. Tuesday or Thursday). The milk samples produced on Monday and Tuesday will be referred to as Ia and Ib respectively, and those produced on Wednesday and Thursday will be referred to as IIa and IIb respectively. The processing steps and measurement intervals for the experiments are given in Tables 6.1a and 6.2a respectively.

In order to investigate the effects of phosphate addition to RO concentrated milks on the storage-induced changes, three more experiments (E, F and G) were performed in June 1996 (Fig 6.3). Raw whole milk was pasteurized and then concentrated to 2.5X by RO. In experiment E, no phosphate was added (control). In experiment F, disodium phosphate (DSP, 0.15% w/w) and in experiment G sodium hexametaphosphate (SHMP, 0.15% w/w) was added to the RO concentrate before UHT treatment. The required amount of phosphate was first dissolved in deionised water and then added to the concentrate, which was mixed for 1 h before UHT treatment. The samples were processed and held as in experiment A.

The processing steps and measurement intervals for experiments E-G are given in Tables 6.1b and 6.2b respectively.

For experiments E, F and G, all analyses were carried out the same way as for experiments A-D, except that viscosity and NPN values were not determined. In addition, the sub-samples from these experiments were ultracentrifuged at 90,000 g for 1 h at 20 °C in a Beckman L2-65B Ultracentrifuge with a Ti- 60 fixed-angle rotor. Top (cream), middle and sediment fractions were collected carefully and then analysed using electrophoresis as described in Chapter 3.

Table 6.1a Processing steps for experiments A-D

Experiments	Dates produced	Processing steps
A	25, 26, 27 and 28 Sept. 1995	P-ROC-UHT-H
B	9, 10, 11 and 12 Oct. 1995	F-ROC-UHT-H
C	24, 25, 26 and 27 Oct. 1995	P-ROC-UHT
D	6, 7, 8 and 9 Nov. 1995	F-ROC-UHT

Table 6.1b Processing steps for experiments E-G

Experiments	Dates produced	Processing steps
E	4 June 1996	P-ROC-UHT-H
F	5 June 1996	P-ROC-A (DSP)-UHT-H
G	6 June 1996	P-ROC-A (SHMP)-UHT-H

(P = pasteurized at 72 °C/15 s, F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized (137.9 bar), A(DSP) = Addition of disodium phosphate (0.15% w/w), A (SHMP) = Addition of sodium hexametaphosphate (0.15% w/w)).

Table 6.2a Measurement intervals for experiments A-D

Experiment	Trial	Measurement interval (weeks)
A	Ia and IIa	1, 9, 17 ,25
	Ib and IIb	5, 12, 21
B	IIa	1, 9, 17 ,25
	IIb	5, 13, 21
C	Ia and IIa	1, 8, 17 ,25
	Ib and IIb	5, 13, 21
D	IIa	1, 9, 17 ,25
	IIb	5, 13, 21

Table 6.2b Measurement intervals for experiments E-G

Experiment	Trial	Measurement interval (weeks)
E	I	1, 5, 9,17, 21, 25
F	I	1, 5, 9,17, 21, 25
G	I	1, 5, 9,17, 21, 25

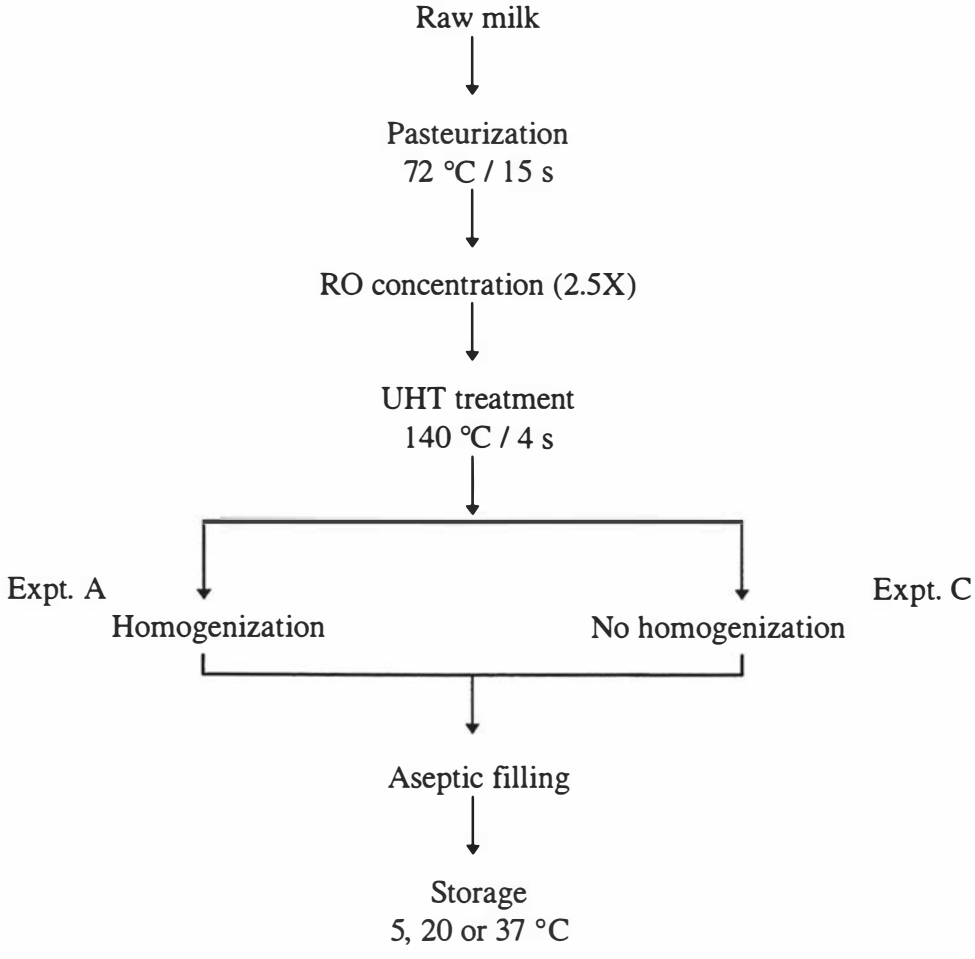


Fig. 6.1 Experimental protocol for the production of samples for Experiment A and C.

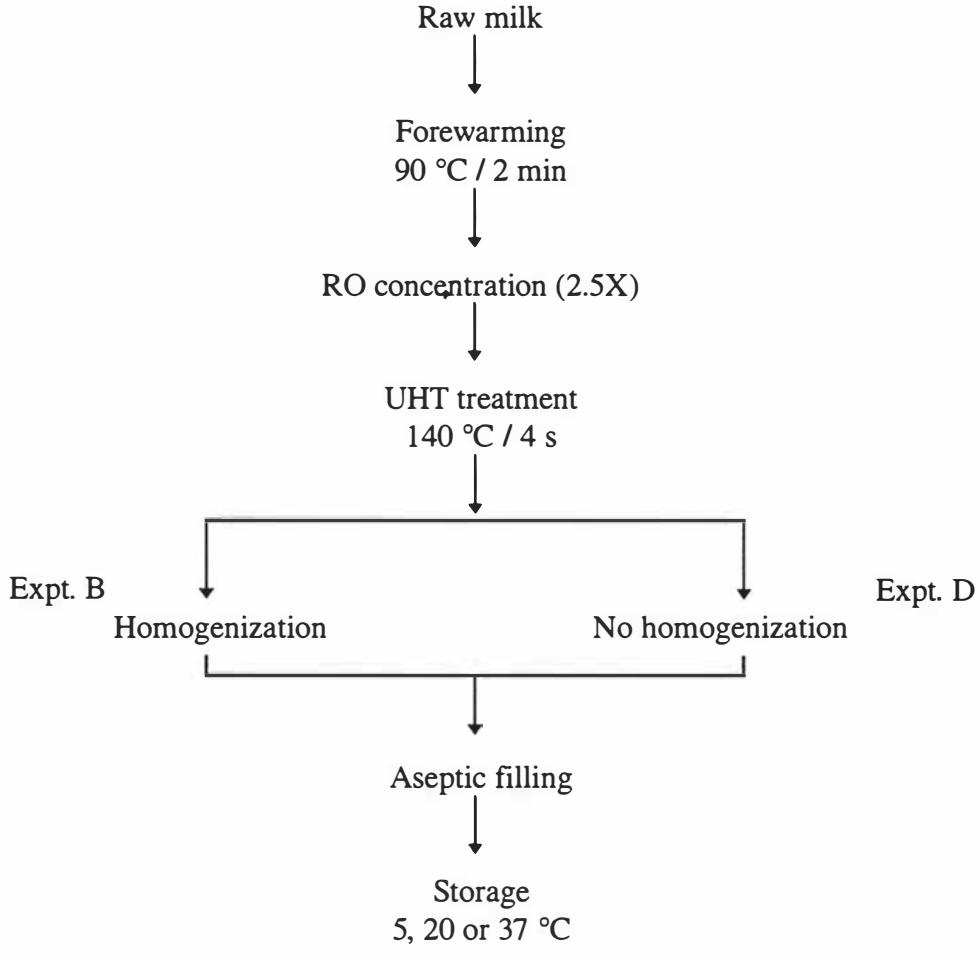


Fig. 6.2 Experimental protocol for the production of samples for Experiment B and D.

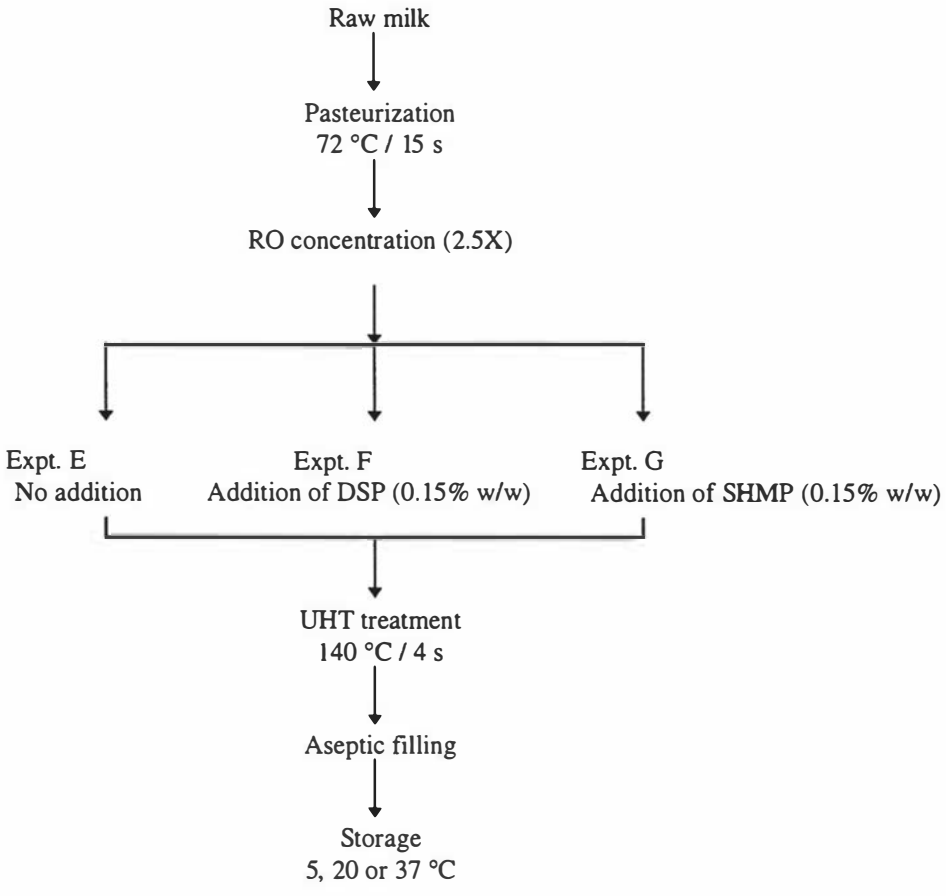


Fig. 6.3 Experimental protocol for the production of samples for Experiment E, F and G.

6.3 Results and discussion

6.3.1 Effects of homogenization after UHT treatment, and forewarming before concentration

6.3.1.1 *Compositions of various milks*

The compositions of the raw milks used and various RO concentrates produced in this study are given in Table 6.3. The concentrates were produced to match the European Standards (10/23, fat/SNF) for evaporated milk. Since the experiments were performed on different days, and the composition of raw milk varies from day to day, the concentration factor (ratio of total solids in concentrate to total solids in raw milk) was controlled closely to 2.5X. The data obtained from trial I for Experiment B and Experiment D have not been included in the results because the raw milks used had low initial solids (though the concentration factor achieved was ~ 2.5 X); this resulted in lower total solids concentrates as compared to other trials for other experiments.

Table 6.3 Compositions of raw milks and various RO concentrates in experiments A-D.

Experiment	Date	Trial	Raw milk		RO concentrated milk		
			Fat (%)	T.S (%)	Fat (%)	T.S (%)	C.F.
A	25.9.95	Ia	4.30	13.15	10.80	33.33	2.53
	26.9.95	Ib	4.30	13.15	10.80	33.33	2.53
	27.9.95	IIa	4.50	13.30	10.70	33.58	2.52
	28.9.95	IIb	4.50	13.30	10.65	33.70	2.53
B	11.10.95	IIa	4.60	13.48	12.20	35.69	2.64
	12.10.95	IIb	4.60	13.48	12.20	35.76	2.65
C	24.10.95	Ia	4.30	13.29	12.60	35.46	2.66
	25.10.95	Ib	4.30	13.29	12.40	35.05	2.63
	26.10.95	IIa	4.25	13.27	12.40	35.32	2.66
	27.10.95	IIb	4.25	13.27	11.60	33.10	2.49
D	8.11.95	IIa	4.40	13.50	11.80	35.03	2.59
	9.11.95	IIb	4.40	13.50	12.00	34.35	2.54

6.3.1.2 Changes in pH

The pH of raw milk used in these studies ranged from 6.60-6.62. After concentration to 2.5X, the pH of the concentrates was found to be in the range of 6.40-6.44. UHT treatment of the concentrates resulted in a further decrease of ~ 0.02 pH units.

The effects of storage time and storage temperature on the pH of samples obtained from experiments A-D are shown in Figs. 6.4-6.7, respectively. Samples stored at 5 °C showed a slight increase in pH during the initial stages of storage (up to 5 weeks) followed by a slight decrease in pH during subsequent storage. The samples stored at 20 °C showed a slight decrease in pH with increase in storage time. Samples stored at 37 °C showed a relatively greater decrease in pH with increase in storage time. The extent of pH drop as related to storage temperature was in the following order: 37 > 20 > 5 °C. Kocak (1985) observed a similar effect of temperature on the pH of UHT-treated RO concentrates (26% T.S) during storage at 2, 10, 20, 30 and 40 °C. There appeared to be little change in pH of samples stored at or below 25 °C, whereas samples stored at higher temperature showed a general decrease in pH during storage. The extent of the decrease was most pronounced in samples stored at 40 °C. Similar results were reported by other workers (Andrews and Cheeseman, 1971, 1972; Andrews, 1975, Zadow and Chituta, 1975; Andrews *et al.*, 1977; Kocak and Zadow, 1985; Manji *et al.*, 1986; Venkatachalam *et al.*, 1993; Celestino e*t al.*, 1997) on UHT sterilized unconcentrated and heat evaporated and UF concentrated milks. They concluded that the level and extent of pH decrease was not related to age gelation because at higher temperatures (40 °C) the samples gelled slower than the samples stored at 30 °C.

It has been suggested that this decrease in pH may be due to loss of positive charges on the protein molecules as a result of the Maillard reaction (Andrews and Cheeseman, 1971; Andrews, 1975; Andrews *et al.*, 1977). However, Venkatachalm *et al.* (1993) showed that the pH of the UHT treated milk with added sucrose also decreased when stored at 35 °C although the samples showed insignificant browning, suggesting that the Maillard reaction may not be the only cause. Another possible explanation for the decrease in pH at higher temperature of storage may be the gradual precipitation of calcium phosphate during storage, shifting the calcium-phosphate equilibrium towards the formation of Ca^{2+} and PO_4^{3-} from other forms of ionic species and releasing protons that decrease pH.

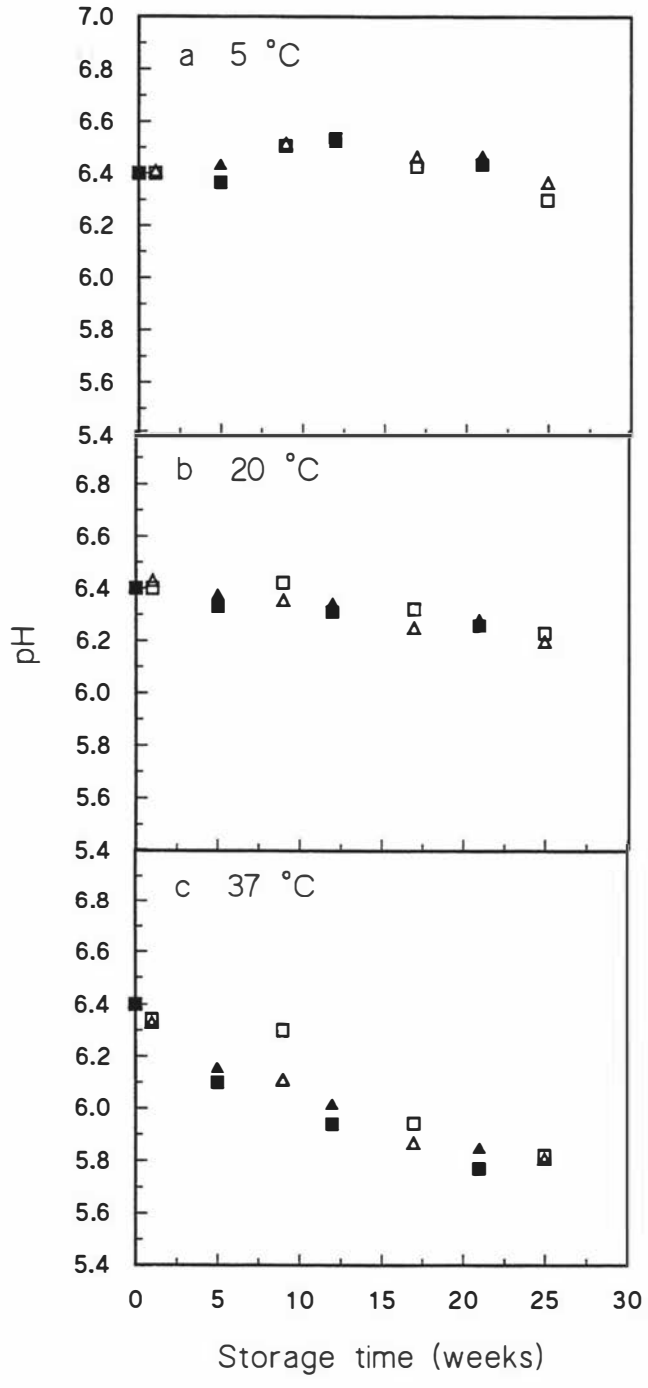


Fig. 6.4 pH changes in Experiment A (P-ROC-UHT-H) milk samples during storage at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

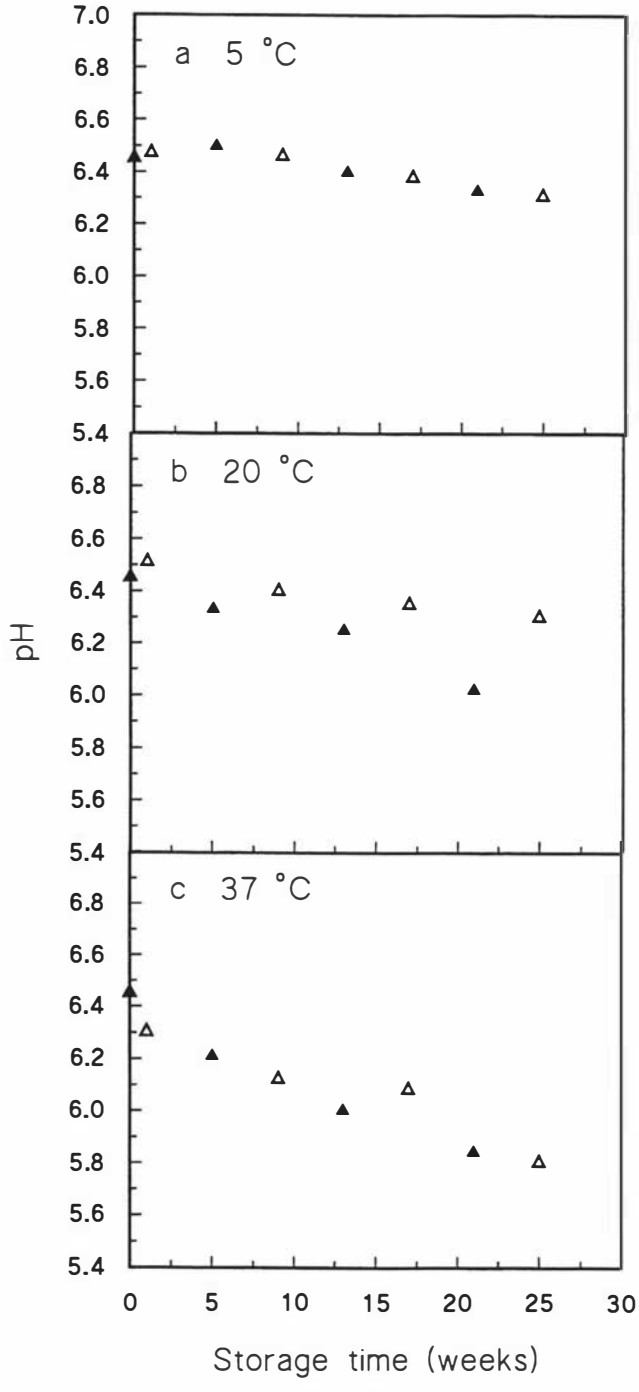


Fig. 6.5 pH changes in Experiment B (F-ROC-UHT-H) milk samples during storage at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

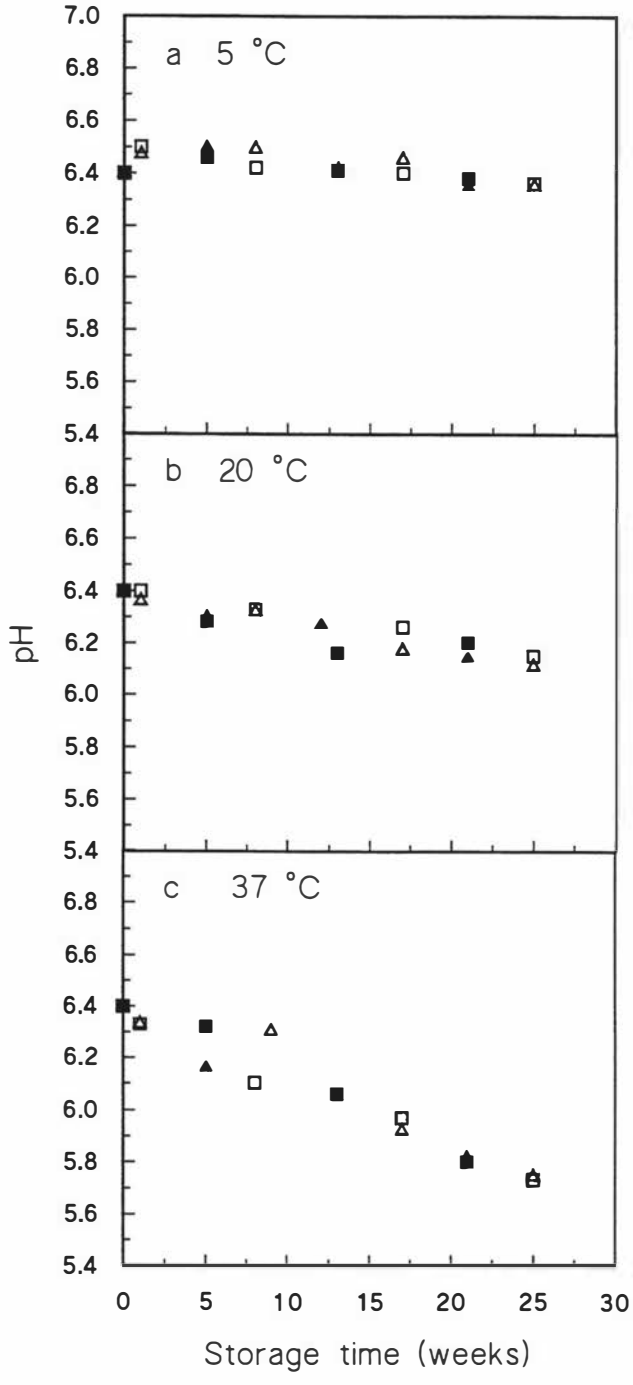


Fig. 6.6 pH changes in Experiment C (P-ROC-UHT) milk samples during storage at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

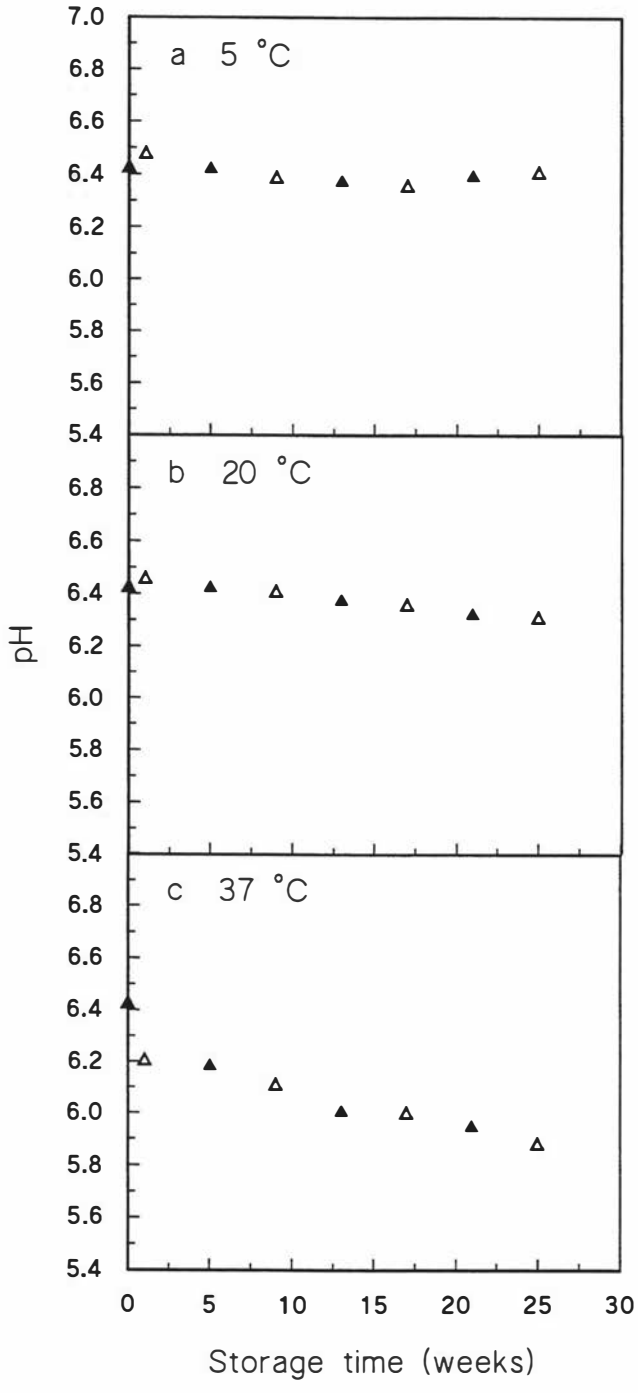


Fig. 6.7 pH changes in Experiment D (F-ROC-UHT) milk samples during storage at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

Venkatachalam *et al.* (1993) suggested that dephosphorylation of caseins during storage would result in the release of free H^+, but Harwalkar and Vreeman (1978a) found no increase in inorganic P during storage of concentrated heat evaporated skim milk (i.e. no dephosphorylation).

The results obtained from experiments A, B, C, and D were similar to each other, indicating that homogenization after UHT treatment, or forewarming of milk prior to RO concentration, had no significant effect on the pH decrease during storage. However, in general there was a significant decrease in pH at 37 °C as compared to 20 or 5 °C because of the fact that chemical reactions proceed at a faster rate at higher temperatures.

6.3.1.3 Particle size distribution

The particle size distributions and d_{43} (weight-average mean diameter) of the samples from experiments A-D are shown in Figs. 6.8, 6.10, 6.12 and 6.14, and Figs. 6.9, 6.11, 6.13 and 6.15, respectively. The particle size distribution represents the initial measurement (no storage) and measurements after 25 weeks storage period with and without the protein dissociating medium.

The particle size distribution of unheated RO concentrated milk is monomodal (see Chapter 5 , Fig. 5. 3a). Fig. 6.8 shows the changes in particle size distribution for experiment A. On UHT treatment, even after subsequent homogenization, the particle size distribution was bimodal (initial measurement). After storage for 25 weeks at 5 °C, the particle size distribution remained mainly bimodal, with the appearance of a partly resolved peak (peak 3) in the size range 7.5-25 μm. Also there was an increase in the proportion of particles in the second peak (peak 2), with a concomitant decrease in the proportion of particles in the first peak (peak 1). After storage for 25 weeks at 20 °C, there was an increase in the population of the particles in peak 3. In contrast, storage for 25 weeks at 37 °C resulted in a complete disappearance of peak 1, and there was a considerable increase in the population of particles in peak 3 (size range 7.5-15 μm). Also, a new partly resolved peak (peak 4) emerged in a size range 15-30 μm. These changes in the particle size distribution with storage temperature clearly revealed the

gradual disappearance of small particles with the formation of large particles, more so at higher storage temperatures.

Dispersion of the samples in a protein dissociating medium changed the particle size distribution to a monomodal distribution for all samples, with all the particles below 2 μm.

The changes in the average diameter (d_{43})of particles as a function of storage time at 5, 20 or 37 °C are shown in Fig. 6.9. The value of d_{43} increased with increase in storage time, especially after 15 weeks. The increase was more pronounced at higher storage temperatures. Dispersion of milk in a protein dissociating medium (2% SDS/ 0.05M EDTA) resulted in a marked reduction in d_{43} at all storage times and temperatures; the d_{43} did not change with storage time at any of the temperatures studied.

Fig. 6.10 shows the particle size distribution for experiment B samples. The changes in particle size distribution with storage time and temperature were qualitatively similar to those observed for experiment A samples. Experiment B samples, however, had wider particle size distributions, particularly at 37 °C.

Again the dispersion of the samples in a protein dissociating medium changed particle size distributions to monomodal with most particles being smaller than 2 μm.

Fig 6.11 shows the changes in d_{43} of experiment B samples with storage time. The values of d_{43} increased with increase in storage times, with the increase being greater at higher storage temperatures. Again, the d_{43} did not change with storage time when the samples were treated with the protein dissociating medium at any of the storage temperatures studied.

On comparing the particle size distribution for experiment A and experiment B, it appears that forewarming the milk at 90 °C for 2 min before RO concentration, resulted in an increase in the average particle size (Fig. 6.9 and 6.11).

Fig 6.12 shows the particle size distributions for experiment C samples. The particle size distribution for the initial measurement was different from that observed in experiments A and B. The population of smaller particles was non-existent (in the size range 0.1-1 μm), and instead there was a higher proportion of larger particles, in the size range 2.5-50 μm. This shift towards larger particle sizes may be due to the fact that these milks had not been homogenized after UHT treatment. After 25 weeks storage at

5 °C, the particle size distribution remained bimodal and became even broader with some particles above 50 μm. On storage for 25 weeks at 20 °C, there was an increase in the population of particles in peak 3 and also an increase in the size of the partially resolved peak 4. The distribution of particles in samples stored at 37 °C was more or less similar to that observed at 20 °C.

Treatment of samples with the protein dissociating medium caused the particle size distributions to become monomodal.

Fig. 6.13 shows changes in d_{43} for experiment C samples with storage time. The values of d_{43} increased with increase in storage time, with a slight increase at lower storage temperatures. Again, the d_{43} did not change with storage time when the samples were treated with the protein dissociating medium at any of the storage temperatures studied.

On comparing the results from experiment A (Fig. 6.9) and experiment C (Fig. 6.13), at all storage temperatures, it appears that particles formed in RO milks homogenized after UHT treatment were smaller as compared with the samples which had not been homogenized after UHT treatment.

Fig 6.14 shows the particle size distribution for experiment D samples. Unlike the particle size distribution for experiment A, B and C, the particle size distribution for samples from experiment D showed a multimodal distribution in the initial measurement, with the appearance of four peaks in the following size ranges: peak 1 = 4-7.5 μm, peak 2 = 7.5-15 μm, peak 3 = 15-30 μm and peak 4 = 30-80 μm. There were a number of particles greater than 100 μm that could not be measured by the Master Sizer. After storage for 25 weeks at 5 °C, there was a slight decrease in the population of particles in peak 1, with corresponding increases in the populations of particles in peaks 2 and 3. Storage for 25 weeks at 20 °C resulted in a decrease in peak 1 and slight increases in peak 2, 3 and 4. The particle size distribution of the sample stored at 37 °C remained unaffected after 25 weeks storage.

From these results, it appears that the combined effects of forewarming the milk prior to concentration, and concentrates not being subjected to homogenization after UHT treatment, could result in the formation of very large particles. These particles do not

appear to change markedly during storage. Again, the treatment with protein dissociating medium, produced monomodal particle size distribution.

As expected, the values of d_{43} of these samples at all the temperatures of storage were greater than for the samples obtained from other experiments. There were relatively minor changes with storage time (Fig. 6.15).

From the above results, RO concentrated pasteurized milks and homogenized after UHT treatment after 25 weeks storage exhibited the formation of smaller aggregates up to size of 25 μm, whereas similar samples not homogenized after UHT treatment showed larger aggregates up to 80 μm. The population of these aggregates was higher at higher storage temperatures. Forewarmed and post-UHT homogenized RO concentrated milks showed the formation of aggregates in the size range 2.5-50 μm, whereas the samples that were forewarmed and not homogenized showed the formation of larger aggregates in the size range > 4 μm, some of which were too large to be detected by the Master Sizer.

It can be inferred that homogenization of the RO concentrate after UHT treatment results in the formation of smaller aggregates as compared with non-homogenized concentrates. Forewarming resulted in an increase in aggregate size as compared with pasteurization. Forewarmed homogenized milks had a lower aggregate size than forewarmed and non-homogenized RO concentrated milks. The sizes of the aggregates formed in RO concentrated milks after storage for 25 weeks were in the following order: pasteurized and homogenized < forewarmed and homogenized < pasteurized and not homogenized < forewarmed and not homogenized.

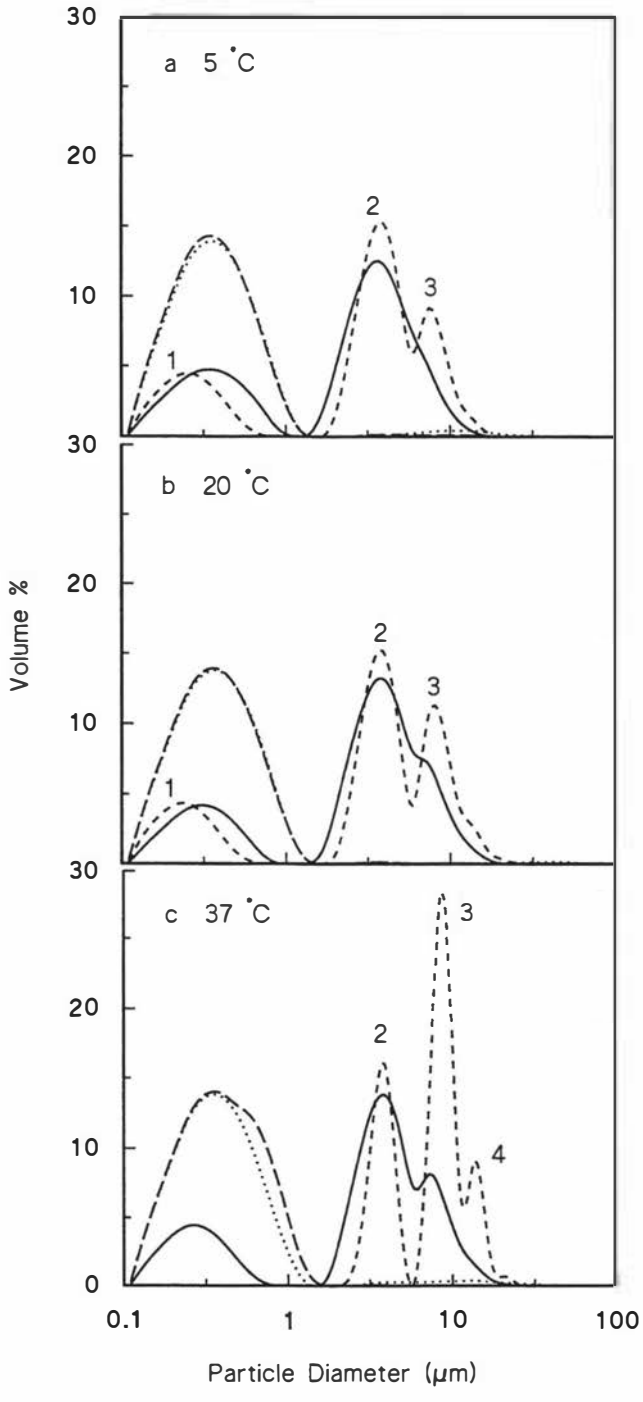


Fig. 6.8 Particle size distribution for Experiment A (P-ROC-UHT-H) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(– – –) initial measurement and (·····) measurement after storage for 25 weeks]. Data shown are from trial II. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

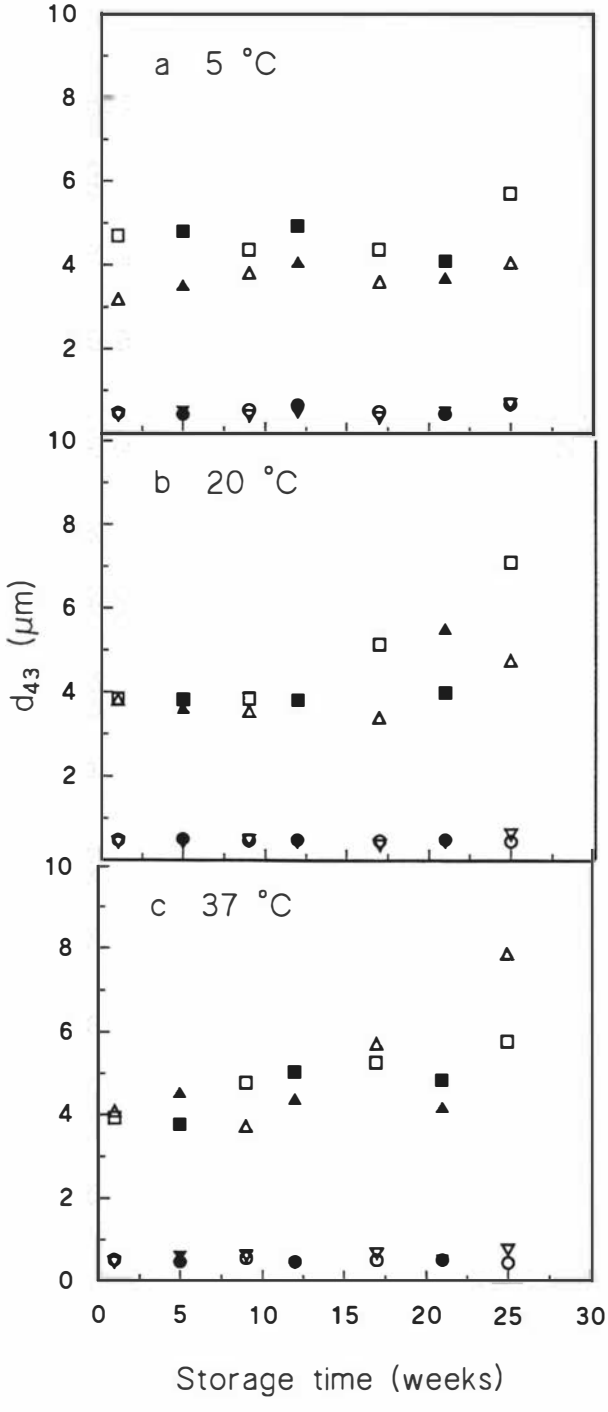


Fig. 6.9 Changes in weight-average diameter (d_{43}) for Experiment A (P-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20, and c) 37 °C. The milk samples were dispersed in Milli Q water for trials Ia □, Ib ■, IIa Δ, IIb, ▲; or dispersed in a SDS/EDTA buffer for trials Ia ○, Ib ●, IIa ∇, IIb ▼. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

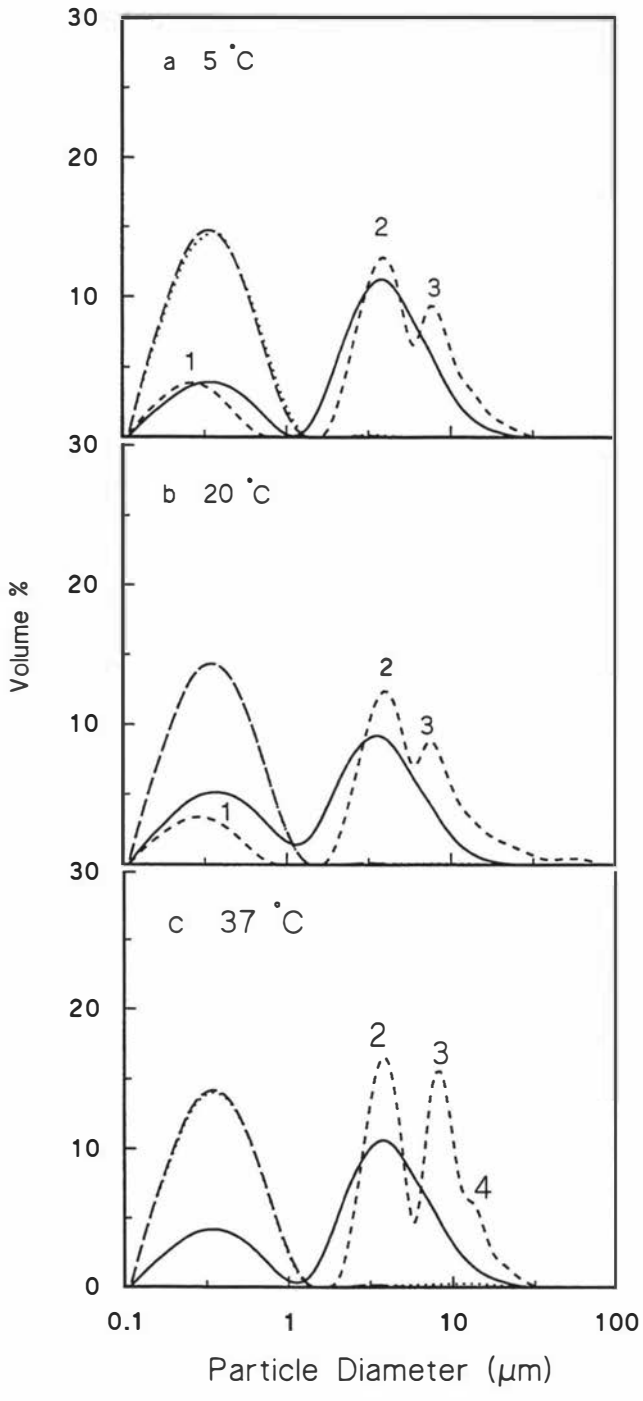


Fig. 6.10 Particle size distribution for Experiment B (F-ROC-UHT-H) milk samples stored at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(— — —) initial measurement and (·····) measurement after storage for 25 weeks]. Data shown are from trial II. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

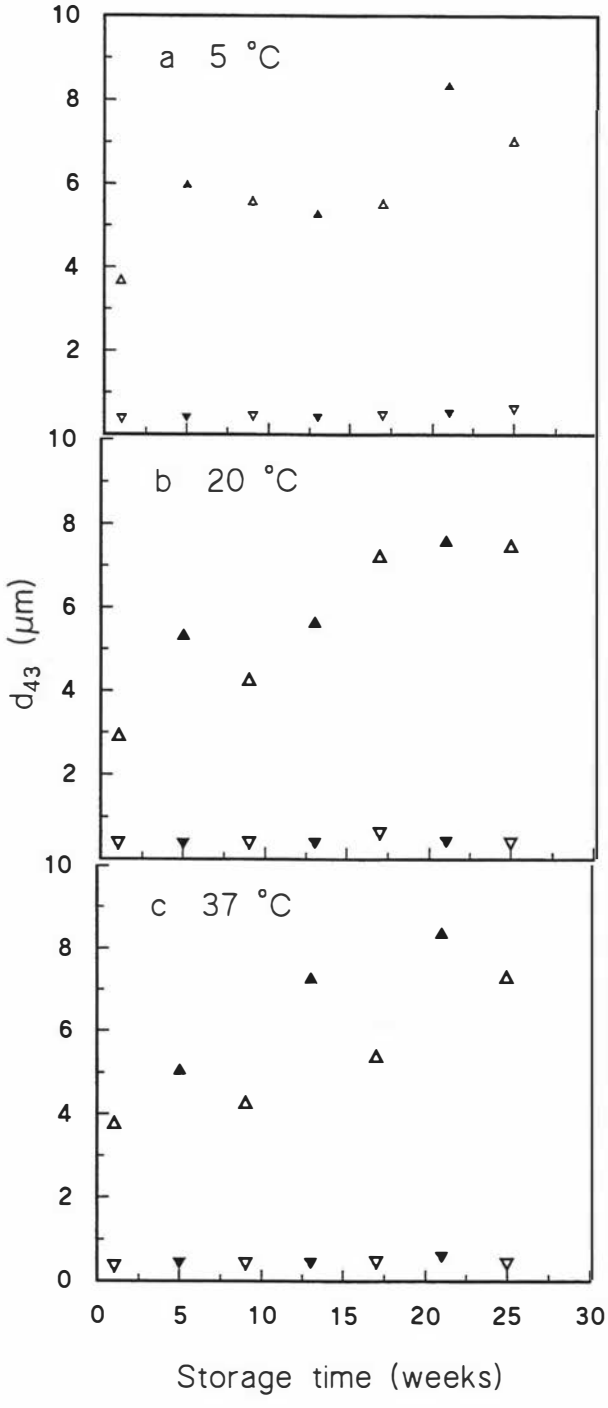


Fig. 6.11 Changes in weight-average diameter (d_{43}) for Experiment B (F-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20, and c) 37 °C. The milk samples were dispersed in Milli Q water for trial IIa Δ, IIb, ▲; or dispersed in a SDS/EDTA buffer for trial IIa ∇, IIb ▼. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

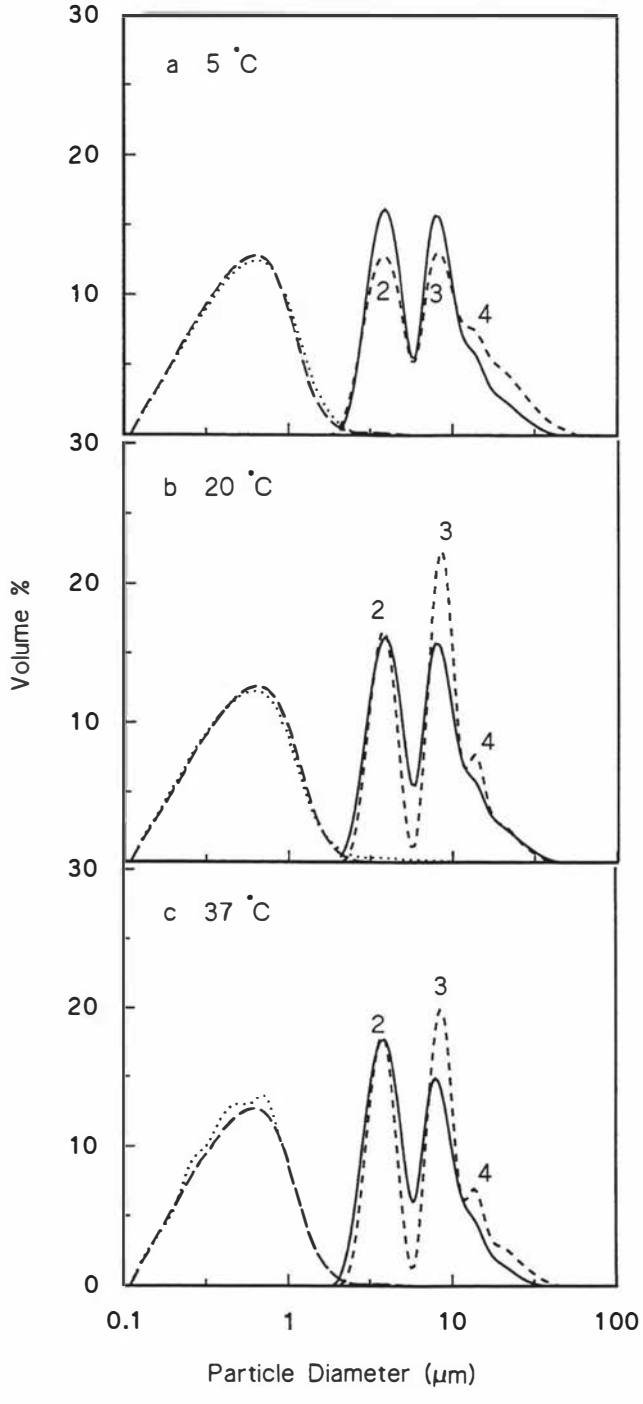


Fig. 6.12 Particle size distribution for Experiment C (P-ROC-UHT) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(— — —) initial measurement and (· · · · ·) measurement after storage for 25 weeks]. Data shown are from trial II. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

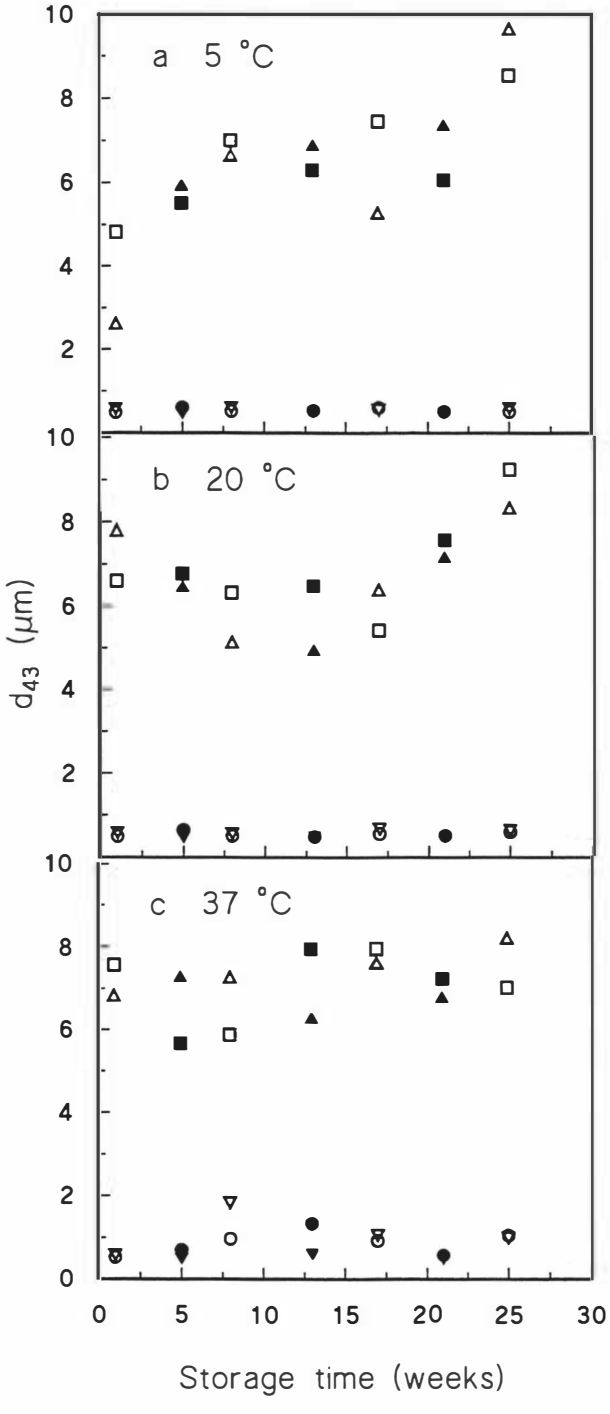


Fig. 6.13 Changes in weight-average diameter (d_{43}) for Experiment C (P-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20, and c) 37 °C. The milk samples were dispersed in Milli Q water for trials Ia □, Ib ■, IIa Δ, IIb, ▲; or dispersed in a SDS/EDTA buffer for trials Ia ○, Ib ●, IIa ∇, IIb ▼. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

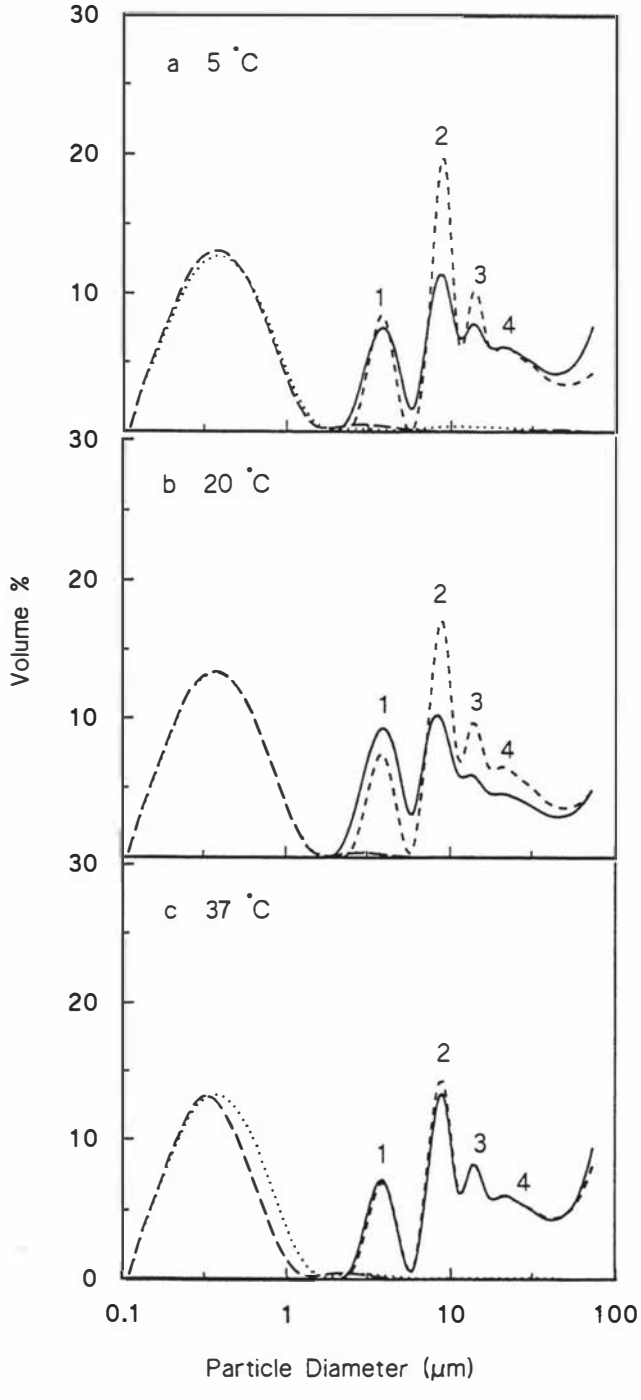


Fig. 6.14 Particle size distribution for Experiment D (F-ROC-UHT) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(– – –) initial measurement and (. . . . . .) measurement after storage for 25 weeks]. Data shown are from trial II. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

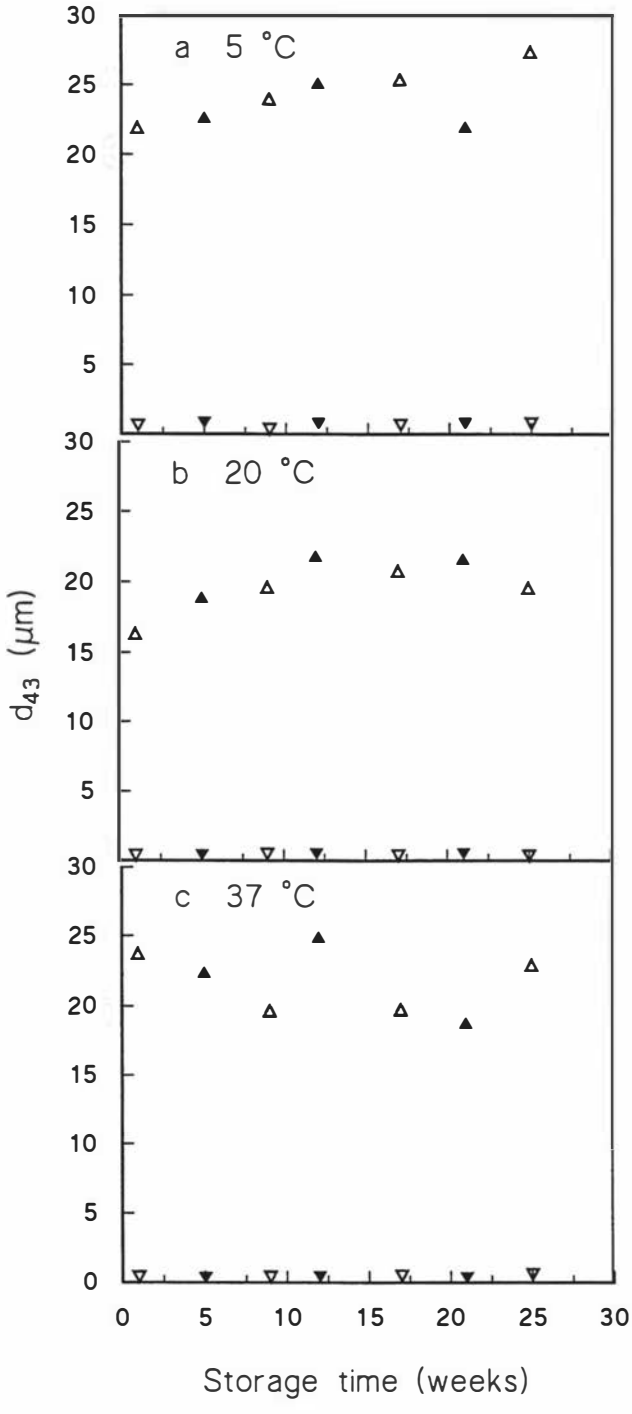


Fig. 6.15 Changes in weight-average diameter (d_{43}) for Experiment D (F-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20, and c) 37 °C. The milk samples were dispersed in Milli Q water for trial IIa Δ, IIb, ▲; or dispersed in a SDS/EDTA buffer for trials IIa ∇, IIb ▼. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

6.3.1.4 Penetration test

Fig. 6.16 shows a typical plot of force (newtons) *versus* distance (mm) obtained with the Instron using a penetration test method for experiment B milk sample stored at 37 °C for 21 weeks. The distance for the force required to cause the first break in the gel was recorded. The result obtained was converted into a ratio of force to distance. It was assumed that the greater was this ratio, the greater was the resistance of the milk gel to probe penetration, or in other words the greater was the gel strength.

The results obtained with the penetration test for experiments A-D are shown in Fig.6.17-6.20. In general, in all experiments, samples stored at 5 °C showed no noticeable change in the ratio of force/distance, which suggests that at 5 °C there was no gel formation in the samples, i.e. they remained liquid throughout the 25 week storage period. However, storage at 20 and 37 °C generally resulted in an increase in the ratio, although there were considerable variations between the samples. A sample with a ratio of more than 0.030 (N/mm) was considered to be gelled. Using this definition, it appears that the samples from experiments A and C, stored at 20 and 37 °C, gelled after 17 weeks storage (Figs. 6.17b c; Figs. 6.19b, c). In experiment B, samples stored only at 20 °C gelled after 25 weeks (Fig. 6.18b). In experiment D, the samples stored at 37 °C gelled after 9 weeks of storage (Fig. 6.20c).

Storage at higher temperatures after the samples had gelled resulted in a decrease in the force to distance ratio because of the fact that gel became weaker with the development of serum (syneresis) (Figs. 6.17 b, c; Figs. 6.19b, c; Figs. 6.20c).

This suggests that post-UHT homogenization had no appreciable effect on the rate of gel formation for pasteurized milk concentrates, but it decreased the rate of gel formation for concentrates that had been forewarmed prior to RO concentration. The effects of forewarming on the rate of gelation was dependent on whether or not concentrates were homogenized after UHT treatment. In the case of concentrates not post-UHT homogenized forewarming increased the rate of gelation, whereas an opposite trend was observed in the case of concentrates that were homogenized after the UHT treatment.

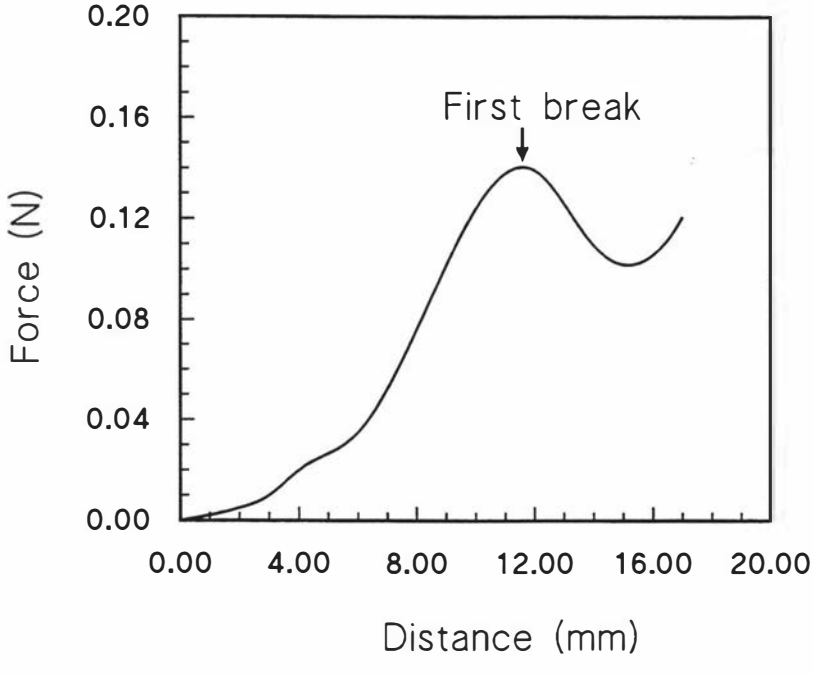


Fig. 6.16 Example showing a typical plot of force (newtons) versus distance (mm) obtained with the Instron using a penetration test for experiment B sample stored at 37 °C for 21 weeks. The arrow on the curve shows the first break (a point at which force to distance ratio was determined).

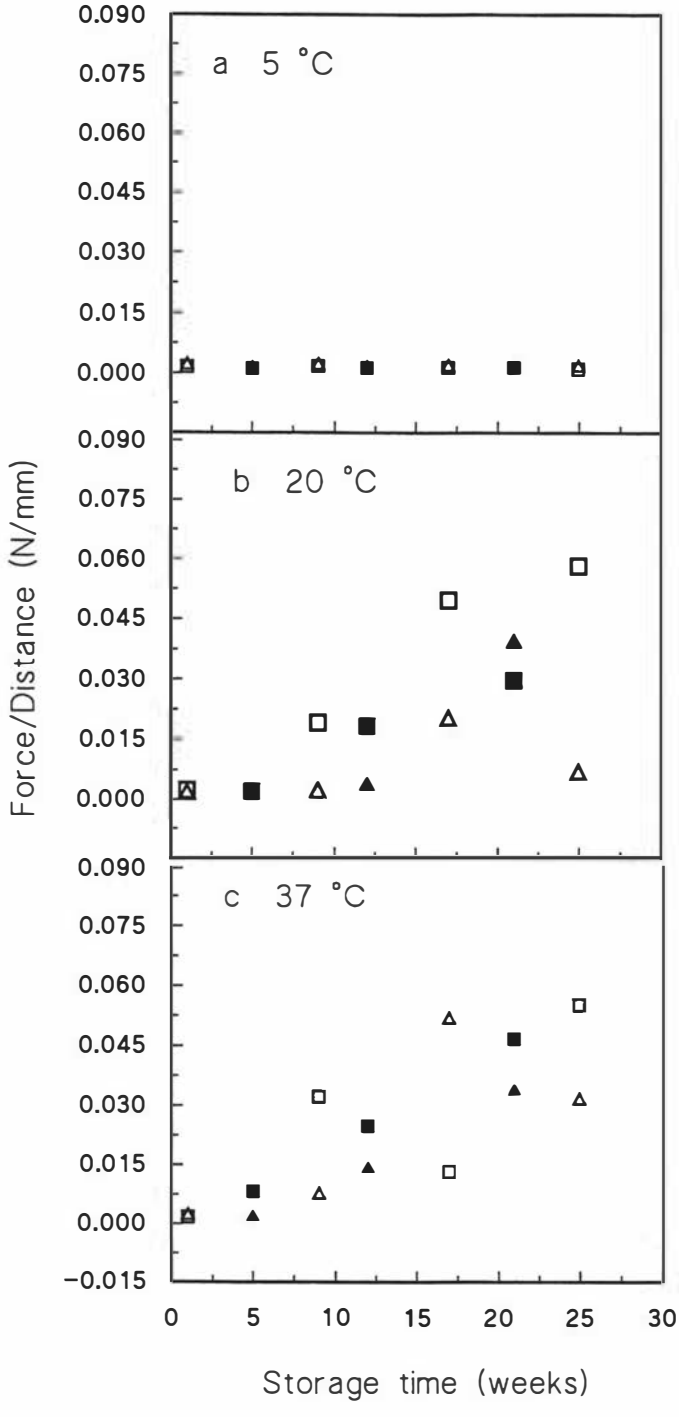


Fig. 6.17 Changes in force/distance ratio for Experiment A (P-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

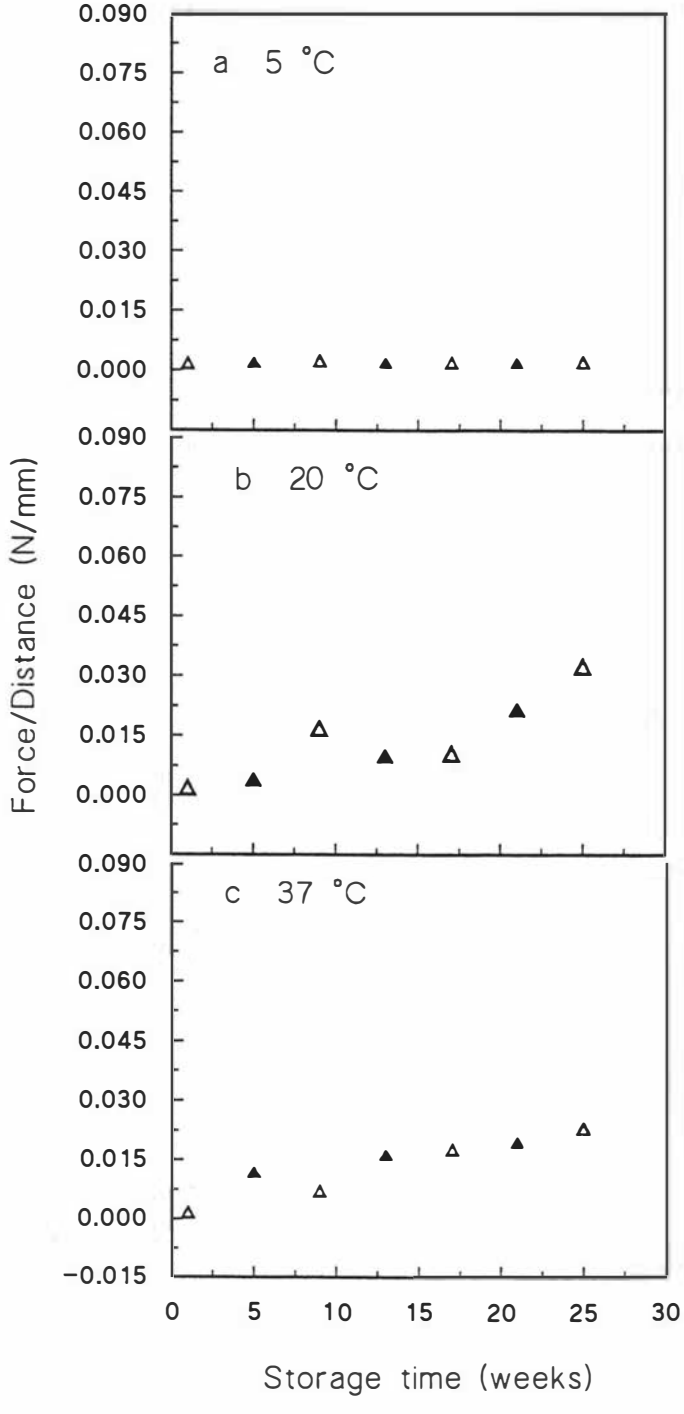


Fig. 6.18 Changes in force/distance ratio for Experiment B (F-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

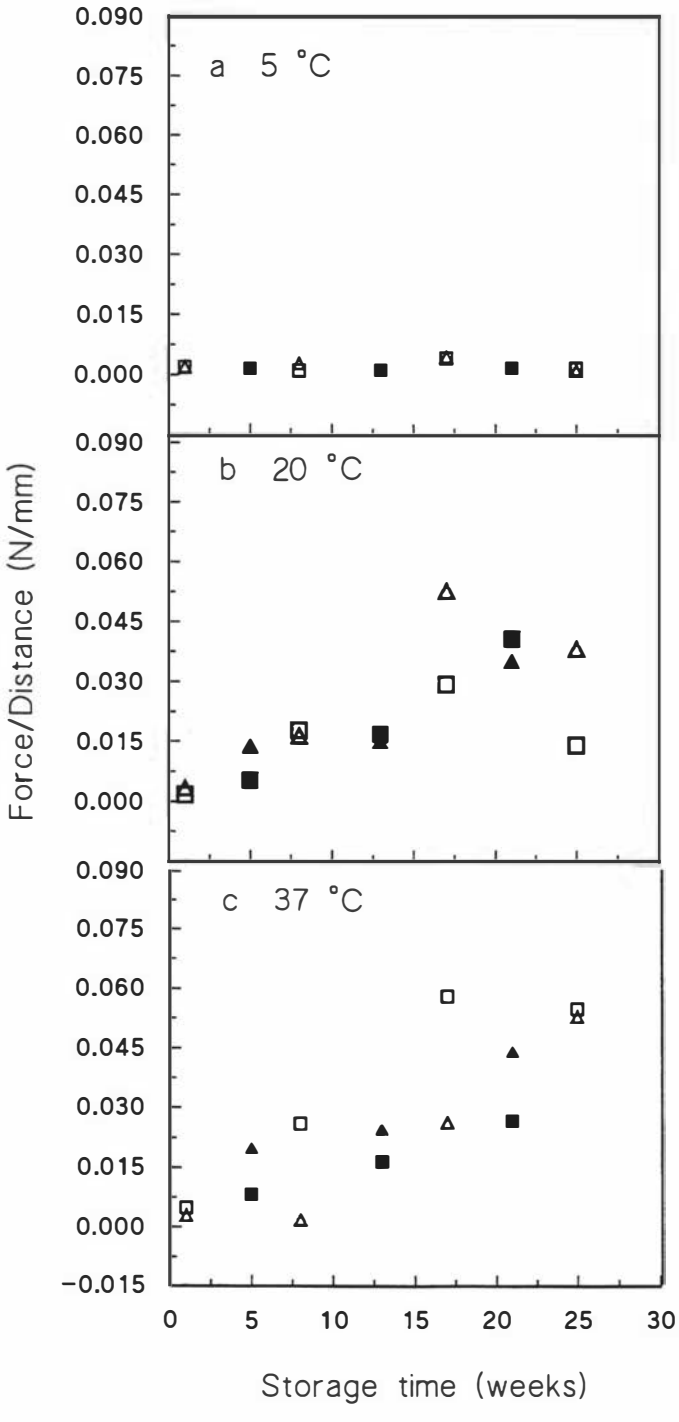


Fig. 6.19 Changes in force/distance ratio for Experiment C (P-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

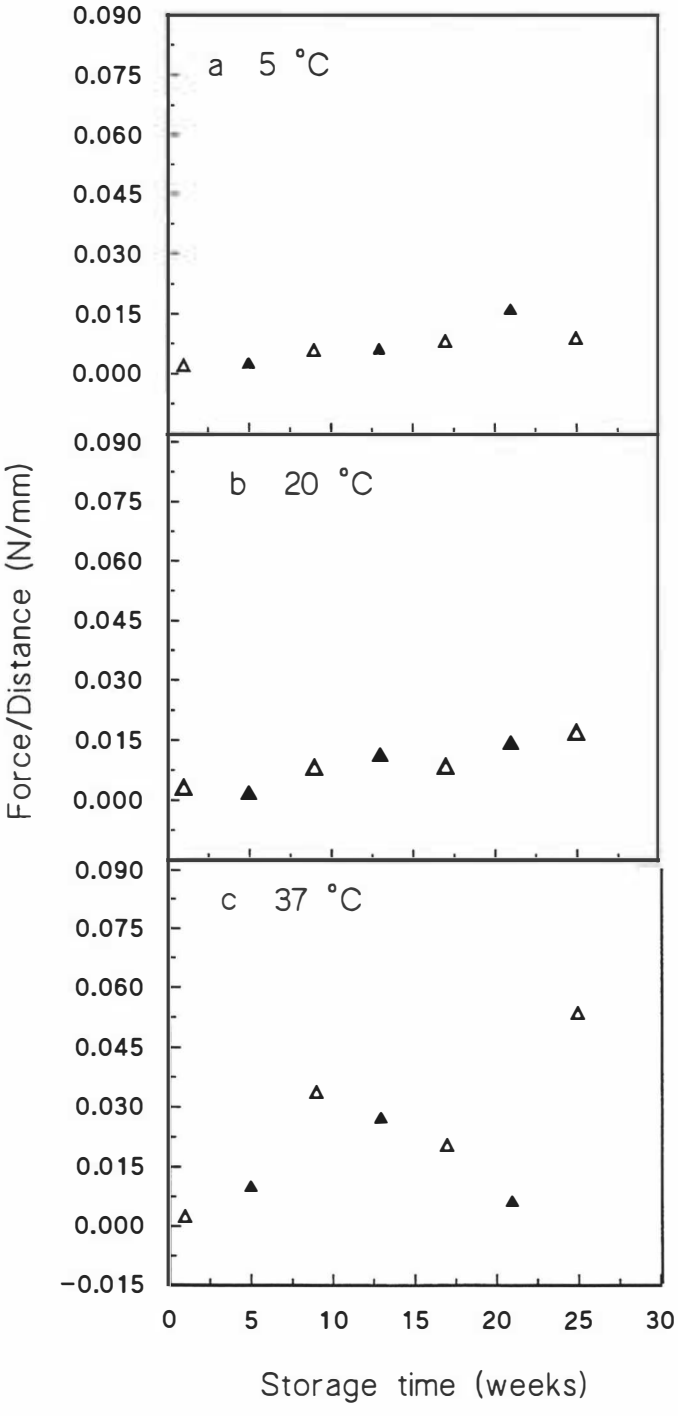


Fig. 6.20 Changes in force/distance ratio for Experiment D (F-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

6.3.1.5 Viscosity

The viscosity of various samples obtained from experiments A, B, C, and D was measured using the Bohlin VOR Rheometer in its viscometry mode (Chapter 4). The viscosity of a sample was measured at the temperature at which the sample had been stored (5, 20 and 37 °C). Samples were first sheared at a high shear rate (921 s^{-1}) for 1 min at the measurement temperature to break reversible structures formed during storage, and then the viscosity was measured at shear rates in the range 29.2 to 367 s^{-1} to obtain up and down flow curves. A typical plot of apparent viscosity versus shear rate is shown in Fig. 6.21. The data represents apparent viscosity (measured at 37 °C) for a experiment C sample stored for 21 weeks at 37 °C. It can be seen that the values of apparent viscosity were slightly lower on the up flow curve than the down flow curve. The value of the apparent viscosity (at shear rate 367 s^{-1}) plotted in Figs. 6.22 to 6.25 is the average of the values from the up and down flow curves at that particular shear rate.

The changes in apparent viscosity for experiment A (P-ROC-UHT-H) samples are shown in Fig. 6.22. At low storage temperature (5 °C), the apparent viscosity of the samples increased from 10 mPas (no storage) to 240 mPas after 25 weeks of storage (an increase of ~ 2300%), and there was considerable variation between the different samples obtained from trials Ia, Ib, IIa, and IIb. At 20 °C, there was a smaller increase in the viscosity of the samples with storage time (from 40 mPas, no storage to 100 mPas after 25 weeks (an increase of ~ 150%)). At 37 °C, a small increase was observed (from 10 mPas, no storage to 60 mPas after 25 weeks (an increase of ~ 500%). At 37 °C, the increase in viscosity was much lower than at 5 °C.

Fig. 6.23 shows the changes in viscosity during storage for experiment B (F-ROC-UHT-H) samples. The viscosity values reported are for trial II only. The viscosity values observed for this set of experiments were considerably higher than those obtained for experiment A samples. As a result the scale on the Y-axis has been changed. There was an appreciable increase in the viscosity during storage at 5 °C (an increase of ~ 100%), whereas at 20 and 37 °C, there was no change in viscosity with increase in storage time. Based on these results, it appears that forewarming of milk prior to concentration markedly increased the initial viscosity values, but the changes in viscosity during storage were less pronounced in the forewarmed milks.

Fig 6.24 shows the changes in the viscosity of experiment C (P-ROC-UHT) samples. The initial viscosity values for these samples were greater than the values obtained for experiment A, but were lower than those obtained for experiment B. In general, there was an appreciable increase in the viscosity of the samples stored at 5 °C for 25 weeks (an increase of ~ 72%), but thereafter viscosity decreased considerably (at 25 weeks storage period); possibly this is a measurement effect caused by syneresis. At 20 and 37 °C there was little change in the viscosity during storage. Homogenized RO concentrates (Fig. 6.22) exhibited higher viscosity values as compared with not-homogenized RO concentrates (Fig. 6.24) at all storage temperatures.

Figs. 6.25 shows the changes in apparent viscosity of the experiment D (F-ROC-UHT) samples. There were higher initial viscosities as compared with samples obtained for experiments A, B and C and therefore the scale for the Y-axis has been changed. The % increase in the viscosity at 5 °C was ~ 200% but at storage temperatures 20 and 37 °C no significant change was observed. The changes in viscosity with storage time followed a trend similar to that observed in experiment C. However, the viscosity values obtained for these samples were higher than for the experiment C samples.

The above results show that the initial viscosity values of various milks were in the order: RO concentrated milks homogenized after UHT treatment (experiment A) < RO concentrated milks forewarmed before concentration and homogenised after UHT treatment (experiment B) < RO concentrated milks not homogenized after UHT treatment (experiment C) < RO concentrated milks forewarmed before concentration and not homogenized after UHT treatment. These differences in viscosity were greater at lower temperatures. However, the trends in viscosity changes during storage were generally similar in all milks.

There are no reports of similar work in the literature with which these results can be compared directly. Previous workers (e.g. Harwalkar and Vreeman, 1978a, Kocak, 1985, de Koning *et al.*, 1992, Gupta and Pal, 1993, Venkatachalam *et al.*, 1993) measured the viscosity at 20 °C on samples stored at other temperatures. In addition, the samples were first sheared at a high shear rate in the present study before the final viscosity measurements. Therefore, in this study largely irreversible changes in viscosity were measured. Kocak (1985) while studying the storage stability of UHT treated RO concentrates (26% T.S) observed that the apparent viscosity (measured at 20 °C) of samples stored at 2 or 10 °C increased linearly, reaching 33 and > 50 mPa.s respectively after 102 days storage.

In contrast, the samples stored at higher temperatures displayed viscosity changes that were more characteristic of gelation (small changes in viscosity followed by a sudden rise). Kocak's UHT treated RO concentrates stored at 30 or 40 °C formed coagula after 102 days of storage, whereas the samples stored at 20 °C remained viscous liquids with an apparent viscosity of > 50 mPa.s. During storage, a significant reduction in apparent viscosity of samples stored at 40 °C was observed before the onset of age gelation. de Koning *et al* (1992) while studying the gelation of in-container sterilized evaporated homogenized milk (31% T.S.) observed that the viscosity of milks stored at 30 °C which were fresh normal sterilized (under usual factory conditions) decreased with storage time from 62 mPas (no storage) to 25 mPas after 280 days storage, whereas milks moderately sterilized (a less intense heat treatment than normal sterilized) showed a decrease from 25 to 20 mPas after a similar period of storage. Also de Koning *et al.* (1992) investigated the effect of cold storing concentrated milk at 4 °C for 96 h, and found that the viscosity for normal sterilized milk increased to 125 mPas in 190 days and then decreased to 72 mPas after 280 days storage; whereas in moderately sterilized milks, the viscosity increased from 25 mPas to 105 mPas in 54 days storage. Harwalkar and Vreeman (1978a) observed an increase with time in the viscosity of UHT sterilized heat evaporated concentrated skim milk (23 % T.S.) stored at 28 °C. The changes in the viscosity were characteristic of gelation. Venkatachalam *et al.* (1993) observed an increase in the viscosity of UF skim milk concentrates stored at 4 and 20 °C (the samples gelled after 21 weeks) whereas at 35 °C no significant increase in viscosity was observed and samples did not gel; instead they showed slight sedimentation after 40 weeks of storage.

On comparing the results from the present study (experiment A) with those of Kocak (1985), it can be said that these results show somewhat different trends in the % increase in the viscosity with storage time. The viscosity of the samples stored at 5°C increased from 10 mPas to 240 mPas (~2300% increase), at 20 °C from 40 mPas to 1000 mPas (~150% increase) and finally at 37 °C from 10 mPas to 60 mPas (~ 500% increase). The present results demonstrate that there is an increase in viscosity with time at lower storage temperatures (5 °C) but the results of Kocak (1985) were that the viscosity

increased at higher storage temperatures (20 and 40 °C). Kocak (1985) observed an increase in the apparent viscosity of RO concentrated UHT milks (26% T.S.). At 2 °C, the viscosity values increased from 13 mPas (no storage) to 33 mPas in 102 days (an increase of 161%); whereas samples stored at 20 and 40 °C showed an increase in viscosity to 52 mPas (an increase of 300%). The reasons for this contradiction may be the higher total solids being used in the current study (more fat and casein), and the fact that the viscosity was always measured at the storage temperature, which removes an expected error of warming up or cooling down the samples when viscosity is measured at room temperature (~ 20 °C).

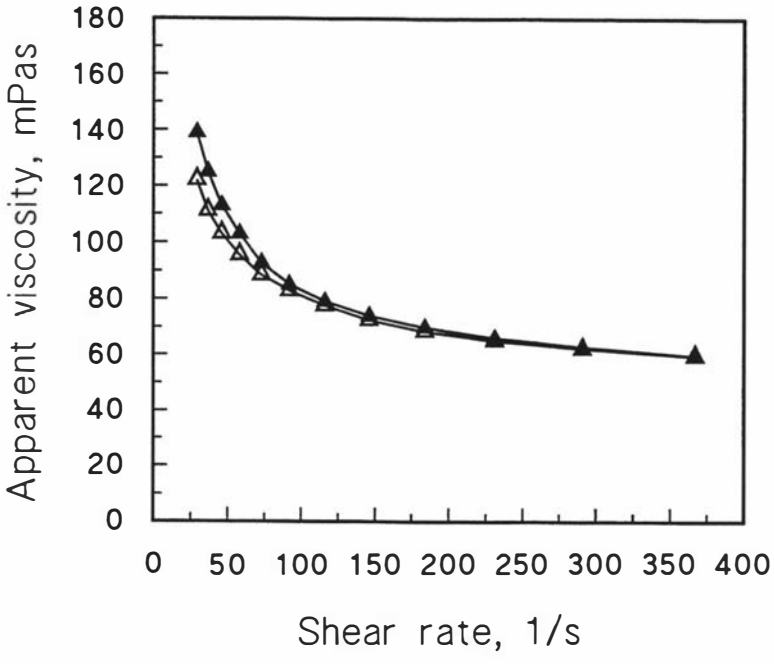


Fig. 6.21 Example showing a typical apparent viscosity *versus* shear rate profile for experiment C sample stored at 37 °C for 21 weeks and viscosity measured at 37 °C. Δ represents up flow curve and ▲ represents down flow curve.

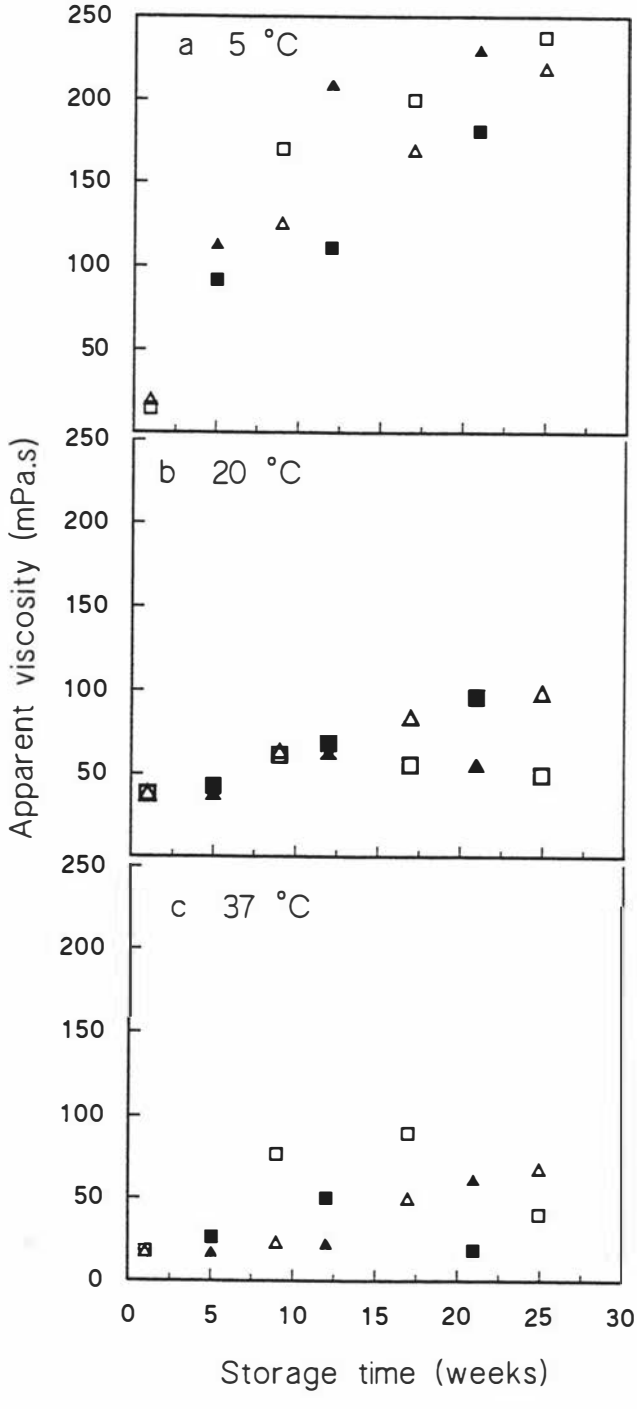


Fig. 6.22 Changes in apparent viscosity at the shear rate of 367 S^{-1} for Experiment A (P-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

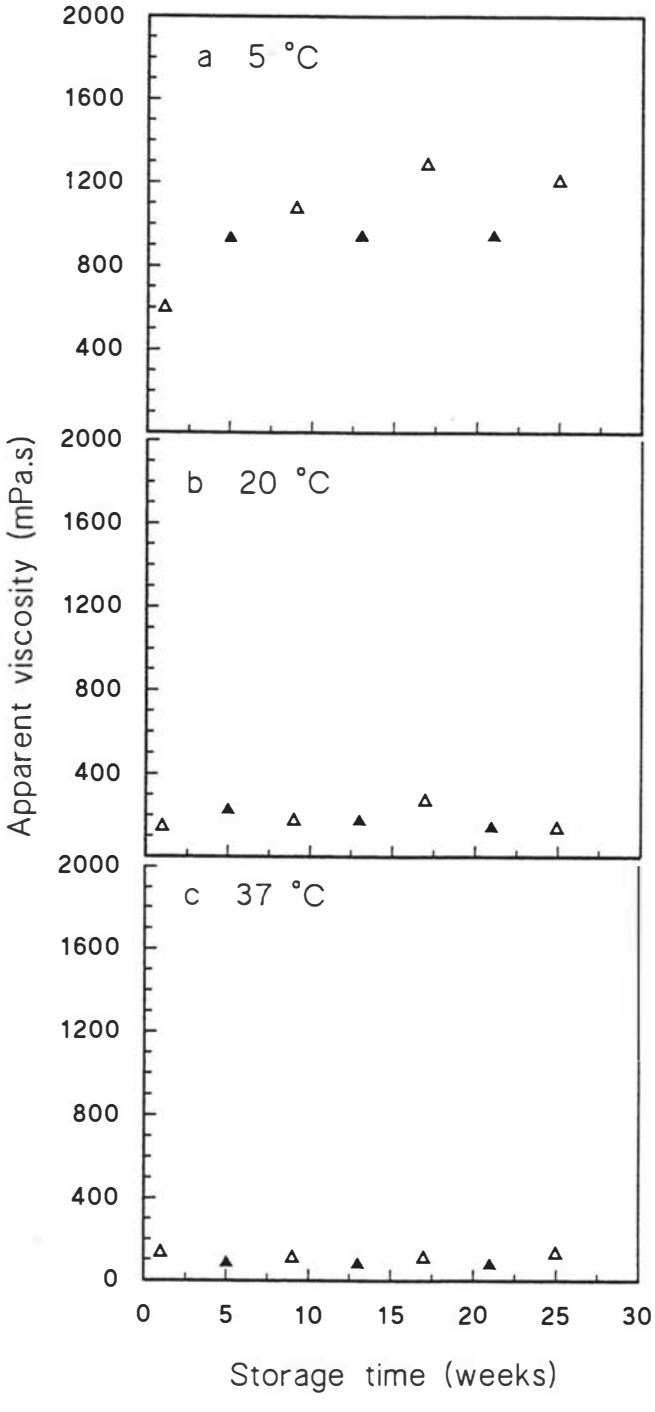


Fig. 6.23 Changes in apparent viscosity at the shear rate of 367 S^{-1} for Experiment B (F-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

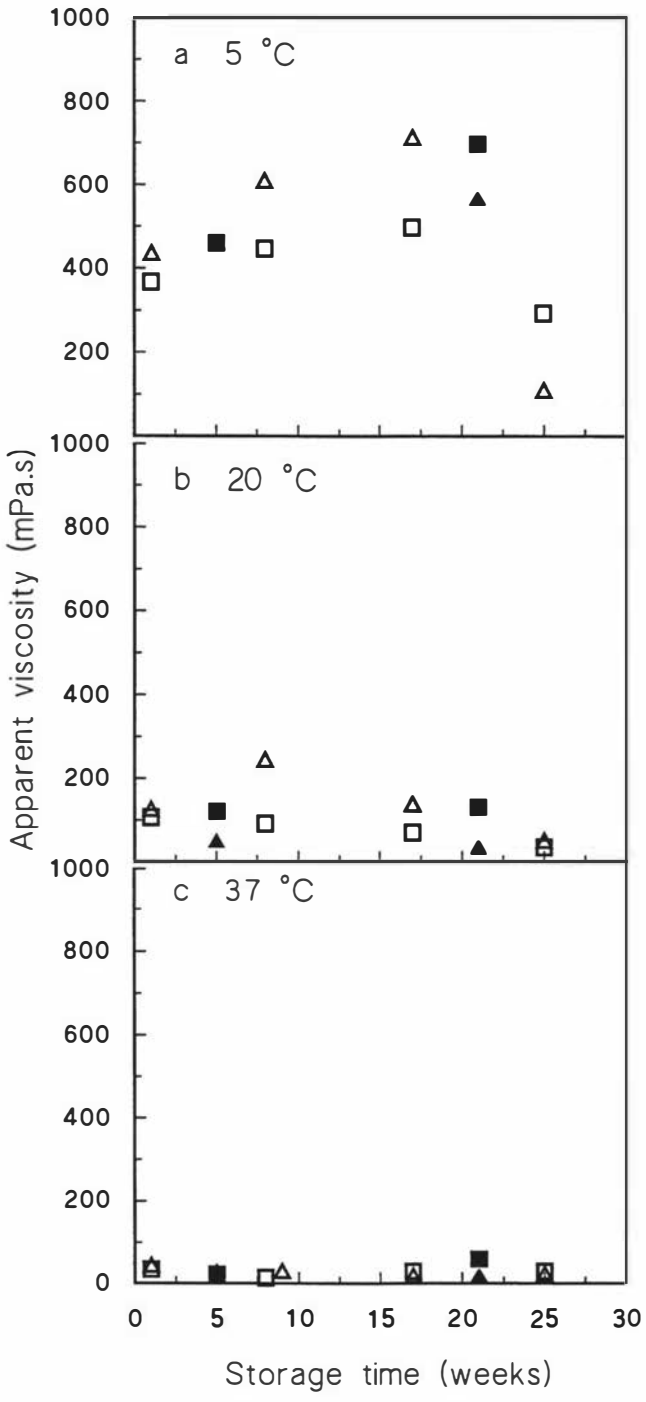


Fig. 6.24 Changes in apparent viscosity at the shear rate of 367 S^{-1} for Experiment C (P-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

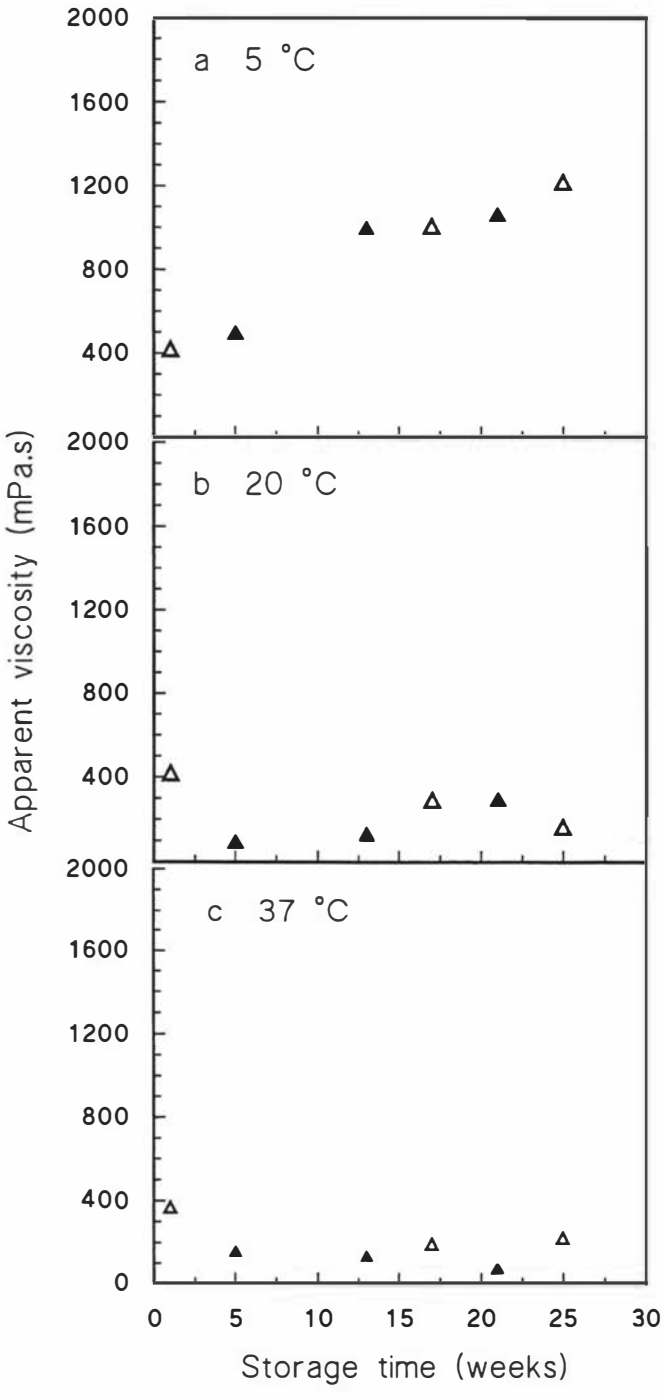


Fig. 6.25 Changes in apparent viscosity at the shear rate of 367 S^{-1} for Experiment D (F-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

6.3.1.6 Non-protein nitrogen

Fig 6.26 shows the changes in the non-protein nitrogen (NPN) content of experiment A samples stored at various temperatures. At 5 and 20 °C there was a slight increase in NPN with storage time, whereas at 37 °C the NPN values increased markedly and almost linearly with storage time.

Similar changes in NPN values were observed for experiment B samples (Fig. 6.27), although the NPN values were slightly lower than the values obtained in experiment A. Results obtained for experiment C and experiment D samples (Figs. 6.28 and 6.29) showed similar trends to those in experiments A and B respectively.

In general, these results show that the increase in NPN during storage was greater at higher storage temperature. Forewarming of milks before concentration resulted in lower NPN values at all storage temperatures, especially at 37 °C. Homogenization of the concentrate after UHT treatment had no effect on the formation of NPN during storage.

There are no reports in literature with which these results can be compared directly. However, Snoeren and Both (1981) found that NPN increased slightly in unconcentrated UHT sterilized milks stored at 28 °C for 32 months. In contrast, de Koning *et al.* (1985) observed that proteolysis was absent in UHT sterilized heat evaporated concentrated milk made from milk forewarmed at 120 °C for 3 min. Guthy *et al.* (1983), using partitioning of protein to determine proteolysis, found NCN and NPN increased continuously with time, and at a higher rate at 35 than at 20 °C.

Kocak (1985) observed during storage of UHT treated RO milks (26% T.S) that samples stored at 30 °C exhibited more proteolysis than the samples stored at 2, 10, 20 and 40 °C. In a way, the present results agree with the results of Guthy *et al* (1983) in that at higher temperatures of storage there was a greater increase in NPN. These results suggest that there is some protein breakdown during storage of RO concentrated milks.

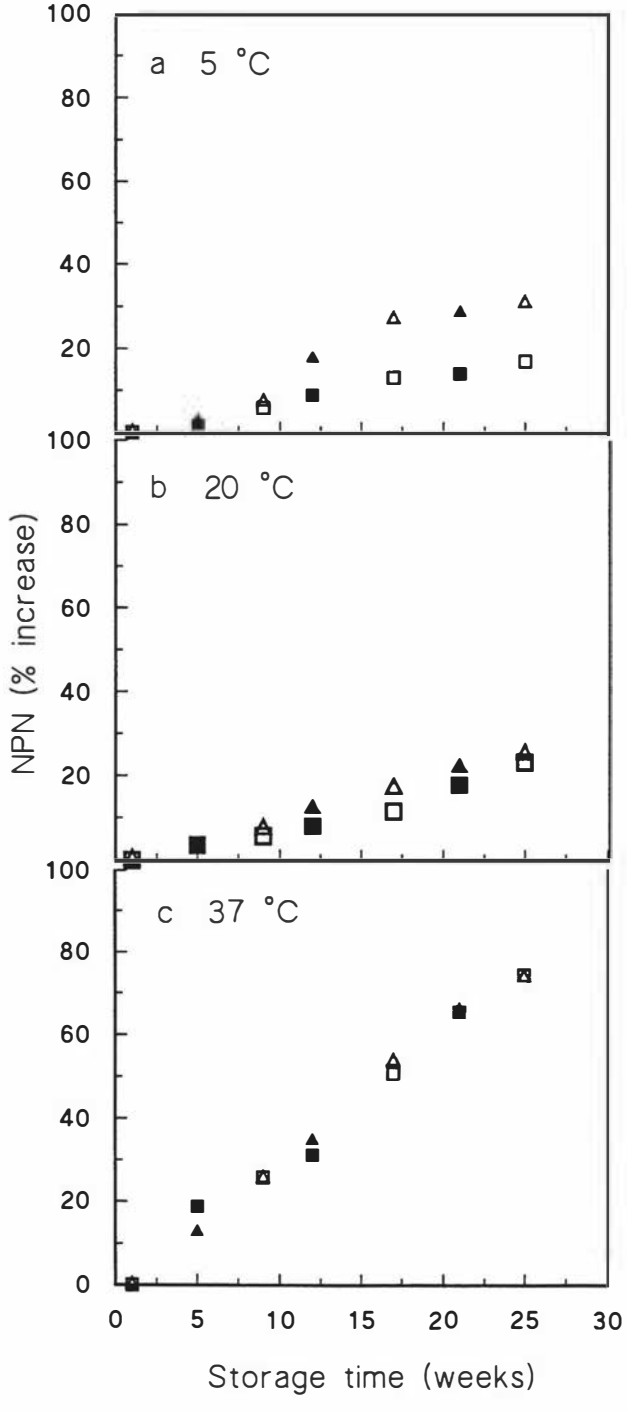


Fig. 6.26 Changes in non protein nitrogen (NPN) in Experiment A (P-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

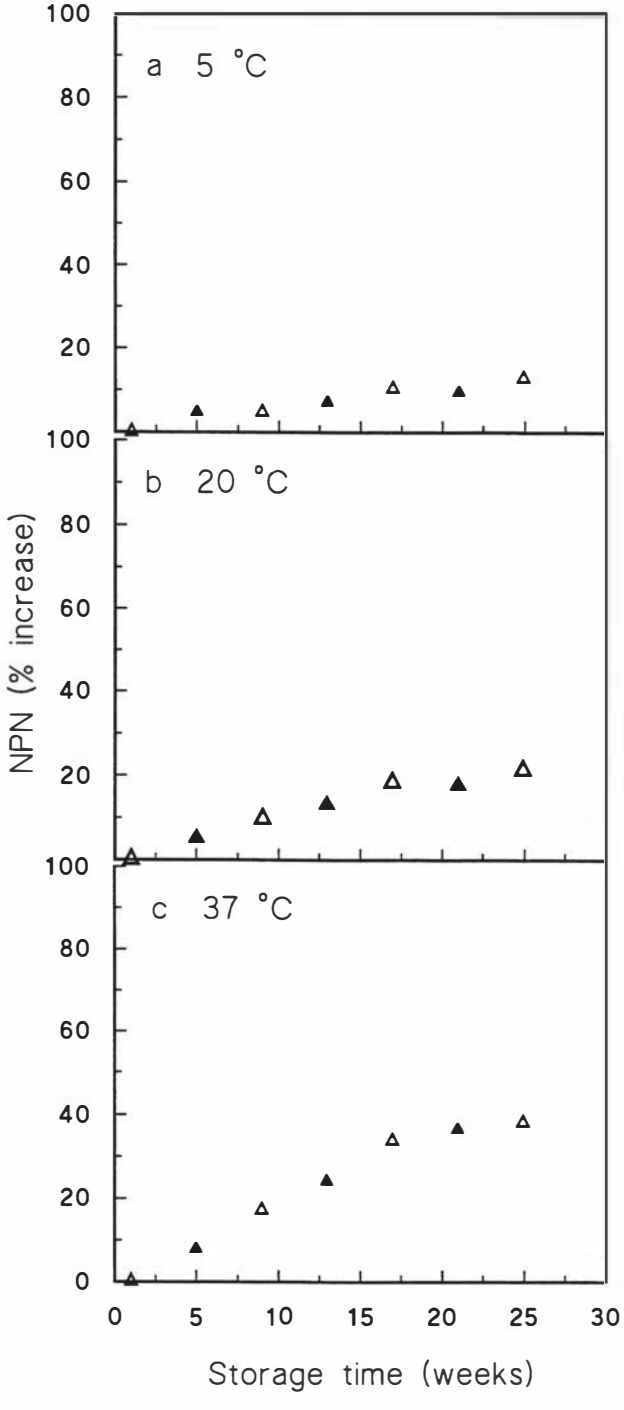


Fig. 6.27 Changes in non protein nitrogen (NPN) in Experiment B (F-ROC-UHT-H) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar).

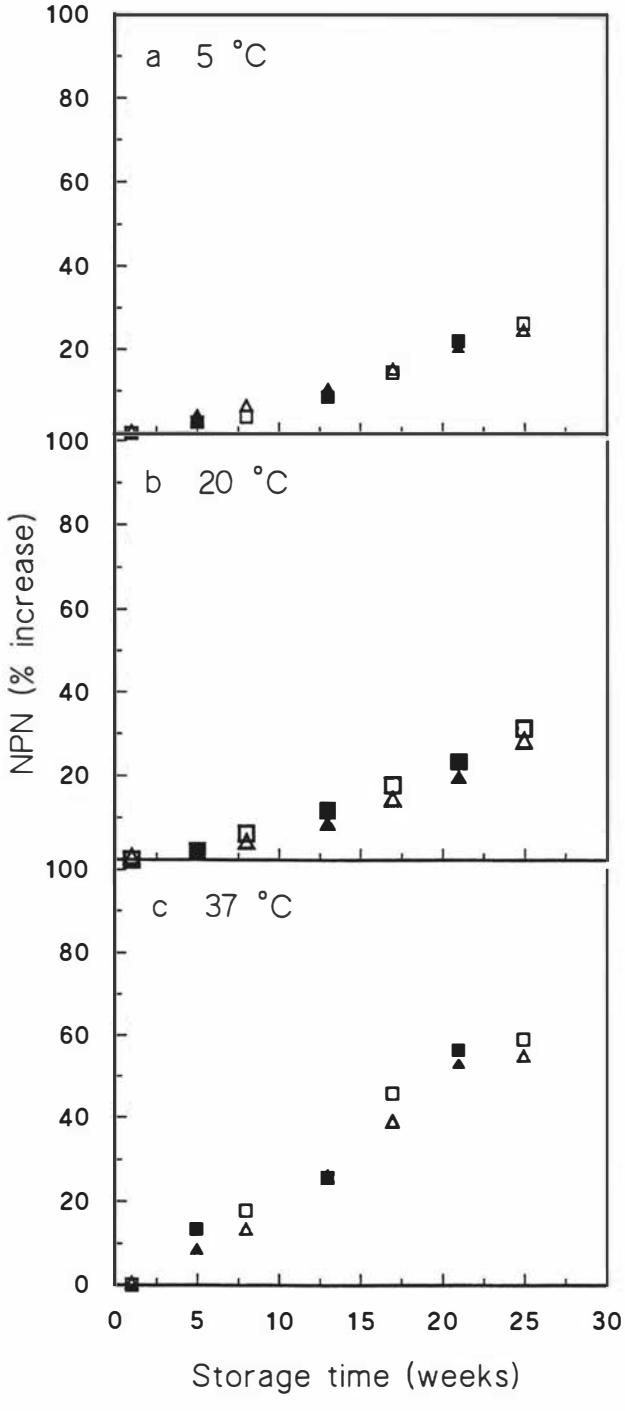


Fig. 6.28 Changes in non protein nitrogen (NPN) in Experiment C (P-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trials Ia □, Ib ■, IIa Δ, IIb, ▲. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

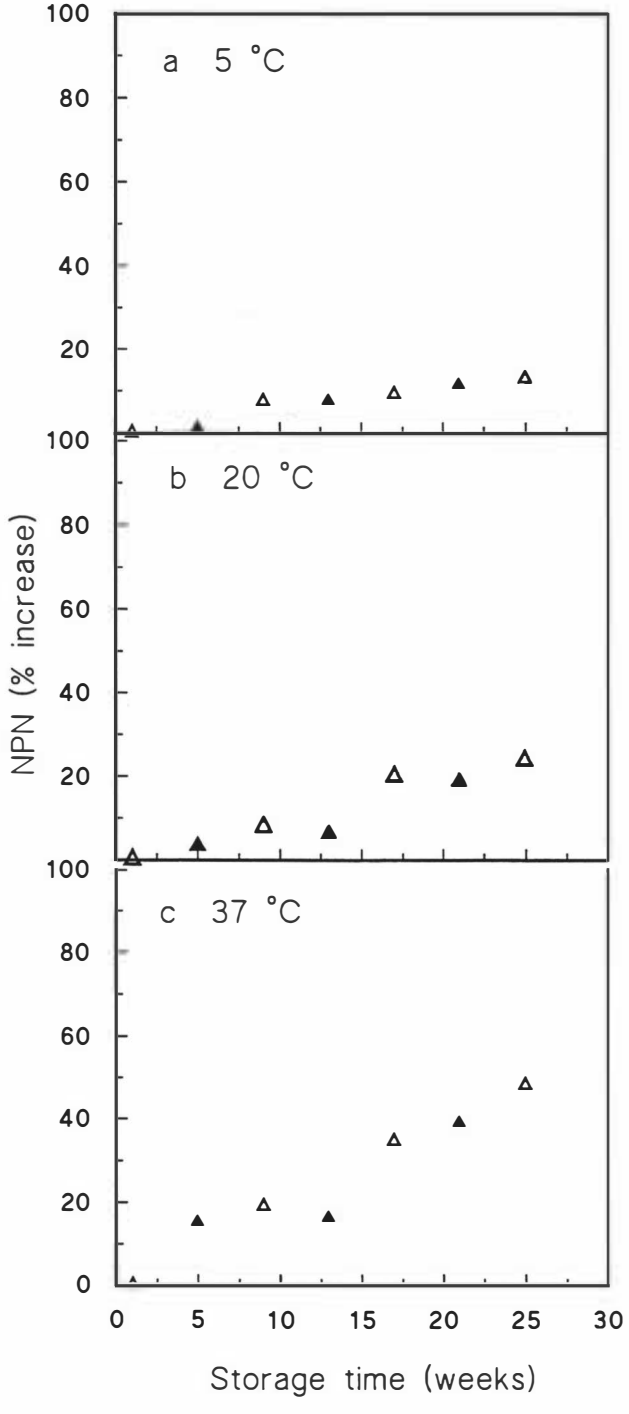


Fig. 6.29 Changes in non protein nitrogen (NPN) in Experiment D (F-ROC-UHT) milk samples as a function of storage time at a) 5, b) 20 and c) 37 °C for trial IIa Δ, IIb, ▲. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

6.3.1.7 Electrophoresis

All samples from experiments A and B (both non-reduced and reduced with mercaptoethanol) were analysed by SDS-PAGE. Both native and corresponding heat-denatured proteins are converted to monomers with the same conformations after SDS and mercaptoethanol treatments and they consequently migrate together. Figs. 6.30 and 6.31 show typical electrophoretic patterns for experiments A and experiment B samples respectively. The results are shown for initial, 12 weeks of storage (13 weeks for experiment B) and 25 weeks of storage at 5, 20 and 37 °C. Reverse osmosis concentrate (not UHT treated) was used as a standard.

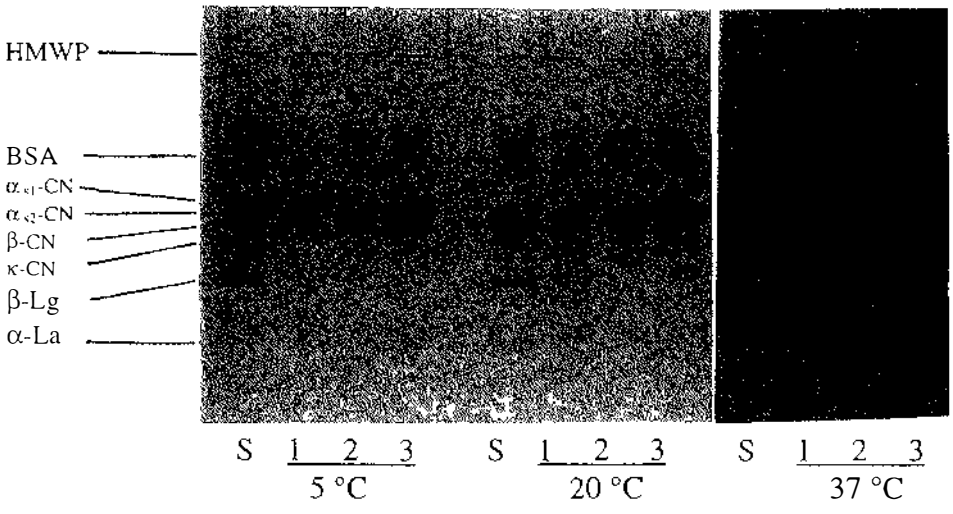
Fig. 6.30a shows the changes in electrophoretic patterns under non-reducing conditions during storage of experiment A samples. The standard (unheated RO concentrate) showed stained bands representing caseins and whey proteins. The whey proteins (β-lactoglobulin and α-lactalbumin) and κ-casein bands were largely absent from the samples that had been subjected to UHT treatment (lane 1). Also, as a result of UHT treatment, there was formation of high molecular weight polymers (HMWP); these polymers remained in the top of the stacking gel and they were presumably linked by disulphide bonds. Samples stored at 5 and 20 °C showed almost similar proteins band patterns with increase in storage time, whereas samples stored at 37 °C, showed blurred pattern after 12 and 25 weeks storage suggesting modification of various proteins (Lanes 2 and 3). When SDS-PAGE was carried out under reducing conditions (i.e. after treatment of samples with 2-mercaptoethanol) (Fig.6.30b), the HMWP dissociated to give whey protein, particularly β-lactoglobulin, and κ-casein bands (lane 1) at all temperatures. During storage at 5 °C, there was not much change in the electrophoretic patterns. The samples stored at 20 °C showed slight loss of sharpness of major protein bands, especially κ-casein and β-lactoglobulin (lanes 2 and 3). No major protein breakdown products were visible.

Samples stored at 37 °C showed blurred protein patterns (lanes 2 and 3) with some protein material not entering the resolving gel even after reduction, suggesting that this material consisted of aggregates linked with non-disulphide covalent bonds (Fig. 6.30b). β-lactoglobulin and α-lactalbumin lost their sharpness and appeared as slower moving diffuse bands. These results are in agreement

with those of de Koning *et al.* (1992) who observed that during storage of heat evaporated concentrated whole milk at 30 °C, there was a decrease in the intensity of α_s- and β-casein, and β-lactoglobulin, bands. Venkatachalam *et al.* (1993) also observed similar trends in caseins and whey protein, and in addition they found the appearance of new bands in the gels of samples of UF concentrated skim milk stored at 4, 20, and 35 °C after storage for 22 weeks. A streaking pattern was prevalent in the samples stored at 35 °C in addition to new bands. Bands in the milks stored at 35 °C were less distinct. Bands corresponding to γ-caseins was more intense in all stored samples, especially in samples stored at 4 and 20 °C. Also some material did not enter the resolving gel and all the samples had a streaky pattern.

Fig. 6.31a shows the changes in the electrophoretic patterns during storage of experiment B samples under non-reducing conditions. The control (standard) used here was the milk which had been forewarmed at 90 °C for 2 min and then RO concentrated to 2.5X (but not UHT treated). Bands of κ-casein and whey proteins were absent in the control, indicating the formation of a complex between these proteins during forewarming. During storage, the sharpness of the bands for caseins (α_s - and β-caseins) decreased, the effect being greater at storage temperatures of 20 and 37 °C than at 5 °C. Fig. 6.31b shows the electrophoretic patterns obtained under reducing conditions. The sharpness of the caseins and β-lactoglobulin decreased with storage time. A blurred protein pattern was observed for the samples stored at 20 °C after 25 weeks of storage and a similar pattern was observed for samples stored at 37 °C after 13 and 25 weeks. The blurred pattern indicates the formation of high molecular weight protein polymers which become linked through non-disulphide covalent bonds during storage. The formation of these covalent bonds is likely to have resulted from Maillard reactions forming lysinoalanine, lanthionine and isopeptides (Singh and Latham, 1993).

a) SDS-NR-PAGE



b) SDS-R-PAGE

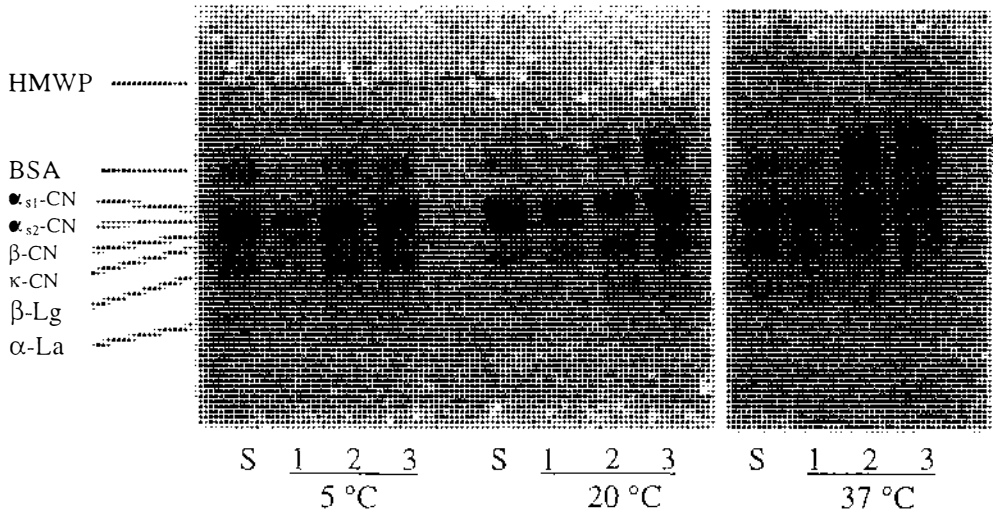
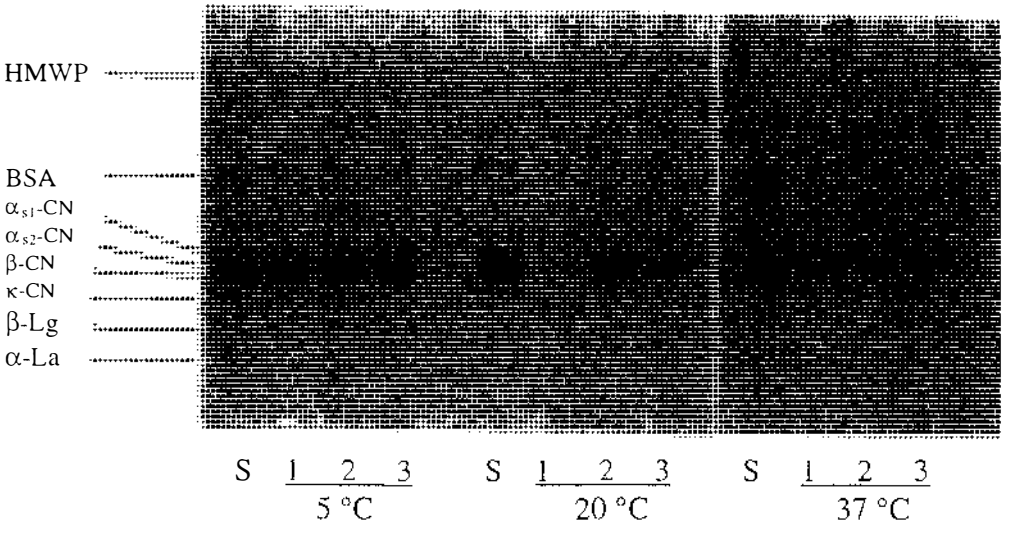


Fig. 6.30 SDS-PAGE gel electrophoretic pattern of Experiment A (P-ROC-UHT-H) milk samples stored at 5, 20 and 37 °C for 1) initial, 2) 12 weeks and 3) 25 weeks. RO concentrate (unheated) was used as standard (S); CN = Casein; α-La = α-lactalbumin; β-Lg = β-lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers. a) non-reducing SDS-PAGE b) reducing SDS-PAGE. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

a) SDS-NR-PAGE



b) SDS-R-PAGE

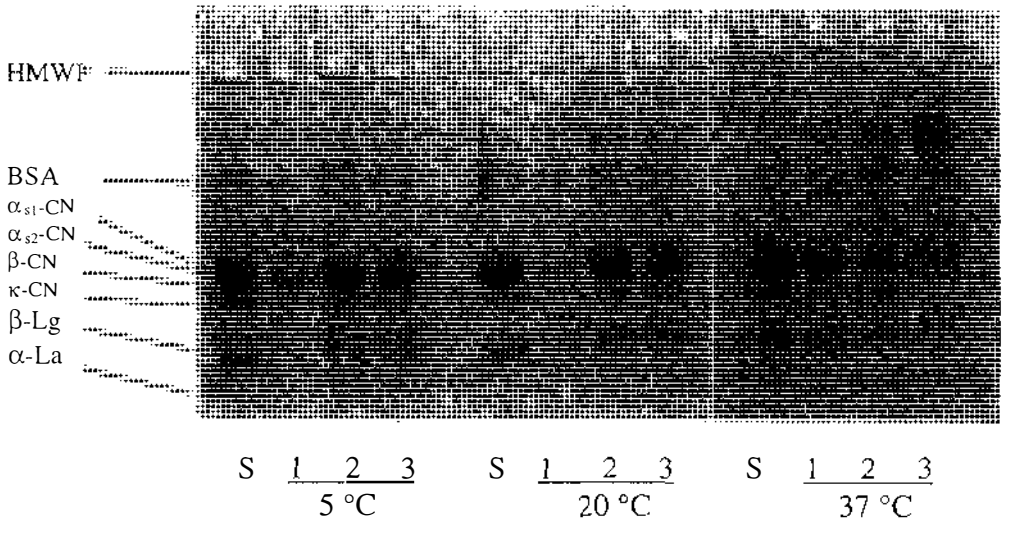


Fig. 6.31 SDS-PAGE gel electrophoretic pattern of Experiment B (F-ROC-UHT-H) milk samples stored at 5, 20 and 37 °C for 1) initial, 2) 13 weeks and 3) 25 weeks. RO concentrate (forewarmed, but not UHT treated) was used as standard (S); CN = Casein; α-La = α-lactalbumin; β-Lg = β-lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers. a) non-reducing SDS-PAGE b) reducing SDS-PAGE. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

These covalent bonds may be formed between different protein molecules adsorbed at fat globule surface or among protein components within the casein micelles or between different dispersed particles (casein micelles and fat globules) which may lead to interparticle cross-linking and aggregation (McKenna and Singh, 1991; Singh and Latham, 1993). The blurred pattern cannot be attributed solely to the Maillard browning reaction because a streaking pattern has been observed in samples of UF skim milk concentrate containing sucrose which apparently showed no brown colour formation (Venkatachalam *et al.*, 1993). High temperatures around 37 °C provide the energy required to activate browning reactions. Other possible reactions with activation energies less than or equal to that of Maillard browning can take place during storage at 37 °C of UHT processed concentrated milk. These may include reactions of ε-amino groups with dehydroalanine and carboxyl side chains to form cross links (iso-peptides).

6.3.1.8 Electron microscopy

The electron micrographs of initial milks and milks stored at 5 and 37 °C for 25 weeks are presented here in Figs. 6.32-6.36. In all the electron micrographs, the presence of fat globules is demonstrated by spherical white spots surrounded by dark layers of casein particles which cover the fat globule surfaces. Casein micelles and protein particles appear as dark spots. The surfaces of the casein particles show thread like protuberances or tendrils, a phenomenon that is only seen after a high temperature heat treatment of milk (see Chapter 5).

The typical microstructure of RO concentrated (unheated) milks as discussed in Chapter 5 (Section 5.3.1.2, Fig. 5.6), can be briefly described as follows: each fat globule had a distinct defined membrane. Also extra bodies of protein were observed (probably derived from casein micelles). The casein micelles held together clumps of the smaller fat globules, the clumps were also attached to parts of larger fat globules. No isolated intact casein micelles could be observed; all were attached to fat globules. On UHT treatment of these concentrates (as discussed in chapter 5, section 5.3.2.2, Fig. 5.16), the casein micelles lost their spherical shape and were fused together, with fat micro-droplets embedded in them. No free micelles were observed in the serum phase

as all the casein particles were involved in aggregate formation. Casein particles appeared as a continuous matrix, with small and large fat globules embedded in them.

Fig. 6.32a, b shows the microstructure of experiment A samples made by the P-ROC-UHT-H process. As observed in Chapter 5, UHT treatment caused extreme aggregation of fat and protein particles; the size of the aggregates ranged from 1 to 6 μm. At higher magnification (Fig. 6.32b), it can be seen that the casein micelles have lost their spherical structure, but the fat globules were spherical and possessed a well defined fat globule surface layer. There were many tiny fat globules entrapped in the casein particles. Casein micelles had fused with each other to form a casein matrix. Fat globules were connected to each other via this casein matrix. Particulate material was observed in the serum phase.

Fig. 6.32c, d depicts electron micrographs of experiment A milk samples stored at 5 °C for 25 weeks. The aggregate size remained almost the same. It can be seen that there was not much change in the structures of fat and casein particles. The casein particles seem to be less electron dense, and most of the casein particles were apparently associated with fat globules, and much of the surface of the fat globules (which seem to be more or less spherical) was, clearly, covered with a secondary membrane of sub micellar or smaller casein particles.

Fig 6.32e, f shows electron micrographs of experiment A milk samples stored at 37 °C for 25 weeks. The size of the aggregates as a result of storage remained unaffected. Some of the fat globules lost their somewhat spherical structure, and the casein particles appeared electron dense. The serum contained small particulate matter. There appeared to be tendril and intermicellar bridge formation, also accompanied by some fragmentation of casein micelles. There was evidence of some sort of coalescence between the casein particles, with many of them linked by protein bridges to other small particles, probably formed by fragmentation of casein micelles. The surfaces of the casein particles were rather rough and there was some tendril formation on these and the smaller particles. These proteinaceous particles were sometimes highly branched and the branches were usually 0.05- 0.2 μm in length. They occurred sometimes in the serum phase unattached to other particles, but more usually were on the surface of casein particles and sometimes between the casein particles. Small fat globules could be

seen apparently buried within the casein matrix. Venkatachalam *et al.* (1993) found that regardless of sugar composition of samples of UF concentrated skim milk, electron micrographs of gelled samples were similar and electron micrographs of all non-gelled samples were similar. In gelled samples, casein particles showed tendrillar or hairy appendages protruding from their surface. Casein particles were connected together by such appendages to form a 3-dimensional network. Particles appeared to be distorted and were not spherical. Some particles were fused with other particles. In non-gelled samples (stored at 35 °C) Venkatachalam *et al.* (1993) observed that casein particles were spherical and had few tendrillar appendages protruding from their surfaces. Most of the particles were separated from each other. de Koning *et al.* (1992) found the presence of fat as white spots-surrounded by a thin layer of casein particles which covered part of the surface of fat globules in in-container sterilised homogenized concentrated milk. Casein micelles appear as dark spots, and consist of complexes of fat globules and casein particles (after storage for 40 weeks at 30 °C). Surfaces of the casein aggregates showed thread-like protuberances. No differences between gelled and non-gelled samples were observed. Thread-like cross links were present between the aggregates. These observations suggest that a fat containing system is different from a skim milk system. Therefore the role of fat globules is of importance for the gelation of whole unconcentrated or concentrated milks

Fig. 6.33a, b shows electron micrographs of forewarmed unconcentrated milk. The milk had been forewarmed at 90 °C for 2 min. As shown by the arrows, there was clear evidence of whey protein-casein micelle interactions as a result of whey protein denaturation. There was little adsorption of casein micelles (or sub-micelles) or fragments thereof on to the fat globule surface, as a result of this forewarming

Fig. 6.33c, d shows electron micrographs of RO concentrated forewarmed milk. On comparing the structures of fat globules and proteins in Fig. 6.33a, b with those in Fig. 6.32c, d, it is clearly seen that as a result of RO concentration, the fat globules had decreased in size, whereas the casein micelles had increased in size. All the fat globules were connected with the casein micelles. On examining these two figures at higher magnification, the structures of the fat globule surfaces were seen to be different. The fat globule surfaces were covered with the casein micelles (or sub-micelles) and

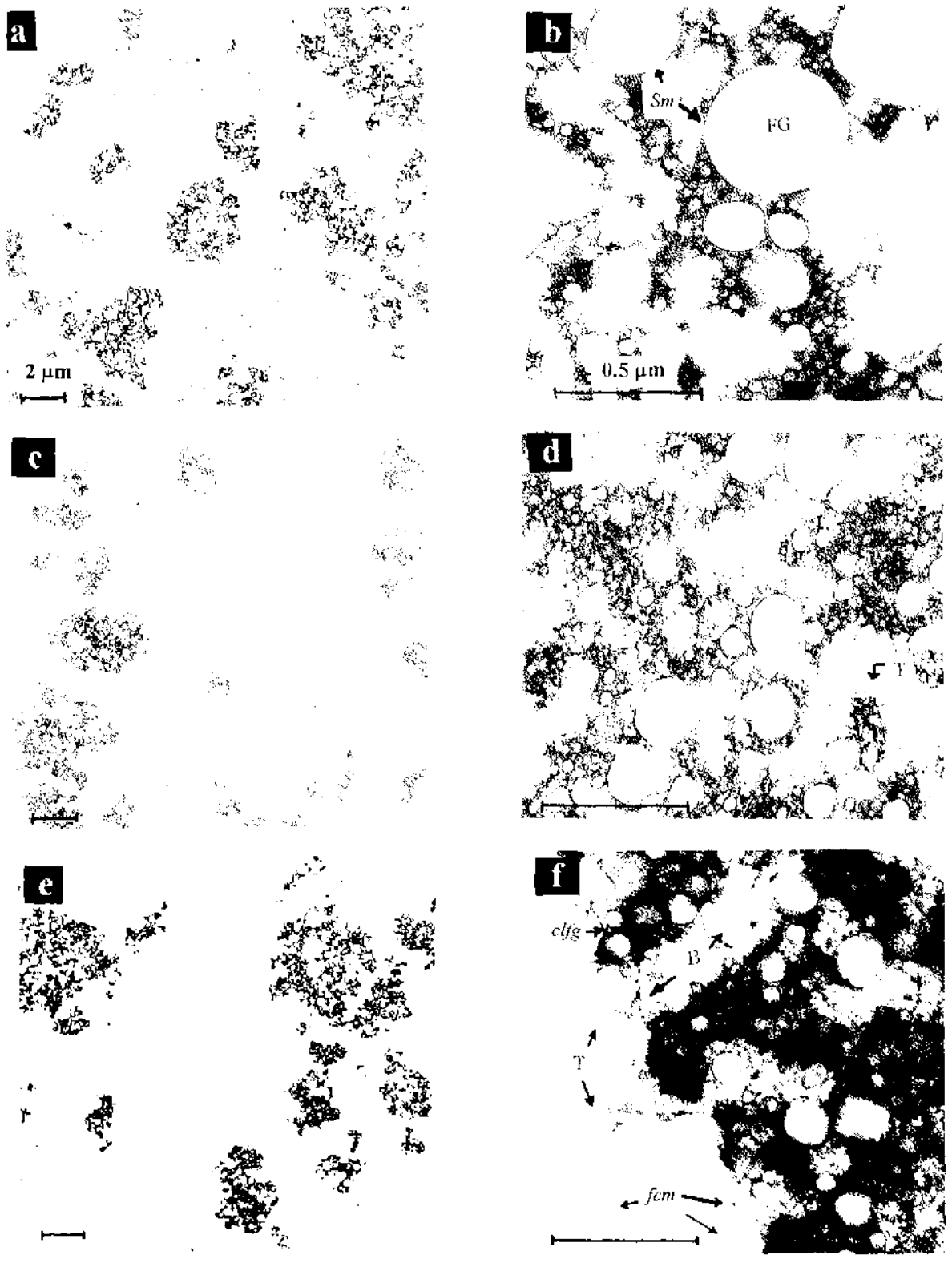


Fig. 6.32 Changes in the microstructure of Experiment A (P-ROC-UHT-H) milk samples stored at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*) tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d, f: 48600X. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

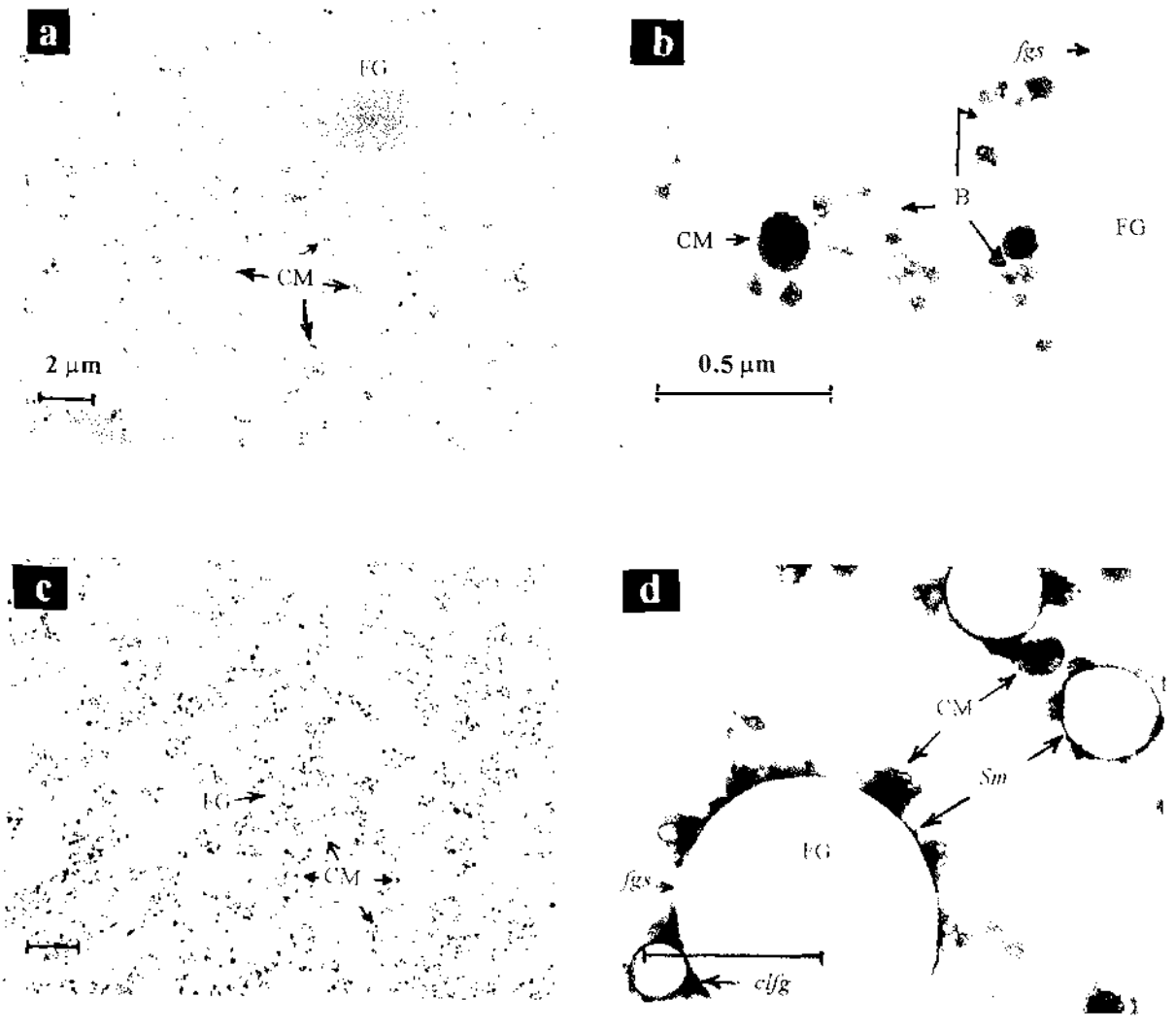


Fig. 6.33 Changes in the microstructure of forewarmed a, b) unconcentrated milk and c, d) subsequently concentrated by reverse osmosis to 2.5X. Magnifications a, c: 3400X; b, d: 48600X, showing casein micelles (CM), submicellar casein (*Sm*), fat globules (FG), fat globule surface (*fgs*), intermicellar bridges (B), casein linking fat globules (*clfg*).

presumably with denatured whey proteins seen as bridges (shown by arrows). The s serum phase was largely devoid of protein particles. The casein micelles in forewarmed unconcentrated milk were more spherical whereas in RO concentrated forewarmed milk, the casein micelles were attached to the fat globule surfaces and had lost their spherical structure. These electron micrographs clearly demonstrate the effect of forewarming and subsequent concentration by RO on the structures of fat and protein particles.

Fig. 6.34a, b shows the microstructure of fat globules and casein micelles of experiment B samples made by the F-ROC-UHT-H process. UHT-treatment resulted in the aggregation of protein and fat particles, and the size of aggregates formed was in the range of 0.5-3 μm. It can be seen in Fig. 6.34b (initial measurement), that the fat globules had well defined and thick fat globule surfaces, probably partly because of the deposition of whey proteins. Casein micelles had lost their spherical structure as compared to unheated (not UHT sterilized) milks (Fig. 6.33d).

On storage at 5 °C for 25 weeks (Fig. 6.34c, d), both the fat globules and the casein micelles appeared similar to those in experiment A (Fig. 6.32d) (aggregate size 2.5-4 μm). The fat globule surfaces were covered with protein particles and the casein matrix was not so dense. Fragmentation of some casein micelles as well as coalescence of most of the casein micelles was observed. Casein often linked a number of fat globules together (clfg). Such bridges were quite distinct from the filamentous bridges formed by tendrils. They can be described as constituting casein glue between the globules.

After storage at 37 °C for 25 weeks (Fig. 6.34e, f) (aggregate size 1-4 μm), the fat globule surfaces had more sub-micellar casein deposited on them and hence the surface layers were thicker that their counterparts in experiment A (Fig. 6.32e, f). The casein particles were not present in a continuos matrix but were attached to the fat globule surfaces. Most of the fat globules were attached to each other either through thread-like cross links or through casein material adsorbed on other small fat globules. Unlike experiment A samples (Fig. 6.32f), not many fat globules were entrapped in the casein matrix. Again some fragmentation of the casein micelles was observed. Some micelles had tendrils. Fat globules were connected to other fat globules via casein linking material, and intermicellar bridges were more prominent.

Fig. 6.35a, b shows the microstructure of fat globules and casein micelles of freshly made experiment C samples made by P-ROC-UHT process. In general, the changes observed here are similar to those observed for experiment A samples. The size of the aggregates formed was in the range of 1-4 μm. On storage for 25 weeks at 5 and 37 °C (Fig. 6.35 c, d, and Fig. 6.35 e, and f respectively), there was an increase in the size of the fat-protein aggregates at 37 °C having a size range 1-8 μm.

Fig. 6.36a, b shows the microstructure of fat globules and casein micelles of freshly made experiment D samples made by the F-ROC-UHT process. The size of the aggregates formed was 1-12 μm. In general, the changes observed here are similar to those observed for experiment B samples for freshly prepared (initial measurement) as well as those samples stored for 25 weeks at 5 (Fig. 6.36c, d) and 37 °C (Fig. 6.36e, f). On storage at 5 °C for 25 weeks, the aggregate size was found to be 1-8 μm, whereas storage at 37 °C for 25 weeks resulted in aggregates of size 1.5-18 μm.

The aggregates in RO concentrates from experiment B (Fig. 6.34e and f) and experiment D (Fig. 6.36e and f) stored at 37 °C showed an open structure with the development of serum in the matrix after storage for 25 weeks.

These results suggest that homogenization of theRO concentrates after UHT treatment does not result in change in the size of the fat-protein aggregates with storage time. Forewarming resulted in a slight increase in the size of aggregates with storage time. RO concentrates not homogenized after UHT treatment showed the formation of larger aggregates than those in homogenized samples with storage time. Forewarmed but not homogenized samples showed a significant increase in the size of aggregates with storage time.

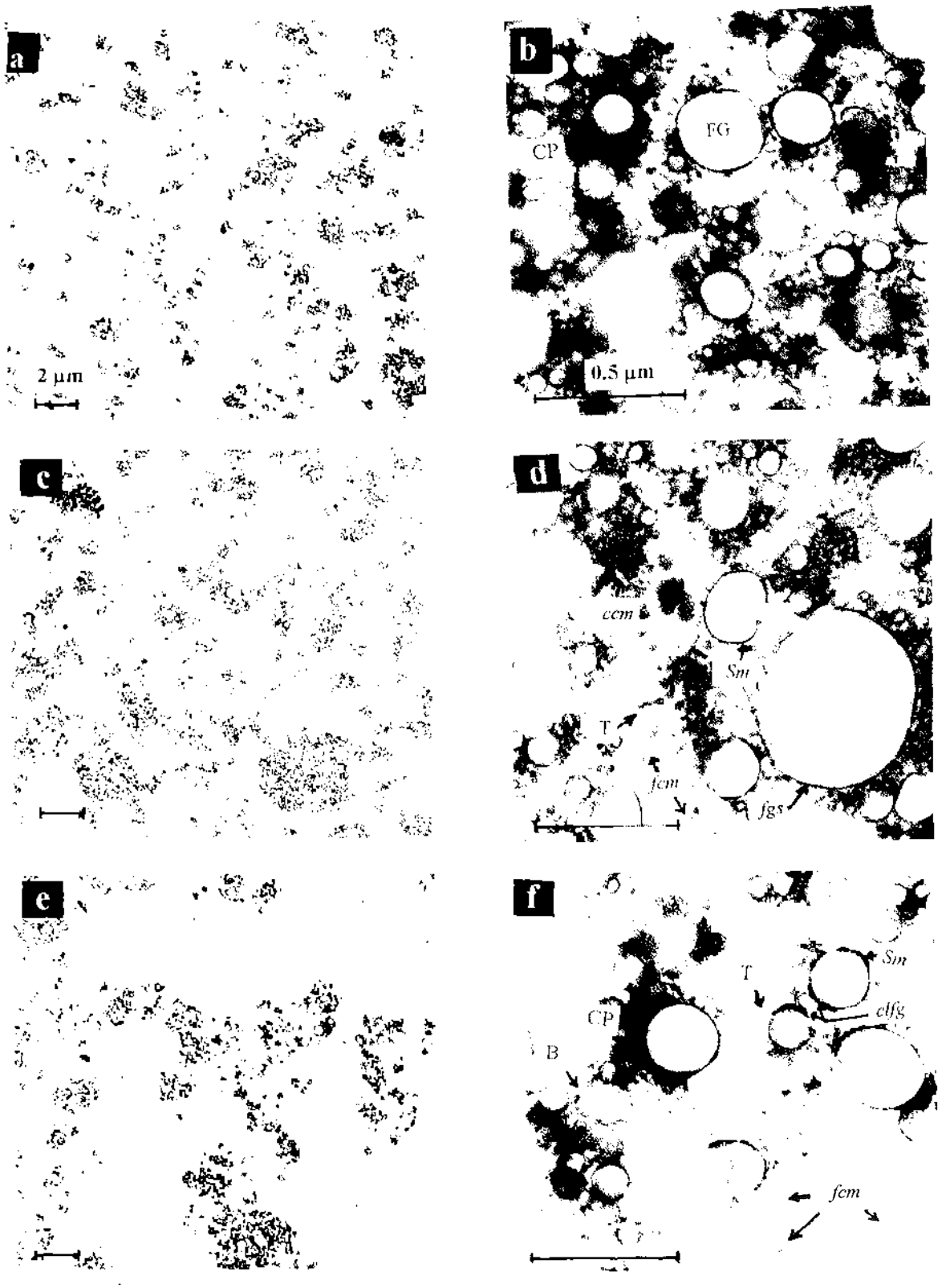


Fig. 6.34 Changes in the microstructure of Experiment B (F-ROC-UHT-H) samples stored at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*) tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d, f: 48600X. F = forewarmed at 90°C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

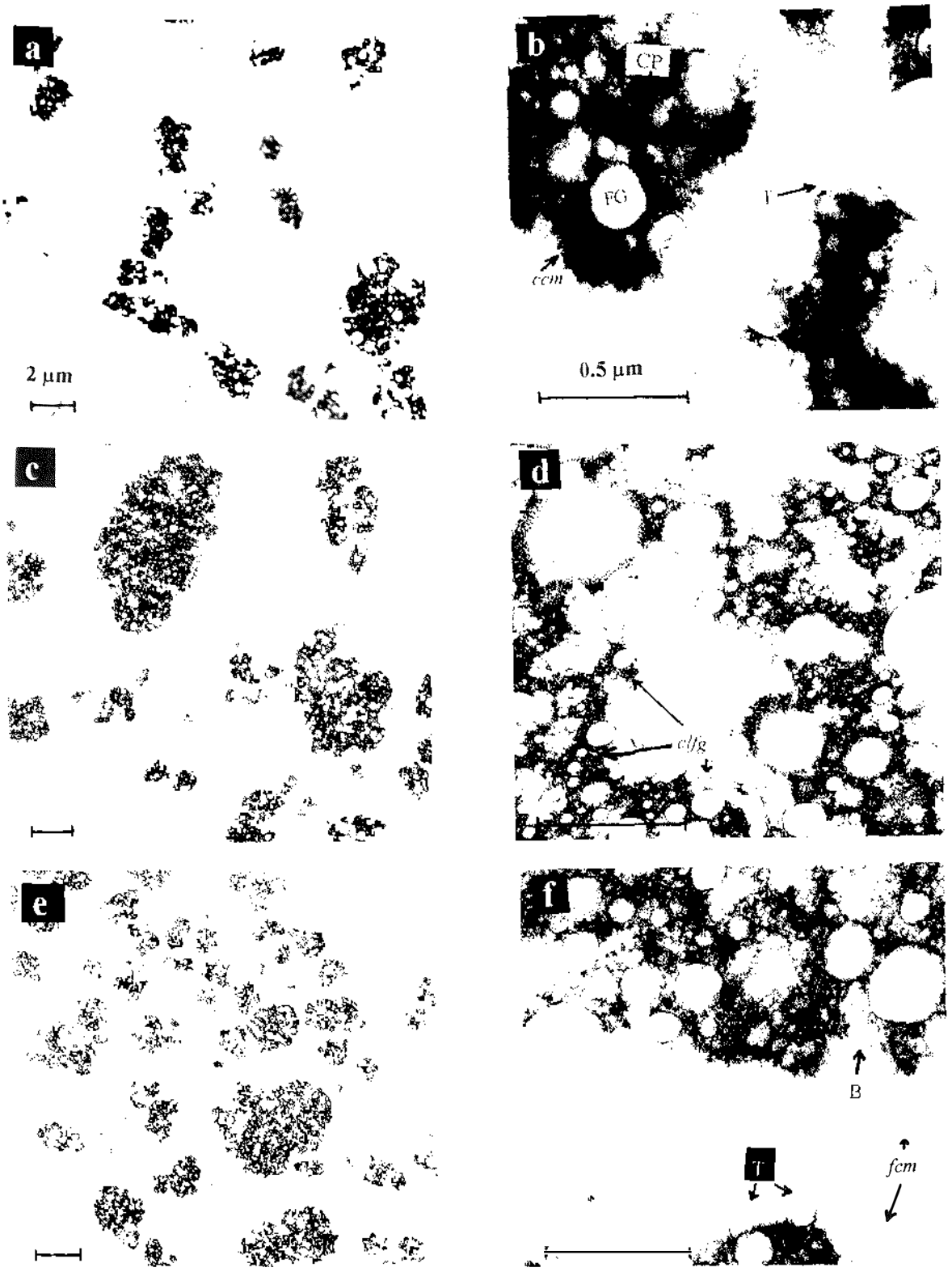


Fig. 6.35 Changes in the microstructure of Experiment C (P-ROC-UHT) milk samples stored at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*), tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d, f: 48600X. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s

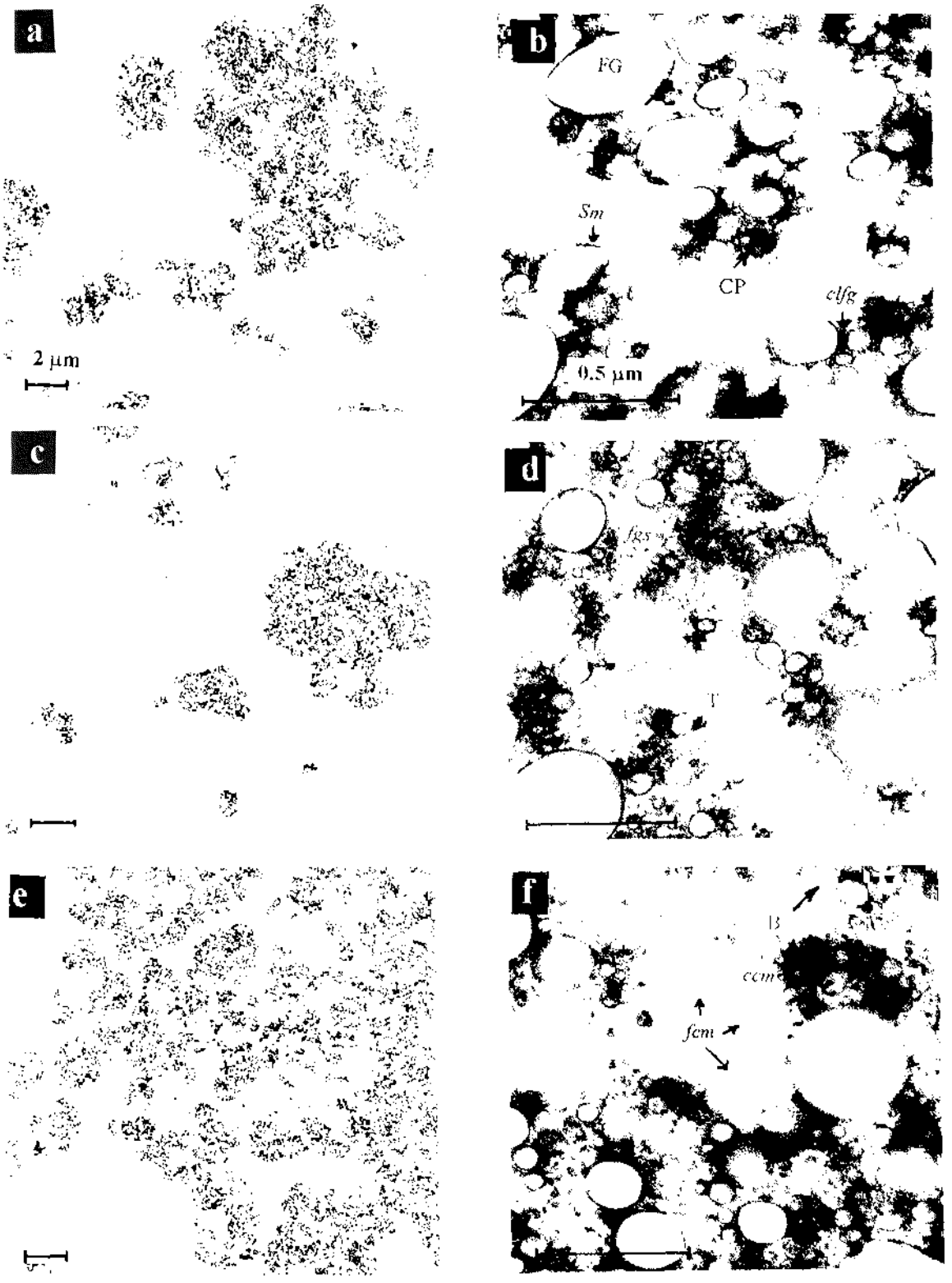


Fig. 6.36 Changes in the microstructure of Experiment D (F-ROC-UHT) milk samples stored at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*) tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d, f: 48600X. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s.

6.3.2 Effect of added phosphate

6.3.2.1 Compositions of various milks

The compositions of the raw milk used and the various RO concentrates produced for this study are given in Table 6.4. Again, the concentrates were produced to match the European Standards (10/23, fat/SNF) for evaporated milk.

Table 6.4 Composition of raw milk and various RO concentrates for experiments E-G.

Experiment	Date	Trial	Raw milk		RO concentrated milk		
			Fat (%)	T.S. (%)	Fat (%)	T.S. (%)	C.F.
E	4.6.96	I	4.10	12.58	11.48	32.00	2.54
F	5.6.96	I	4.10	12.58	11.26	31.35	2.49
G	6.6.96	I	4.10	12.58	11.42	32.09	2.55

6.3.2.2 Changes in pH

Fig. 6.37a, b, c shows the changes in pH of samples from experiment E, F and G, on storage at various temperatures. As observed earlier (Section 6.3.1.2), there was a considerable decrease in pH with storage time at higher storage temperatures. For experiment E, the samples stored at 5 and 20 °C, showed a slight decrease of pH with increase in storage time, whereas storage at 37 °C resulted in a gradual but greater decrease in pH with storage time. Samples from experiment F and G showed similar results, but the decrease in pH at 37 °C in experiment F was greater than in experiments E and G. In other words, the addition of DSP to concentrates before UHT treatment resulted in the greatest decrease in pH during storage at 37 °C. DSP acts as a sequesterant of calcium, which lowers the Ca^{2+} ion activity and therefore results in the release of more protons from CCP during storage at higher temperatures.

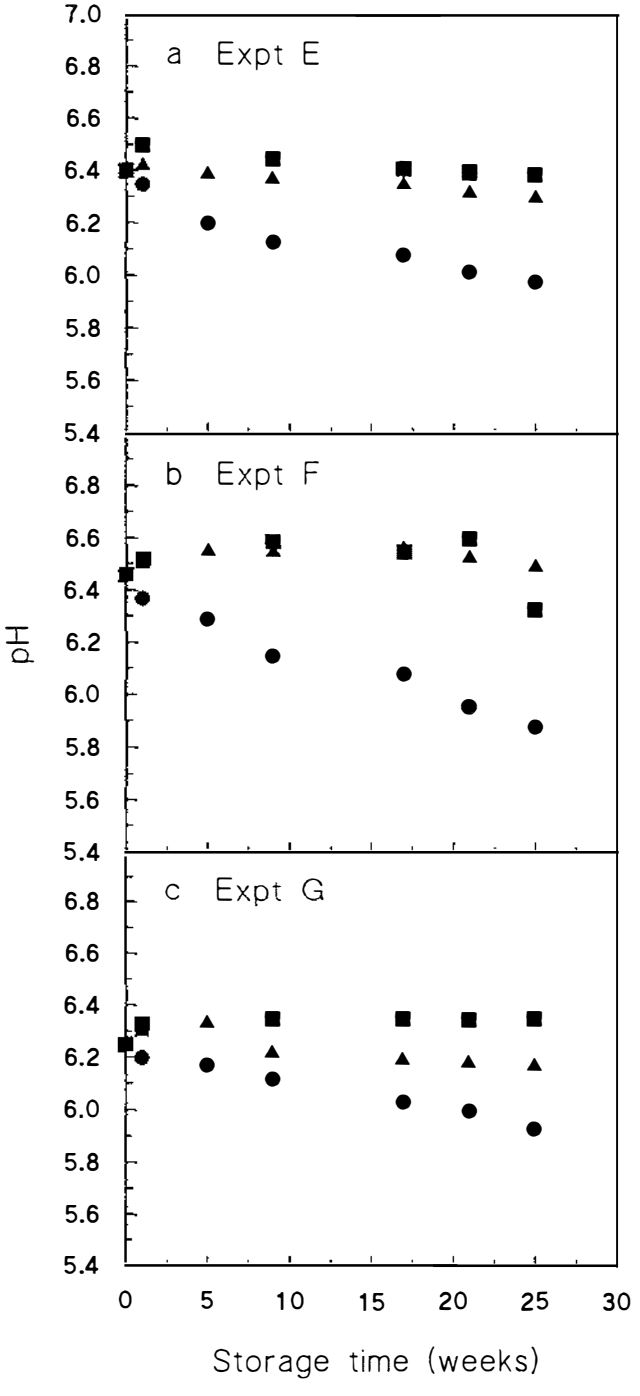


Fig. 6.37 pH changes in a) Experiment E (P-ROC-UHT-H), b) Experiment F (P-ROC-A(DSP)-UHT-H) and c) Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples as a function of storage time at 5 ■, 20 ▲, and 37 °C ● respectively. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar. A(DSP) = Addition of disodium phosphate (0.15% w/w), A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w).

6.3.2.3 Particle size distributions

The particle size distributions for experiments E samples are shown in Fig. 6.38. Storage for 25 weeks at 5 °C had no major effect on the particle size distribution; it remained mainly bimodal with most of the particles < 10 μm. There was a slight decrease in the proportion of particles in peak 2 (size range 1-7.5 μm), and a slight increase in the population of particles in the size range 10-50 μm. On storage for 25 weeks at 20 °C, peak 1 disappeared and there was a marked decrease in the population of particles in peak 2 (5-7.5 μm). Correspondingly, the populations of particles in peaks 3 (7.5-15 μm) and 4 (15-70 μm) increased. Some particles were too large (>80 μm) to be measured by the Master Sizer using a 45 mm lens. On storage for 25 weeks at 37 °C, there were marked decreases in the populations of particles in peaks 1 and 2, with a corresponding increase in peak 3 (7.5-50 μm). In addition, there was a considerable population of particles with sizes above 80 μm which were not measurable by the Master Sizer. Treatment with protein dissociating medium changed the particle size distribution to monomodal with all particles below 2 μm (Figs. 6.38 a-c) at all storage temperatures and times.

The differences in the particle size distributions of samples from experiment A (Fig. 6.8) and experiment E can be attributed to differences in milk composition; experiment E was performed in June (winter) and experiment A was performed in October (spring).

Fig. 6.41a shows the changes in d_{43} with storage time for samples from experiment E. The d_{43} increased with storage time. There was no noticeable increase in d_{43} at 5 °C; but a very large increase in d_{43} was observed at 37 °C. On treatment with protein dissociating medium, the d_{43} of all samples dropped to a low value which was invariant with storage time.

Fig. 6.39 shows the particle size distributions of samples from experiment F. In this case, DSP was added to the concentrates before UHT treatment. The particle size distribution of the samples for the initial measurement was bimodal and peaks 1 and 2 were partially resolved, whereas the particle size distribution peaks of the initial sample from experiment E were fully resolved. On storage for 25 weeks at 5 °C the particle size distribution remained mainly bimodal with peaks 1 and 2 fully resolved. There was

a significant increase in the population of the particles in peak 2. Storage for 25 weeks at 20 °C resulted in a slight decrease in the population of particles in peaks 1 and 2, and the appearance of peak 3 in the size range 10-80 μm. However, storage for 25 weeks at 37 °C appeared to have little effect on the proportion of particles in peak 1, but a slight increase in peak 2. There was no peak 3. Treatment with protein dissociating medium changed the particle size distributions to monomodal in all cases with all particles below 2 μm. Storage temperature and time had no effect on the particle size distribution after treatment with protein dissociating medium.

Fig. 6.41b shows the changes in d_{43} with storage time for samples from experiment F. There were no increase in d_{43} at 5 °C. There was a slight increase in d_{43} at 20 °C during the first 17 weeks, followed by a considerable increase after 21 weeks and followed by a decrease after 25 weeks. However, on storage at 37 °C there was an increase in d_{43} during the first 9 weeks followed by a gradual decrease to 25 weeks. On treatment with protein dissociating medium, the d_{43} of the samples remained unchanged for all the measurement intervals at all storage temperatures.

Fig. 6.40 shows the particle size distribution of samples from experiment G. In this case, SHMP was added to the concentrate before UHT treatment. The particle size distributions of the samples remained largely unaffected by storage for 25 weeks at 5, 20 or 37 °C except that there was a slight shift of peak 2 towards the lower size range.

Fig. 6.41c shows the changes in d_{43} with storage time for samples from Experiment G. There was no change in d_{43} with storage time at any storage temperatures. On treatment with protein dissociating medium, the d_{43} of the samples remained unchanged for all the measurement intervals at any storage temperatures

From the foregoing results it is apparent that changes in particle size distribution during storage are affected to some extent by the addition of phosphates. RO concentrated milks with no added phosphate (control) showed the formation of large aggregates at 20 and 37 °C but not at 5 °C (Fig. 6.38). Addition of DSP resulted in a slight increase in the particle size at 5 °C and at 20 °C there was a sharp increase in the population of large particles (Fig. 6.39b). During storage for 25 weeks at 37 °C (Fig. 6.39c), it is surprising that there were no very large particles.

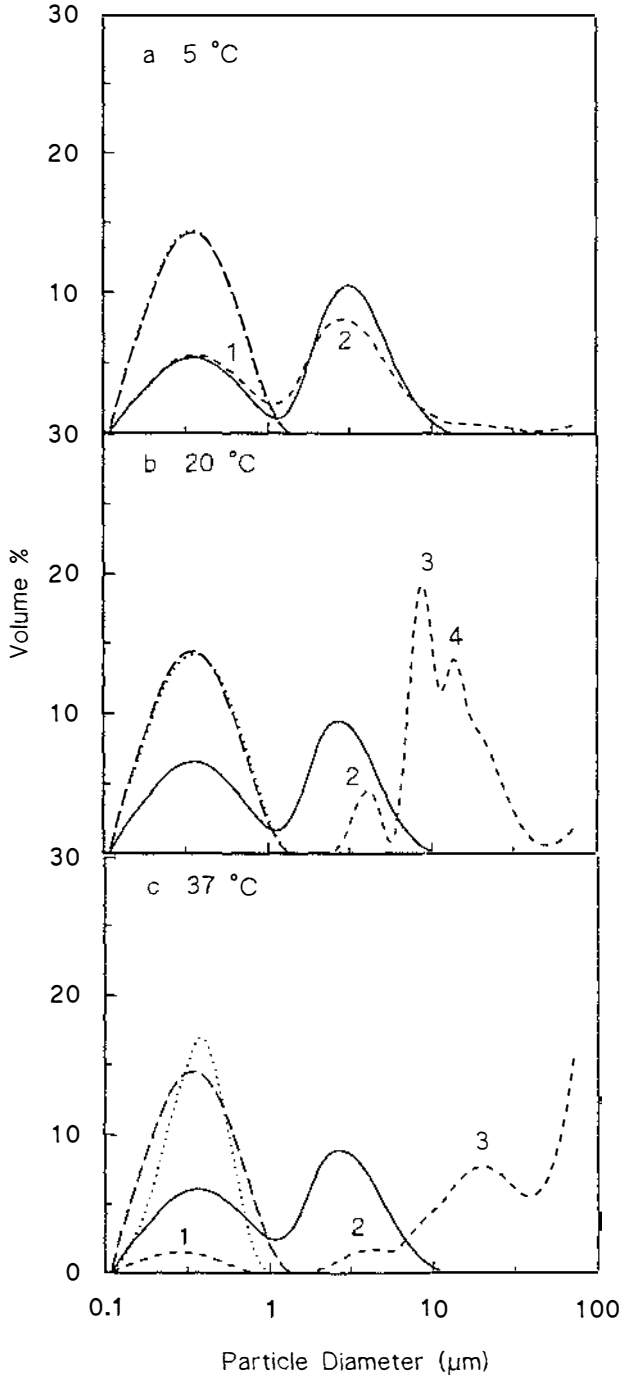


Fig. 6.38 Particle size distribution for Experiment E (P-ROC-UHT-H) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(– – –) initial measurement and (· · · · ·) measurement after storage for 25 weeks]. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

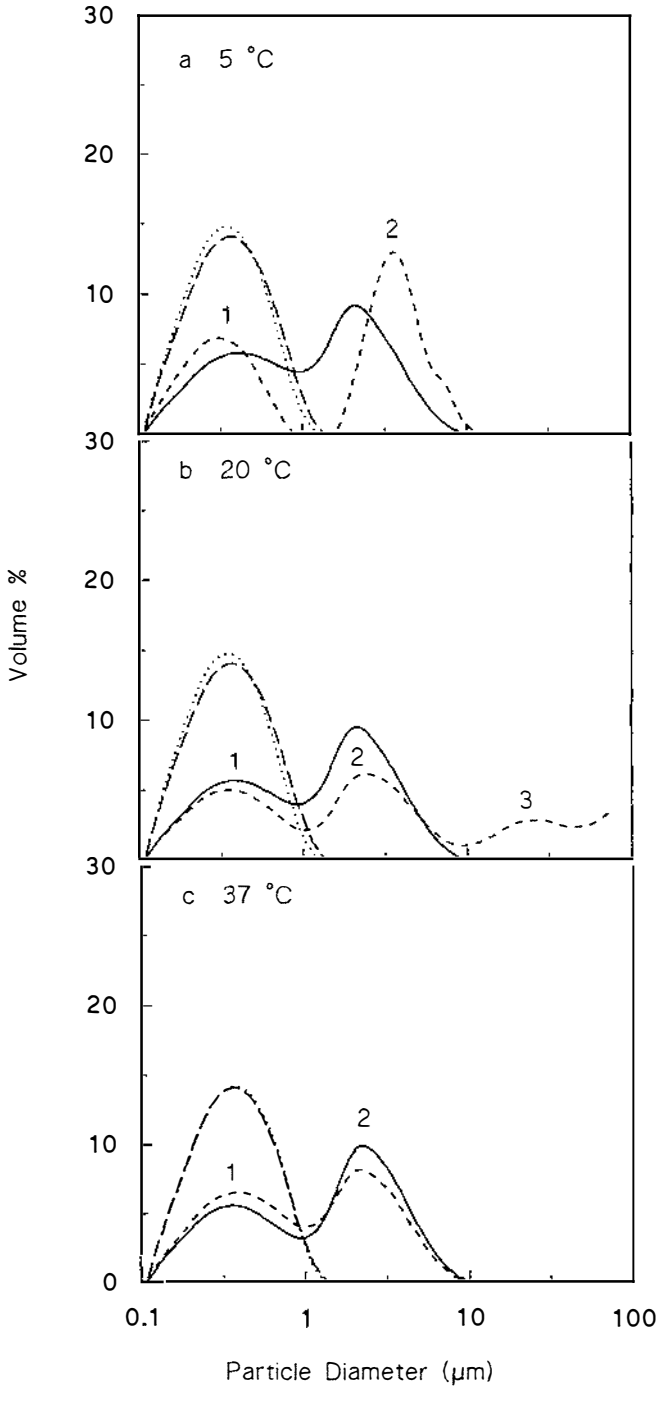


Fig. 6.39 Particle size distribution for Experiment F (P-ROC-A(DSP)-UHT-H) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(– – –) initial measurement and (· · · ·) measurement after storage for 25 weeks]. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, A(DSP) = Addition of disodium phosphate (0.15% w/w), UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

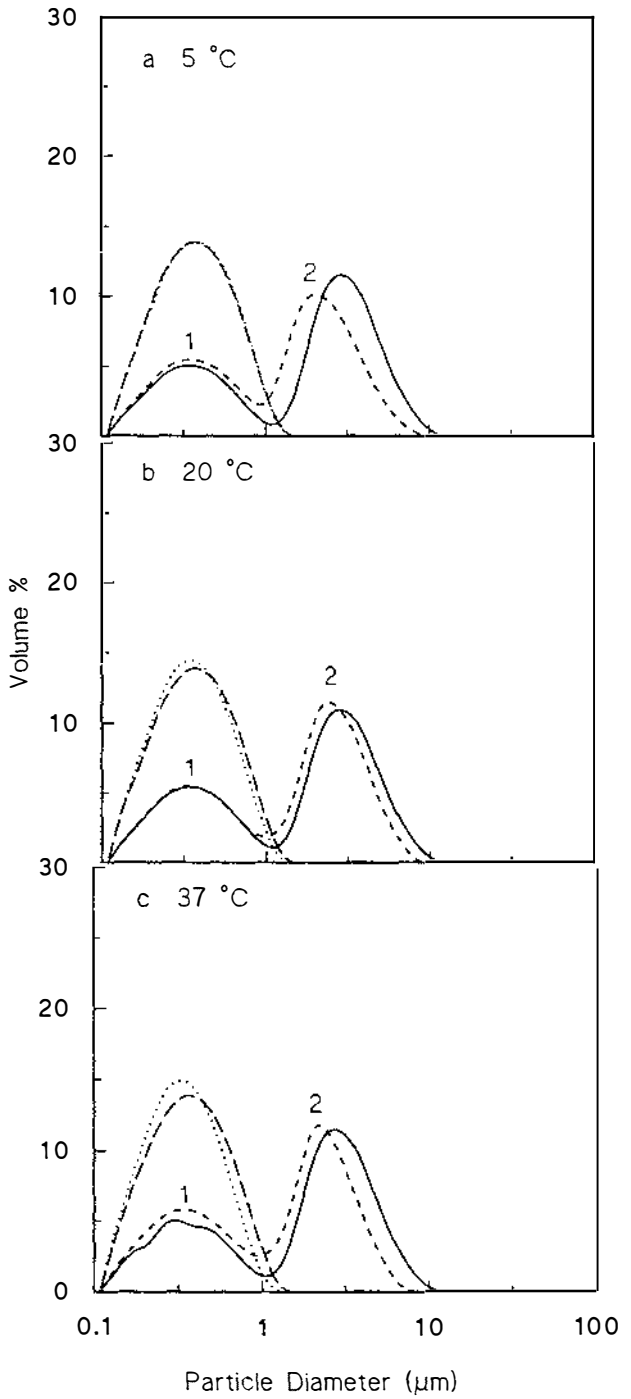


Fig. 6.40 Particle size distribution for Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples at a) 5, b) 20 and c) 37 °C. The milk samples were dispersed in Milli Q water [(——) initial measurement and (------) measurement after storage for 25 weeks] or dispersed in a SDS/EDTA buffer [(– – –) initial measurement and (· · · · ·) measurement after storage for 25 weeks]. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w),UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

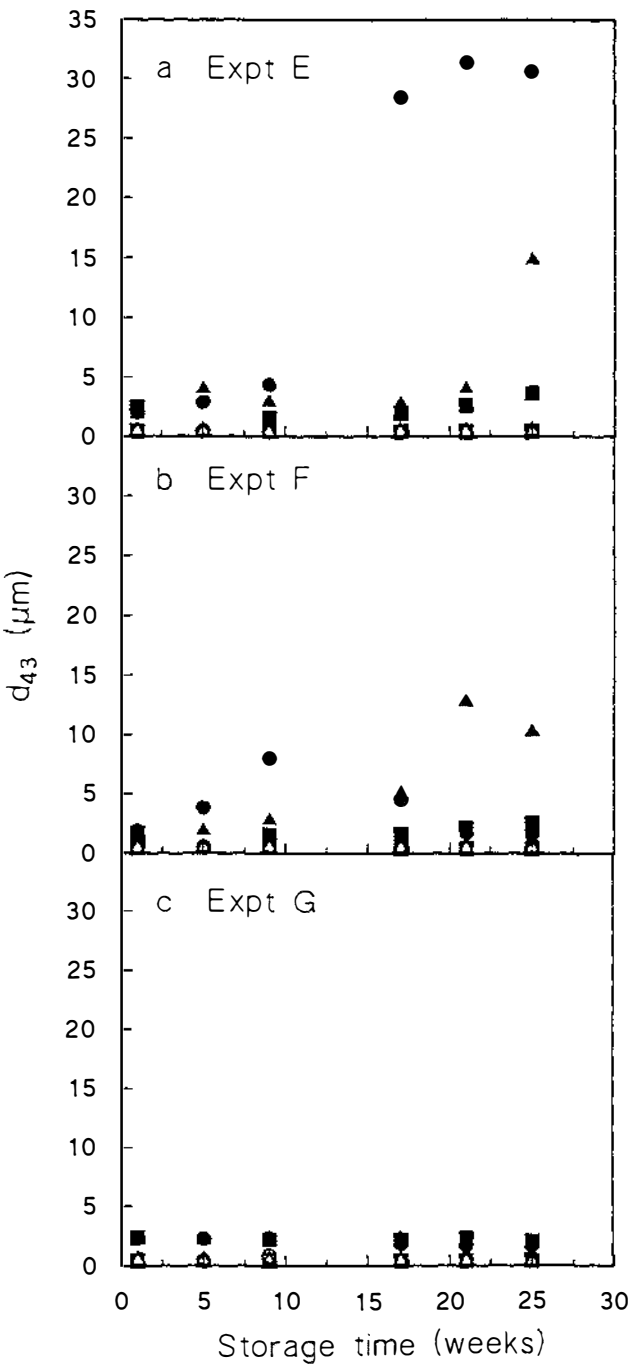


Fig. 6.41 Changes in weight-average diameter (d_{43}) for a) Experiment E (P-ROC-UHT-H), b) Experiment F (P-ROC-A(DSP)-UHT-H), and c) Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples as a function of storage time at 5, 20, and 37 °C. The milk samples were dispersed in Milli Q water at 5 ■, 20 ▲, and 37 °C ● or dispersed in a SDS/EDTA buffer at 5 ., 20 ▼, and 37 °C ● respectively. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar. A(DSP) = Addition of disodium phosphate (0.15% w/w), A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w). The d_{43} values for 37 °C may be somewhat problematic because of the experimental limitation in the range of particle sizes measurable (see page 236).

Their absence could be due to difficulties in obtaining a representative sample, perhaps because of the phase separation. The addition of SHMP to RO concentrated milks resulted in a slight shift of the peaks to lower sizes after storage at 5, 20 and 37 °C, and there was no formation of large particles.

From these experiments, it may be inferred that the addition of SHMP to concentrates before UHT treatment stabilises the samples at all storage temperatures, whereas the addition of DSP before UHT treatment does not stabilise the samples compared to the control (experiment E), where aggregates were formed at 20 and 37 °C storage temperatures after 25 weeks storage.

6.3.2.4 Penetration test

Fig 6.42 a, b, and c shows the changes in force to distance ratio with storage time for experiments E, F and G. Using the definition of gelation in section 6.3.1.4, it can be observed that in experiment E, the samples stored at 20 and 37 °C gelled after 21 weeks storage, and the sample stored at 5 °C gelled after 25 weeks. In experiment F, the samples stored at 20 °C gelled after 21 weeks whereas the samples stored at 37 °C showed a highest force to distance ratio (0.022 N/mm) after 9 weeks storage, and this ratio decreased with subsequent storage. For experiment G, gel formation was slow; at the end of 25 weeks storage period, the milks had not completely gelled when stored at 20 and 37 °C, and the sample stored at 5 °C was still liquid.

At higher storage temperatures, once the sample had gelled there was a decrease in the force to distance ratio mainly because the gel gradually became weak (loosening of casein particles) with the development of some serum in the gel matrix which resulted in syneresis. Also, in some of the samples, owing to the development of syneresis (at higher temperatures) in the post-gelation period, it was observed while performing the penetration test that there was a slight displacement of the whole block of gel in the bottle as the probe penetrated through the milk sample owing to displacement of serum from beneath the gel. This might have resulted in the decrease in the force to distance ratio.

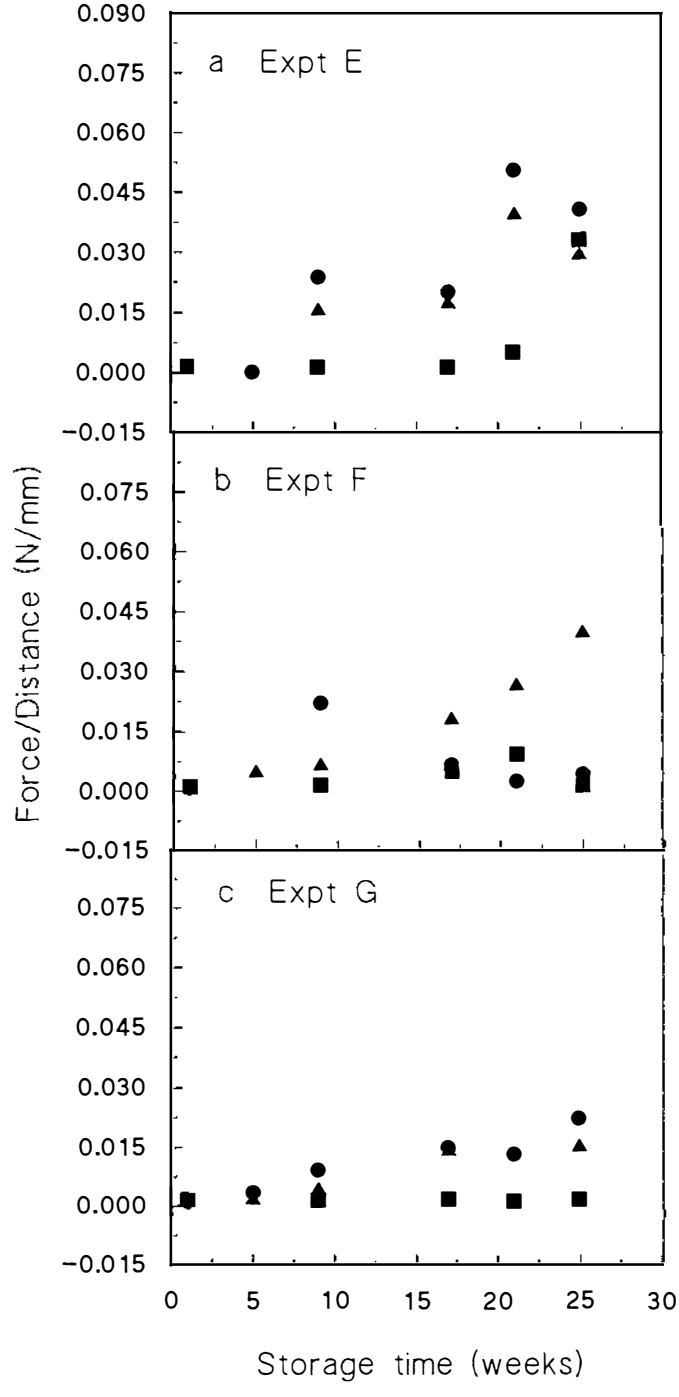


Fig. 6.42 Changes in force/distance ratio for a) Experiment E (P-ROC-UHT-H), b) Experiment F (P-ROC-A(DSP)-UHT-H) and c) Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples stored at 5 ■, 20 ▲, and 37 °C ● respectively. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar. A(DSP) = Addition of disodium phosphate (0.15% w/w), A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w).

From the above results, the addition of SHMP did not cause appreciable increases in the force to distance ratio whereas the addition of DSP did cause an increase in this ratio especially when the samples were stored at 37 °C (i.e. the sample gelled more quickly than at 20 °C). RO concentrated milk with no addition had higher force to distance ratio and resulted in a decrease in gelling time at 20 and 37 °C after 21 and 25 weeks storage. Based on these results it can be speculated that the addition of SHMP to RO concentrated milk prior to UHT treatment provides some stability towards gelation, whereas the addition of DSP to the concentrates before UHT treatment makes them prone to gelation, more so at a storage temperature of 37 °C than at 20 °C.

6.3.2.5 Electrophoresis

All samples from experiments E, F and G were analysed by SDS-PAGE under reducing and non-reducing conditions. Figs. 6.43-6.45 show typical electrophoretic patterns obtained for initial, 9, 17 and 25 weeks storage at 5, 20 and 37 °C. Reverse osmosis concentrate (not UHT treated) was used as the standard.

Fig. 6.43a shows the electrophoretic pattern changes during storage of experiment E samples under non-reducing conditions. The pattern of the protein bands in the control and UHT treated samples stored at different temperatures was similar to that of experiment A samples. As a result of UHT treatment, there was formation of high molecular weight polymers (HMWP); these polymers were presumably linked by disulphide bonds. When PAGE was carried out under reducing conditions (Fig.6.43b), the HMWP dissociated into monomeric protein bands. Whey protein bands, particularly β-lactoglobulin and α-lactalbumin, are visible. During storage at 5 °C, there was not much change in the electrophoretic patterns, although the casein band intensity appears to decrease. The samples stored at 20 °C showed slight loss of sharpness of major protein bands, especially that for κ-casein, but no clear protein breakdown products were identifiable. Samples stored at 37 °C showed blurred protein patterns with some protein material not entering the resolving gel, suggesting that these aggregates were linked via non-disulphide covalent bonds (Fig. 6.43b). β-lactoglobulin and α-lactalbumin lost their sharpness and appeared as slower moving diffuse bands.

Milk samples from experiment F (with added DSP, 0.15% w/w) (Fig. 6.44) and milk samples from experiment G (with added SHMP, 0.15% w/w) (Fig. 6.45) showed electrophoretic pattern changes similar to the samples containing no additive (experiment E, Fig. 6.42). Similar observations have been made by McKenna and Singh (1991) for UHT-processed reconstituted concentrated skim milk. They observed that milk samples with added hexametaphosphate (0.15% w/w) showed electrophoretic changes similar to those in samples containing no hexametaphosphate. Harwalkar and Vreeman (1978a) using starch-gel electrophoresis observed that the rate of protein breakdown was unaffected by the addition of orthophosphate or hexametaphosphate in UHT sterilized heat evaporated concentrated skim milk stored at 28 °C for 17 weeks. With storage time, they observed that breakdown of α_{s1}- casein resulted in fast moving bands whereas breakdown of β-casein resulted in slower moving bands and also whey proteins (β-lactoglobulin and α-lactalbumin) lost their sharpness. However, these changes occurred to approximately the same extent in all samples after any given storage period regardless of whether or not phosphates were added.

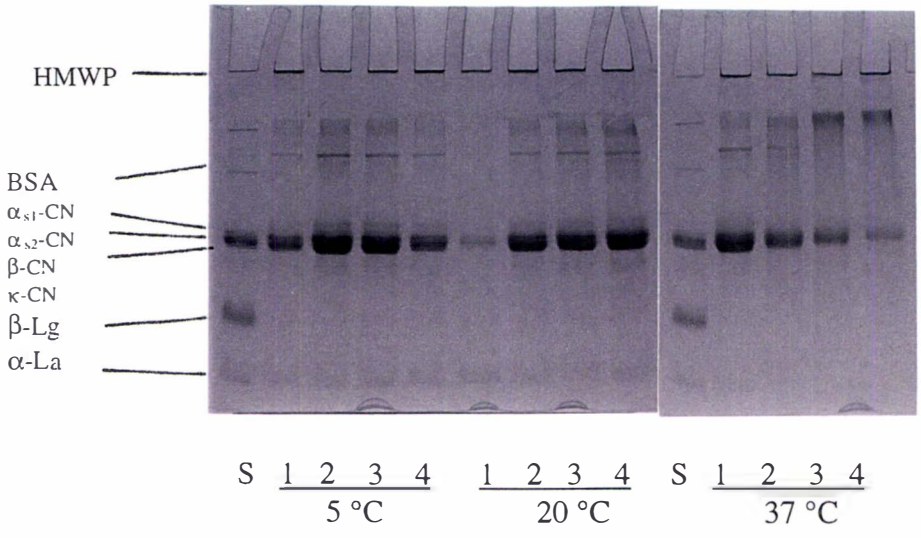
From the results of present study, it can be inferred that addition of DSP or SHMP has no appreciable effect on the electrophoretic patterns of the various milk samples although these additions influenced gelation behaviour.

6.3.2.5.1 Ultracentrifugation

Stored samples from experiments E, F, and G were ultracentrifuged at 90,000 g for 1 h at 20 °C to obtain a top (cream) layer, a middle fraction and a sediment fraction. Gel electrophoresis of these three fractions that had been dispersed in SDS buffer with 2-mercaptoethanol was carried out to obtain information on the presence of individual protein components among these fractions.

The cream layer obtained from experiment E (Fig 6.46a) initial measurement which had been UHT treated, had relatively greater intensities of β-lactoglobulin and α-lactalbumin bands as compared to the standard (unheated RO concentrate) probably because heating caused increased association of these proteins with the fat globules.

a) SDS-NR-PAGE



b) SDS-R-PAGE

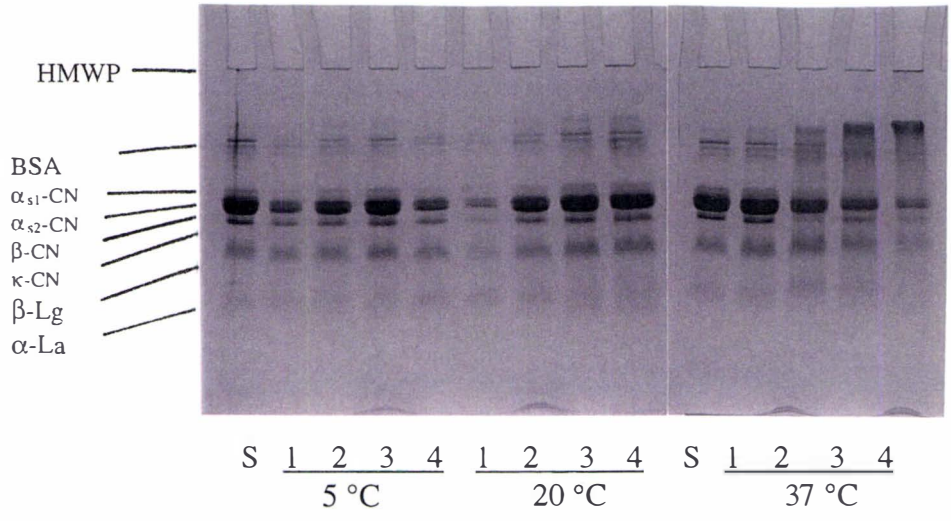
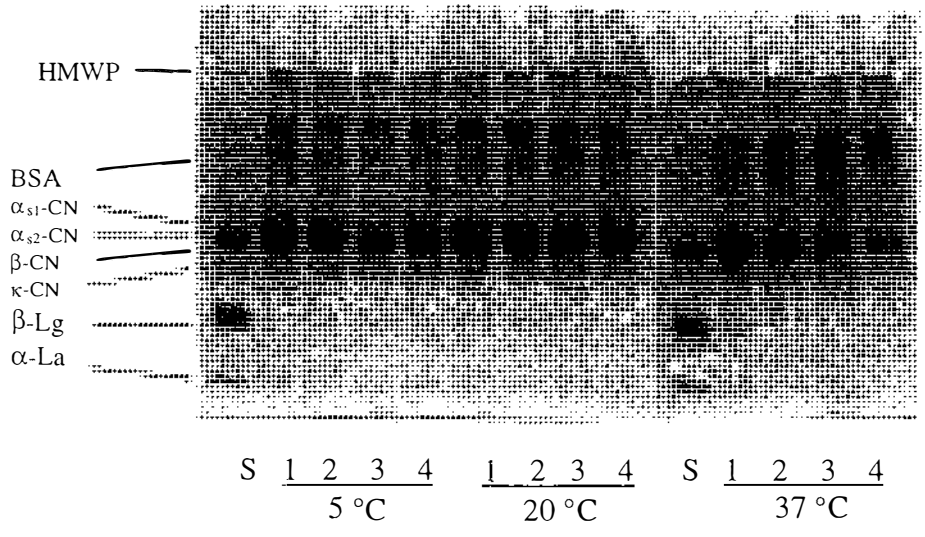


Fig. 6.43 SDS-PAGE gel electrophoretic pattern of Experiment E (P-ROC-UHT-H) milk samples stored at 5, 20 and 37 °C for 1) initial, 2) 9 weeks, 3) 17 weeks and 4) 25 weeks. RO concentrate (unheated) was used as standard (S); CN = Casein; α-La = α-lactalbumin; β-Lg = β-lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers. a) non-reducing SDS-PAGE b) reducing SDS-PAGE. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

a) SDS-NR-PAGE



b) SDS-R-PAGE

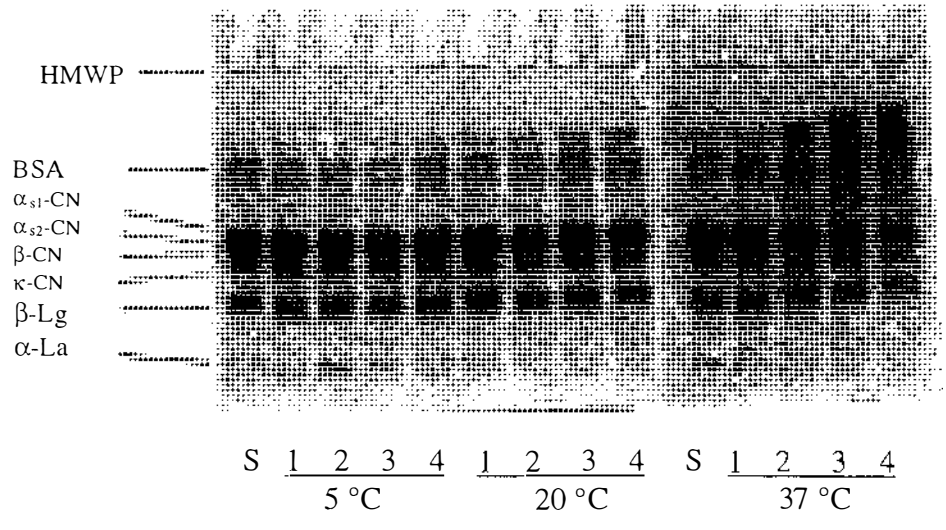
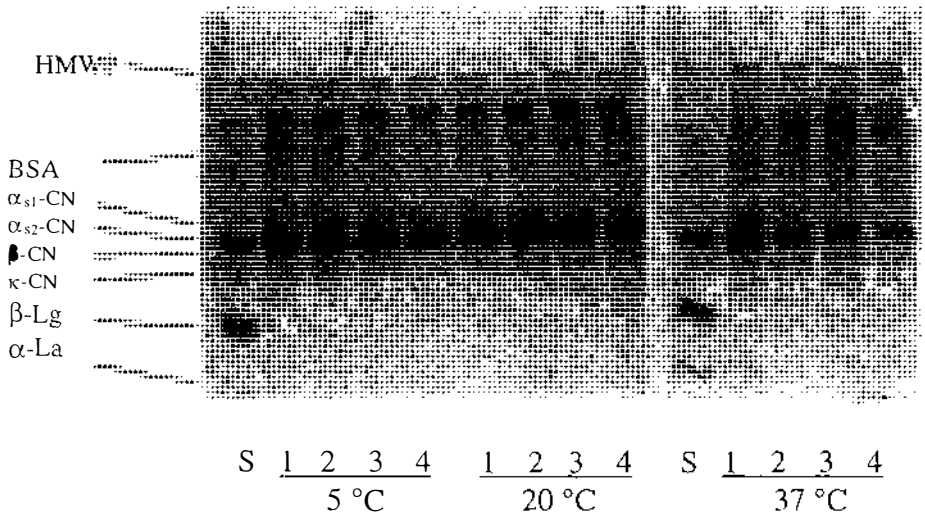


Fig. 6.44 SDS-PAGE gel electrophoretic pattern changes for Experiment F (P-ROC-A(DSP)-UHT-H) milk samples stored at 5, 20 and 37 °C for 1) initial, 2) 9 weeks, 3) 17 weeks and 4) 25 weeks. RO concentrate (unheated) was used as standard (S); CN = Casein; α-La = α-Lactalbumin; β-Lg = β-Lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers. a) non-reducing SDS-PAGE b) reducing SDS-PAGE. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, A(DSP) = Addition of disodium phosphate (0.15% w/w), UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

a) SDS-NR-PAGE



b) SDS-R-PAGE

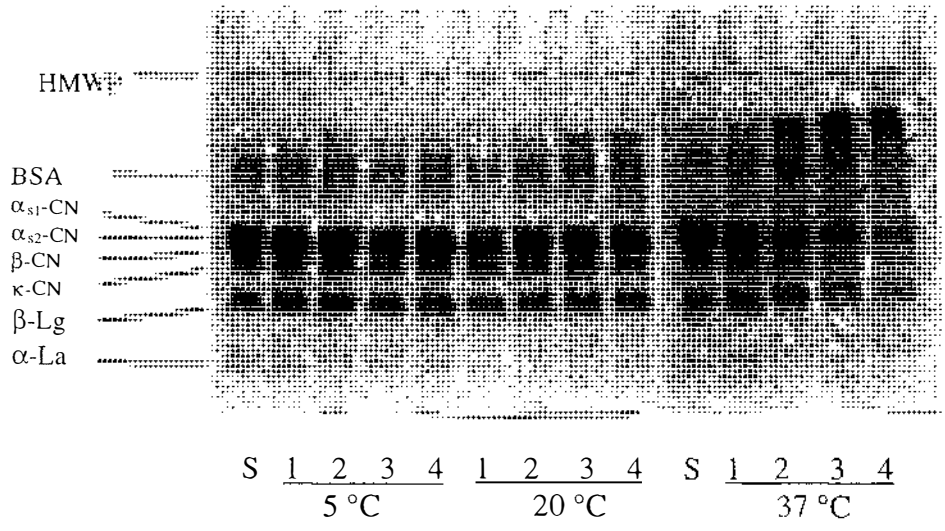


Fig. 6.45 SDS-PAGE gel electrophoretic pattern of Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples stored at 5, 20 and 37 °C for 1) initial, 2) 9 weeks 3) 17 weeks and 4) 25 weeks. RO concentrate (unheated) was used as standard (S); CN = Casein; α-La = α-Lactalbumin; β-Lg = β-Lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers. a) non-reducing SDS-PAGE b) reducing SDS-PAGE. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w), UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

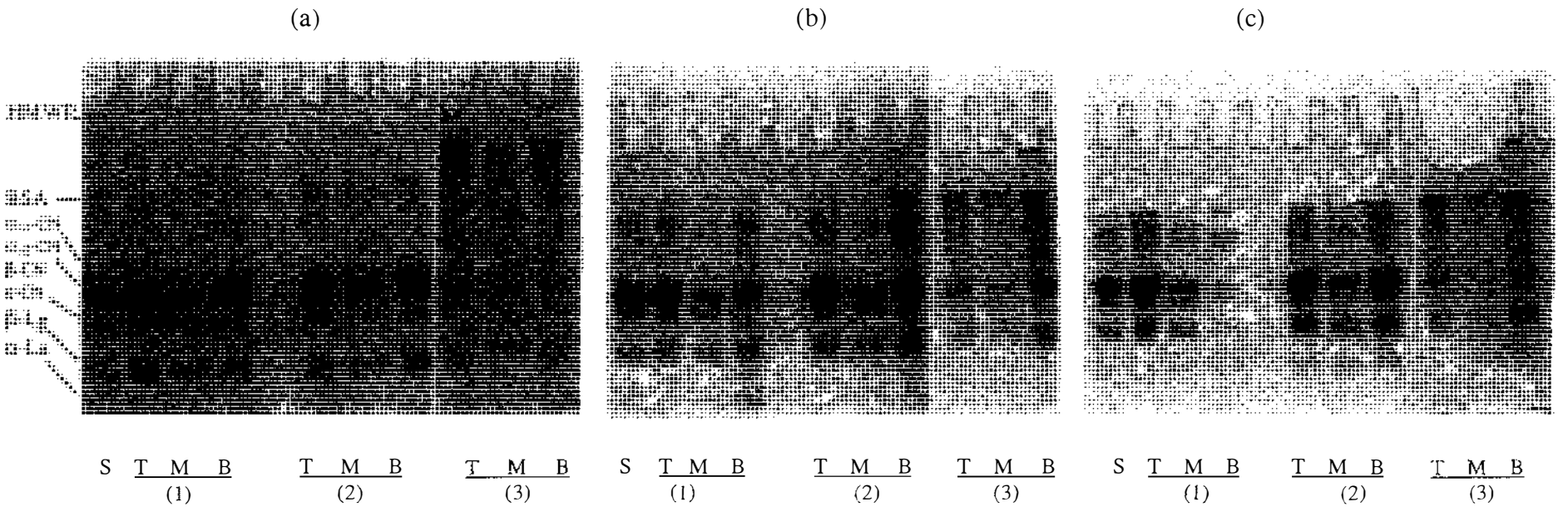


Fig. 6.46 SDS-reducing PAGE gel electrophoretic pattern of the ultracentrifugal fractions obtained from a) Experiment E, b) Experiment F and c) Experiment G milk samples after 1) initial, 2) 5 °C for 25 weeks, 3) 37 °C for 25 weeks storage. RO concentrate (unheated) was used as standard (S); T = Top layer; M = Middle fraction; B = Sediment fraction. CN = Casein; α-La = α-Lactalbumin; β-Lg = β-Lactoglobulin, BSA = Blood serum albumin; HMWP = High molecular weight polymers.

The electrophoretic patterns of the samples for initial measurement showed the same pattern in all the three fractions. On storage at 5 °C for 25 weeks the pattern remained unchanged and did not show any protein breakdown, whereas the samples stored at 37 °C for 25 weeks showed blurred patterns in the three fractions, particularly in the cream and sediment fractions. The intensity of casein bands was less in the middle (soluble) fraction. These results do not indicate protein breakdown. Previous studies have shown that the polymerisation of casein and whey proteins owing to Maillard type reactions occurs during extended storage of unconcentrated and concentrated milks (Andrews and Cheeseman, 1971, Andrews, 1975). After storage for 25 weeks at 37 °C, the intensities of the individual whey proteins and caseins decreased markedly and most of the protein material remained at the top of the resolving and stacking gels. Changes observed in the cream fraction would have been occurring in the proteins adsorbed irreversibly at the fat globule surfaces. The middle fraction and sediment fraction showed changes qualitatively similar to those in the cream layer. After storage for 25 weeks at 37 °C, the sediment fraction showed no clear protein bands in the resolving gels. It appears that storage for 25 weeks at 37 °C caused proteins in the serum, as well as those adsorbed on to fat globule surface to polymerise in such a way that reduction with 2-mercaptoethanol did not return them to the monomeric form. Similar results were obtained for experiment F (Fig. 6.46b) and experiment G (Fig. 6.46c) for initial, storage for 25 weeks at 5 °C and storage for 25 weeks at 37 °C.

From these results, it can be inferred that in the initial measurement (no storage), the protein bands were well separated on SDS-PAGE gels after treatment with 2-mercaptoethanol. Therefore, it appears that disulphide bonds were involved in the heat-induced formation of aggregates in RO concentrated milk. Also, addition of DSP or SHMP did not change the pattern of various protein bands in the three fractions obtained after ultracentrifugation in the samples stored at three temperatures and with increase in storage time.

In SDS reducing gels of milks stored at 37 °C for 25 weeks, the cream and sediment fractions showed formation of HMWP, involving both casein and whey proteins, linked through non-disulphide covalent bonds. The protein adsorbed at the fat

globule surface (protein in cream fraction) were polymerised in a similar manner to the proteins in serum and sediment fractions. Similar polymerization reactions can be induced on on heating reconstituted skim milk at 140 °C for extended periods (Singh and Latham,1993) during which the protein was polymerised gradually, via the formation of covalent bonds between polypeptide chains in the order of dimeric, trimeric and then polymeric aggregates. The nature of these covalent bonds is not known and may be the result of Maillard reactions, the formation of isopeptides, etc. These covalent bonds may be formed between protein molecules adsorbed at the fat globule surface or proteins within the casein micelles.

6.3.2.6 Electron microscopy

Fig. 6.47a, b shows the microstructure of fat globules and casein micelles of freshly made experiment F samples made by the P-ROC-A(DSP)-UHT-H process. In general, the changes in the microstructures of casein and fat globules were similar to those in samples in experiment A. With storage at 37 °C for 25 weeks (Fig. 6.47f), intermicellar bridges linking different casein micelles can be seen. Also, some fragmentation of the micelles was observed and the casein matrix was found to be more electron dense than the sample stored at 5 °C for 25 weeks (Fig 6.47d).

Fig. 6.48a, b shows the microstructures of fat globules and casein micelles of freshly made experiment G samples made by the P-ROC-A(SHMP)-UHT-H process. It can be seen that there is no tendril formation on the casein micelles (Fig. 6.48b) and the serum phase is devoid of any particulate matter, but during prolonged storage of these samples at 5 °C, the casein micellar particles undergo progressive changes. After 25 weeks (Fig. 6.48d), the casein particles have become spiky in appearance with a number of electron dense tendrils protruding from them. Also, more sub-micellar casein was adsorbed on the fat globule surfaces. With storage at 37 °C for 25 weeks (Fig. 6.48f) there was tendril formation, but not many intermicellar bridges were present. More sub-micellar casein was deposited on the surfaces of the fat globules. The serum phase contained some particulate matter possibly formed by the fragmentation of casein micelles.

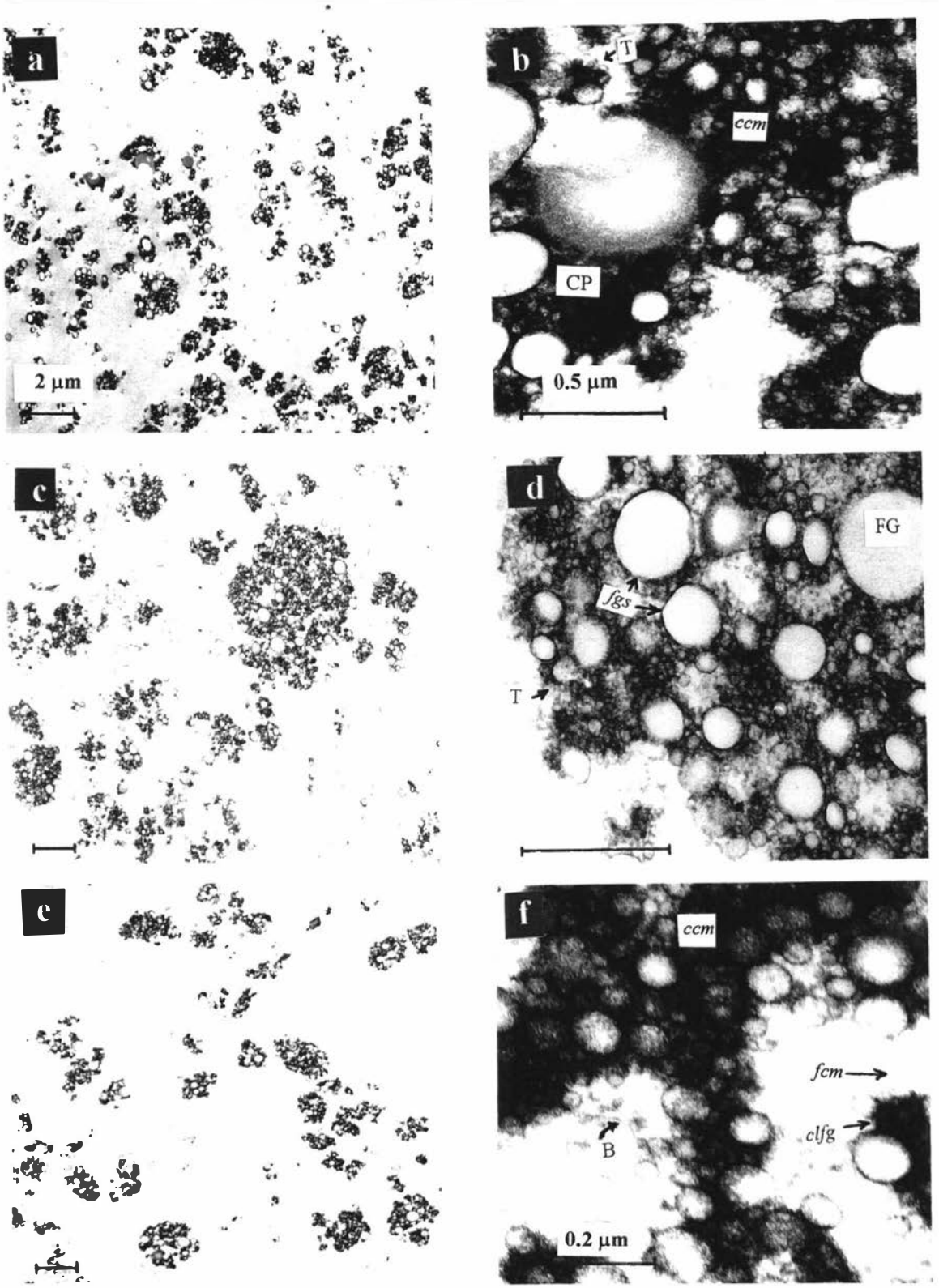


Fig. 6.47 Changes in the microstructure of Experiment F (P-ROC-A(DSP)-UHT-H) milk samples stored at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*) tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d: 48600X; f: 103000X. F = forewarmed at 90 °C/2 min, ROC = reverse osmosis concentrate 2.5X, A(DSP) = Addition of disodium phosphate (0.15% w/w), UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

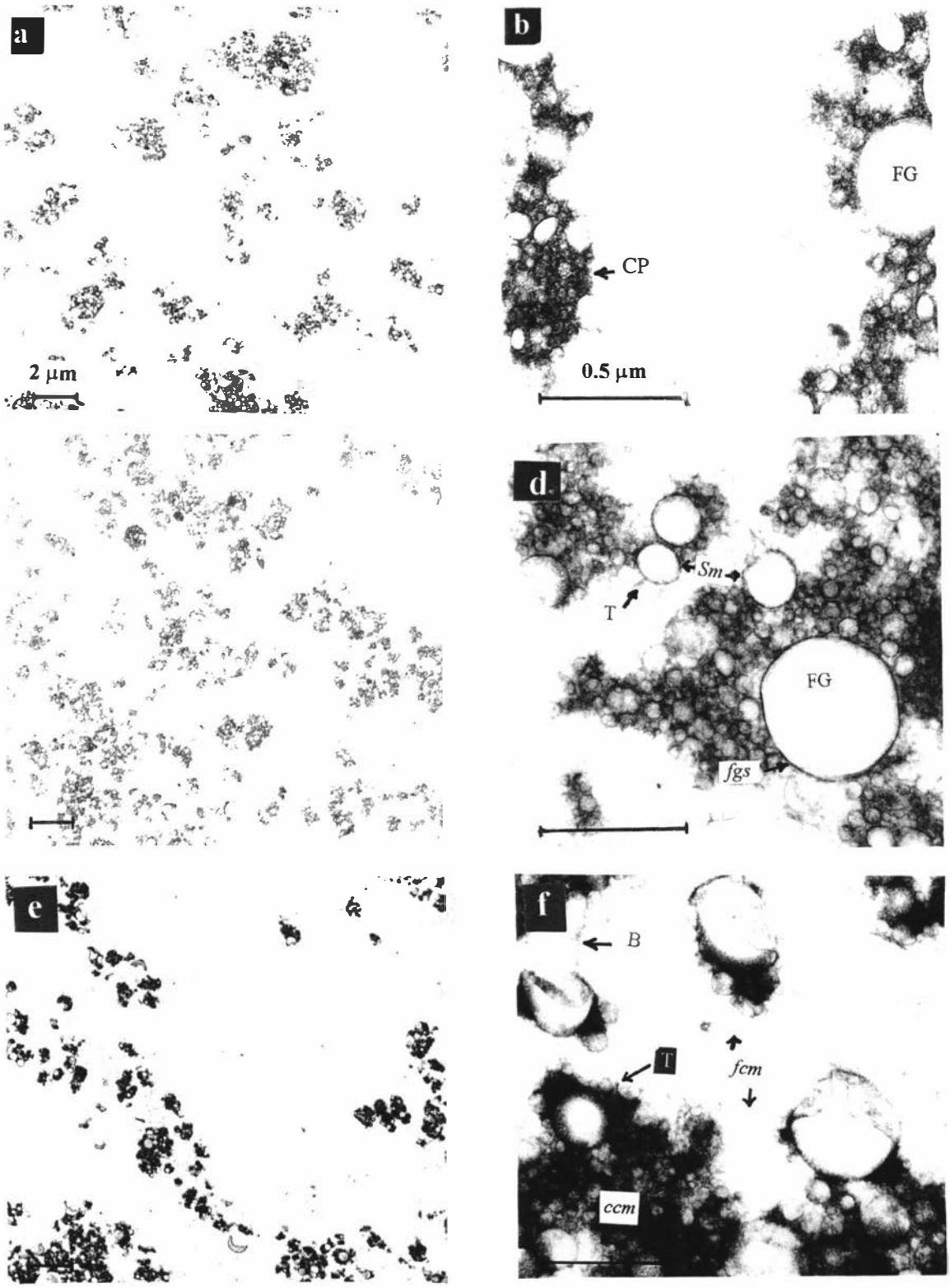


Fig. 6.48 Changes in the microstructure of Experiment G (P-ROC-A(SHMP)-UHT-H) milk samples during storage at a, b) initial measurement, c, d) 5 °C for 25 weeks and e, f) 37 °C for 25 weeks showing casein particles (CP), submicellar casein (*Sm*), fat globule (FG), fat globule surface (*fgs*), intermicellar bridges (B), fragmentation of casein micelles (*fcm*) tendril formation on casein micelles (T), coalescence of casein micelles (*ccm*), casein linking fat globules (*clfg*). Magnification a, c, e: 3400X; b, d, f: 48600X. P = pasteurized at 72 °C/15 s, ROC = reverse osmosis concentrate 2.5X, A(SHMP) = Addition of sodium hexametaphosphate (0.15% w/w), UHT = UHT treatment at 140 °C/4 s, H = Homogenized at 137.9 bar.

These results suggest that the presence of DSP or SHMP did not cause any obvious alteration in the nature and distribution of the micellar particles. The addition of DSP to the concentrates prior to UHT treatment might have altered the micelle surface, whereas the addition of SHMP to the concentrates prior to UHT treatment, may have minimised alteration of the micelle surface, the appearance of bridging material and subsequent gelling, and hence may have had a stabilizing effect to some extent. The addition of polyphosphates delays gelation but does not prevent it. The reason is that with time the polyphosphate hydrolyses and loses its ability to prevent the micelles from interacting leading to network formation. Kocak (1985) observed that the level of proteolysis during storage at 25 °C was not affected to any great extent by the addition of SHMP (0.1% w/w) to UHT sterilized RO concentrates (25% T.S). Harwalkar and Vreeman (1978a) also reported no difference in the extent of protein breakdown of UHT sterilized evaporated skim milk containing SHMP (0.15% w/w) compared to controls during storage at 28 °C. The stabilizing activity of SHMP has also been shown to be independent of proteolysis in unconcentrated UHT whole milks (Snoeren *et al* , 1979; Kocak and Zadow 1985b). Kocak and Zadow (1985a) have suggested a two-stage mechanism for gelation i.e. proteolysis of the milk protein followed by physico-chemical changes which effect the aggregation of the destabilised casein micelles. SHMP is thought to interfere with the process of casein aggregation during the second stage of gelation (Morr, 1975).

6.3.2.7 Bacteriological quality

All RO concentrated UHT treated milk samples proved to have remained sterile during the whole storage period of 25 weeks at all storage temperatures (5, 20 and 37 °C).

6.3.3 Possible Gelation Mechanism

Since the last review on this subject (Harwalkar, 1992; Nieuwenhuijse, 1995), there still remains gaps in our knowledge of the mechanisms of age gelation, particularly in UHT treated concentrated milks. It is generally accepted that gelation of UHT concentrated skim milk during storage involves modification of the surface properties of casein

micelles, which initiates interactions between casein micelles, eventually resulting in the formation of a three-dimensional network.

The colloidal stability of casein micelles in milk is maintained by κ-casein, CCP, a high negative surface zeta potential (-18 mv) and steric effects caused by the protruding chains of κ-casein (Singh *et al.*, 1989). Many physical and chemical changes occur in UHT concentrated milk during storage which may be responsible for the destabilization of the casein micelle. The surface zeta potential of casein micelles (and hence electrostatic repulsions between micelles) is likely to decrease during storage, as a result of decrease in pH. Dephosphorylation of caseins during storage may also cause decrease in electrostatic repulsions between casein micelles. Dissociation of casein micelles, especially of micellar κ-casein from the micelle surface, during storage of UHT treated milk may result in loss of stability. This dissociation may be caused by changes in the salt equilibrium, especially calcium and phosphate during storage. A chemical modification of casein micelle by Maillard type reactions may also alter the charge on the casein micelles, but its significance in the stability of micelles is not clear. The relative contributions of these changes in age gelation is not known.

Harwalkar (1992) suggested that the modification of surface properties of casein micelles may be caused by partial dissociation of the micelles during storage. This process of dissociation could expose regions on the surface of casein micelles that promote interaction between casein micelles. Singh *et al.* (1989) suggested that κ-casein (probably with attached whey protein) may dissociate from the casein micelles during storage, and this dissociation may trigger the loss of micelle stability. There is some evidence (Schmidt, 1968; Aoki and Imamura, 1974) to show that casein micelles dissociate with the formation of soluble protein, rich in κ-casein, during storage of UHT concentrates.

McMahon (1996) suggested that β-lactoglobulin/κ-casein complex dissociates from the micelles during storage of UF concentrated UHT treated skim milk. These large complexes accumulate in serum phase until they reach a critical level and eventually form a gel. Consequently, gelation takes place in serum phase, rather than as aggregation of casein micelles. But what causes the dissociation of β-lactoglobulin/κ-casein complexes is not known as yet. He did not explain why casein micelles, having

lost their protective component (κ-casein), do not aggregate. McMahon used skim milk concentrated by UF; in these milks the concentrations of calcium, magnesium and inorganic phosphate would be lower than in normal heat-evaporated or RO concentrated milks. Hence, it is uncertain whether or not similar mechanisms will operate in heat evaporated or RO concentrated milks.

Most of the previous work has focussed on skim milk systems and little information is available on fat-containing homogenized systems. The results from the present study showed that the RO whole milk concentrates were different than the heat evaporated or UF skim milk concentrates in terms of their compositions and microstructure of casein and fat particles. In milks concentrated by RO, the types of casein and fat particles are very distinct from heat evaporated homogenized concentrated milks. All the fat globules, especially larger ones, are surrounded by a well-defined membrane of proteins around them, these layers are presumably formed by extensive fragmentation of casein micelles. The location of whey proteins and κ-casein in these particles is uncertain. Small fat globules appear to be embedded in casein micelles and in many cases a number of globules are embedded in to a single casein micelle. The conformation of caseins in these very complex structures is not known. It is conceivable that casein particles with embedded small fat globules behave like large casein micelles. UHT treatment of RO milk causes aggregation of these particles, which appears to occur via interactions of protein adsorbed at the fat globule surfaces, involving whey proteins. How whey proteins attach to fat globules in these systems is not clear as the location of κ-casein is not known. The interactions of denatured whey proteins with the fat globules may occur through the intermolecular disulphide bond formation with the κ-casein of intact casein micelles and/or large fragments of micelles adsorbed at the surface of fat globules. Also, in addition whey proteins may interact with other whey protein that are already adsorbed at the fat globule surface. These complexes appear, under the electron microscope, as appendages protruding from the surface of the casein particles which are adsorbed on to the fat globule surface.

Electron micrographs of RO concentrated milks also showed the formation of proteinaceous material (possibly formed as a result of dissociation of caseins and whey proteins from the fat-protein aggregates) in the serum, and fibre-like material protruding

from the surface of the casein particles during storage. These fibre-like appendages appear to connect different casein particles within the aggregates; in some instances, this protein material also appears to be involved in joining the aggregates together. Harwalkar and Vreeman (1978b) and Andrews *et al.* (1977) also observed an abundance of non-micellar particles which increased during storage of concentrated skim milk and unconcentrated whole milk, respectively. The exact reason for the formation of dissociated material is not known as yet, but it is possible that the dissociation of casein particles may be due to the solubilization of colloidal calcium phosphate (CCP) during storage caused by the decrease in pH of RO concentrates during storage. It may be speculated that these dissociated proteinaceous material and the material protruding from the aggregates as fibre-like structures are involved in gel formation. Covalent cross-linking between various protein species could be responsible for the formation of a three-dimensional network. This has been shown by SDS-PAGE.

SHMP presumably binds with the proteinaceous particles in the serum thus providing additional negative charge; this probably reduces the encounter frequency and rate of aggregation of dissociated proteaceous particles. Addition of DSP to milk probably reduces the concentration of calcium in the serum. This causes more CCP to solubilize from the micelles to maintain calcium equilibrium, which in turn would result in disintegration of the micelles, and an increase in dissociated proteinaceous material. Forewarming of milk prior to concentration may affect the rate of dissociation of the casein micelles. The formation of dissociated proteinaceous particles could be less and hence the gelling of such systems would be slower.

7. GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

The main purpose of this study was to examine in detail the effects of RO concentration on the states of protein and fat globules in milk, the stability of RO concentrates to UHT processing, and changes in the UHT treated product during storage. The rheological properties of unheated and UHT treated concentrates at various concentration factors was also investigated. This chapter provides general conclusions arrived at on various aspects of RO concentrated milks.

7.1 Rheological properties of milks

A basic understanding of rheological behaviour of milks and milk concentrates is essential for process design and evaluation (designing pumps, piping systems, and heat and mass transfer operations), quality control and finally consumer acceptability. Therefore, initial work was focussed on the characterisation of the rheological behaviour of milks concentrated by RO. Whole milk, homogenized milk, skim milk, and UHT-treated whole milk were examined. The milks were analysed fresh and after storage for 48 h at 5 °C to determine the effects of cold storage on the rheological behaviour. Using the power law equation as a model of flow properties, it was observed that various milks showed Newtonian behaviour at low total solids concentrations, but exhibited shear thinning behaviour at high total solids. The value of n was not significantly affected by either temperature or total solids, but the values of k increased with increase in total solids concentration and decreased with increase in temperature.

As n remained essentially constant with temperature and k decreased with increasing temperature, an Arrhenius type equation was used to quantify the effect of temperature on k alone. (Eq. 4.5). Arrhenius plots (Fig. 4.5) were found to be linear for unheated RO concentrated whole milk samples which suggests that the state of the fat and therefore the deformability of fat globules seemed not to matter. For UHT treated whole milk, the plots (Fig. 4.5d) showed no definite trend which might have been due to the formation of aggregates during UHT treatment.

As expected, the apparent viscosity (at a shear rate of 367 s^{-1}) of the various concentrates increased with increase in the total solids content and decreased with increase in temperature (Fig 4.4). On storage for 48 h at 5 °C, there was an increase in the viscosity at 5 °C of all milks; whole milk concentrate samples showed a much greater increase in viscosity, particularly when measured at 5 °C, than the skim milks. UHT treated whole milk concentrates showed even an larger increase in viscosity after storage at 5 °C for 48 h. These results showed that the combined effects of UHT treatment, and storage for 48 h at 5 °C, together with a higher total solids content, could result in a considerable increase in the viscosity of the milk. It appears that during storage at low temperatures, milk fat plays an important role in the increase in the viscosity at 5 °C (owing to time-dependent solidification of fat at low temperatures). The increase in viscosity owing to UHT treatment indicated that the denaturation of whey proteins, interaction between κ-casein and whey proteins, and fat-protein interactions might have contributed to the increase in viscosity. These interactions would have influenced the voluminosity and sizes of various protein particles in the system resulting in an increase in viscosity.

A shift factor approach was used to quantify the rheological data. Eq. 4.9 can be used to predict values of k in terms of temperature and total solids content. The values of k obtained with this equation, together with the values of n in Table 4.10, can be used to predict the flow properties of RO concentrates at temperature between 5-60 °C, and total solids contents of 13-40% for whole milks and 9-26% for skim milks.

Another way used to quantify the data was the approach used by Fernandez-Martin (1972), where a general expression relating apparent viscosity, total solids and temperature can be derived. As n remained constant, values of k were used for regressions. Eq. 4.12 can be used to calculate the value of k, which together with the values of n, provides a means of predicting the flow properties of RO concentrates.

Using these two approaches, predicted values of k were calculated. On comparing predicted and experimental values, it was found that the Fernandez-Martin approach gave the smallest % differences in the k values and had higher r^2 values than the shift factor approach.

A hydrodynamic approach, using Eilers' equation (Eq. 2.5), was used to calculate the basic viscosity of the RO concentrated milks, since in a typical concentrated system, hydrodynamic interactions take place between the various dispersed particles. The data here was analysed using two schemes. For Scheme A, lactose was included in η_{ref} (the viscosity of continuous phase) and in Scheme B, lactose was included in volume fraction (ϕ). The experimental values for the basic viscosity of various milks using Scheme A were higher than the values predicted by Eilers' equation. The values for whole milks differed significantly, whereas the values for skim milk differed only slightly. The basic viscosity increased with increase in the volume fraction for all unheated milks, whereas in the case of UHT treated whole milks there was an increase in basic viscosity up to a volume fraction of 0.35, but thereafter the viscosity remained more or less constant (Fig. 4.11). On storage for 48 h at 5 °C, there was an increase in the basic viscosity of all milks.

It was found that a closer relationship existed between the experimental values and the predicted by Eilers' equation using Scheme B (where lactose was included in volume fraction) (Fig. 4.12). Based on these results, it is recommended to include lactose in the volume fraction rather than in the calculation of the viscosity of the continuous medium.

Overall, it can be concluded from the Chapter 4 results that the rheological properties of RO concentrated milks are very complex and depend on temperature, concentration, storage and the physical states of milk fat and proteins. Mathematical relationships developed in this work can be used to accurately predict the flow properties and viscosity of various types of milks concentrated by RO for engineering design purposes.

7.2 Effects of RO concentration and UHT treatment on the states of proteins and fat globules

It is known that the RO process damages fat globules, but no information is available on the nature and mechanism of this damage. Chapter 5 focuses on the study of the structures of fat globules and casein micelles in RO concentrated milk, the nature of the adsorbed protein, and the modes of interactions between fat globules and casein

particles. The effects of RO concentration on the stability of milk towards subsequent UHT sterilization were also explored.

The results presented here clearly showed that the RO process, in addition to concentrating the milk, had a homogenizing effect on the fat globules. It was interesting to note that (Fig. 5.4) the fat globules were disrupted mainly at the beginning of the RO concentration process and that continuous recycling of the concentrate caused only slight further disruption of fat globules. Moreover, it was clear that the structures of fat globules and casein particles in RO concentrated milks were very different from those found in milks homogenized by a conventional valve homogenizer. The RO concentrated milks contained some very small fat particles compared with milks homogenized in a valve homogenizer. Specifically, in RO concentrated milks, the membranes around the fat globules were different and there were very few intact or semi-intact micelles adsorbed at the surfaces of fat globules. Very few distinct casein micelles could be observed in the serum. There were a number of very small fat globules grouped together, apparently held together by quantities of protein. A number of these fat globules appeared to be embedded in casein particles. These protein bodies (presumably derived from casein micelles) were unique to the RO system as they were not observed in conventionally homogenized unconcentrated whole milks or reblended milks.

When RO milks were heated in a UHT plant, large aggregates of intact fat globules were formed. There was no evidence of coalescence. Aggregation was protein mediated and presumably resulted from interactions of protein adsorbed at the fat globule surfaces. It was found that about ~ 65% of whey protein denaturation was necessary for the formation of these large aggregates. On treatment with protein dissociating medium (EDTA and SDS buffer), the aggregates dispersed completely, suggesting no coalescence of fat globules.

When non-homogenized reblended milk was UHT treated (in this system fat globules retained their original natural membrane material), large particles were also formed but these particles consisted of protein alone, with no incorporation of fat globules (Fig. 5.18c). The casein micelles were connected to each other in chains and clusters. Micelles had developed tendrils, which were possibly denatured whey proteins

attached to casein micelles via the β-lactoglobulin/κ-casein interaction. When reblended milk was first conventionally homogenized and then UHT treated, large aggregates were formed, (Fig. 5.18e) which involved the fat globules, but the structures of these fat and protein particles were different than those observed in RO concentrated milks.

The pH of the RO concentrate prior to UHT treatment affected the extent of aggregate formation and the size of the aggregates. The size of the aggregates decreased as the pH of the concentrate increased from 6.3 to 7.1 prior to UHT treatment. When the pH of the concentrate was adjusted to 7.2 prior to UHT treatment, very few aggregates were formed. Adjustment of the pH of the concentrate after UHT treatment had no effect on the size of aggregates formed and the extent of aggregation.

Addition of DSP (0.20% w/w) resulted in a decrease in aggregate size, and this was not due to the pH change caused by the addition of DSP. The effects of phosphate addition prior to UHT treatment on aggregate formation in RO concentrated milk were however similar to those of a pH increase prior to UHT treatment. This suggests that a reduction in calcium ion activity may be another essential factor for protein/fat aggregation during heating in RO concentrated milk. This may arise because the fat globules may act as κ-casein-depleted micelles, as a result of spreading of the casein micelles at the fat globule surfaces. These globules may be sensitive to calcium ions in the serum, because of the high calcium ion sensitivity of α_{s1}- and β-caseins. Hence, calcium bridging between these fat particles is possible. Therefore, two types of fat particles may emerge during UHT heating of RO concentrates. In one type, denatured whey proteins are deposited on these globules and are actively involved in joining them together. The other type of fat particles may be sensitive to calcium ion concentration, because of an insufficient concentration of κ-casein on its surface. This was supported by results obtained on the effects of pH and phosphate (DSP) addition prior to UHT treatment, both of which decrease calcium ion activity, and the observation that ~ 65% of whey protein denaturation was required to initiate aggregation of protein-fat particles.

7.3 Storage induced changes in UHT treated RO concentrated milks

The work described in Chapter 6 focussed on exploring the storage-induced physical and chemical changes in RO concentrates (32-35% T.S.) at different storage temperatures. The effects of storage temperature, homogenization after UHT treatment, forewarming of milk prior to concentration, and the addition of phosphates to concentrates prior to UHT treatment were investigated.

The results showed that, irrespective of the processing treatments, there was a decrease in the pH with storage time (Figs. 6.4-6.7), the extent of pH drop being greater at higher storage temperatures.

Penetration test results showed that the higher the storage temperature the shorter was the gelation time (Figs. 6.17-6.20). The changes in the particle size distribution during storage were generally consistent with the gel formation results. Homogenization had no appreciable effect on the gelation time for pasteurized milk concentrates but it decreased the rate of gel formation for concentrates that had been forewarmed prior to concentration. While forewarming decreased the rate of gelation for homogenized milks, an opposite trend was observed for concentrates that had not been homogenized.

The changes in the viscosity of RO milk samples during storage did not relate to the time of gelation of the samples.

The NPN of all the samples increased with both storage time and temperature (Fig. 6.26-6.29); the increase was higher at higher storage temperatures. Forewarming of milks before concentration resulted in lower NPN values at all storage temperatures, which may be due to the binding of the denatured whey protein on to the casein particles, thus inhibiting the breakdown of peptide bonds. Homogenization of the concentrates had no effect on the formation of NPN.

Electrophoresis results (under non-reducing conditions), irrespective of whether the milks had been forewarmed or not, showed that the formation of HMWP on UHT treatment involved disulphide bonds (Fig. 6.30a, Fig. 6.31a). The samples stored at higher temperature developed a blurred pattern (under reducing conditions), suggesting the formation of non-disulphide covalent cross-links during storage (Fig. 6.30b, Fig. 6.31b).

Electron microscopy showed that at a low temperature of storage (5 °C) there was little change in the structures of fat and casein particles (Fig. 6.32c, d), whereas storage at higher temperatures (e.g. 37 °C) (Fig. 6.32e, f) resulted in an increase in fibre-like material and proteinaceous particles (probably dissociated casein micelles) in the serum. Forewarming of concentrates prior to concentration (Fig. 6.34) resulted in similar trends with storage except that the fat globules in such milks had a thicker surface layer than in milks that had not been forewarmed.

The addition of SHMP to concentrates before UHT treatment resulted in the formation of smaller aggregates during storage, whereas the addition of DSP before UHT treatment resulted in the formation of larger aggregates, suggesting some kind of stabilizing action by SHMP. This was confirmed by penetration test results which showed that the addition of SHMP to RO concentrates prior to UHT treatment provided some stability towards gelation, whereas the addition of DSP made samples more prone to gelation, the more so at higher storage temperatures (Fig. 6.42).

Electrophoresis results showed that addition of DSP or SHMP had no appreciable effect on the electrophoretic patterns of the various milk samples (Figs. 6.43-6.45). Electron microscopy showed that the presence of DSP or SHMP did not cause any obvious changes in the nature and distribution of the micellar particles (Figs. 6.47-6.48).

Gelation in RO concentrated milks may be the result of increase in the formation of proteinaceous particles (presumably from dissociation of casein micelles) in the serum during storage at higher storage temperatures, as seen by electron microscopy. The dissociation of casein particles may be caused by the solubilization of the CCP during storage as a result of the decrease in pH of RO concentrate during storage. These dissociated proteinaceous materials, together with the fibre-like structures protruding from the aggregates, are involved in the gel formation. The three-dimensional network might be formed owing to covalent cross linking between various proteins, SDS-PAGE showed that covalent cross links existed after extended storage.

The results from Chapter 6 confirm that higher temperatures of storage are not ideal for the shelf life of UHT treated RO concentrated milk. Storage at refrigeration temperatures gave the best results, but this is expensive and often impractical. SHMP

treatment appears to be of practical value in terms of prolonging the shelf life of these milks.

7.4 Recommendations for further work

The work described in this thesis has contributed to an understanding of the properties of RO milk concentrates made under different processing conditions. The characterisation of the rheological properties of RO concentrates (both unheated and UHT treated) (Chapter 4) should help in process design and control as well as in the quality control of final product. The unique structures of the fat and casein particles observed in RO concentrated milks (Chapter 5) open up exciting opportunities for producing a range of new products, or for modifying the functional properties of existing products. For example, if RO concentrates are used to manufacture yoghurt, it would be expected that the structure of the gel matrix formed, and hence its texture and sensory properties, would be different from that of yoghurt made by conventional methods. The present work also investigated the effects of various processing variables on the interactions of fat and protein. By choosing the appropriate processing sequences, products with improved functionality and shelf life can be produced. Since there is a growing trend towards the manufacture of UHT products, and a possibility of using RO concentrates in the manufacture of these product, further work involving the areas mentioned below should be done to gain more insights into RO milk systems.

1. More detailed investigations into determining the nature of fat-protein aggregate material in RO concentrated systems, and the mechanisms of its formation.
2. Investigation of the implications of the unique fat and protein structures for other products, such as ice cream, yoghurt, cheese, low fat dairy desserts made using unheated RO concentrates as base materials.
3. Investigation of the effects of a range of forewarming regimes for milk prior to concentration by RO on subsequent UHT treatment and storage.
4. Investigation of the effects of addition of SHMP in various proportions prior to concentration of milk.
5. Investigation of the effects of addition of phosphatides (lecithins) to RO concentrates prior to UHT treatment.

6. Investigation of the use of loose RO membranes to determine the effects of minerals on the heat-induced association of fat and protein particles and their interactions during storage.

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