DEPARTMENT OF FOOD TECHNOLOGY
MASSEY UNIVERSITY

PHYSICAL AND RENNET COAGULATION PROPERTIES OF RECOMBINED CHEESE MILK MADE FROM MILK PROTEIN CONCENTRATE (MPC-56)

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

VISAKA POMPRASIRT

1996

ABSTRACT

The effects of heat treatment and homogenization on the physical and rennet coagulation properties of recombined cheese milk (40% total solids) made from reconstituted milk protein concentrate (MPC-56) (20%) and fresh frozen milkfat for recombination (FFMR) (20%) have been investigated. The effects of heat treatment of the concentrate prior to drying were also studied.

Heat treatment, either during MPC powder manufacture or after reconstitution and recombination of MPC powder had a significant influence on whey protein denaturation, viscosity, and rennet coagulation properties of recombined cheese milk.

The degree of whey protein denaturation, as determined by the decrease of soluble whey protein at pH 4.6, increased with increasing severity of heat treatment, and β-lactoglobulin A was more heat sensitive than β-lactoglobulin B and α-lactalbumin.

Recombined cheese milk showed shear thinning behaviour, e.g. recombined cheese milks behaved as pseudoplastic materials. The viscosity of recombined cheese milk determined at a shear rate of 18.5 - 731 s^{-1} increased with increasing severity of heat treatment, indicating aggregation of protein and fat particles. The changes in viscosity were related to the degree of whey protein denaturation and the interactions between whey proteins and casein micelles.

The rennet coagulation properties of recombined cheese milk were determined in terms of gelation time (GT), storage modulus (G'), and the force required to fracture the renneted gels (yield force). In general, G' and yield force decreased with increasing severity of heat treatment. Gelation time appeared to remain unaffected by heat treatment, either of the recombined cheese milk or during MPC manufacture. There was an almost linear inverse relationship between G' or yield force and whey protein denaturation of up to ~ 60%. Further denaturation had no further effect. It is likely that denaturation and complex formation between whey proteins and casein micelles sterically interferes with the aggregation of altered casein micelles, resulting in slower increases in G' and yield force values.

Compared to heat treatment, the degree of homogenization appeared to have a minor effect on the physical and rennet coagulation properties of recombined cheese milk. Increased homogenization pressure resulted in a decrease in average fat globule diameter and an increase in viscosity. Rheological parameters, i.e. G' and yield force of renneted-induced gels decreased as the homogenization pressure was increased. The changes in milk fat globule diameter and its surface composition are probably involved in this phenomenon.

Microstructure examination and permeability measurements of renneted gels indicated that the casein networks were very strong and dense with limited porosity.

Since increasing heat treatment and homogenization reduced the strength of renneted gels, with only a small effect on gelation time, it might be possible to use these two processes to counter the gel firmness problem of recombined cheese made from MPC powder.

ACKNOWLEDGMENTS

I gratefully acknowledge my chief supervisor, Associate Professor Harjinder Singh, for his excellent supervision, understanding, warm encouragement throughout my study, especially for his enthusiasm to help, constructive criticism, endless patience in discussion and correction of my English. Without him, this work would not have been possible.

My gratitude and appreciation are extended to Dr. John Lucey for his help during the experimental work and also for reading my manuscripts.

I also wish to express my sincere gratitude to my co-supervisors, Mr Rod Bennett, for his encouragement, invaluable suggestions, guidance in the planning and conduct of the experimental work and advice during the preparation of this manuscript.

Most warm thanks to Mr Bing Soo for introduce me to this project, providing the samples, advice and discussion during the experimental work.

My gratitude are also extent to Anthony McKenna (NZDRI) for his assistance with the confocal microscopy.

I am very grateful to June Lathum, Steve Glasgow, Alistair Young, Gary Redford for their excellent technical assistance, friendship and encouragement.

Thanks to all the staff and postgraduate students in Food Technology Department at Massey University for their help friendship and support which has made my study in New Zealand pleasant and memorable.

Finally, and most important, my special thanks go to my family for their encouragement and constant moral support during this study.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

In cheese research, considerable attention has been focused on understanding the milk coagulation process as well as trying to improve cheese quality and production yield through the utilization of heat treatment, homogenization and ultrafiltration technology. Although it has been well established that these processing variables have significant influence on the physical and rennet coagulation properties of normal milk (Walstra & Jenness, 1984; Singh *et al.*, 1988; Dalgleish, 1992; McMahon *et al.*, 1993), the studies on the effects of these processing variables on recombined cheese milk (40% total solids) made from milk protein concentrate (MPC) powder are limited.

Milk protein concentrate (MPC) is a high protein containing skim milk powder manufactured from skim milk using the combined technology of heat treatment and ultrafiltration. It contains casein and whey protein in their original form in the ratio of approximately 82:18. MPC powders are widely use to standardize the protein content of cheese milk and also in the recombined cheese industries, such as in the production of soft cheese varieties, e.g. Feta cheese, Process cheese and Mexican cheese (Novak, 1992; Soo, 1994). The utilization of MPC powder to substitute skim milk in recombined industries appears attractive due to the simplification in the production process, increase production yield by the incorporation of whey protein and also lower production cost due to the reduction in process requirement (Novak, 1992). However, there are major problems encountered in the manufacture of hard cheeses from MPC powder, which include reduced coagulation times and high gel firmness of the renneted gel which makes it difficult to handle (e.g. cut) in the conventional cheesemaking equipment. By using heat treatment and homogenization with the utilization of MPC powder, it might be possible to correct the coagulation time and gel firmness problems encountered with the use of MPC powder in the recombined cheese.

The objectives of this study were to investigate the effects of process variables, namely the preheat treatment used during the manufacture of MPC powder, and the heat treatment and homogenization of the recombined cheese milk on the physical, rheological properties and microstructure of rennet gels. A better understanding of the influence of these process variables

on its physical properties and rennetability should provide useful information on the utilization of MPC in recombined cheese manufacture and suggest ways to produce new varieties of fresh cheese.

CHAPTER 2

LITERATURE REVIEW

2.1 General characteristics of milk proteins

Milk is a complex system of protein aggregates, soluble proteins, salts, lactose, fat and water. It is a very versatile raw material used for the manufacture of a wide variety of products due to the functional properties of its components. Cheese is one of the main dairy products and its manufacture is dependent on the milk proteins, especially the caseins and to a lesser extent the milk salts.

Milk proteins can be divided into two major groups, namely the caseins and the whey or serum proteins (Walstra & Jenness, 1984, Dalgleish, 1992). Of the two, caseins are the most important for the manufacture of cheese. Caseins are more abundant in milk, and they are also the proteins which form the gel matrix on which cheese manufacture is based. The whey proteins are of little relevance in normal cheese manufacture, but may become important if cheese milk is ultrafiltered or severely heated, i.e. to the temperatures greater than 60°C. In such circumstances, whey proteins are denatured and can be incorporated into the curd (Banks, 1988).

2.1.1 Casein micelles

Caseins, a group of phosphate-containing milk proteins that precipitate upon acidification to pH 4.6, represent about 80 % of total proteins in milk (Schmidt, 1982; Walstra & Jenness, 1984). They are not homogeneous, but composed of at least four different protein types, known as α_{s1}-, α_{s2}-, β- and κ-caseins in the ratio of 4:1:4:1 (Davis & Law, 1983). The caseins do not exist as individual molecules, but form large aggregates of submicelles, which also contain insoluble calcium phosphate called "micellar" or "colloidal" calcium phosphate to form the particles known as casein micelles (Schmidt, 1982; Walstra & Jenness, 1984). These casein micelles, range in diameter from 50 - 300 nm (Schmidt *et al.*, 1973) and consist of approximately 92 % protein and 8 % inorganic salts, mainly calcium, phosphate and citrate (Schmidt, 1980; Whitney, 1988).

Although the exact structure of the casein micelle has not fully resolved, the casein micelle is generally considered to be approximately spherical and composed of smaller units called sub-micelles. Although, there is no clear agreement on their size; a diameter of 10 - 20 nm and an average monomer molecular weight of 23,300 Da for casein have been reported (Farrell, 1988). Individual sub-micelles are believed to be linked together by colloidal calcium phosphate (CCP) to form the casein micelles (Schmidt, 1982). Even though the nature of the bonds that enable CCP to link adjacent sub-micelles are not known, it is presumed to be electrostatic. Hydrophobic interaction and hydrogen bonds are also thought to contribute to micelle stability.

The different caseins, α_{s1}-, α_{s2}-, β- and κ-casein, are not evenly distributed throughout the micelles; in particular, κ-casein is located mainly at the surface of the micelles (Dalgleish, 1992), so it can exercise a stabilizing effect upon the native micelles and prevent them from coagulation. This stability effect arises because κ-casein can be divided into two distinct regions, namely the hydrophobic para-κ-casein (residues 1 - 105) and the hydrophilic macropeptide or glycomacropeptide (CMP or GMP, residues 106 -169) (Holt, 1985; Dalgleish, 1992). In its normal position on the surface of casein micelles, κ-casein is probably linked to the remainder of the micelles via the hydrophobic para-κ-casein part of the molecule, allowing the macropeptide to protrude from the surface into the surrounding solution and interact with the solvent to stabilize the micelles (Dalgleish & Holt, 1988; Walstra, 1990; Dalgleish, 1992). The model for the structure of casein micelles proposed initially by Schmidt (1982) and modified slightly by Walstra and Jenness (1984) is shown in Figure 2.1.

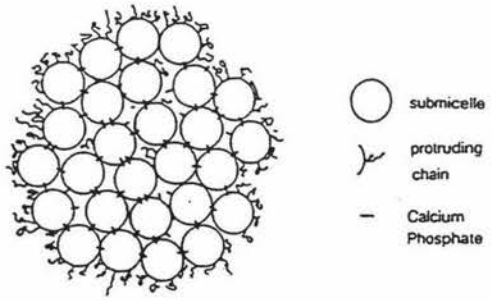
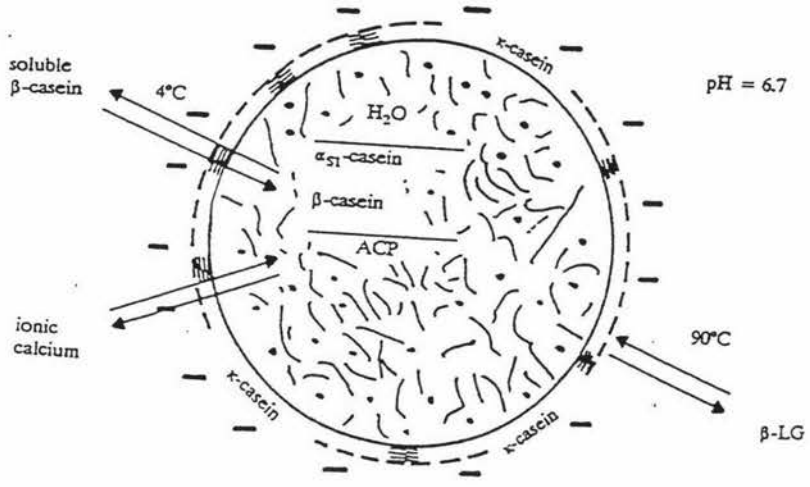


Figure 2.1 A model of the structure of the casein micelles (Walstra & Jenness, 1984)

However, Visser (1992) claimed that the sub-micellar model proposed by Walstra and Jenness (1984) is over simplification and can not explain some phenomenon that occurs upon the casein micelles. He proposed that the casein micelle is not based on the sub-micelles but is an aggregate of the individual caseins, in which κ-casein is located at the surface to stabilize the micelles by its charges and surface hydration layers. α_{s1}-, α_{s2}-, β-Casein are randomly aggregated with the preference for α_{s1}-casein to form a skeleton of the overall structure probably in combination with the minor protein. Whereas, β-casein are aggregated and entrapped within the skeleton. He used this model as shown in Figure 2.2 to explained the phenomenon that occurs on casein micelles, which included renneting.



(ACP = amorphous calcium phosphate, β-LG = β-lactoglobulin)

Figure 2.2 A novel model of casein micelles proposed by Visser (1992)

2.1.2 Whey proteins

Whey proteins are those milk proteins which remain in the serum phase after precipitation of the caseins at pH 4.6 (≥ 20°C). In contrast to the caseins, whey proteins, which comprise about 20 % of the proteins in milk, are globular and exhibit well developed secondary and tertiary structures, so they are susceptible to protein denaturation (Brown, 1988). Whey proteins may be subdivided into different groups, including β-lactoglobulin (β-Lg), α-lactalbumin (α-La),

bovine serum albumin (BSA), immunoglobulins (Ig) and small molecular weight peptides, some of which are derived from proteolysis of caseins (Walstra & Jenness, 1984). The various properties of whey protein have been reviewed by Walstra and Jenness (1984) and Dalgleish (1992).

β-lactoglobulin

β-Lactoglobulin (β-Lg), the most abundant whey protein, comprises about 50 % of the total whey proteins in bovine milk. It exhibits well developed secondary, tertiary and quaternary structures with a monomeric molecular weight of 18,000 Da. β-Lg contains five cystine residues per mole, of which four are involved in disulphide linkages. It has a single free thiol group that is of great importance for changes occurring in milk during heating since it can interact with other proteins, notable κ-casein and α-La (Walstra & Jenness, 1984). The physico-chemical properties of β-Lg have been extensively reviewed by Green *et al.* (1979) and Hambling *et al.* (1992).

α-Lactalbumin

α-Lactalbumin (α-La) accounts for 20% of whey proteins. Like other whey proteins, α-La is a small compact globular protein, with a molecular weight of 14,000 Da (Brew & Grobler, 1992). α-La contains four interchain disulphide bonds, but has no sulphydryl groups.

Bovine serum albumin

Bovine serum albumin (BSA) which is identical to the serum albumin found in the blood system, has a molecular weight of 66,260 Da (Eigel *et al.*, 1984). BSA represents about 10 % of total whey proteins. It has one free thiol and 17 disulphide linkages, which hold the protein in a multiloop-type of structure.

Immunoglobulins

Immunoglobulins are antibodies synthesised in response to stimulation by macromolecular antigens foreign to the animal. They account for up to 10% of the whey proteins and are formed from two different types of polypeptide chains called light (MW 22,400 Da) and heavy

(MW 50 - 60,000 Da). These light and heavy chains are joined by disulphide linkages to form the basic immunoglobulin structure (Walstra & Jenness, 1984).

Other whey proteins

Several other whey proteins are found in small quantities in serum, including two iron binding proteins, lactoferrin and transferin, and a group of acyl glycoproteins.

2.1.3 Effect of heat treatment on milk proteins

A number of physical and chemical changes occur in the milk protein system during heating which affect their functionality in milk products (Singh *et al.*, 1988; van Boekel & Walstra, 1989). Heat-induced changes in the milk system have been extensively reviewed by Fox (1981) and Singh and Creamer (1992). The effects depend on the intensity of heat treatment, both heating temperature and holding time (Harper, 1981; Singh & Fox, 1989). Significant changes occur at temperatures > 60°C including whey protein denaturation, interactions between denatured whey proteins and casein micelles, and the conversion of soluble calcium and phosphates to the colloidal state. Heating milk at temperatures greater than 70°C causes progressive denaturation of most of the whey proteins, some of which interact preferentially with the micelle via the formation of intermolecular disulfide bridges (Singh *et al.*, 1988).

Generally, casein micelles are very stable at high temperatures owing to their lack of secondary or tertiary structure (Walstra & Jenness, 1984). They can withstand commercial heat treatments, although some changes such as dephosphorylation and proteolysis, as well as changes in micellar structure (zeta potential, hydration changes and association-dissociation reaction) do occur with very severe heat treatment (Fox, 1981; Singh & Creamer, 1992). The most pronounced effect on casein micelles during severe heat treatment is an increase in micelle size with a concomitant decrease in the number of small micelles (Creamer & Matheson, 1980; McMahon *et al.*, 1993), which is probably due to deposition of denatured whey proteins on the micellar surface and also the precipitation of calcium phosphate.

In contrast to caseins, whey proteins are sensitive to heat and undergo denaturation at temperatures > 60°C. Denaturation involves the unfolding of the globular conformation, an increase in surface hydrophobicity and the formation of disulphide linkages with other proteins.

Whey protein denaturation begins with an initial swelling of the protein structure when it is first exposed to heat. As the intensity of the heat treatment increases, whey proteins unfold, aggregate, sediment and also form gels (whey protein gelation) through multiple-reaction processes. Following or possibly during denaturation, whey proteins often interact either with themselves or with other molecules to form aggregates, precipitates or gels which are virtually irreversible. In heated milk, the denatured whey proteins become closely associated with the micelles through disulphide and hydrophobic interactions (Law *et al.*, 1994; Singh & Creamer, 1992).

If the heat treatment is sufficiently severe, these changes become irreversible, and the denatured whey proteins precipitate along with the caseins at pH 4.6. The rate of protein denaturation is highly dependent on temperature and the severity of heat treatment; the rate increases between 20 - 30 times for every 10°C rise in temperature (McMahon *et al.*, 1993). Thermal denaturation of whey proteins has been extensively reviewed by Mulvihill and Donovan (1987). The order of sensitivity of various whey proteins to heat has been reported to be: immunoglobulins > BSA > β-lactoglobulin (variant A > B) > α-lactalbumin (Dannenberg & Kessler, 1988a, b).

Although α-lactalbumin can be denatured at a lower temperature, i.e. 62°C compared to 64, 72 and 78°C for BSA, Ig and β-lactoglobulin, respectively (Brown, 1988; Dannenberg & Kessler, 1988a, b), it is considered to be more heat-resistant than the other whey proteins because its denaturation is reversible. In contrast, β-lactoglobulin is denatured irreversibly. β-Lactoglobulin denatures more rapidly than α-lactalbumin in heated milk and whey systems and the overall effects of heating are greater on β-lactoglobulin than on α-lactalbumin.

The formation of complexes between denatured whey proteins and κ-casein affects the steric stabilization of casein micelles (Mohammad & Fox, 1987). Moreover, other interaction forces, such as van der Waals attraction and electrostatic interactions, are also affected, which in turn affect the overall colloidal stability of micelles. Electron microscopy indicates that the structure of rennet or acid-induced milk gels prepared from unheated and heated milk differ in size of the primary protein aggregates, the thickness of the protein strands and the size of the holes

between strands (McMahon *et al.*, 1993). The rennet coagulation properties of milk also differ depending on the extent of β-lactoglobulin denaturation (Green & Grandison, 1993).

2.2 Rennet coagulation properties of milk

The unique characteristic step in cheese manufacture involves the coagulation of casein micelles by enzymatic proteolysis by the rennet enzyme. The rennet coagulation process may be divided into (i) primary enzymatic proteolysis phase where κ-casein which stabilizes the casein micelles is attacked by proteolytic enzyme contained in rennet, (ii) secondary aggregation phase which is the subsequent coagulation of the renneted-altered micelles and possibly (iii) tertiary stages (Green *et al.*, 1983; McMahon & Brown, 1984; Fox, 1987).

Primary phase

During the primary stage, rennet (chymosin) splits κ-casein at the junction between para-κ-casein and macropeptide moieties, i.e. at the Phe_{105}-Met_{106} bond, to yield two peptides with markedly different properties, caseinmacropeptide and para-κ-casein (Castle & Wheelock, 1972). The macropeptide moiety is hydrophilic and soluble, so it diffuses away from the micelles into the serum after hydrolysis. Whereas, para-κ-casein is strongly hydrophobic and remains attached to the micelles. As a result, the stability of casein micelles is reduced and the micelles can begin to coagulate once sufficient κ-casein has been hydrolysed (Cheryan *et al.*, 1974; Culioli & Sherman, 1978; Garnot *et al.*, 1982). Dalgleish (1983) reported that when about 85 % of the total κ-casein has been hydrolysed, the micelles begin to aggregate but an individual micelle can not participate in gelation until approximately 97 % of its κ-casein has been hydrolysed.

Secondary phase

The secondary stage of the rennet coagulation process, aggregation, is known to involve interactions between calcium ions and the rennet-altered micelles, resulting in the formation of coagulum. The initial stages of gel formation involve the formation of small aggregates with micelles linked in chain-like structures, which eventually join to form a gel network (Green *et al.* 1978; Fox, 1987). The process probably progressively strengthens the junctions between micelles, explaining the rise in gel firmness after coagulation. Calcium ions are necessary for

gel formation; their role may be attributed to the precipitation in the phenomenon, perhaps in the form of calcium "bridges" between aggregating micelles and reducing the net negative charge of casein.

The rate of coagulation of rennet-altered casein (in the presence of Ca^{2+}) is markedly affected by temperature, pH, the presence of other divalent ions and interactions of casein with other milk components as a result of processing, such as homogenization and heat treatment (Mehaia & Cheryan, 1983).

Tertiary phase

The rennet coagulation process can also be described as having a tertiary stage which involves the changes in the properties and structural rearrangements of the renneted-gel network once it has been formed (Dalgleish, 1983; Lucey, 1995).

These rennet coagulation processes have an important effect on the physical properties of the final gel, particular on its porosity, permeability and thickness of strands, and on the further stages of curd and cheese development.

2.2.1 Rennet coagulation time

The rennet coagulation time (RCT) is defined as the time between rennet addition and the visible coagulation of particles or the sum of the time required to obtain a critical degree of κ-casein hydrolysis and the time for at least partial aggregation of hydrolysed casein before visible flecks are formed (Sharma *et al.*, 1994). The RCT is influenced by three factors: (i) the rate of enzymatic reaction; (ii) the rate of aggregation and (iii) the degree of proteolysis before the aggregation starts (Dalgleish, 1980; Garnot & Corre, 1980).

RCT is generally used to characterize the changes in the physical state of coagulating milk in its transition from a sol to gel (Sharma *et al.*, 1990). The RCT depends on a number of factors, including milk composition, pH, temperature, rennet concentration, and also processing factors such as heat treatment and homogenization. It can be measured in a number of ways, ranging from simple observation of the formation of visible particles to more sophisticated methods that record changes in the apparent viscosity or rheological properties (e.g. the storage modulus, G′,

or phase shift, δ) of a coagulating sample (Bohlin *et al.*, 1984; Guinee, *et al.*, 1994; Sharma *et al.*, 1994). However, the RCT gives no direct information about the kinetics of gel formation and textural characteristics or the firmness of the gel (Culioli & Sherman, 1978).

2.2.2 Gel strength or firmness

Gel strength is a useful description for describing the rheological properties of milk. The speed at which the milk coagulum forms and its firmness are factors that considerably affect the texture and yield of cheese. Consequently, these factors are frequently used as parameters to evaluate the suitability of milk, which has been submitted to physical treatments such as heat treatment, homogenization and concentration by UF, for cheesemaking (Masi *et al.*, 1988).

The gel starts to form at the gelation point and the process can be followed quantitatively thereafter by measuring the increase in firmness. Initially, micelle chains are formed and these begin to aggregate into a loose network. The network then extends and becomes more differentiated, so that the chains of micelles appear to strengthen. Initially, many micelles are joined by bridges, but later these appear to contract, bringing the micelles into closer contact and eventually causing partial fusion. The increase in gel firmness is due to increase in both number and strength of bonds between micelles (Green & Grandison, 1993).

Casein gels are viscoelastic, i.e. they have both viscous (liquid-like) and elastic (solid-like) components. Gel strength can be described by the storage modulus (G') and loss modulus (G'') (Bohlin *et al.*, 1984). G' represents the elasticity or stiffness of the gel while G'' is related to the viscous contribution. Both G' and G'' increase after the RCT resulting in an increase in gel firmness. Milk composition and processing treatments, such as heat treatment, homogenization and UF have been reported to influence the firmness of rennet gels (Green *et al.*, 1983; Dalgleish, 1993; McMahon *et al.*, 1993).

2.3 Factors effecting rennet coagulation

Several factors such as milk composition and processing treatments have been reported to influence the rennet coagulation of milk (Green *et al.*, 1983; Dalgleish, 1992; McMahon *et al.*, 1993; Lucey, 1995). These effects may be on the enzymatic primary phase, or on the aggregation secondary phase or both. Some of these effects are summarized below.

2.3.1 Effects of heat treatment on rennet coagulation

It is well known that heat induced interactions occur between denatured whey proteins and κ-casein (Mohammad & Fox, 1987; van Hooydonk *et al.*, 1987; Singh *et al.*, 1988), which causes the casein micelles to become (at least partly) covered with denatured β-lactoglobulin (and α-lactalbumin). Milk that has been heated at temperatures above 70°C has a longer coagulation time and forms a weaker gel than the original unheated milk (McMahon *et al.*, 1993; Lucey, 1995). Consequently, milk that has been heated to beyond pasteurization temperatures is not normally suitable for cheese making (McMahon *et al.*, 1993).

Normally, milk used in the manufacture of most cheese varieties is not heat-treated to any significant extent since the rennetability of milk is impaired and a weak curd is formed. The main reason for heating milk severely for cheese making is to incorporate denatured whey proteins into the cheese, which offers an attractive means to increase cheese yield (Banks, 1988). However, it has proved difficult to produce good quality cheese from severely heated milk.

(i) Effect of heat treatment on rennet coagulation time

Several researchers (Wilson & Wheelock, 1972; Wheelock & Kirk, 1974; van Hooydonk *et al.*, 1987; Lucey *et al.*, 1993; McMahon *et al.*, 1993) reported that the RCT of milk increases with the severity of the heat treatment. However, there are conflicting reports about whether the increased RCT is due to impairment of the enzymatic or aggregation reactions, or both. Wheelock and Kirk (1974) concluded that the effect on RCT was by inhibition of the primary action of rennet. Wilson and Wheelock (1972) suggested that not only the rate of the primary phase might be reduced by the formation of complexes, but also this phase might be limited by a reduction in the amount of peptides, which were released by rennin. van Hooydonk *et al.* (1987) found also that heating decreased both the initial velocity of κ-casein hydrolysis and the

amount of hydrolysable κ-casein in milk. However, Marshall (1986) reported that the enzymatic stage is hardly affected by heating and that the major effect of heating is to hinder the aggregation stage.

It is generally agreed that the principal factor responsible for the increased RCT of heated milk is the extent of denaturation of β-lactoglobulin and the complex formation between κ-casein and β-lactoglobulin (Morrisey, 1969; Singh *et al.*, 1988, Dalgleish, 1990). Dalgleish (1990) reported that the effect of protein denaturation on the RCT is dependent on the extent to which the whey protein had been denatured, not the temperature at which the denaturation had occurred.

Alternatively, heating may also cause more extensive changes in the micellar structure itself. It is, for example, possible that heating causes changes in the distribution of calcium phosphate in the micelles and serum, which render the renneted micelles less susceptible to aggregation (van Hooydonk *et al.*, 1987; Dalgleish, 1993). Heating causes the precipitation of calcium phosphates with a concomitant reduction in soluble calcium, resulting in inhibition of the aggregation reaction which is very sensitive to changes in Ca^{2+} concentration (Casiraghi *et al.*, 1987). Lucey *et al.* (1993) reported that the structure of heated-precipitated calcium phosphate may differ from that of indigenous colloidal calcium phosphate (CCP), especially in milk heated at very high temperatures (e.g. 120°C). Furthermore, heat treatment of milk at temperatures in the range 80 to 150°C induces changes in casein itself, such as (1) dephosphorylation, (2) proteolysis, (3) covalent bond formation, and (4) changes in casein micellar structure. Green and Grandison (1993) reported even if the level of whey protein denaturation was kept constant cheesemaking parameters appear to be affected more at higher heating temperatures.

(ii) Effect of heat treatment on gel strength

The strength of renneted milk gels is also adversely affected in heated milk (van Hooydonk *et al.*, 1987; Singh *et al.*, 1988, Lucey *et al.*, 1993; Waungana, 1995). The reduction of gel strength is presumably caused by the disruption of the continuity of the gel network caused by attachment of denatured whey proteins onto the casein micelles. The denatured whey proteins

may sterically hinder the close approach and contact between micelles, resulting in a weaker, looser network due to reduced crosslinking (van Hooydonk *et al.*, 1987; McMahon *et al.*, 1991; Lucey, 1995; Waungana, 1995). Lucey (1995) reported that the aggregation rate of heated milk was lower than unheated milk, which means that its gel strength increases at a lower rate than in unheated milk; thus the strength of the rennet-induced gels from heated milk, determined at any particular time after renneting, will be lower than that from unheated milk. McMahon *et al.* (1993) also found that in severely heated milk the extensive coverage of the micellar surface by denatured whey proteins altered the mechanism governing aggregation, resulting in a softer gel.

2.3.2 Effects of homogenization on rennet coagulation

Homogenization is necessary for recombining skim milk powder and milk fat and therefore it is an integral part in the manufacture of recombined cheese (Mulder & Walstra, 1974; Gilles & Lawrence, 1981). The objective of homogenization is to achieve a homogeneous mix between the reconstituted skim milk and milk fat, but there is still considerable debate as to how this process can best be carried out. However, it is generally agreed that the homogenization temperature must be greater than 40°C, i.e. above the melting point of milk fat (Mulder & Walstra, 1974). Homogenization can be carried out either before or after the heat treatment of milk by subjecting milk to high pressure. The process causes major physical changes in milk fat globules, including a reduction in fat globule diameter, an increase in surface area and number of fat globules, as well as an alteration in fat globule membrane composition by adsorption of casein micelles and serum proteins onto the newly created milk fat globule surface (Mulder & Walstra, 1974; Walstra & Jenness, 1984).

The size of fat globules is reduced, due to the intense turbulence and cavitation that occurs during homogenization, from the average diameter of 5-6 μm in native milk to < 3 μm after homogenization. This increases the fat globule interfacial surface area by a factor of 5-6. Simultaneously, fat globules become coated with a layer of casein and other milk proteins creating a new membrane at the interfacial between fat droplets and the serum phase of milk (Oortwijn *et al.*, 1979). This interfacial membrane consist of a protein composite containing casein micelles, casein subunits, and some serum proteins (Darling & Butcher, 1978). The presence of these micellar subunits at the interface implies that the creation of the new fat

globule membranes disrupts some casein micelles into subunits. This is not caused by the pressure drop that milk undergoes during homogenization but rather by the interfacial forces generated by the creation of the new interface. These subunits, however, appear to remain intact on the interface (Darling & Butcher, 1978). Thus, owing to homogenization fat globules acquire surface properties somewhat similar to casein micelles (Dalgleish, 1984). The role of fat globules during milk coagulation is thus different when homogenized milk is renneted compared to unhomogenized milk (McMahon *et al.*, 1993).

The effects of homogenization on milk components and rennet coagulation properties have been extensively reviewed by Peters (1964), Harper (1976), Walstra and Jenness (1984) and Jana and Upadhyay (1992). The amount of adsorbed materials, fat globule size and membrane composition are influenced by the conditions of homogenization such as homogenization pressure, temperature and fat concentration (Mulder & Walstra, 1974). High homogenization pressure causes increased adsorption of protein onto fat globule surfaces.

When homogenization is used to produce recombined milk for cheesemaking, a low homogenization pressure of 34.5 bar is recommended to minimize the undesirable effects on gel structure. For most natural rennet cheese varieties, high homogenization pressures adversely affects the RCT, curd strength, curd melting and the characteristics of the resultant products. When cheese is made from whole milk, fat globules are trapped within the casein matrix of the curd, and the desired properties of the cheese can be achieved by the normal processing steps for that cheese varieties. Whereas, when homogenized milk is used, the new artificial fat globule membrane participates in the casein matrix and often prevents the development of the desired properties in the cheese by acting as a permanent cross-link (Xiong & Kinsella, 1991a, b). Cheese curd becomes difficult to fuse together, resulting in impaired cheese texture. Consequently, cheese manufactured from homogenized milk has different rheological properties from that made from non-homogenized milk.

The differences in the rennetability of homogenized milk compared to that of normal milk is due mainly to the differences in composition of the fat globule membrane. The composition of the artificial fat globule membrane in homogenized milk is very different from that of the natural membrane of native milk fat globules (Mulder & Walstra, 1974). In natural milk, the

fat globule membrane consists mainly of specific proteins (almost half of the membrane material) and phospholipids (roughly one-third) (Dalgleish, 1993), while homogenized fat globule membranes that are formed during homogenization contain primarily casein and some serum proteins, mainly β-lactoglobulin (McPherson *et al.*, 1984; Dalgleish, 1992). McMahon *et al.* (1993) also found that homogenization cause the fat globule droplets to be coated with casein micelles and become an integral part of the protein network.

When rennet was added to homogenized milk, it coagulated more quickly than non-homognized milk (Green *et al.*, 1983; Robson & Dalgleish, 1984). Robson and Dalgleish (1984) reported that the coagulation of homogenized milk was controlled by the same factors as skim milk even through the coagulation time is shorter for homogenized milk compared to skim milk. They found that homogenization does not make κ-casein more susceptible to enzyme hydrolysis and the maximal rate of aggregation for both skim milk and homogenized milk is achieved after about the same exposure to rennet.

(i) Effect of homogenization on rennet coagulation time

Information in the literature on the effect of homogenization on RCT is conflicting. El-Salam and El-Shibiny (1982) reported that homogenization of milk caused no increase in RCT, while Green *et al.* (1983), Robson and Dalgleish (1984) and McMahon *et al.* (1993) found that homogenization slightly reduced the RCT, with the effect being more marked with increasing homogenization pressure. They attributed this effect to the structural alterations in casein micelles. Casein micelles become less stable as a result of being adsorbed as part of the new membrane around fat globules. Altered casein micelles are immobilized and more susceptible to coagulation by rennet (Oortwijn *et al.*, 1979). Furthermore, McMahon *et al.* (1991) reported that a smaller extent of hydrolysis was needed to initiate aggregation of homogenized milk compared to non-homogenized milk because the casein is spreaded thinly over the surface of homogenized fat globules, so its stabilizing power is reduced. However, Lapshina *et al.* (1978) reported that the RCT is delayed when milk is homogenized at very high pressures.

(ii) Effect of homogenization on gel strength

It has long been known that the use of homogenized milk for cheesemaking leads to the formation of weak curd. The aggregation of the casein particles and curd fusion are all slower than normal (Green *et al.*, 1983), and its structure is altered (Culioli & Sherman, 1978; Jana & Upadhyay, 1992). Alteration in the fat globule size distribution may also affect curd formation and structure during initial cheesemaking (Mackey *et al.*, 1991).

To date, no single explanation for the weakening of gel strength by homogenization of milk has been accepted. Several researchers found that homogenization of milk reduced the curd tension resulting in a weaker coagulum (Maxcy *et al.*, 1955; Sasaki & Miyasawa, 1955; McMahon *et al.*, 1993) and the effects became more pronounced with increasing homogenization pressure. The weaker coagulum from homogenized milk has been attributed to (i) greater dispersion of fat in the curd serving as points of weakness in the gel structure (Doan 1954; Peters, 1964; Harper, 1976), (ii) reduction of number of free casein particles available to form a strong gel network since some caseins are absorbed onto the interface of the newly formed fat globules (Doan, 1954; Maxcy *et al.*, 1955; Harper, 1976; Mackey *et al.*, 1991), and (iii) physical breakdown of casein during subsequent cheesemaking (Nicholas, 1947; Iwaida & Tsugo, 1961). These factors may be cooperative and their effect may be additive. Another possible cause may be the release of the lecithin-protein complex from the original fat surface and the interaction of casein with these complexes, resulting in a change in the action of rennet on homogenized milk (Peters, 1964).

Other reports claim that the firmness of the coagulum (Eisele & Bundy, 1964) increases with homogenization pressure. Storey *et al.* (1983) observed that homogenization at 46°C, with a pressure of $\geq$ 70 bar caused an increase in coagulum strength compared to non-homogenized milk. Proteins adsorbed on fat particles disrupt the continuity of the gel structure and increase the volume of network strands and hinders strand rearrangements which help reinforce the network and increase the firmness of the gel, once if has formed (Paters, 1964; Green *et al.*, 1983; McMahon *et al.*, 1993)

2.3.3 Effects of ultrafiltration on rennet coagulation

Ultrafiltration is a useful technique for increasing the production yield, through the recovery of whey proteins in the cheese. The most significant effect of ultrafiltration on the milk system is an increase in protein concentration, an increase in the number of casein micelles per given volume of milk and simultaneous decrease in the aqueous phase, which have major influences on the rennet coagulation properties.

It is generally agreed that concentration by ultrafiltration results in a decrease in the degree of κ-casein proteolysis during the enzymatic phase (Garnot & Corre, 1980; Garnot *et al.*, 1982; Mehaia & Cheryan, 1983; van Hooydonk *et al.*, 1987). The number of "reactive sites" created by rennet also increases with concentration and as well the mean free path between micelles is decreased. This may resulted in an increase in the number of effective collisions (i.e. those between reactive sites leading to bridging) between para-casein micelles (Sharma *et al.*, 1990; McMahon *et al.*, 1993, Waungana, 1995). The number and strength of bonds within the gel network and the rate at which they are formed also increases resulting in higher rates of gel firming and stronger gels. Dalgleish (1993) reported that the aggregation phase of ultrafiltered milk commences at a lower degree of proteolysis (about 50%) than that observed in unconcentrated milk (80 - 90%).

(i) Effect of ultrafiltration on rennet coagulation time

Some researchers (Darling & van Hooydonk, 1981; Green & Morant, 1981; Reuter *et al.*, 1981; Mehaia & Cheryan, 1983; Lucisano *et al.*, 1985) have reported a reduction in RCT for ultrafiltered milk. Others (Chaplin & Green, 1980; Garnot & Corre, 1980; Garnot *et al.*, 1982; Payens, 1984) obtained an increase in coagulation time after milk was ultrafiltered. However, Dalgleish (1981) reported that ultrafiltration has no effect on the RCT. It is evident that the different methods used to determine the RCT and the different experimental conditions (e.g. type of enzyme used and renneting pH) and also the precision and sensitivity of the methods used to detect the RCT, contribute to the reported discrepancies.

(ii) Effect of ultrafiltration on gel strength

It is generally accepted that there is a rapid increase in the rate of coagulation and final gel strength following renneting of ultrafiltered milk; the higher the protein concentration, the firmer the coagulum (Dalgleish, 1980; Green *et al.*, 1981; Payens, 1984; Waungana, 1995). They attributed the increased rate of aggregation and final gel strength to an increased protein concentration (5% protein), reduced pH (Guinee *et al.*, 1994) and increased total as well as ionized calcium.

(iii) Effect of heat treatment of ultrafiltered milk

Green (1990) and McMahon *et al.* (1993) observed a significant greater whey protein denaturation in ultrafiltered concentrated milk than in normal milk. However, Waungana (1995) found that whey protein denaturation in ultrafiltered concentrated milk was comparable to that in normal milk. McMahon *et al.* (1993) further reported that more protein material was adhered to the casein micelles after heat treatment of concentrated milk as compared to normal milk. They attributed this to a higher level of whey protein denaturation in ultrafiltered milk as well as the availability of less water for dispersion of casein micelles. The basic mechanism of the formation of rennet gels in heated ultrafiltered milk is presumably similar to that proposed for heated milks except that the adverse effects of heat treatment are compensated by the increased casein concentration.

As ultrafiltration and heat treatment have opposite effects on the cheesemaking properties of milk, it may be advantageous to combine these two processes. Furthermore, the two process increase cheese yields by different means; the ultrafiltration process offers the possibility of transferring the native whey proteins to the cheese while heat treatment transfers the denatured whey protein (Novak, 1992). Combining these two processes should give additional yield increases (Green, 1990). Sharma *et al.* (1990) found that heat treated ultrafiltered milk retained its coagulation and gel firming properties, despite a slight decrease in the rate of κ-casein hydrolysis. Anis and Ernstrom (1984) recommended heating ultrafiltered milk to 82°C for 30 min prior to cheesemaking to produce best quality Domiati cheese. Green (1990) suggested that heating ultrafiltered milk may have some beneficial effects, in particular in allowing yield to be maximized.

Waungana (1995) reported that heat treatment of ultrafiltered concentrated milk at the temperature in the range of 80 to 140°C for 4 s resulted in lower gel strengths, while the gelation time did not significantly changes at those temperature, except at the severe heat treatment, i.e. at the temperature of 140°C the GT increase. He attributed this to the extent of denaturation of β-lactoglobulin and its association with the casein micelles. However, insufficient information is available on the effect of heat treatment of UF milk on its rennet coagulation properties. The role of the whey proteins in ultrafiltered cheeses is more complex if the milk is heated, since the extent of heat treatment has a great effect on the denaturation of the whey.

(iv) Effect of homogenization of ultrafiltered milk

Green *et al.* (1983) found that some of the undesirable renneting characteristics of homogenized milk were improved when ultrafiltered milk was used. The aggregation of casein micelles during coagulation of homogenized ultrafiltered milk was slower and a finer structured gel was formed in comparison with non-homogenized milk. In contrast, McMahon *et al.* (1993) observed no differences in the firmness of gel made from non-homogenized and homogenized concentrated milk. They attributed this phenomenon to the increased solids content of concentrated milk, which produces a gel network of much greater volume density so that any further changes because of incorporation of fat into the network are not significant in increasing gel firmness. However, more information is required on the effect of homogenization on the rennet coagulation properties of ultrafiltered milk.

2.4 Milk protein concentrate (MPC) and recombined cheese

Milk protein concentrate (MPC) is a high protein spray-dried milk powder manufactured from skim milk by means of membrane separation. The manufacture of MPC is based on protein concentration by ultrafiltration (UF), preservation by heat treatment, and water removal by evaporation and drying. By using UF technology at relative low temperatures, milk proteins are concentrated at the natural pH (~ 6.6), which means that caseins and whey proteins are present in their native form except for those produced using severe heat treatments to denature whey proteins (Novak, 1992; Soo, 1994).

MPC-56 contains 56% protein in which casein and whey proteins are present in their original proportions in milk, i.e. approximately 82% caseins and 18% whey proteins. It can be used as an alternative to skim milk powder in countries with insufficient milk production, particularly where long distance transport is involved. MPC-56 has been used extensively to standardize the protein content in normal milk and also in the recombined cheese industry. A variety of recombined cheeses, such as Feta cheese, Processed cheese and Mexican-style cheese, can be successfully manufactured using MPC powder (Soo, 1994). A process of manufacturing recombined cheeses from MPC is based on recombination of the powder with milk fat, homogenization, pasteurization and rennet addition.

Gilles and Lawrence (1981) reported that a critical factor for recombined cheese manufacture is the type of milk powder used. Heat treatment during the manufacture of milk powder not only denatures whey proteins but also decreases the ionic calcium level which in turn affects milk coagulation. Soo (1994) also reported that some denaturation of whey proteins occurs during the manufacture of MPC powder due to the preheat treatment step after ultrafiltration. This alteration in the milk system may affect the functional properties of MPC powder and also its rennetability. Gilles (1974) reported that the firmness of the renneted gel made from recombined milk decreased as the severity of the heat treatment that the milk powder had undergone increased. However, Novak (1992) suggested that the use of MPC powder, prepared in a suitable way to retain good rennetability, presented a good possibility for substitution of skim milk powder in the manufacture of recombined cheese. Consistent quality, less water addition and higher production yields, due to the incorporation of whey protein, can be obtained. The production process of recombined cheese manufacture has been extensively reviewed in IDF Special Issue No. 9001 (1990).

Even though, the use of MPC powder in the recombined cheese manufacture appears attractive, no systematic studies have been carried out on the factors that determine the physical and rennet coagulation properties of cheese milks made using MPC. It is expected that preheat treatment given to the milk during MPC production, heat treatment and homogenization of cheese milk will have an influence on the rennetability of recombined cheese made from MPC powder. The study of these factors may improve the utilisation of MPC in the recombined cheese industry and suggest ways to produce new varieties of cheese.

CHAPTER 3

OBJECTIVES

- To determine the influence of heat treatment of recombined cheese milk made from MPC-56 and anhydrous milk fat on:
  - (i) denaturation of whey proteins
  - (ii) viscosity and shear stress
  - (iii) rennet coagulation properties

- To determine the influence of preheat treatment during MPC-56 powder manufacture on the above changes

- To determine the influence of homogenization on rennet coagulation properties of recombined cheese milk made using MPC-56 and anhydrous milk fat

- To relate the denaturation of whey proteins to the viscosity and rennet coagulation properties of recombined cheese milk

- To investigate the permeability and microstructure of rennet-induced gels made from the above milks

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Milk protein concentrate powder (MPC-56)

Commercial MPC-56 powder, manufactured by Kiwi Co-operative Dairies, Hawera, New Zealand, was obtained from the New Zealand Dairy Broad. Some MPC-56 powders were produced using different preheat treatments at the New Zealand Dairy Research Institute (NZDRI), Palmerston North, New Zealand. The general MPC powder manufacturing process is shown in Figure 4.1.

For the manufacture of commercial powder, whole milk was thermalized at 65°C for 15 s, cooled to 58°C and the cream was separated. The skim milk was then cooled to 10°C and ultrafiltered. The UF concentrate was subjected to a heat treatment at 85°C for 40 s and evaporated to 36% total solids using a four-effect falling film evaporator. The concentrates were then dried to approximately 4% moisture content using a spray drier and fluidized bed drier.

In the case of powders manufactured using various preheat treatments, skim milk was ultrafiltered to a volume concentration factor of 2.3X with a recirculation temperature of approximately 50°C using a Koch pilot plant (Koch S-2-4″ spiral membranes, Auckland, New Zealand). The retentate was then directly heated to the required temperature and holding time to achieve the specified heat treatment as shown in Table 4.1. Evaporation was carried out in a falling film evaporator (three-effect, Wiegand Gmbh, Karlsruhe, Germany). The concentrate was then spray dried to approximately 4% moisture in a Delaval spray drier (Delaval Separator Company, River Falls, Wisconsin, USA). The compositions of the MPC powders, both commercial and those made using various preheat treatments are shown in Table 4.1.

4.1.2 Fresh frozen milkfat for recombination (FFMR)

Fresh Frozen milkfat for recombination (FFMR), which contained 99.95 % milkfat and 0.05% moisture, was obtained from the New Zealand Dairy Board, Wellington, New Zealand.

Table 4.1 Compositions of milk protein concentrate MPC-56

Composition	Preheat Treatment used in the MPC Manufacture							
	Commercial	Preheat Treatment Powders						
	85°C for 40 s	75°C for 15 s	85°C for 15 s	85°C for 60 s	85°C for 120 s	85°C for 180 s	85°C for 240 s	120°C for 120 s
Main Components (g/100 g)								
Protein (N x 6.38)	56.2	55.7	55.1	55.5	55.3	55.8	55.5	55.3
Lactose	32.0	33.4	33.4	32.8	33.2	33.2	33.5	33.4
Fat	1.1	1.2	1.2	1.2	1.2	1.4	1.2	1.0
Ash	7.6	7.8	7.9	7.9	7.9	7.9	7.9	7.9
Moisture	3.2	4.0	4.0	3.6	3.6	3.6	4.2	4.0
Minerals (mg/100 g)								
Calcium	2032	1972	1908	1980	1986	1928	1928	1960
Phosphorus	1242	-	-	-	-	-	-	-

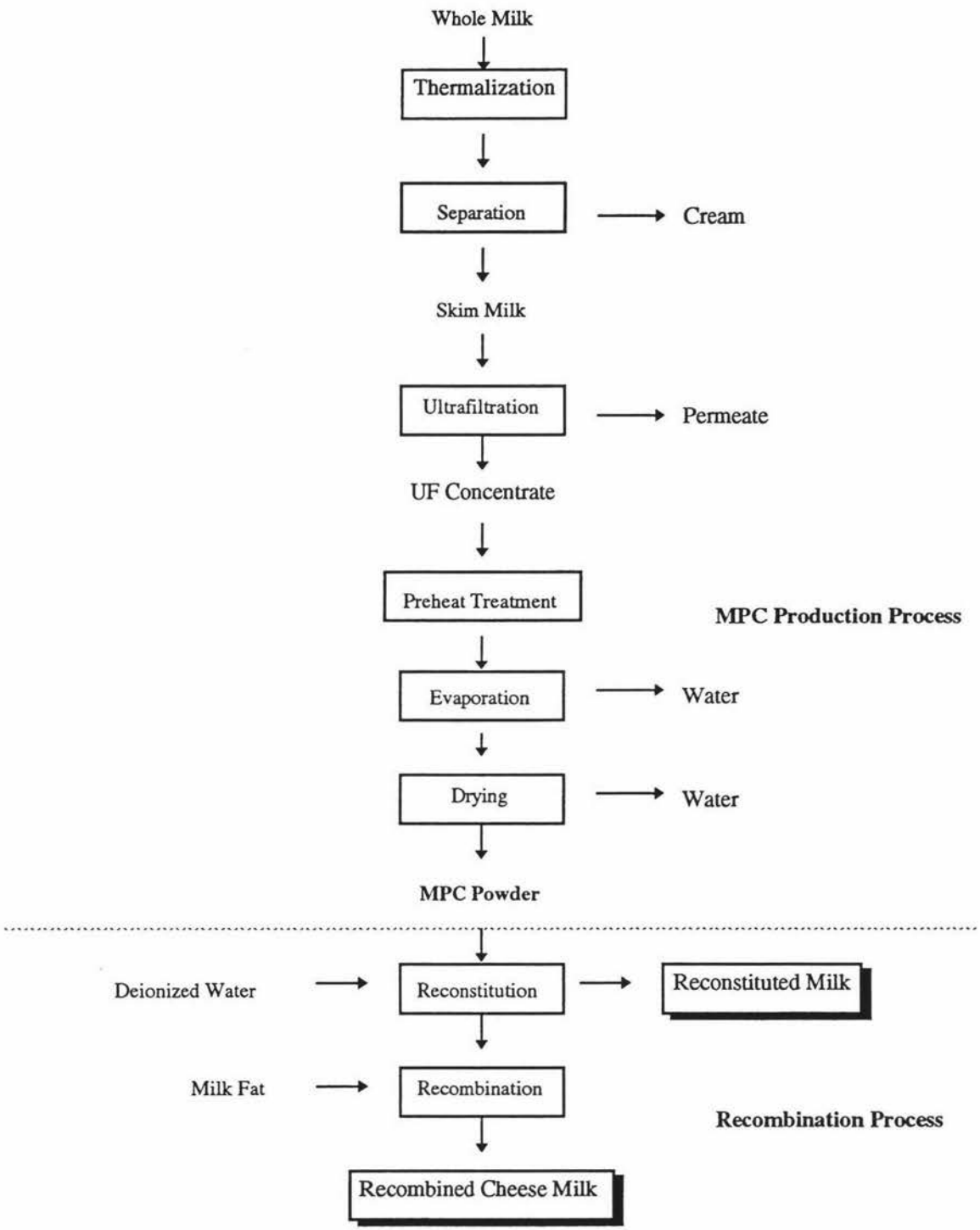


Figure 4.1 Schematic diagram of the MPC manufacture and recombination process used in the study.

4.1.3 Rennet source

Commercial calf rennet which contained 59 RU/ml was obtained from the New Zealand Co-operative Rennet Company (Eltham, New Zealand). This rennet was freshly diluted 1:10 with deionized water and used at the rate of 160 µl/100 ml milk.

4.1.4 Sodium azide

An antibacterial agent, sodium azide (Fisher Scientific Co., Don Mills, Intro, Canada), was added to all the milk samples at the rate of 0.02% before the sample were stored in a chilled room at 5°C.

4.2 Processing methods

4.2.1 Preparation of reconstituted skim milk (20% total solids)

Reconstituted skim milk was prepared by dissolving MPC-56 powder in deionized water at 45°C to give 20% total solids (w/w) concentration. Mixing was continued for 20 min using an overhead stirrer to ensure that all the powder had been dissolved.

4.2.2 Preparation of recombined cheese milk (40% total solids)

To prepare 1000 g recombined cheese milk, 200 g of MPC powders were reconstituted with 600 g deionized water at 45°C using an overhead stirrer. Fresh frozen milkfat for recombination (FFMR) (200 g) was then added to the reconstituted skim milk to give the final concentration of 40% total solids (w/w). The mixture was heated to 60°C and homogenized. Sodium azide (0.02%) was added and the samples were kept overnight at 5°C before analysis. The experimental process is shown in Figure 4.1

4.2.3 Homogenization

Homogenization was carried out in a two-stage homogenizer (Rannie, Model Lab 100, Cophenhagen, Denmark).

The milk sample was heated to approximately 60°C and fed through the homogenizer. Particular care was taken to avoid air inclusion and the samples were taken only when the homogenization pressure was constant.

Standard recombined cheese milks were homogenized at 120 and 50 bar for the first and second stage, respectively. To study the effect of homogenization, the pressure was varied from 100 to 250 bar for the first stage, while the second stage was kept constant at 50 bar.

4.2.4 Heat treatment

Recombined cheese milk was heated at temperatures in the range 75 - 85°C using a mini UHT plant (Spiral flow indirect UHT plant, Alfa-Laval, Australia). The UHT plant used indirect heating through a spiral heat exchanger to heat the samples to the required processing temperature. After the product reached the required temperature, it was then transferred to a controlled temperature water bath and held for the desired holding time. After heating, milks were cooled rapidly to approximately 20°C by immersion in an ice-water bath.

4.2.5 pH Adjustment

The pH of milk was adjusted to 6.5 by the slow addition of 1N HCl or 1N NaOH at 20°C with vigorous stirring. The pH was measured using a combined glass-calomel electrode and Orion 720 A pH meter (Orion Research, Boston, MA 02129 USA) calibrated at pH 4 and 7.

4.3 Analytical methods

4.3.1 Whey protein denaturation

To determine total nitrogen, heated milk samples and unheated controls were adjusted to pH 4.6 with acetic acid/acetate buffer (10% acetic acid and 1N sodium acetate) to precipitate the casein and denatured whey protein. The solution was then filtrated through a Whatman No. 1 filter paper (Fisher Scientific, Pittsburgh, PA). The non-protein nitrogen (NPN) was measured as nitrogen soluble in 12% trichloroacetic acid.

Total nitrogen and NPN contents of the supernatants were determined using the Kjeldahl method (IDF, 1964). The supernatants were digested using a Kjeltec Digester (Kjeltec 1007 Digester, Tecator, Sweden) and distilled using a Kjeltec system (Kjeltec 1026 Distilling unit, Tecator, Sweden). The concentrations of whey protein nitrogen in the supernatants were calculated by subtracting the NPN values from the Total N value. An empirical factor of 6.38 was used to convert whey protein nitrogen to whey protein concentration (WP).

$$WP = (Total\ N\ in\ the\ supernatant - NPN\ in\ the\ supernatant) \times 6.38$$

The percentage of whey protein denaturation in each sample was then calculated using the following formula:

$$\%\ Denaturation\ of\ whey\ protein = \frac{WP_{unheated} - WP_{heat}}{WP_{unheated}} \times 100$$

The powder which had been given the lowest heat treatment (75°C for 15 s) was considered to be the control sample, i.e. virtually no whey protein denaturation. This was because the original unheated milk used for MPC production was not available.

4.3.2 Fat globule size measurement

The size distributions of the fat globule in recombined cheese milk were determined using a Malvern MasterSizer (Model MS 20, Malvern Instruments Ltd., Malvern, Worcestershire, UK) at the New Zealand Dairy Research Institute (NZDRI), Palmerston North. Measurement were made in two different media; deionized water and dissociating solution (2% SDS and 0.5 M EDTA dissolved in deionized water). The dissociating solutions can be used to measure the "primary" size of the fat globule after most of the casein micelles had been dissolved, so it allowed the evaluation of the average volume-weighted fat globule diameter without the contribution of protein aggregates (Robin & Paquin, 1991).

In this method, a low power laser beam is diffracted by a diluted dispersion of the milk sample and is collected over a range of scattering angles by a series of semicircular photo-electric diodes. The volume size distribution is calculated from the intensity of light diffracted at each angle using the Lorenz-Mie theory. To calculate size distribution data successfully the

refractive index and absorbance of the particle being measured and the refractive index of the medium in which the particles dispersed are required by the MasterSizer programme.

The MasterSizer divides the size distribution into 22 classes across the submicron range (0.1-1.0 microns). In this study, the fat globule diameter was reported as the volume-moment average diameter, d_{43}, which was calculated based on the following equation:

$$d_{43} = (\Sigma n_i d_i^4 / \Sigma n_i d_i^3)$$

where n_i is the number of globules with a diameter d_i

A typical analysis of fat globule size distribution and the parameters obtained from Malvern MasterSizer MS 20 are shown in Appendix IV.

4.3.3 Polyacrylamide gel electrophoresis

Electrophoresis exploits the amphoteric nature of proteins which enables them to migrate under the influence of an electric field. This migration is influenced by the pH and differs for each protein due to the differences in their net charges and molecular weights. Therefore, if an electric field is applied to a mixture of proteins, they migrate at different rates dependent on their charge-to-mass ratio.

For the analysis of the residual native proteins, Native-PAGE was used. This uses a non-dissociating and non-reducing buffer system, so only proteins which still in their native monomeric form are separated. The procedure described by Andrews (1983) was used for Native-PAGE.

Native Polyacrylamide Gel Electrophoresis (Native-PAGE)

Undenatured whey protein in unheated and heated samples were separated by native-PAGE using the Bio-Rad Protean II system (Bio-Rad Laboratories, Richmond, Ca, USA) and Bio-Rad power supply unit (Model 1000/500, Bio-Rad, Richmond, Ca, USA). The native-PAGE

sample buffer contained 20% 0.5M Tris-HCl buffer, 10% glycerol and 1.25 mg of bromophenol blue (tracking dye).

Preparation of solutions

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

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

Resolving gel buffer

TRIS (Trishydroxymethylaminoethane, 36.3 g) was added to 90 ml of deionized water. The pH was then adjusted to 8.8 with 6M HCl. The solution was adjusted to 200 ml with deionized water and stored at 4°C.

Stacking gel buffer

TRIS (6.0 g) was dissolved in 60 ml deionized water. The pH was then adjusted to 6.8 with 1M HCl and the solution adjusted to 100 ml with deionized water. The buffer was stored at 4°C.

Sample buffer

500 ml of sample buffer was prepared by mixing 100 ml of stacking gel buffer with 300 ml of deionized water, 10 ml of 0.10% bromophenol blue and 40 ml of glycerol. The pH was adjusted to 6.8 and the buffer stored at 4°C.

Electrode buffer

TRIS (7.5 g) and glycine (36.0 g) were added to about 400 ml deionized water. The pH was adjusted to 8.3 after which the solution was made up to 500 ml with deionized water. For each electrophoresis run, 80 ml of this stock solution was diluted with 320 ml deionized water.

Gel Preparation

The resolving gel was prepared by mixing 10 ml of a 30% stock solution of *bis*-acrylamide mixture, 2.5 ml of resolving gel buffer and 7.5 ml of deionized water. The gel solution was degassed at 20°C for 15 min with continuous stirring. After which 10 µl of *N*, *N*, *N'*, *N'*

tetramethylenediamine (TEMED) and freshly made 10% (w/v) ammonium persulphate (100 µl) were carefully added and thoroughly mixed. The gel solution (3.3 ml) was then poured between two glasses casting plates (Bio-Rad Mini Protean, Bio-Rad Richmond, CA, USA). The deionized water was poured over the gel solution which was then left to polymerize at 20°C. The water was drained and the polymerised resolving gels were dried with filter paper before pouring the stacking gel.

The stacking gel was prepared by adding 1.25 ml of acrylamide stock solution to 2.5 ml of stacking gel buffer and 6.25 ml of deionized water. The gel solution was degassed for 15 min after which 10 µl of TEMED and 10% ammonium persulphate (50 µl) solutions were added and gently mixed prior to applying the solution to the top of the resolving gel. A 10-slot comb was then inserted. The gel was allowed to set before the comb was removed. The gel slots were washed with deionized water to remove any unpolymerized gel solution and the excess was removed with filter paper.

Gel electrophoresis

A pair of gels was fitted into a Mini-Protean II slab Electrophoresis System (Bio-Rad, Richmond, CA, USA). A stock solution of electrode buffer (400 ml) was then used to completely fill the inner buffer chamber and partially fill the outer buffer chamber. The sample to be analysed was diluted 1:1 (v/v) with Native-PAGE sample buffer and thoroughly mixed using a vortex mixer. 10 µl of sample was applied to the slots in the gel. The gels were run using approximately 200 V for about 75 - 90 min, until the Bromophenol blue tracking dye was close to the bottom of the slab. The gels were then removed from the plates and transferred to plastic containers in which the staining solution was present.

Staining/Destaining

After electrophoresis, the gel was stained for 1 h in 50 ml of Coomassie brilliant Blue R solution (1 g brilliant blue R dissolved in 500 ml of isopropyl alcohol and 200 ml acetic acid and the solution made up to 2 litres with distilled water). The gels were rocked to ensure uniform staining. After staining for 1 h, the staining solution was carefully drained and replaced with destaining solution which was made up of 100 ml isopropyl alcohol and 100 ml acetic acid which was diluted to 1 litre with distilled water. The destaining solution was

replaced after 1 h with fresh destaining solution and destaining was continued for another 19 h with rocking to ensure uniform destaining of the gels. After destaining, the gels were then scanned and photographed.

Scanning

The gels were scanned on a UltraScan XL model laser densitometer (LKB Produkter AB, Bromma, Sweden) immediately after the destaining procedure ended. In the densitometer, the protein bands on the stained gel were scanned by a narrow beam of laser light and the absorbance at 522 nm was plotted as a function of track distance. The output from the densitometer was quantified by measuring the areas under the individual peaks. The results were plotted as a graph of individual peaks and a table of individual peak areas was plotted by a printer attached to the densitometer. The amount of residual native whey proteins in each sample was expressed as a percentage of the original amount of native proteins in an unheated control.

4.4 Experimental procedures

4.4.1 Rennet coagulation properties

Renneting

A sample of recombined cheese milk was adjusted to pH 6.5 using 1M HCl or 1M NaOH and equilibrated at 34°C for 30 min in a controlled temperature water bath. It was then thoroughly mixed with diluted rennet (1:10) at the rate of 1.6 ml/l milk for 30 s.

Dynamic low amplitude oscillation test

(a) Theoretical background

The viscoelastic properties of the renneted milks were determined by sinusoidal oscillation in a controlled strain Bohlin VOR Rheometer (Bohlin Rheologi, Lund, Sweden) using low amplitude oscillation as described by Bohlin *et al.* (1984). The Bohlin VOR Rheometer is a computer controlled instrument working in three different mode performing; oscillation, viscometry and relaxation. The oscillation and viscometry modes were used in the present study.

The measuring system consisted of a Couette type cup and a fixed bob system. The bob is suspended using a torsion bar and a torque shaft is suspended on an air bearing. The viscoelastic properties of the gel, including the elastic (storage) modulus G', the viscous (loss) modulus G'' and the ratio of G'' to G' called the phase angle (tan δ) were measured.

During measurement, the cup is oscillated so that the sample is subjected to a harmonic, low amplitude shear strain, γ, of angular frequency ω and

$$\gamma = \gamma_o \cos \omega t$$

Where γ is shear strain, γ_o is the strain amplitude, ω is the angular frequency (i.e. $2\pi f$), f is the oscillation frequency, t is time in s and cos ω is a simple harmonic function. The applied shear strain results in an shear stress, σ, of the same angular frequency, but which is out of phase by the angle δ

$$\sigma = \sigma_o \cos (\omega t + \delta)$$

The storage (elastic) modulus, G', is a measure of the energy stored per deformation cycle. It is determined from the component of stress which is in phase with the strain and can be given by the equation

$$G' = (\sigma_o/\gamma_o) \cos \delta$$

The loss modulus, G'', the viscous part of stress, which is the part of stress out-of-phase with the strain, is defined as:

$$G'' = (\sigma_o/\gamma_o) \sin \delta$$

The phase angle (tan δ), which gives the ratio of the elastic and viscous elements of the gel is defined as:

$$\tan \delta = G''/G'$$

The tan δ is an important parameter indicating changes in the nature of bonds and their relative importance. The larger the value of tan δ, the more liquid-like the material is behaving and *vice versa* (van Vliet *et al.*, 1991).

For a perfectly elastic material, all the energy is stored and the stress and strain will be in-phase and G'' will be zero. For a liquid possessing no elastic character, all the energy is dissipated as heat and G' is zero and the stress and strain are out-of-phase. When viscoelastic gels are subjected to a sinusoidally oscillating strain, the stress is neither completely in-phase nor completely out-of-phase (Figure 4.2).

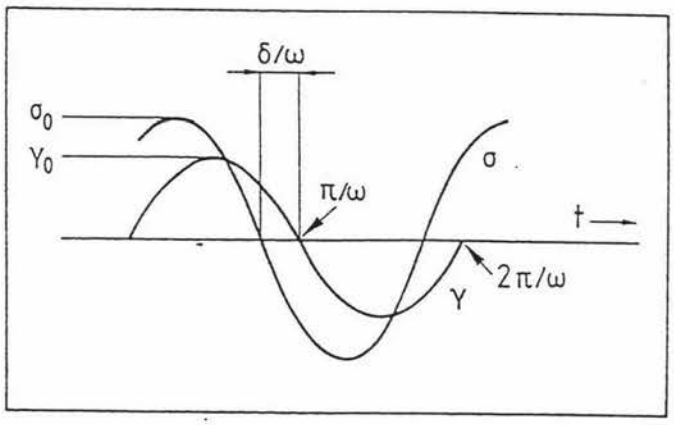


Figure 4.2 Shear strain (γ) and shear stress (σ) as a function of time during the dynamic rheological measurement of a viscoelastic material.

Dynamic oscillatory measurements can monitor changes in the rheological properties of samples during gelation. Oscillatory strain must be kept sufficiently low (within the linear viscoelastic region), in order to prevent disruption of the gel structure during measurement.

The gelation process is a transition phenomenon from a sol solution to an infinitely large network (gel) (Zoon *et al.*, 1988). The gelation time, GT, which marks the transition of milk from a sol to a gel, was evident from the sudden decrease of the phase angle. The storage modulus, G′, increases continuously after the gelation point.

(b) *Parameters used to monitor rennet coagulation properties*

Immediately after the addition of rennet, the milk solution (~13 ml) was carefully placed into the measuring system consisting of a C 25 cup (diameter 27.5 mm) and a bob (diameter 25 mm). A torsion bar of 315 g cm^{-1} was used. The bob was then slowly lowered into the cup until the milk solution just covered the top of the bob, a few drop of vegetable oil layered onto the surface of the sample to prevent evaporation during measurement.

To ensure that measurements were in the viscoelastic region, a low amplitude shear strain of 0.0206 was applied at a frequency of 0.1 Hz. Measurements were started 120 s after the addition of rennet and were continued with measurements every 60 s for 2 h. Table 4.2 shows the parameters selected to follow the rheological characteristics of rennet-induced gels.

Table 4.2 Dynamic oscillation parameters of the Bohlin VOR Rheometer used for gelation studies

Measuring System	C 25
Cup	27.5 mm Diameter
Bob	25 mm Diameter
Temperature	34°C
Frequency	0.1 Hz
Amplitude	10%
Strain	0.0206 rad
Torsion bar	315 g cm^{-1}
Sensitivity	1 x

In this study, the gelation time (GT) was determined as the point when the phase angle, δ, decreased suddenly and the G' had a value greater than 1 Pa. The storage modulus G', which represents the stiffness of the gel (van Vliet *et al.*, 1991), was used as an indication of gel strength and its value at 1 or 2 h after the addition of rennet was reported.

4.4.2 Frequency sweep

A frequency sweep was carried out after the oscillation test to determine how the rheological properties vary with the time scale of the applied deformation. This measures the gel properties at a wide range of frequencies.

In this study the frequency was varied from 0.001 to 1 Hz (ω from 0.006283 to 3.283 rad s^{-1}) with a strain of 0.0206 at 34°C. The frequencies were increased in seven steps from 0.001 Hz to 1 Hz and then subsequently decreased to 0.001 Hz. The rheological parameters G', G'', and phase angle, δ, were determined in all cases.

4.4.3 Viscosity test

The viscosity of recombined cheese milk (no rennet addition) was determined at 34°C using the Bohlin VOR Rheometer (Bohlin Rheologi AB, Lund, Sweden) with Couette cylinder by using a shear rate sweep from 18.5 to 731 s^{-1}. The C 25 concentric cylinders measuring system, consisting of a 25 mm diameter fixed bob and a 27.5 mm diameter rotating cup were used in the study. The temperature was maintained at 34°C and the sample (~13 ml) was carefully loaded into the cup until the sample just covered the top surface of the bob. The samples were equilibrated for one minute before initiating a shear rates sweep.

4.4.4 Penetration test

Coagulation of milk

50 ml samples of recombined cheese milk which had been adjusted to pH 6.5 were equilibrated at 34°C for 30 min in 100 ml glass beaker (internal diameter of 53 mm). The milk was gently stirred while 80 µl of diluted rennet (1:10) were added. The beakers were then covered with aluminium foil and held in a controlled temperature water bath at 34°C for 1.3 h before analysis.

Determination of yield force at yield point

The yield force at yield point was determined 1.3 h after rennet addition using an Instron Universal Testing Machine (Table Model 4502, Instron Corporation, USA).

The beaker containing the gel was placed on the compression plate of a load cell (compression type - 10 N) mounted at the base of the instrument. A 20 mm cylindrical probe was mounted underneath the crosshead. The crosshead was positioned so that the probe was within 0.5 mm of the surface of the milk gel. The position of the probe was set to zero before the crosshead was lowered. The crosshead was lowered at the rate of 10 mm/min into the renneted sample. The force exerted on the probe was measured by the load cell and recorded continuously by a computer data recorder. The yield force was defined as the maximum force (using N units) at the yield point (point on the curve with a zero slope). Four replicates were performed for each sample and the average of the results was taken.

4.4.5 Permeability

Preparation of rennet whey

Twenty litres of whole milk was warmed to 50°C in a controlled temperature water bath and the fat was removed using a pilot-scale centrifugal separator (Alfa-Laval; Model 103 AE, Hamilton, New Zealand). The skim milk was immediately cooled to 34°C. Diluted rennet (1:10) was then added to the skim milk at the rate of 1.6 ml/l milk and the renneted milk was allowed to gel for 1 h. After which, the curd was separated from the whey by centrifugation at 13,400 g at 4°C for 20 min. Rennet whey was filtered through a Whatman No.1 filter paper (Fisher Scientific, Pittsburgh, PA).

Permeability measurement

The permeability coefficient, B, was determined using the technique of van Dijk and Walstra (1986). Clean glass tubes (3.7 mm internal diameter and 25 cm length) with open ends were inserted into milk to which rennet had just been added at 34°C. After 1 h, the tubes were withdrawn, cleaned and transferred to a whey bath. The level of the rennet whey was higher than the height of the gel so that a pressure gradient was present in the gel and caused the serum to permeate upward through the gel as shown in Figure 4.3. The temperature of the milk serum was kept at 34 °C.

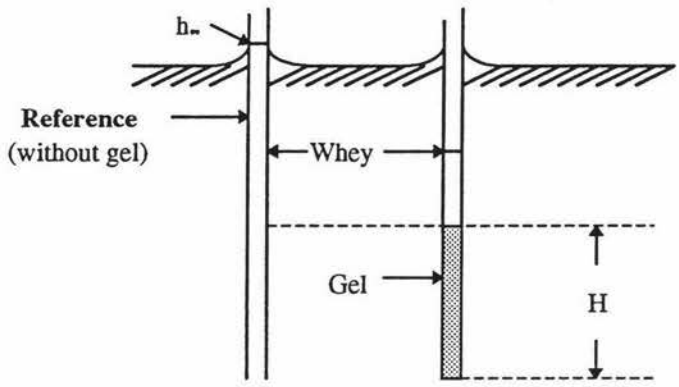


Figure 4.3 Schematic diagram of permeability measurement

The level of the serum in the tubes was measured over a period of time (usually for up to 1 day) and for each tube B was calculated between each subsequent measuring times using the following equation:

$$B = \frac{-\ln\left(\frac{h_\infty - h_{t2}}{h_\infty - h_{t1}}\right)\cdot\eta\cdot H}{\rho\cdot g\cdot(t_2 - t_1)}$$

Where:

B	=	permeability coefficient (m^2)
$h_{(\infty)}$	=	height of serum level in the empty reference tube (m)
$h_{(t)}$	=	height of serum level in the gel tube (m) at time t
H	=	length of the gel (m)
g	=	gravitational acceleration (ms^{-2})
η	=	viscosity of the serum (Pa s)
ρ	=	density of the serum (kg/m^3)

4.4.6 Microstructure

The microstructure of renneted-induced gel made from recombined cheese milk whcih was prepared from commercial (preheat treatment at 85°C for 40 s) and high-heat treatment powders (preheat treatment at 120°C for 180 s), homogenized at 120/50 bar without any additional heat treatment was investigated using the Confocal Scanning Laser Microscopy (CSLM).

A small quantity of fast green (BDH Chemicals) florescent protein dye was added to 100 ml recombined cheese milk. The milk solutions were then warm to 34°C for 30 min to allow the dyes to disperse. 160 µl of diluted rennet (1:10) was then added and a few drops of the rennted milk was added to special microscope slides with a concave hollow. A cover slide was placed over the milk sample and microscope slides were placed in a petri dish which was placed in an incubator at renneting temperature (i.e. 34°C). Approximately 1 h after the addition of rennet the microsteucture of the rennet-induced gels were examined by the Leica Confocal Microscopy (Medical School, University of Auckland) which has a PL Apo 63x oil immersion objective (numerical aperture N.A. = 1.4). The CLSM had a Argon/Krypton laser which was used with an excitation wavelength of 488 nm and detectery florescence at > 550 nm.

CHAPTER 5

EFFECT OF HEAT TREATMENT ON THE PHYSICAL AND RENNET COAGULATION PROPERTIES OF RECOMBINED CHEESE MILK

It is well established that heat treatments influence the functional properties of milk and milk products. Milk that has undergone heat treatment at temperatures greater than pasteurization tends to have longer gelation times and produce weaker gels (McMahon *et al.*, 1993; Lucey, 1995). Although the effects of heat treatment on milk are well established, there do not appear to be any published studies on the effects of heat treatment on the physical and rennet coagulation properties of recombined cheese milk made from milk protein concentrate (MPC) powder. This chapter describes the effects of various heating times and temperatures on the average fat globule diameter (d_{43}), denaturation of whey proteins, viscosity and shear stress, and rennet coagulation properties of recombined cheese milk made from MPC-56 powder.

RESULTS AND DISCUSSION

5.1 Effect of various heating times at three different heating temperatures

The effects of heating time were investigated on recombined cheese milks made from commercial MPC-56 powder, which had undergone a preheat treatment at 85°C for 40 s during the powder manufacturing process. Heat treatment was carried out after homogenization as described in Chapter 4. The heating temperatures were 75, 80, or 85°C for holding times of 3, 5, 7, and 10 min. Recombined cheese milk made from low-heat treatment powder that has not been heated to any extent after homogenization was used as the control. All the results are shown in Appendix II, Table 1 and the replicates are shown in Appendix III. Data for each of the individual sections is given in Tables or graphs in the following sections.

5.1.1 Effect on average fat globule diameter ($d_{4,3}$)

The effect of heating time on the average fat globule diameter, $d_{4,3}$ is shown in Table 5.1. The results show that heating time did not have a significant effect on the $d_{4,3}$ measured after dispersion of cheese milk in deionized water and dissociating solution, except when severe heat treatment was applied. The $d_{4,3}$ of recombined cheese milk, made using a homogenization pressure of 120/50 bar, were in the range 1.09 to 1.14 μm when measured using either deionized water or dissociating solution.

Table 5.1 Effect of various heating temperatures and times on average fat globule diameter, $d_{4,3}$, of recombined cheese milk. The $d_{4,3}$ was measured after dispersion of recombined cheese milk in deionized water or dissociating solution (SDS-EDTA).

Heat Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)	
	Deionized water	Dissociating solution
no-heat treatment	1.11	1.09
75°C for 3 min	1.11	1.10
75°C for 5 min	1.11	1.10
75°C for 7 min	1.10	1.09
75°C for 10 min	1.13	1.12
80°C for 3 min	1.12	1.11
80°C for 5 min	1.12	1.12
80°C for 7 min	1.12	1.11
80°C for 10 min	1.13	1.12
85°C for 3 min	1.13	1.12
85°C for 5 min	1.11	1.11
85°C for 7 min	1.14	1.13
85°C for 10 min	1.13	1.11

Severe heat treatment, at temperature of 80 and 85°C for 7 or 10 min, resulted in slight increase in $d_{4,3}$ of recombined cheese milk. To see whether there was an effect of heat treatment on the particle size distribution of recombined cheese milk, the volume-weighted diameter versus the volume frequency for recombined cheese milk without any heat treatment and with heat treatment at 80 for 5 min and 85°C for 10 min measured after dispersion of sample in deionized water were plotted (Figure 5.1).

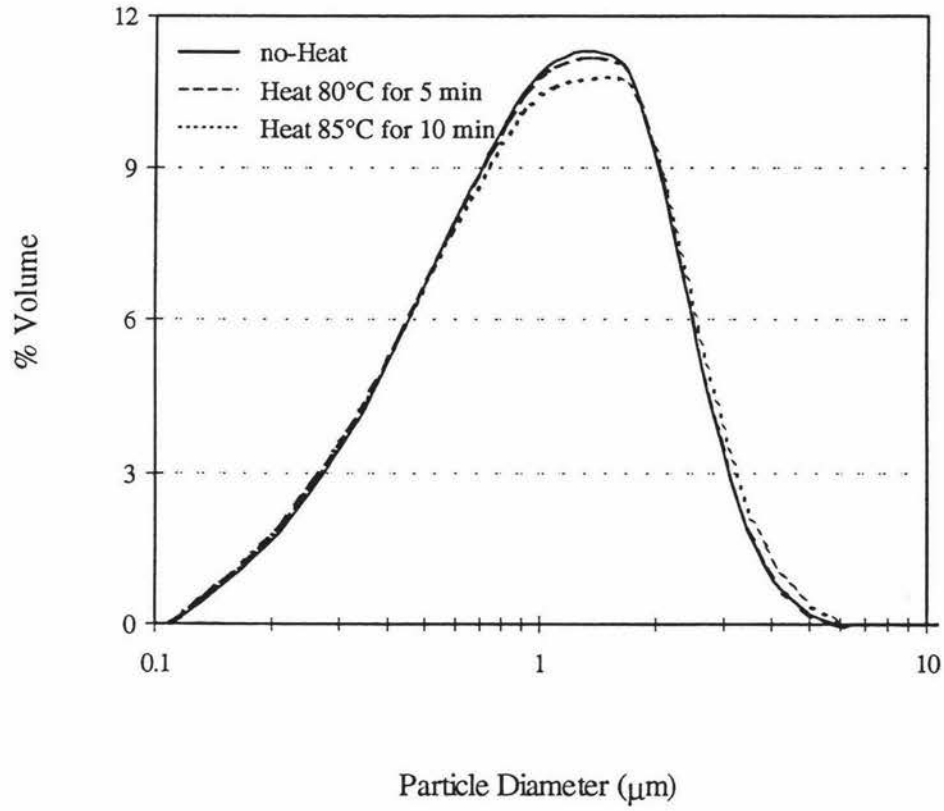


Figure 5.1 Fat globule size distribution in recombined cheese milks homogenized at 120/50 bar, without any heat treatment (——) and with heat treatment at 80°C for 5 min (— -) and 85°C for 10 min (-----). The samples were diluted with deionized water.

It was found that the particle size distribution was slightly altered when recombined cheese milk had undergone severe heat treatment, resulting in a shift in the fat globule distribution towards the slightly larger diameter.

Although it has been reported that heat treatment of fresh whole milk after homogenization influences the physical and chemical properties of the milk fat globule membrane (van Boekel & Walstra, 1989), no data has been reported in the literature on the recombined milk system which contains high total solids. However, experiments on heated homogenized cream (38% fat) showed that heating had almost no effect on the $d_{v,s}$ and the fat globule size distribution (van Boekel & Flokerts, 1991). Melsen (1989) also reported that heating of recombined cream (30% fat) caused no change in $d_{v,s}$ and globule size distribution. However, Fink and Kessler (1985) observed a small increase in d_{43} of 30% homogenized cream heated at temperature higher than 95°C, and the effect was dependent on heating temperature and holding times. The shift in the particle size distribution toward a larger particle diameter was also observed in their experiments. They attributed this effect to the coalescence of the fat globules and also the aggregation of milk proteins, which resulted in the formation of fat globule aggregates.

A slight increase in particle size observed in recombined cheese milk may be related to the higher concentration of fat and protein which favour protein aggregation or protein-fat cluster formation during heating. It may be possible that after homogenization, some of the fat globules formed casein-fat aggregates. Melsen (1989) and van Boekel and Walstra (1991) also observed the aggregation of casein at the fat globule surface in microscopic examination of the heated cream.

5.1.2 Effect on whey protein denaturation

The denaturation of total whey proteins in heated samples was examined by investigating changes in the amount of nitrogen soluble at pH 4.6 by Kjeldahl analysis. The extent of individual whey protein denaturation was also determined using Native-PAGE on the pH 4.6 supernatant derived from recombined cheese milk. Results were reported in terms of % denaturation of whey protein in the samples. Recombined cheese milk made from low-heat treatment powder without any additional heat treatment after homogenization was used as a

control sample, i.e. 0% denaturation, even though this powder probably had a small amount of whey protein denaturation due to heating during the powder production process.

The effect of heating time at the temperatures 75, 80, or 85°C for 3, 5, 7 and 10 min on the % denaturation of total and individual whey proteins are shown in Figures 5.2 and 5.3, respectively. As expected, the % denaturation of total whey proteins increased with increased heating time. The extent of denaturation of total whey proteins at 85°C was greater compared to lower temperatures, i.e. 75°C.

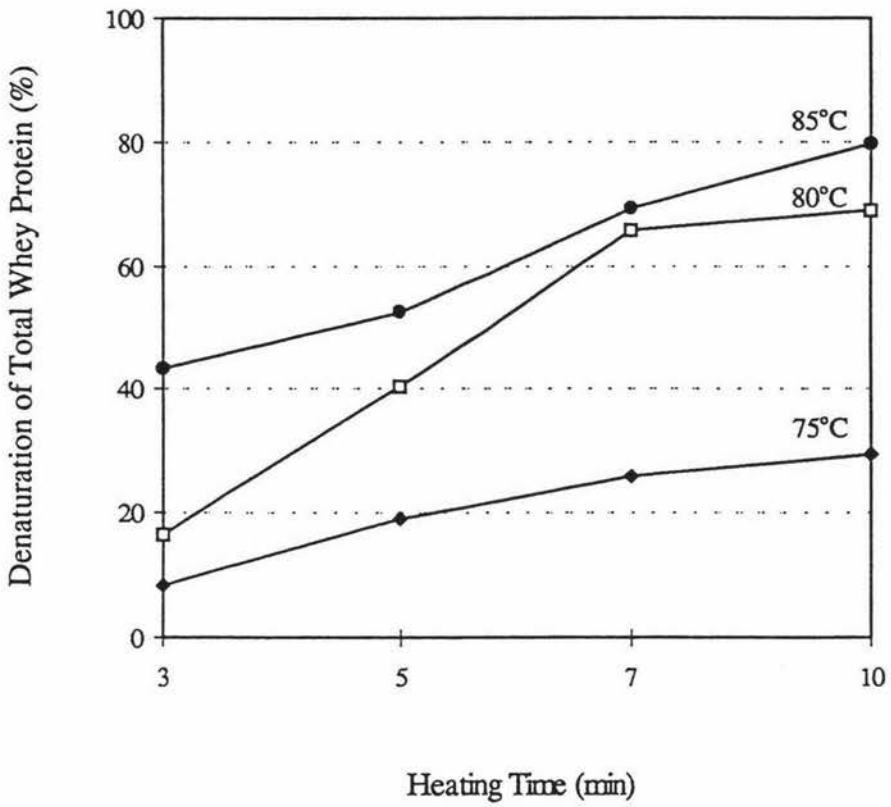


Figure 5.2 Effect of heating time on the % denaturation of total whey proteins in recombined cheese milk: heating at 75 (◆), 80 (□), and 85°C (●) for 3, 5, 7, and 10 min.

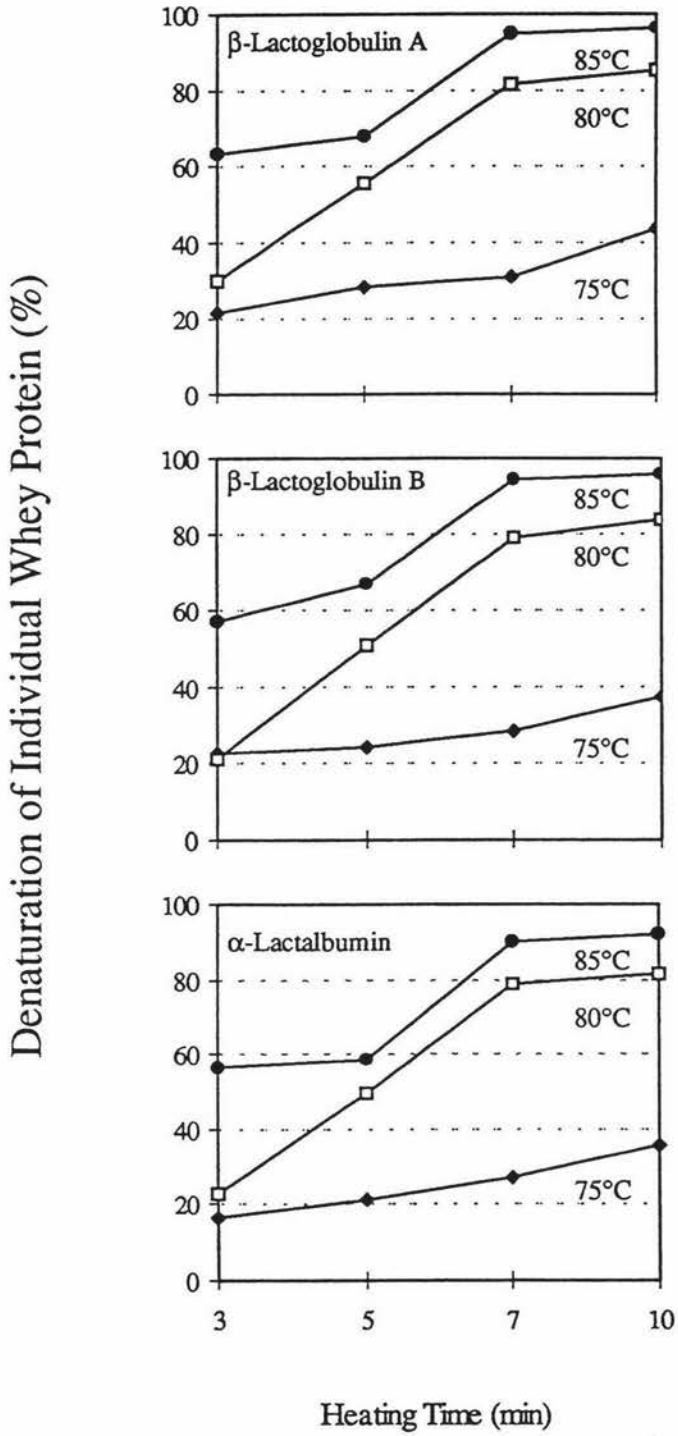


Figure 5.3 Effect of heating time on the denaturation of individual whey proteins in recombined cheese milk, heating at 75 (◆), 80 (□), and 85°C (●) for 3, 5, 7, and 10 min.

When the heating time was < 7 min, there was a significant increase in the % denaturation of total whey protein with increasing heating time. Heating for 10 min resulted in only a slight further increase in % denaturation of whey protein. The level of denaturation of individual whey proteins also increased with heating time, with β-lactoglobulin A being more affected by heat treatment than β-lactoglobulin B and α-lactalbumin (Figure 5.3). Each whey protein was denatured more rapidly and to greater extent at 85°C than at 75°C. For example, after heating for 10 min, 96.5, 96.1 and 92.3 % of β-lactoglobulin A, B and α-lactalbumin were denatured at 85°C.

The results obtained indicated that heat treatments of 40% total solids recombined cheese milk above pasteurization conditions, i.e. at temperatures in the range 70 - 85°C for 3 - 10 min, have significant effects on the levels of total and individual whey protein denaturation and the effect was more pronounced as the severity of heat treatment was increased. The order of heat sensitivity of whey proteins was β-lactoglobulin A > β-lactoglobulin B > α-lactalbumin, which was in good agreement with those reported by others researchers for heated skim milk (Dannenberg & Kessler, 1988; Law *et al.*, 1994). Comparison of the degree of denaturation shown in Figure 5.3 with that of Dannenberg and Kessler (1988) and Law *et al.* (1994) suggested that the denaturation of whey protein in recombined cheese milk was slightly higher than that observed in skim and whole milk. Law *et al.* (1994) observed 42% and 18% denaturation of β-lactoglobulin and α-lactalbumin when whole milk was heated at 80°C for 5 min. Whereas, 55% of β-lactoglobulin and 43% of α-lactalbumin were denatured in recombined cheese milk heated at those heating conditions. The higher total solids content (40% total solids), the present of milk fat globules and its composition may have contributed to this phenomenon. Walstra (1980) and McMahon *et al.* (1993) reported that rather than heat denatured, some whey proteins may adhere and unfold at the newly formed fat-water interfaces during homogenization, thus when casein is precipitated at pH 4.6, the whey proteins would be carried with the fat globules entrapped within the casein precipitate. This may explain why more denaturation of whey protein was observed in homogenized milk than non-homogenized milk or skim milk.

5.1.3 Effect on viscosity and shear stress of recombined cheese milk

The effect of heating time at 75, 80 and 85°C on the viscosity and shear stress of recombined cheese milk was investigated at 34°C by a shear rate sweep from 18.5 to 731 s^{-1}. It was found that heating time had a significant effect on the viscosity and shear stress of recombined cheese milk and that effect was more pronounced at high temperatures (Figures 5.4 and 5.5).

At low heat treatments, i.e. heating at 75°C, both the viscosity and shear stress of recombined cheese milk, determined at any shear rate, slightly increased as heating time increased. The viscosity and shear stress of recombined cheese milk heated at 75°C for 10 min were approximately twice those of unheated samples. Increasing the heating temperature to 80 or 85°C resulted in significant increase in viscosity and shear stress. The viscosity and shear stress of recombined cheese milk that had undergone heat treatment at 85°C for 10 min was about 20 times that of the corresponding unheated sample (see Appendix II, Table 5).

In all cases, the viscosity of recombined cheese milk decreased and the shear stress increased with increasing shear rate, i.e. recombined cheese milk exhibited shear thinning behaviour. In other words, recombined cheese milk behave as pseudoplastic material. To determined if and how the flow behaviour of recombined cheese milk altered as a function of heat treatment, the power law was used to calculate the flow behaviour index (n) and the consistency index (k) as follow:

$$\tau = k(\gamma)^n$$

i.e.

$$\log \tau = \log k + n \log \gamma$$

where τ = Shear stress (Pa)

γ = Shear rate (s^{-1})

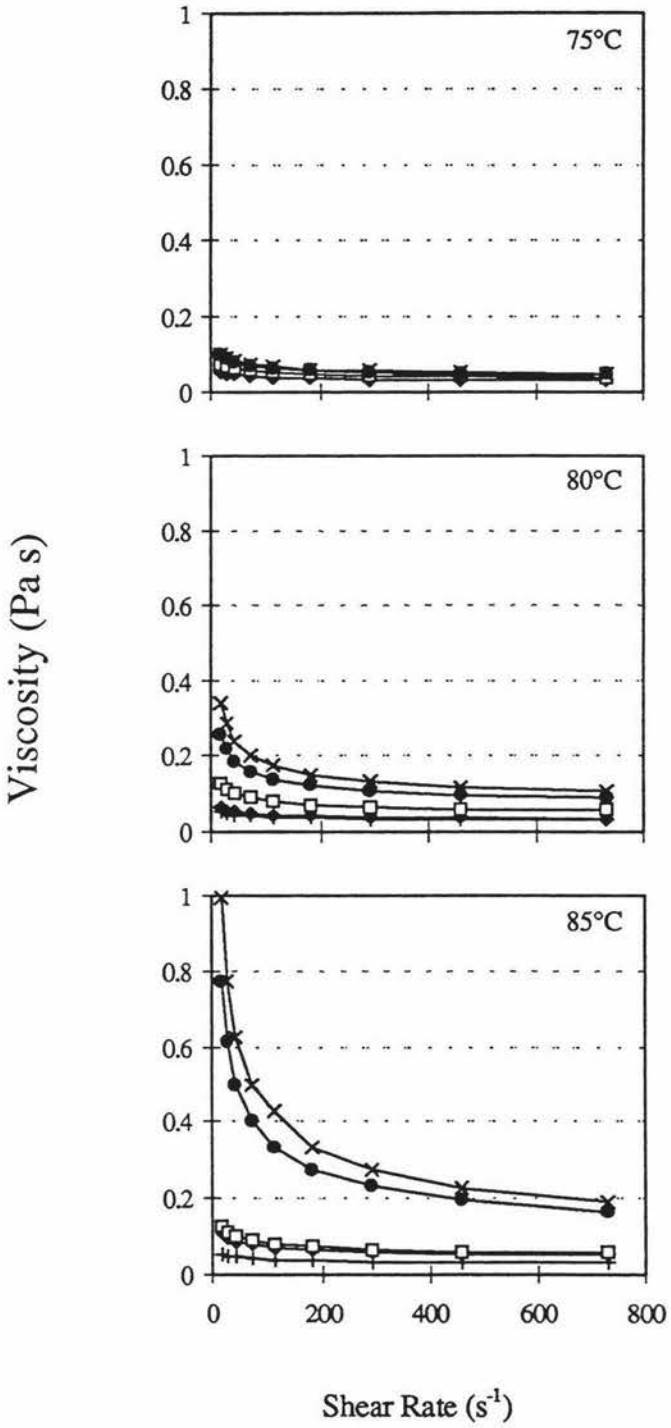


Figure 5.4 Viscosity as a function of shear rate at 34°C of recombined cheese milk that had undergone different heat treatments: no-heat treatment (+), heating at 75, 80 and 85°C for 3 (◆), 5 (□), 7 (●) and 10 min (×).

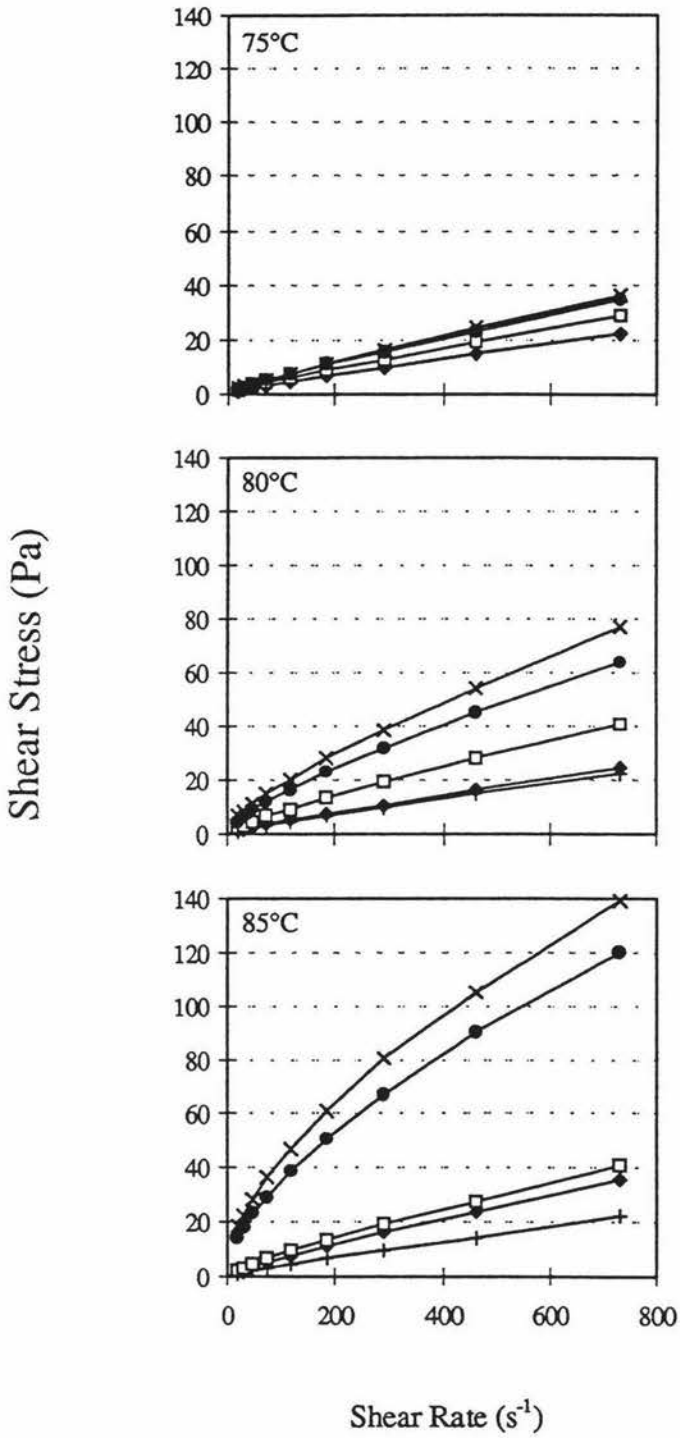


Figure 5.5 Shear stress as a function of shear rate at 34 °C of recombined cheese milk that had undergone different heat treatments: no-heat treatment (+), heating at 75, 80 and 85°C for 3 (◆), 5 (□), 7 (●) and 10 min (×).

The slope of a plot of log τ versus log γ gives the values of the flow behaviour index (n), whereas the intercept at log $\gamma = 0$ (i.e. $\gamma = 1$) is the value of log k. For all treatments, there was a linear relationship between τ and γ, in which the values of τ increased with increase in the severity of heat treatment. The values of n and k of recombined cheese milk which had undergone different heat treatment are shown in Table 5.2.

Table 5.2 The flow behaviour index, determined at 34°C, of recombined cheese milk (40% total solids) which had undergone different heat treatment.

Heat Treatment	Flow Behaviour Index (n)	Consistency Index (k)
no-Heat	0.8424	0.0824
Heat 75°C for 3 min	0.8352	0.0868
Heat 75°C for 5 min	0.8175	0.1249
Heat 75°C for 7 min	0.7981	0.1722
Heat 75°C for 10 min	0.7974	0.1787
Heat 80°C for 3 min	0.8247	0.1015
Heat 80°C for 5 min	0.7762	0.2356
Heat 80°C for 7 min	0.7086	0.5760
Heat 80°C for 10 min	0.6766	0.8417
Heat 85°C for 3 min	0.7039	0.1831
Heat 85°C for 5 min	0.7788	0.2339
Heat 85°C for 7 min	0.5811	2.5048
Heat 85°C for 10 min	0.5536	3.4829

In all cases, the n value was less than 1 indicating that the recombined cheese milk did not behave as a totally Newtonian behaviour ($n = 1$), but can be described as pseudoplastic behaviour ($n < 1$). It was found that the values of n and k were significantly affected by the heating process. The n value decreased and the k value increased as the intensity of heat treatment increased. These values changed rapidly when recombined cheese milk had

undergone severe heat treatment at 85°C for 7 or 10 min; i.e. the n value decreased from 0.8424 to 0.5536 and the k value increased from 0.0824 to 3.4829.

The n and k values obtained for recombined cheese milk which contained 40% total solids (20% milk fat and 20% MPC powder) observed in this experiment were slightly different from those reported on ultrafiltered concentrated milk by another researchers. Kaw *et al.* (1994) observed an n value of 0.92 and a k value of 0.0431 at 40°C for whole milk concentrated by reverse osmosis (3X) containing 40% total solids (13% fat content) homogenized at 150/50 bar without any additional heat treatment. The difference may be attributed to the differences in milk composition and also the differences between the fat globule membrane materials and their interactions with milk proteins.

As shown in Figures 5.4 and 5.5, it was evident that the viscosity and shear stress of recombined cheese milk, determined at any particular shear rate, increased as the heat treatment increased and the effect was dependent on the intensity of heating process. All recombined cheese milks were shear thinning (pseudoplastic behaviour) when subjected to shear rates ranging from 18.5 to 731 s^{-1}, i.e. the viscosity decreased and the shear stress increased as the shear rate increased. Furthermore, it was found that heated milk demonstrated more shear thinning than its corresponding unheated milk.

The similarity in the effect of heat treatment on whey protein denaturation and the increase in viscosity of recombined cheese milk suggested that there might be a close relationship between these two parameters. The relationship between denaturation of total whey proteins and viscosity of recombined cheese milk is shown in Figure 5.6. It was evident that the increase in viscosity was possibly due to the increase in the levels of whey protein denaturation. The relationship between denaturation of whey protein and viscosity of recombined cheese was not linear. The plots of viscosity as a function of total whey protein denaturation (Figure 5.6) can be divided into two main regions. In region I, denaturation up to 60% of total whey proteins caused a slight increase in viscosity (from 0.0383 to 0.1400 Pa s). Whereas, in region II, a further denaturation of the total whey proteins caused marked increase in viscosity of recombined cheese milk (up to 0.4600 Pa s). This suggested that there might be some unknown

interactions occurring in the milk system with severe heat treatment, which resulted in a sharp increase in viscosity.

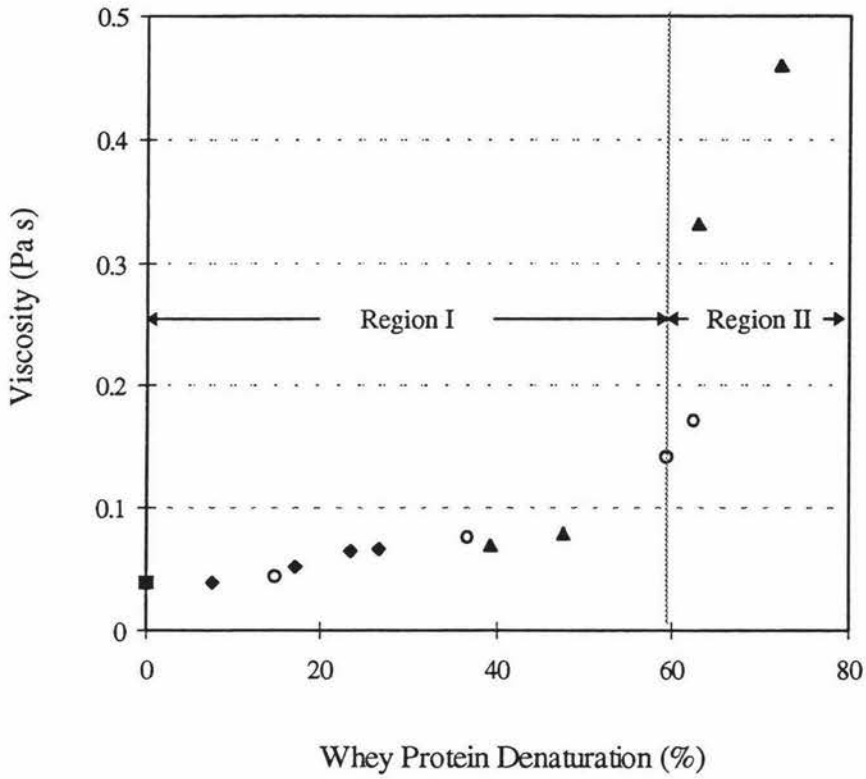


Figure 5.6 The changes in viscosity of recombined cheese milk as a function of the extent of denaturation of the total whey proteins. Heating temperatures were 75 (◆), 80 (○) and 85°C (▲). ■ represents the recombined cheese milk without any additional heat treatment.

5.1.4 Effect on rennet coagulation properties

Rennet coagulation properties of recombined cheese milk were determined as described in Chapter 4. The storage modulus (G'), loss modulus (G'') and tan δ were determined as a function of time after rennet addition. The time when tan δ decreased rapidly and the G' started to increase was considered to be the gelation time (GT). The GT and G' values at 1 and 2 h

after rennet addition were reported. A typical curve during the rennet coagulation of recombined cheese milk is shown in Figure 5.7.

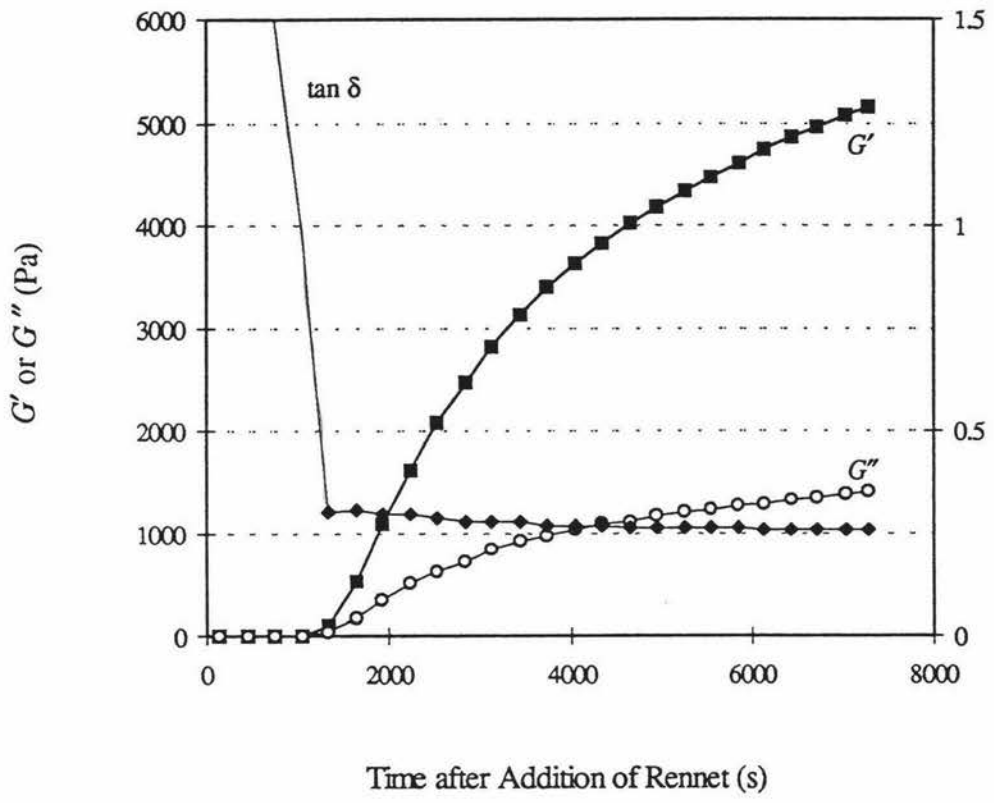


Figure 5.7 The rheological properties which occur during the rennet coagulation of recombined cheese milk, homogenized at 120/50 bar without any additional heat treatment. Storage modulus G', (■), loss modulus G'', (○), and tan δ, (◆).

The changes in dynamic rheological parameters, G', G'' and tan δ, as a function of time after rennet addition represent the development of a rennet gel structure. During the gelation process G' and G'' increased with renneting time and in all cases, G' was greater than G''. There appeared to be a lag period where neither G' nor G'' was detectable, i.e. the values were less

than 0.1 Pa. The lag period occurred because rennet-altered casein micelles do not begin to aggregate until about 80% of the κ-casein on the surface of a micelle has been hydrolysed (Dalgleish, 1990).

Approximately 20 min after the addition of rennet, G' and G'' began to increase, while tan δ decreased rapidly, indicating the beginning of gel formation. After this point, both moduli initially increased rapidly, thereafter there was a slower rate of increase and no constant or plateau value was observed over the time measurements (2 h). The increase in G' values after gelation was attributed to an increase in the number of bonds caused by the network formation, which includes incorporation of rennet-altered micelles and dangling strands, and by the network aging, i.e. rearrangement of strands and fusion of micelles (Zoon *et al.*, 1988). After gel formation, tan δ remained approximately constant.

G' as a function of time after renneting of milk samples that had undergone different heat treatment is shown in Figure 5.8. It was clearly shown that heat treatment, both heating time and temperature, had significant influences on the gelation process of recombined cheese milk. The shape of the G' curves for heated cheese milks was typical of that observed during renneting of the unheated control but the slope was lower, suggesting that the extensive coverage of the micelle surface by denatured whey proteins altered the mechanism of micelle aggregation and gelation. The final G' values decreased as the intensity of heat treatment increased. The G' values of recombined cheese milk, which had been heat treated, had a much slower initial rate of increase compared with those of unheated milk. The G' values of heat treated milk at 1 and 2 h after addition of rennet were also lower.

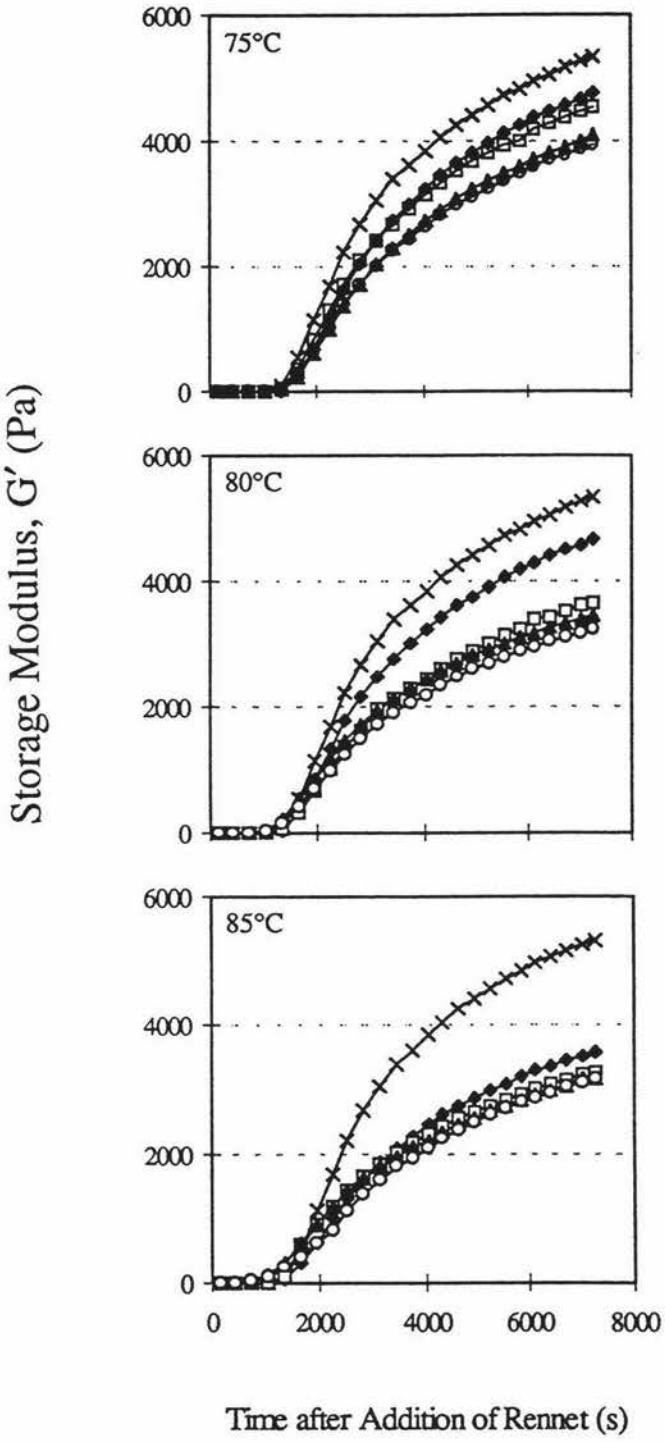


Figure 5.8 Effect of heat treatment on the storage modulus (G') of renneted recombined cheese milk; without heat treatment (×) and with heat treatment at 75, 80, and 85°C for 3 (◆), 5 (□), 7 (▲) and 10 min (○).

Effect on gelation time (GT)

The gelation time (GT), which is comprised of both enzymatic and aggregation phases, was determined and the effect of heating time at 70, 80 and 85°C on the GT of recombined cheese milk is shown in Figure 5.9. It was found that heating at 75°C for 3, 5 and 7 min and at 80°C for 3 min, slightly increased the GT of recombined cheese milk. Severe heat treatment at 80°C for more than 3 min or at 85°C resulted in significantly decreased GT. The GT of recombined cheese milk, without any additional heat treatment after homogenization, was 18 min and the GT was decreased to about 5 min when recombined cheese milk was heated at 85°C for 10 min.

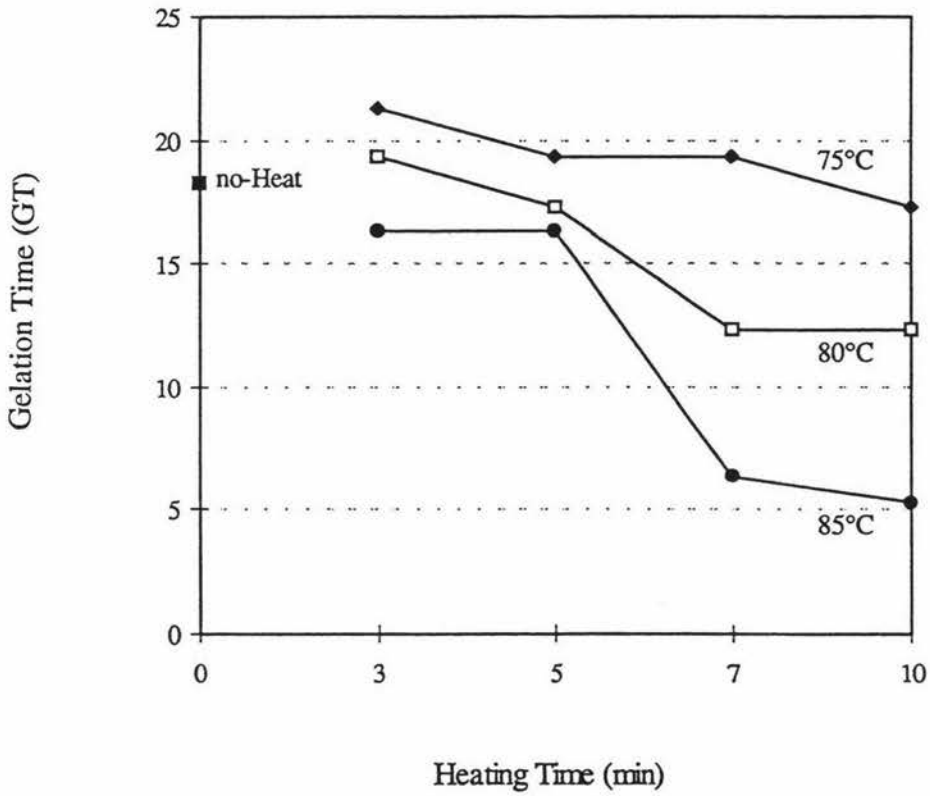


Figure 5.9 Influence of heating time on the gelation time (GT) of recombined cheese milk; no heat treatment (■) and with heat treatment at 75 (◆), 80 (□), and 85°C (●) for 3, 5, 7, and 10 min.

The results on the effects of severe heat treatment on the GT obtained here were in contrast to those observed on normal milk by another researchers (Wheelock & Kirk, 1974; van Hooydonk *et al.*, 1987; Reddy & Kinsella, 1990; McMahon *et al.*, 1993, Waungana, 1995). They observed an increase in GT with increasing the severity of heat treatment in normal and ultrafiltered milk. The discrepancy may be due to the fact that the recombined cheese milk used in this study had a high solid content, i.e. 20 % milk powder and 20% milk fat. When recombined cheese milk was heat treated to high temperatures, the effect of heat treatment was more pronounced and whey proteins were denatured quickly, resulting in significantly increased viscosity of heated milk. It is also possible that heated milks had undergone considerable heat-induced aggregation, which resulted in a decrease in GT when rennet was added. In other words, the gelation process was not only due to the rennet addition but was also due to heat-induced aggregation.

Effect on storage modulus (G')

The effect of heating time on the G' of rennet-induced gels, made from recombined cheese milk, is shown in Figure 5.10. It was found that G', determined at 1 and 2 h after the addition of rennet, decreased as the severity of heat treatment increased and the effect of heating time was more pronounced at lower heating temperatures. The G' value, which is related to the stiffness of the gel, made from recombined cheese milk that was heated at 85°C for 10 min, was about half of the unheated sample. At the same heating time, recombined cheese milk that had undergone heat treatment at higher temperatures produced weaker gels than those that had undergone lower heat treatment.

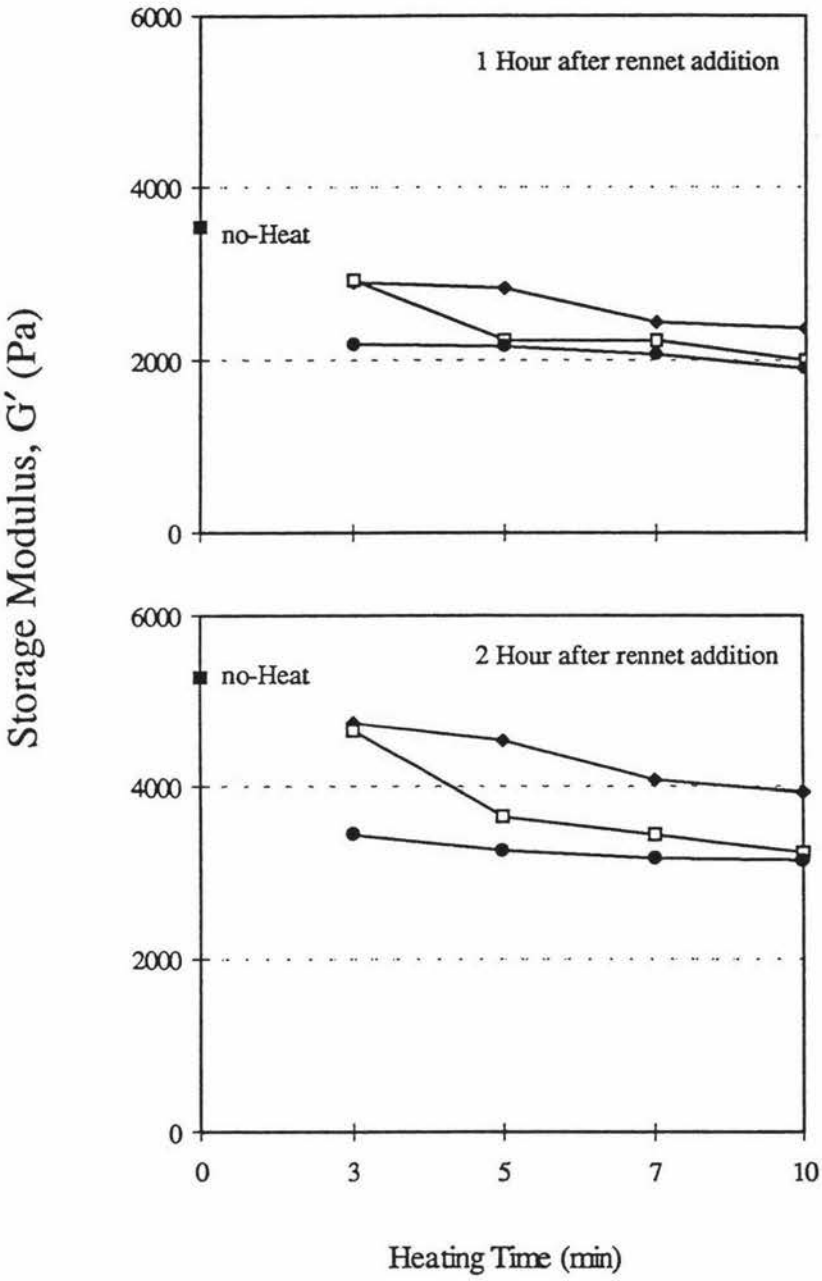


Figure 5.10 Effect of heating time on the storage modulus (G') of recombined cheese milk determined 1 and 2 h after rennet addition; heating at 75 (◆), 80 (□), and 85°C (●) for 3, 5, 7, and 10 min. ■ represents the G′ value of recombined cheese milk without any heat treatment.

The results obtained demonstrated that heat treatment, both heating time and temperature after homogenization, had significant effects on the rennetability of recombined cheese milk made from MPC-56 powder. The effect of heat treatment was, of course, dependent on the intensity of heat treatment. The GT, rate of aggregation and G' value determined at any particular renneting time of heated milk was lower than those of the corresponding unheated milk.

The reduction in G' values have also been observed for normal and ultrafiltered milk by several researchers (Singh *et al.*, 1988; Lucey *et al.*, 1993; McMahon *et al.*, 1993). They attributed the reduction in G' values of heated milk to the disruption of the continuity of the gel network caused by attachment of denatured whey proteins to the casein micelles. The denatured whey proteins may sterically hinder the close approach and contact between casein micelles, resulting in a weaker, looser network due to reduced aggregation rate of heated milk. Lucey *et al.* (1993) reported that the gel strength of heated milk increases at a slower rate than unheated milk. Thus, the strength of rennet-induced gels made from heated milk, determined at a particular time after renneting, will be lower than from unheated milk.

The reduction in G' values of heated milk may be related to the denaturation of whey proteins observed in the milk sample. The relationship between denaturation of total whey proteins and G' values, determined at 2 h after the addition of rennet, is shown in Figure 5.11.

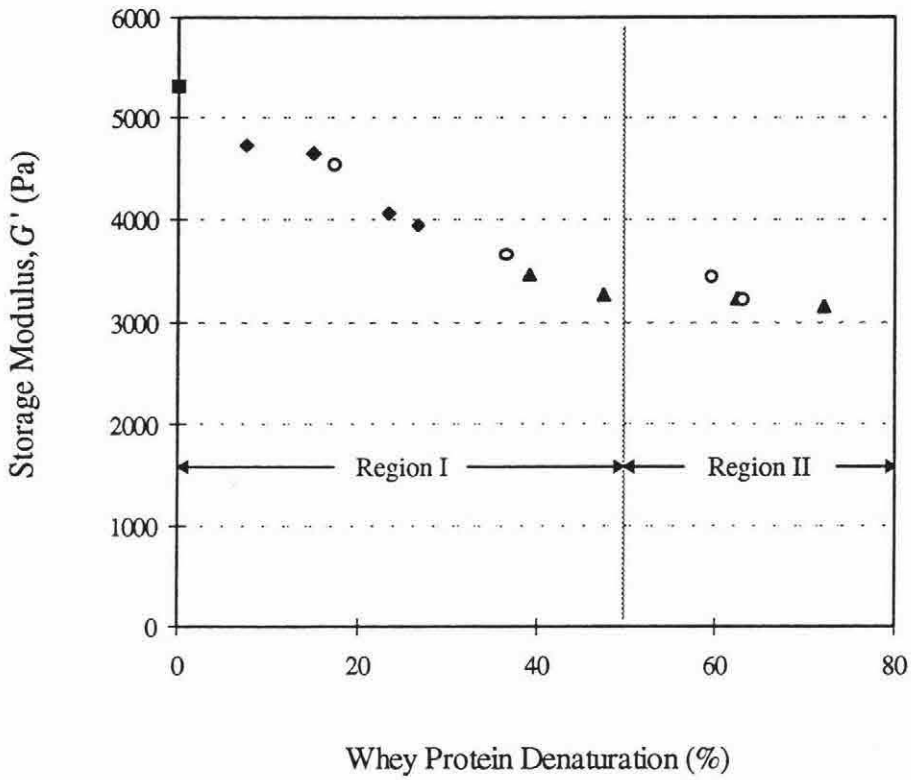


Figure 5.11 The G' values, determined at 2 h after the addition of rennet, of recombined cheese milk as a function of the extent of denaturation of the total whey proteins. Heating temperatures were 75 (◆), 80 (O) and 85°C (▲). ■ represents the recombined cheese milk without any additional heat treatment.

It evident that there was a close relationship between reduction in G' values observed in heated milk and increased in the level of denaturation of totals whey proteins (Figure 5.11). G' values decreased almost linearly with increase in denaturation up to ~ 50% (Region I). Further denaturation had no effect on G' (Region II).

5.1.5 Effect on yield force at the yield point

The effect of heating time on the force required to fracture the rennet-induced gels (yield force) was determined 1.3 h after renneting and the results are shown in Figure 5.12. These results showed the same trend as the effect of heating time on G', i.e. the yield force decreased with increasing heating time.

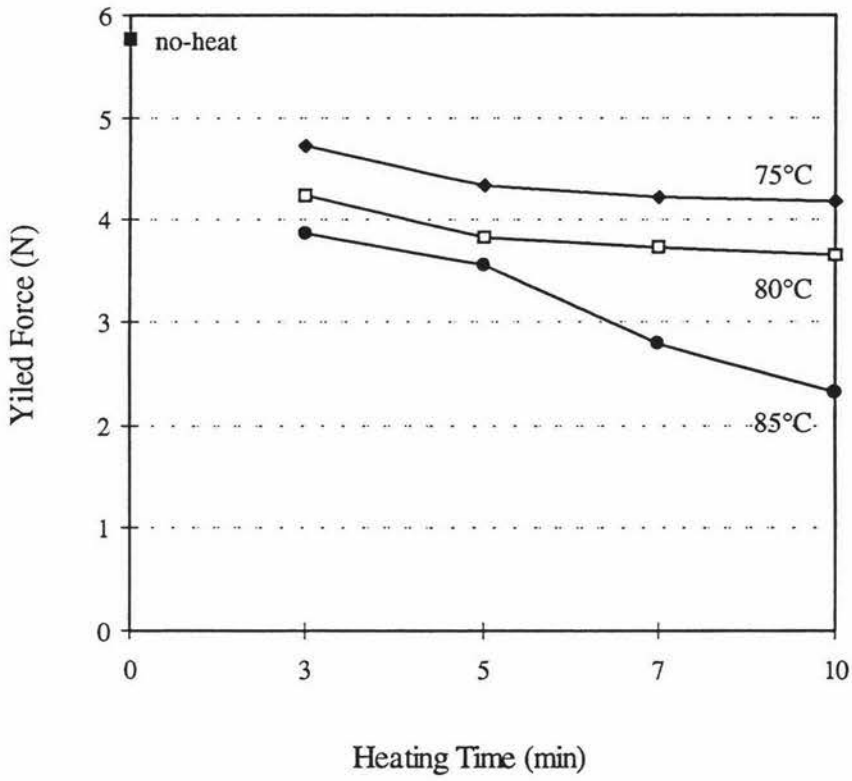


Figure 5.12 Effect of heating time on the yield force require to fracture rennet-induced gels made from recombined cheese milk, determined 1.3 h after the addition of rennet; heating at 75 (◆), 80 (□), and 85°C (●) for 3, 5, 7, and 10 min. ■ represents the no-heat treatment sample.

It was found that recombined cheese milks that had undergone high heat treatment had lower yield force required than samples that had undergone lower heat treatments. The yield force

needed to fracture the renneted-induced gel made from recombined cheese milk that had undergone heat treatment at 85°C for 10 min was about half that needed to fracture the gel made from unheated control milk.

The reduction in the force required to fracture can also related to the level of whey protein denaturation as shown in Figure 5.13. The higher the level of whey proteins denaturation, the lower the force required to fracture the gel.

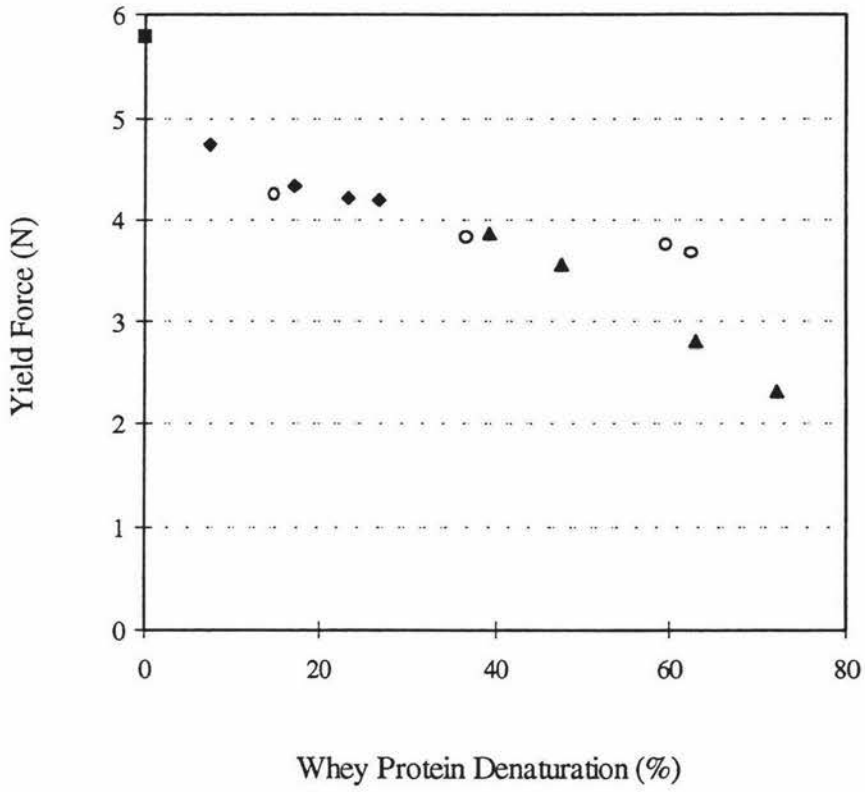


Figure 5.13 The yield force required to fracture the renneted gel, determined at 1.3 h after the addition of rennet, of recombined cheese milk as a function of the extent of denaturation of the total whey proteins. Heating temperatures were 75 (◆), 80 (O) and 85°C (▲). ■ represents the recombined cheese milk without any additional heat treatment.

Severe heat treatment, i.e at 85°C for > 5 min, which resulted in high levels of whey protein denaturation markedly reduced the force required to fracture the gel.

5.2 Effect of various heating temperatures at 3 min holding time

The effect of various heating temperatures for a constant holding time of 3 min on the physical and rennet coagulation properties of recombined cheese milks was investigated using two different types of MPC-56 powders. One powder had a very low heat treatment during its manufacture and the second was the commercial MPC-56 powder. The low-heat treatment powder was produced by heating at 75°C for 15 s after ultrafiltration, whereas the commercial powder was produced using the preheat treatment of 85°C for 40 s. These powders had different levels of whey protein denaturation and the recombined cheese milk made from the low-heat treatment powder was used as a control unheated sample.

All milk samples were homogenized at 120/50 bar before any heat treatment. The milks were heated to defined temperatures between 70 and 83°C in a UHT Plant and held for 3 min using a controlled temperature water bath. The effects of heating temperature on the average fat globule diameter ($d_{4,3}$), whey protein denaturation, viscosity and shear stress, and rennet coagulation properties were investigated.

5.2.1 Effect on average fat globule diameter ($d_{4,3}$)

The effect of various heating temperatures for a 3 min holding time on the average fat globule diameter ($d_{4,3}$) of recombined cheese milks is shown in Table 5.3. It appeared that heating temperature for 3 min holding time does not have a significant effect on the $d_{4,3}$ of recombined cheese milk, measured after dilution of samples in deionized water and dissociating solution. The fat globules of recombined cheese milk made from both low-heat treatment and the commercial powders had the same average diameter.

Table 5.3 Effect of heating at various temperatures for 3 min on the average fat globule diameter ($d_{4,3}$) of recombined cheese milk made from low-heat treatment and commercial powder, homogenized at 120/50 bar before performed any heat treatment. The measurement was carried out after dilution of samples with deionized water or dissociating solution (SDS-EDTA).

Heat treatment	Average fat globule diameter ($d_{4,3}$, μm)			
	Deionized water	Dissociating solution	Deionized water	Dissociating solution
	Low-heat treatment powder		Commercial powder	
no-Heat	1.09	1.10	1.09	1.07
70°C for 3 min	1.08	1.08	1.10	1.08
73°C for 3 min	1.10	1.10	1.10	1.09
75°C for 3 min	1.09	1.09	1.10	1.10
78°C for 3 min	1.10	1.08	1.08	1.10
80°C for 3 min	1.09	1.08	1.09	1.10
83°C for 3 min	1.10	1.09	1.11	1.08

The particle size distributions of recombined cheese milk without any additional heat treatment and with heat treatment at 75 and 83°C for 3 min, measured after dilution of sample in deionized water, are shown in Figure 5.14. It was found that the particle size distribution was slightly altered as the heating temperature increased and the effect was more pronounced in recombined cheese milk made from commercial powder.

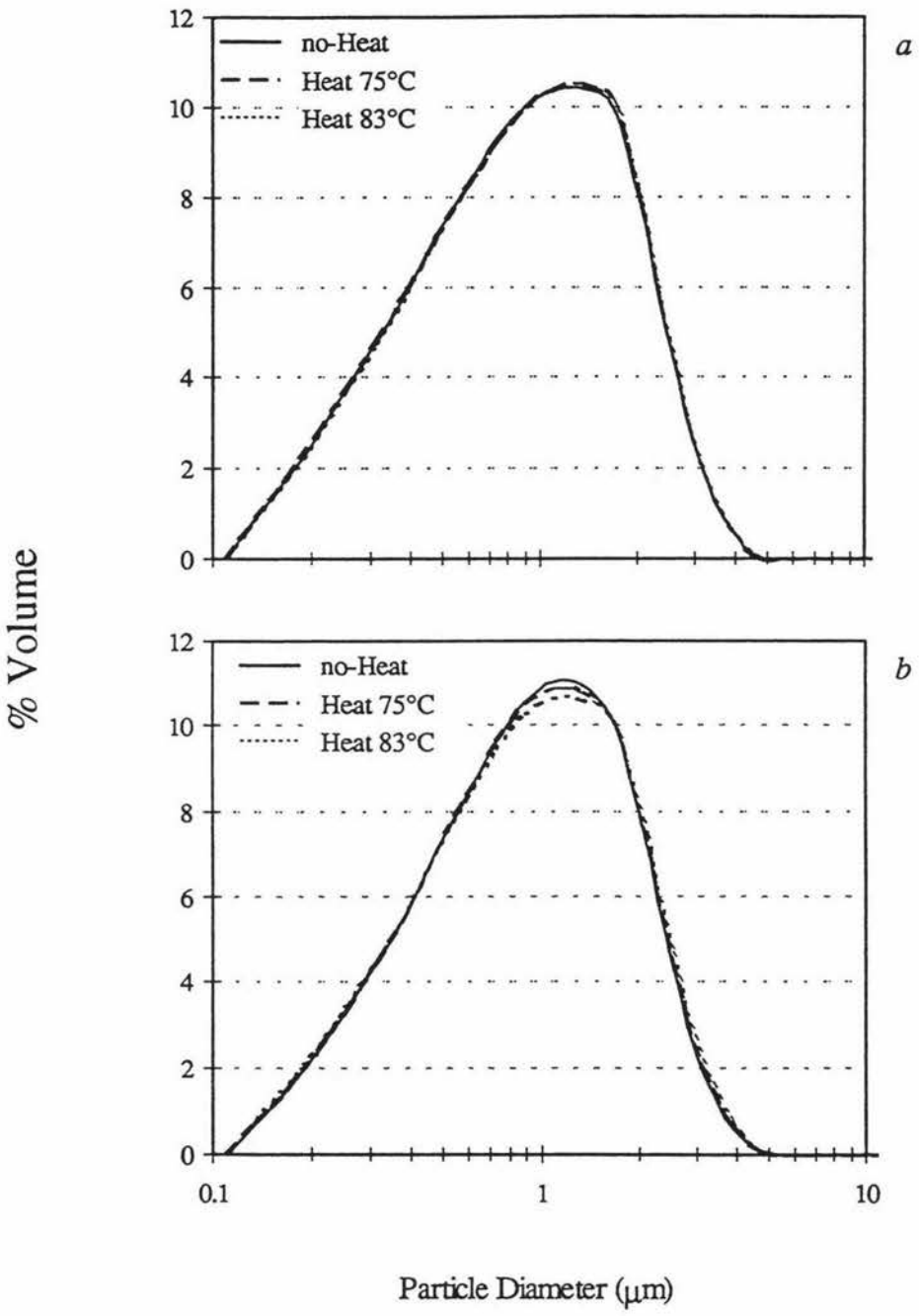


Figure 5.14 The profiles of fat globule size distribution in recombined cheese milks made from low-heat treatment (*a*) and commercial MPC powder (*b*), homogenized at 120/50 bar without any heat treatment and with heat treatment at 75 and 83°C for 3 min.

The reason why the effects of heat treatment on the $d_{4,3}$ and particle size distribution were more pronounced in recombined cheese milk made from commercial MPC powder may be attributed to the higher lever of whey protein denaturation compared to those of low-heat treatment powder. Consequently, greater interactions of the denatured whey proteins either with milk fat globule membrane materials or adsorbed caseins onto the milk fat globule membrane, resulted in greater aggregation of protein and fat globule.

5.2.2 Effect on whey protein denaturation

As expected, more whey protein was denatured when recombined cheese milk was heated to higher temperature (Figure 5.15). The effect of heating temperature was slightly more pronounced in recombined cheese milk made from commercial MPC powder than that of low-heat treatment powder. It was apparent that the recombined cheese milk made from commercial powder had higher levels of denaturation of whey protein.

Denaturation of total whey protein at 73°C observed in this experiment was 25 and 32% for recombined cheese milk made from low-heat treatment and commercial powders, which was different from that reported on normal and ultrafiltered milk (McMahon *et al.*, 1993, *Law et al.*, 1994). McMahon *et al.* (1993) studied the effect of heat treatment on homogenized ultrafiltered whole milk (3X) in the temperature ranging from 72 to 140°C and found that the denaturation of whey proteins increased with heating temperature. They observed 24% denaturation of total whey protein when ultrafiltered milk was heated at 72°C for 109 s. Law *et al.* (1994) studied the denaturation of whey protein in normal milk in the temperature range from 72 to 140°C for 1 min holding time and reported that the denaturation of whey protein increased with increasing heating temperature. They observed ~ 10% denaturation of total whey proteins at heating temperature 72°C for 1 min. Differences in the composition of milk and the method of heat treatment may have contributed in the discrepancy.

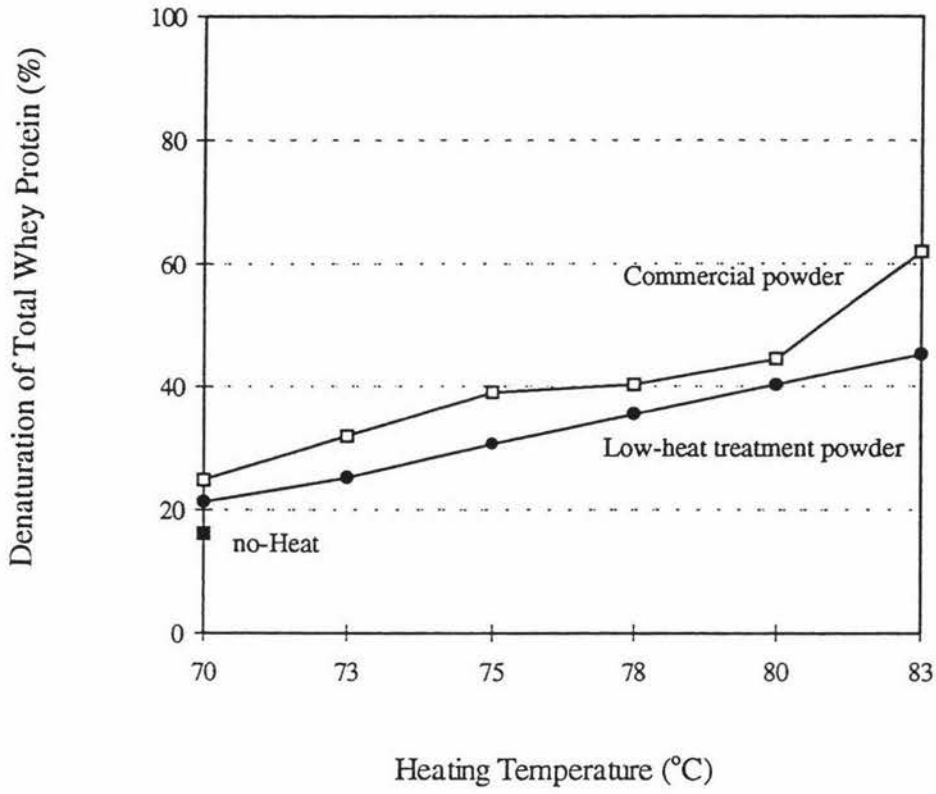


Figure 5.15 The effect of heating temperatures, at a holding time of 3 min, on the extent of denaturation of total whey proteins in recombined cheese milk made from low-heat treatment (●) and commercial powder (□). ■ represents recombined cheese milk made from commercial powder without any heat treatment.

5.2.3 Effect on viscosity and shear stress of recombined cheese milk

The effect of heating at various temperatures for 3 min on the viscosity and shear stress of recombined cheese milk, made from low-heat treatment and commercial powders, is shown in Figures 5.16 and 5.17, respectively. It was found that at the same heat treatment the viscosity and shear stress of recombined cheese milk made from commercial MPC-56 powder were higher than those made from low-heat treatment powder. Increasing the heating temperature seems to have a larger effect on viscosity and shear stress in recombined cheese milk made from commercial MPC-56 powder. In all cases, both the viscosity and shear stress increased with an increase in the intensity of heat treatment.

To check how the flow behaviour of recombined cheese milk changed as a function of heating temperature at 3 min holding time, the flow behaviour index (n) and consistency index (k) values were determined and the results are shown in Table 5.4.

Table 5.4 The flow behaviour index, determined at 34°C, of recombined cheese milk (40% totals solids) made from low-heat treatment and commercial powders which had undergone different heat treatments.

Heat Treatment	Low-heat treatment powder		Commercial powder	
	n	k	n	k
no-Heat	0.9144	0.0309	0.8422	0.0804
Heat 70°C for 3 min	0.9102	0.0325	0.8316	0.0913
Heat 73°C for 3 min	0.8972	0.0352	0.8339	0.0920
Heat 75°C for 3 min	0.8948	0.0373	0.8392	0.0929
Heat 78°C for 3 min	0.8923	0.0385	0.8241	0.1167
Heat 80°C for 3 min	0.8904	0.0410	0.8174	0.1225
Heat 83°C for 3 min	0.8723	0.0490	0.8008	0.1633

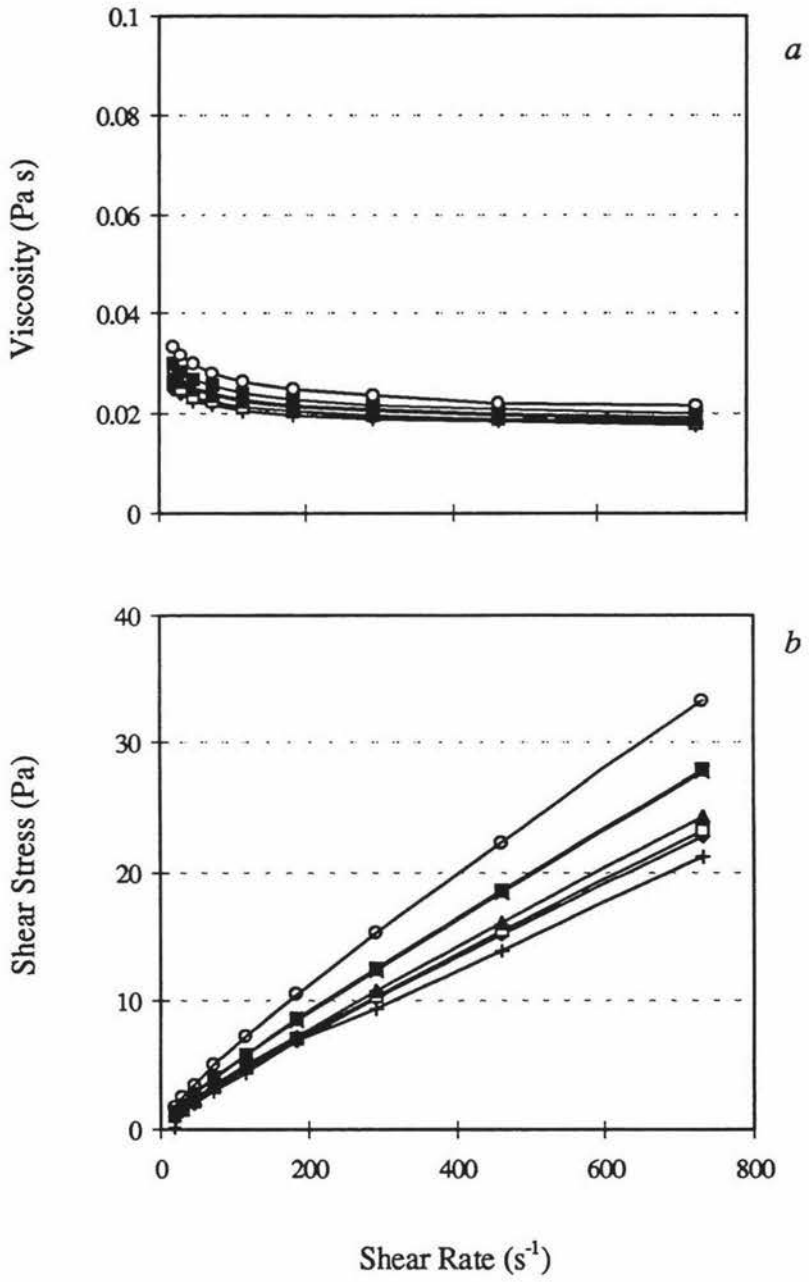


Figure 5.16 Effect of heating at various temperatures for 3 min on the viscosity (*a*) and shear stress (*b*) of recombined cheese milk (without rennet addition) made from low-heat treatment powder without heat treatment (+) and with heat treatment at 70 (◆), 73 (□), 75 (▲), 78 (×), 80 (■) and 83°C (○) for 3 min.

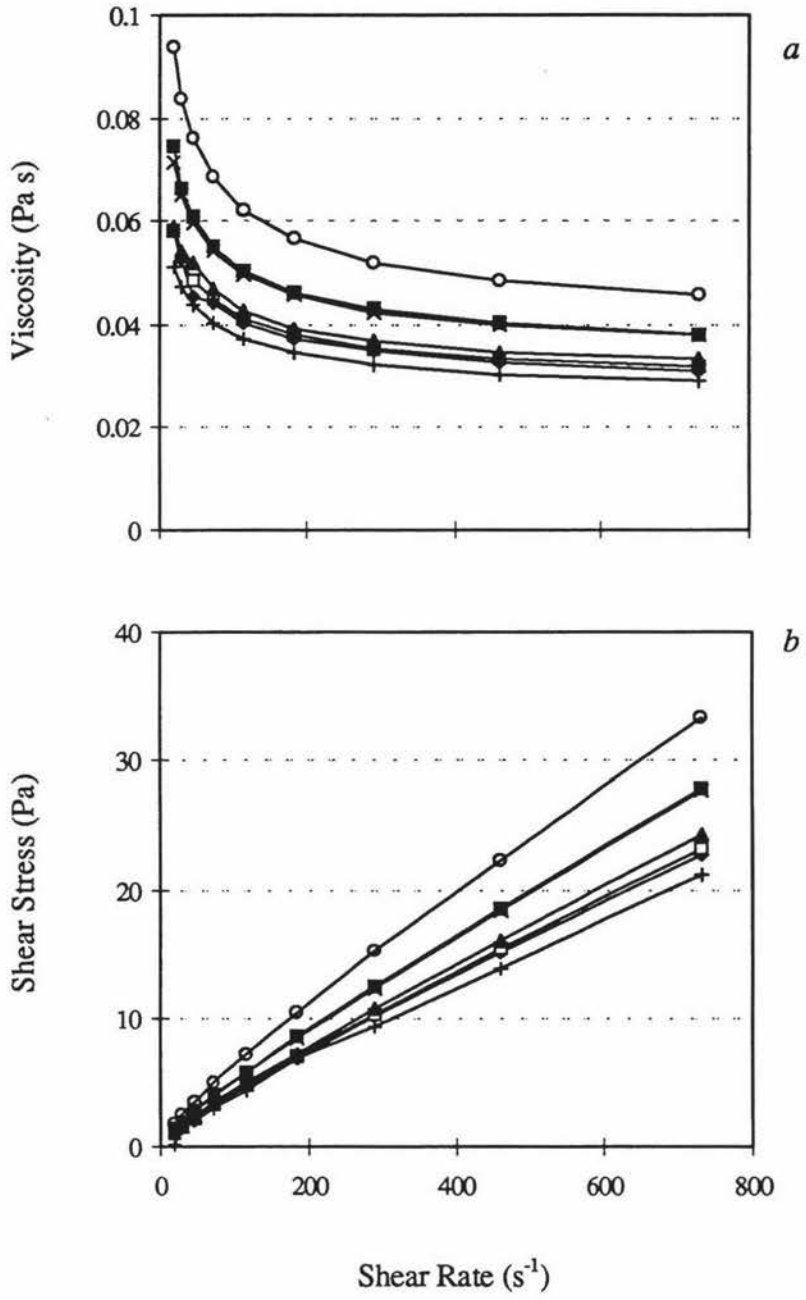


Figure 5.17 Effect of heating at various temperatures on the viscosity (*a*) and shear stress (*b*) of recombined cheese milk (without rennet addition) made from commercial powder without heat treatment (+) and with heat treatment at 70 (◆), 73 (□), 75 (▲), 78 (×), 80 (■) and 83°C (○) for 3 min.

It was found that the n and k values of recombined cheese milk made from both types of MPC powders were dependent on the heating temperature. At all heat treatments, the n values of recombined cheese milk made from low-heat treatment powder were higher than that of commercial powder. The n value slightly decreased as the intensity of heat treatment increased, i.e. the n value decreased from 0.9144 to 0.8723 in recombined cheese milk made from low-heat treatment powder and decreased from 0.8422 to 0.8008 in recombined cheese milk made from commercial powder when the heat treatment increase from no-heat to heated at 83°C for 3 min. Whereas, the k values increased with the severity of heat treatment. The k values ranged from 0.0309 to 0.0490 and from 0.0804 to 0.1163 in recombined cheese milk made from low-heat treatment and commercial powder, respectively. The changes in n and k values were more pronounced when recombined cheese milk was made from commercial powder. The change in viscosity and shear stress as a function of shear rate and flow behaviour index observed in this experiment indicated that recombined cheese milks made from both types of MPC powders exhibit pseudoplastic behaviour ($n < 1$). The n and k values observed on the effect of heating temperature were in the same range with that observed on the effect of heating time, indicating that the flow behaviour index were dependent on the severity of heating process, i.e. the n and k values depend on the degree of denaturation of whey protein.

The change in viscosity as a function of whey protein denaturation is shown in Figure 5.18. It was found that heat treatment at the temperature ranging from 70 to 83°C for 3 min, which resulted in up to 45% and 62% denaturation of total whey proteins of recombined cheese milk made from low-heat treatment and commercial powder, resulted in a slight increase in viscosity (from 0.0372 to 0.0621 and 0.0205 to 0.0264 Pa s in recombined cheese milk made from low-heat treatment and commercial powder, respectively). This is consistent with the results reported in Figure 5.6 which showed that denaturation of over 50% was required to cause a major increase in viscosity.

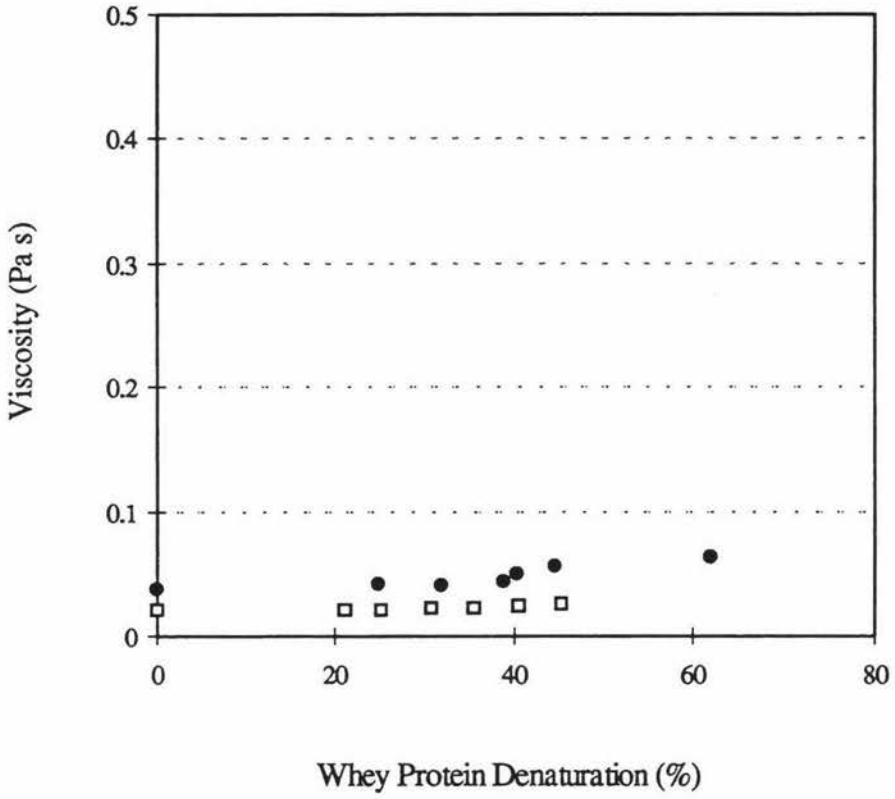


Figure 5.18 The changes in viscosity of recombined cheese milk, made from low-heat treatment (□) and commercial powders (●), as a function of the extent of denaturation of the total whey proteins. Heating at temperatures ranging from 70 to 83°C for 3 min.

5.2.4 Effect on the rennet coagulation properties

The effect of various heating temperatures on the rennet coagulation process of recombined cheese milk made from low-heat treatment and commercial powders are shown in Figure 5.19. Heating temperature, at constant holding time of 3 min, resulted in a reduced G' value and a reduced rate of aggregation of renneted milk, but did not seem to have a significant effect on GT of recombined cheese milk. The effect of heating temperature was greater in recombined cheese milk made from commercial powder compared to that of low-heat treatment powder.

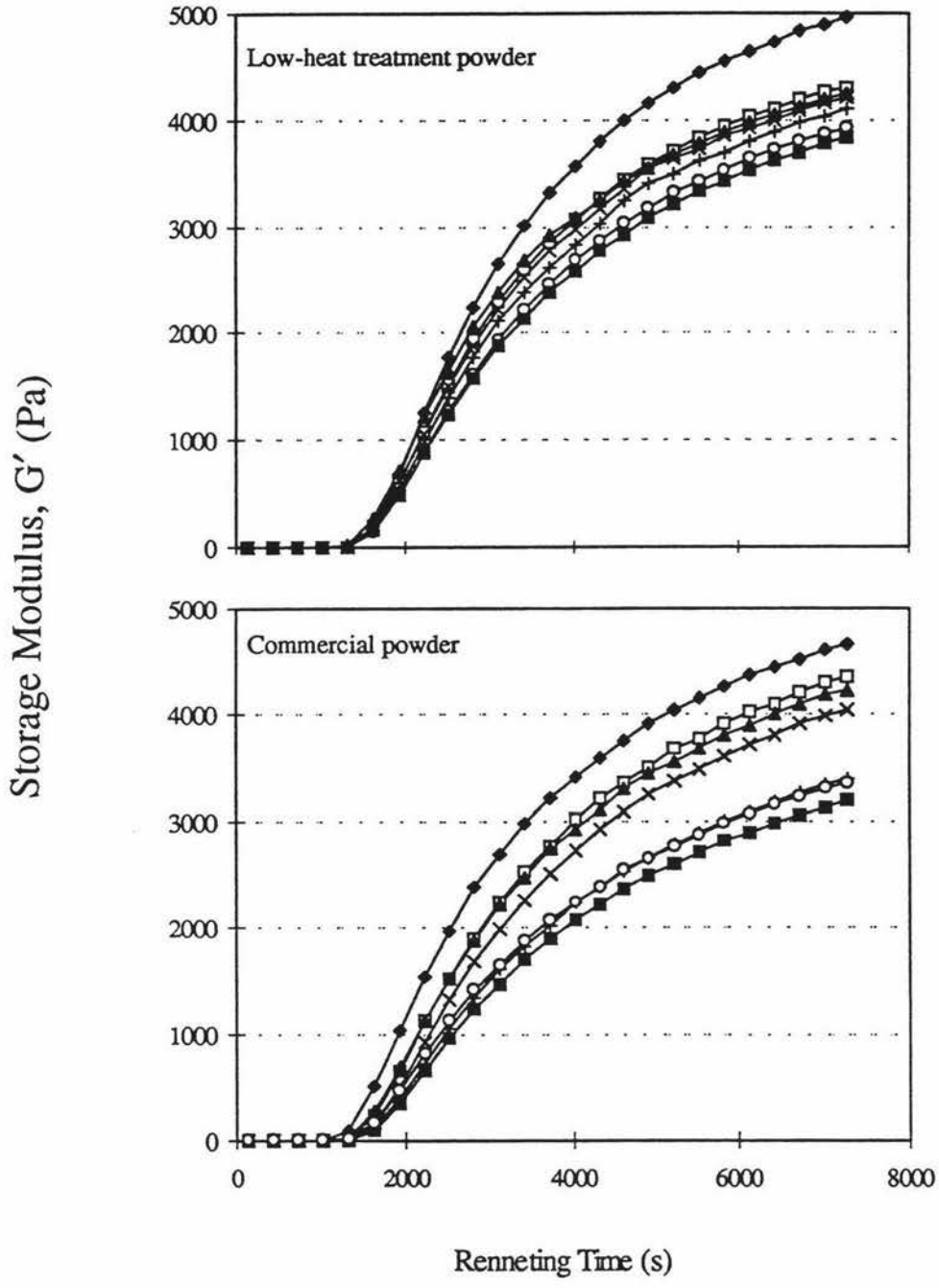


Figure 5.19 The effect of various heating temperatures for 3 min on the storage modulus (*G'*) of recombined cheese milk made from the low-heat treatment and commercial powders without any heat treatment (◆) and with heat treatment at 70 (□), 73 (▲), 75 (×), 78 (+), 80 (○) and 83°C (■) for 3 min.

The G' value of recombined cheese milk made from low-heat treatment powder, determined at any particular time after the addition of rennet, was higher than that made from commercial powder when the milk had undergone the same heat treatment. The GT was also shorter in recombined cheese milk made from commercial MPC powder. In all cases, a maximum or plateau value of G' was not reached 2 h after rennet addition.

Effect on gelation time (GT)

Heat treatment of recombined cheese milk made from commercial powder for 3 min resulted in a slight increase in GT compared to that of corresponding unheated control (Figure 5.20).

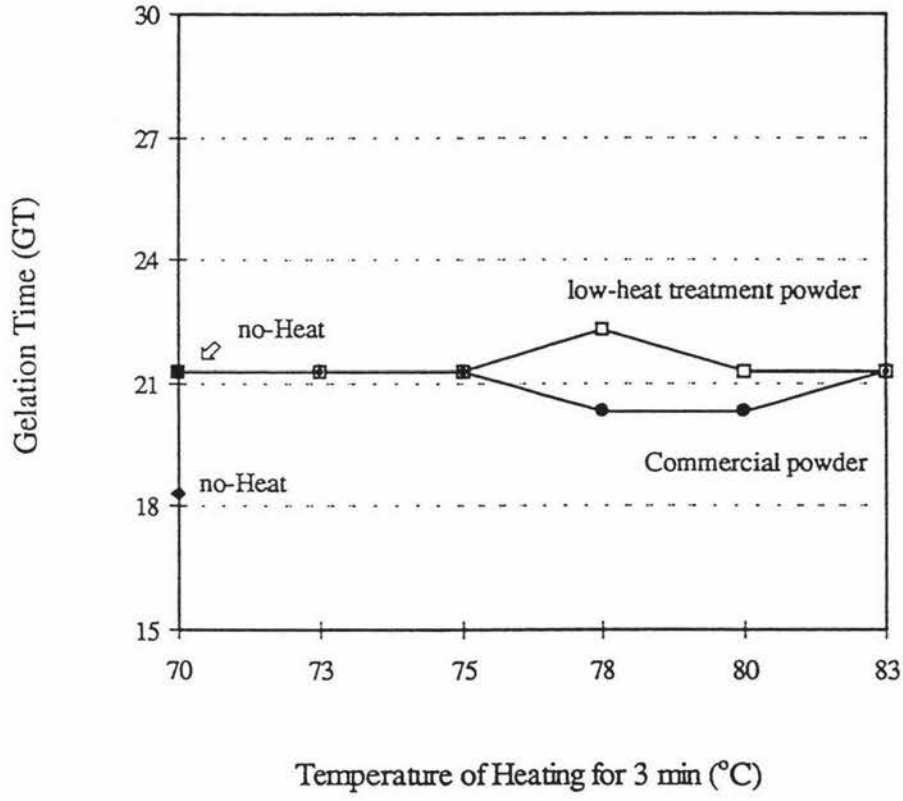


Figure 5.20 The effect of heating at various temperatures for 3 min on the gelation time (GT) of recombined cheese milk made from low-heat treatment (□) and commercial powder (●). ■ and ◆ represented no-heat treatment samples made from low heat treatment and commercial powders, respectively.

The GT of recombined cheese milk made from commercial powder increased from 18 min to 20 - 21 min when the milk had undergone heat treatment for 3 min. In contrast, the GT of recombined cheese milk made from low-heat treatment powder remained constant at approximately 21 - 22 min for all milk samples. Comparison of GT, between recombined cheese milk made from two types of MPC powders, revealed that heating temperature seems to have more effect on recombined cheese milk made from commercial powder. In all cases, the GT of recombined cheese milk made from commercial powder was not greater than those made from low-heat treatment powder. However, GT was not significantly affected by increasing heating temperature in the range of 70 - 83°C for 3 min for both types of recombined cheese milk.

The increase in GT of heated recombined cheese milk made from commercial powder, compared to its unheated control, may be attributed to the interactions between denatured whey proteins, especially β-lactoglobulin, and κ-casein, which rendered the casein micelles less susceptible to coagulation by rennet. Wilson and Wheelock (1972), Wheelock and Kirk (1974) and van Hooydonk *et al.* (1987) reported that heating decreased both the rate and the extent of the enzymatic hydrolysis of the κ-casein, which resulted in longer GT.

Effect on storage modulus (G′)

The G' values of renneted milk gels, determined 1 and 2 h after the addition of rennet, were affected by heating temperature as shown in Figure 5.21. As the heating temperature increased, the G' of renneted milk gels made from both types of MPC-56 decreased and the effect was greater for the recombined cheese milk made from commercial powder. In all cases, the G' values, determined at any particular time after renneting, of renneted milk gels made from low heat treatment powder were slightly higher than those of commercial powder.

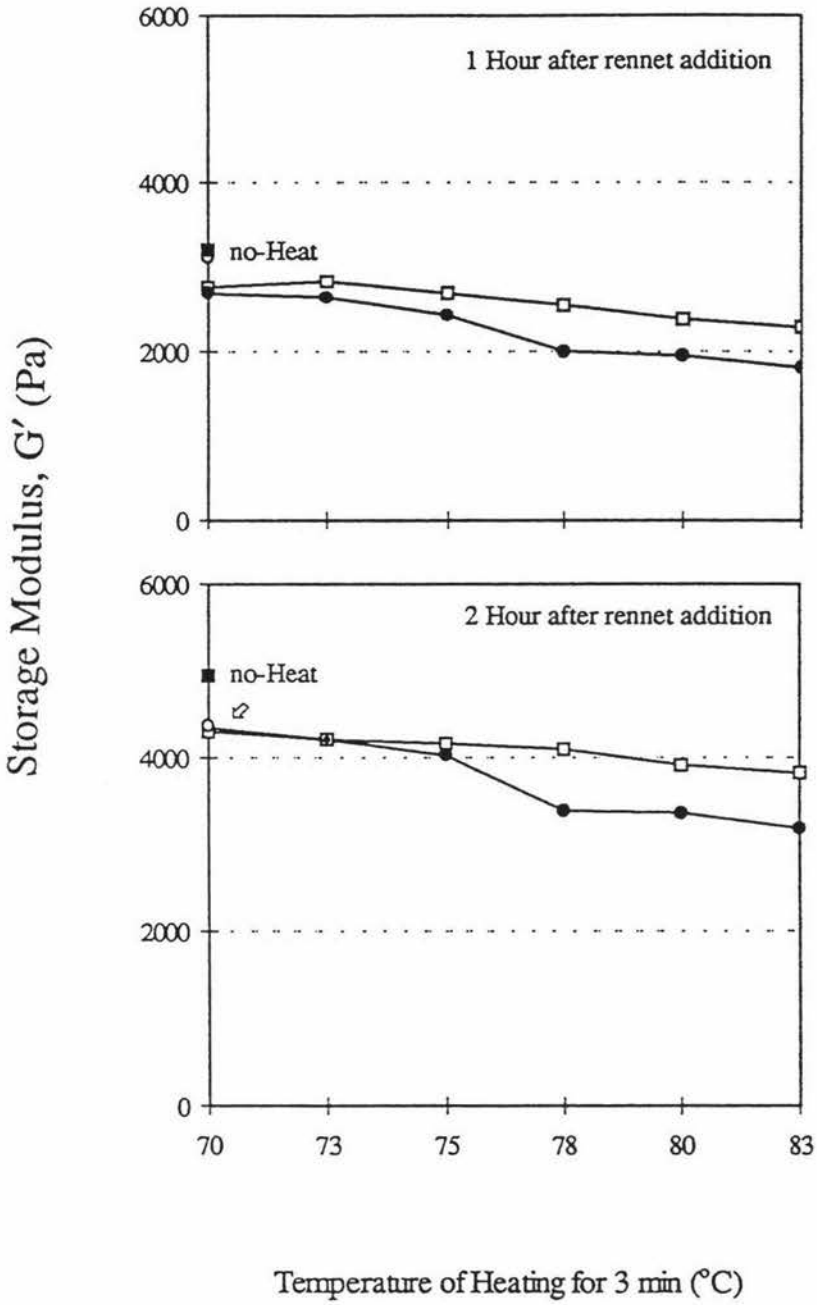


Figure 5.21 The effect of heating at various temperatures for 3 min on the storage modulus (G') of recombined cheese milk made from low-heat treatment (□) and commercial powder (●) determined 1 and 2 h after the addition of rennet. ■ and ○ represent no-heat treatment sample made from low-heat treatment and commercial powders, respectively.

Heating at 83°C for 3 min reduced the G' values of renneted gel determined at 2 h after the addition of rennet from 4943 to 3820 Pa and from 4637 to 3173 Pa in recombined cheese milk made from low-heat treatment powder and commercial powder, respectively. The reduction in G' values of heated milk may be related to the higher level of whey protein denaturation observed in heated samples. The plot between the extent of whey protein denaturation and G' values determined at 2 h after the addition of rennet is shown in Figure 5.22

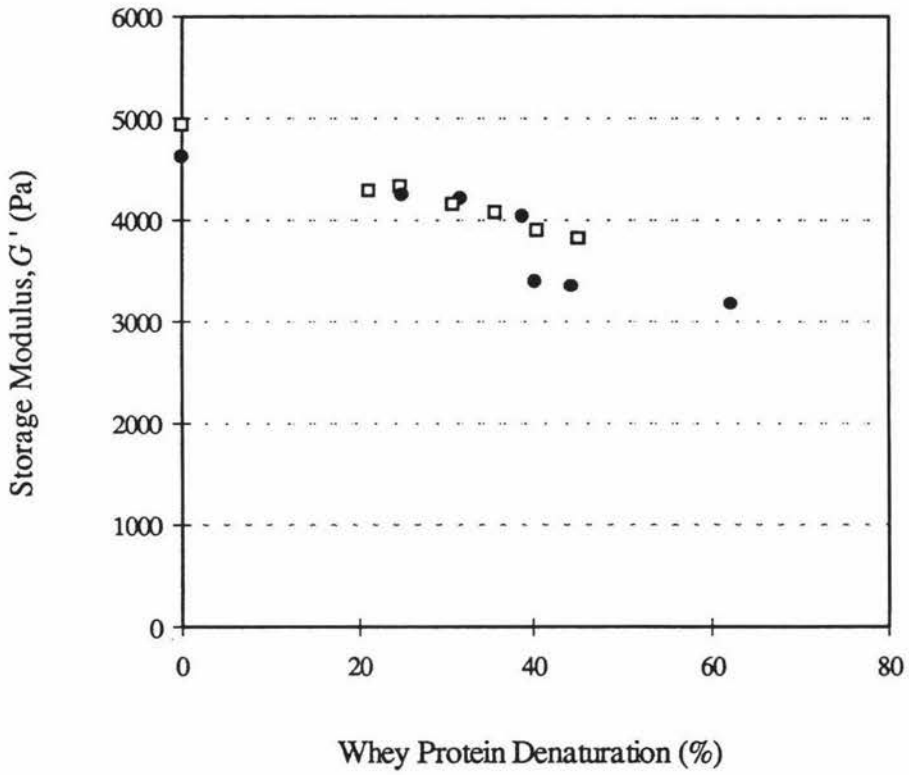


Figure 5.22 The G' values, determined at 2 h after the addition of rennet, as a function of the extent of denaturation of the total whey proteins. Recombined cheese milk made from low-heat treatment (□) and commercial powders (●) with heat treatment at heating temperatures ranging from 70 - 83 °C for 3 min.

It was found that there was a close correlation between the denaturation of whey proteins and the reduction in G' of renneted milk gel made from both types of MPC powders. McMahon *et al.* (1993) also found that the gel strength of rennet-induced milk gels made from ultrafiltration concentrated milk slightly decreased with increased milk processing temperature.

5.2.5 Effect on yield force

The effect of heating for 3 min at various temperatures on the yield force required to fracture the renneted-induced gel determined 1.3 h after the addition of rennet is shown in Figure 5.23.

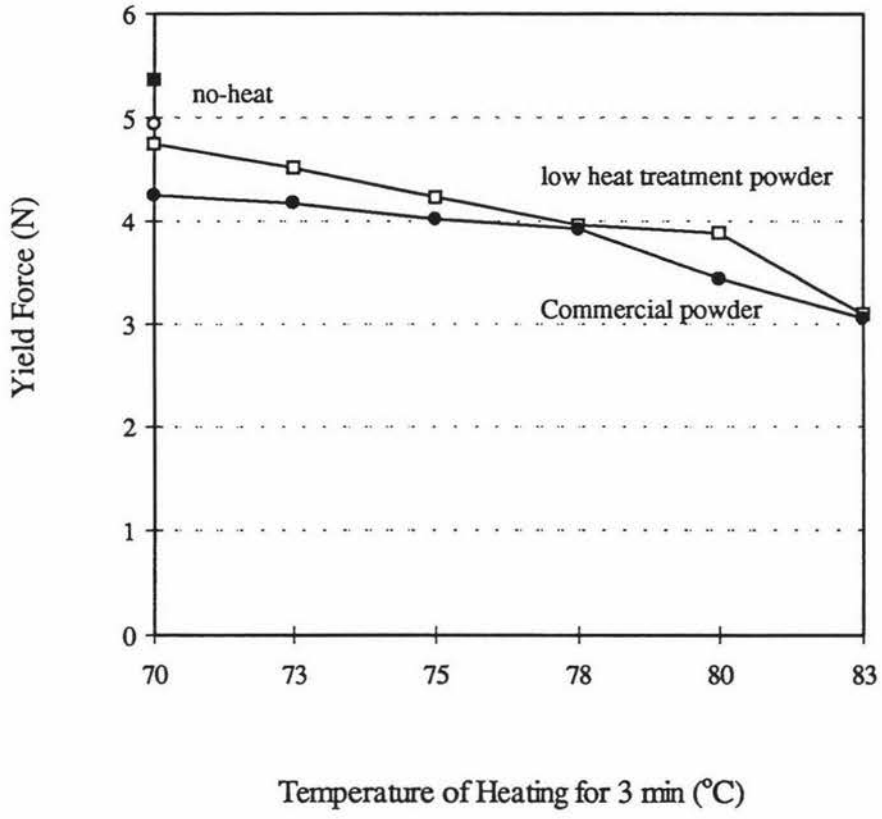


Figure 5.23 The effect of heating for 3 min at various temperatures on the yield force determined at 1.3 h after the addition of rennet for recombined cheese milk made from low heat treatment (□) and commercial powder (●). ■ and ○ represent no-heat treatment samples made from low-heat treatment and commercial powder, respectively.

It is clear that heat treatment reduced the yield force required to fracture renneted gels made from both types of MPC powders. The yield force decreased as the severity of heat treatment increased, i.e. with increasing temperature of heating. At all heat treatments, recombined cheese milks made from low-heat treatment powder produced rennet gels with higher yield forces than renneted gels made from commercial powder, even when they were given the same heat treatment. The relationship between the level of whey proteins denaturation and yield force is shown in Figure 5.24. A higher level of whey protein denaturation resulted in a lower yield forced required to fracture the renneted gel.

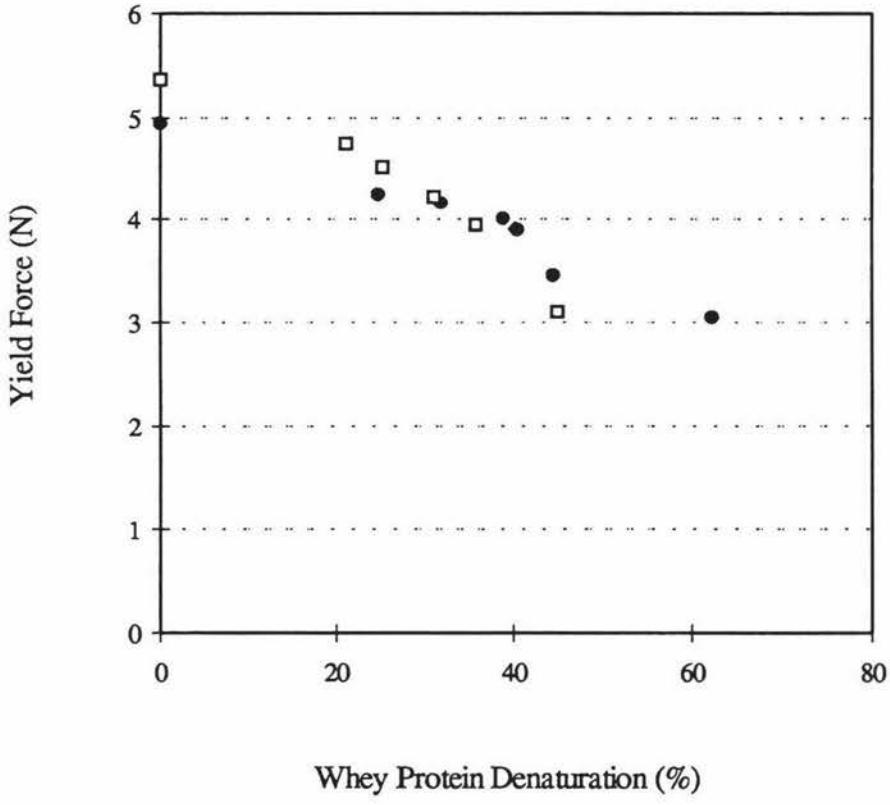


Figure 5.24 The yield force required to fracture the renneted gel, determined at 1.3 h after the addition of rennet, made from recombined cheese milk as a function of the extent of denaturation of whey proteins. Recombined cheese milk made from low-heat treatment (□) and commercial powder (●) heated at temperature ranging from 70 to 83°C for 3 min.

5.2.6 Frequency Sweep

Frequency sweeps were carried out after oscillation measurements were finished in order to characterize the effect of the time scale of the applied deformation on the rheological characteristics of rennet-induced gels. G' and tan δ as a function of angular frequency of renneted milk gels made from low-heat treatment and commercial powder are shown in Figures 5.25 and 5.26, respectively.

The G', determined at any particular frequency, decreased with increased heating temperature. Decreases in G', which is strongly dependent on the density of the gel network, indicated a decrease in the elastic structure of renneted gel. A decrease in G' was also observed in oscillation experiments (frequency of 0.1 Hz). In all cases, the G' values of renneted gels made from recombined cheese milk decreased with decreasing angular frequency. The slope of the curve between G' and angular frequency varied between 0.2270 - 0.2403 and 0.2418 to 0.2450 for recombined cheese milk made from commercial and low-heat treatment powder, respectively. The slope value was higher than that observed on rennet milk gel made from normal milk (Zoon *et al.*, 1988).

Tan δ, which is a measure of the type of link that forms the gel network, decreased as the angular frequencies and heat treatment increased. A increase in tan δ implies that the gel has relatively more elastic characteristic (Zoon *et al.*, 1988). Renneted gels made from recombined cheese milk that had undergone a low heat treatment had higher tan δ, indicated that the gel was more elastic compared to that made from cheese milk that had undergone a high heat treatment (Figure 5.25 and 5.26).

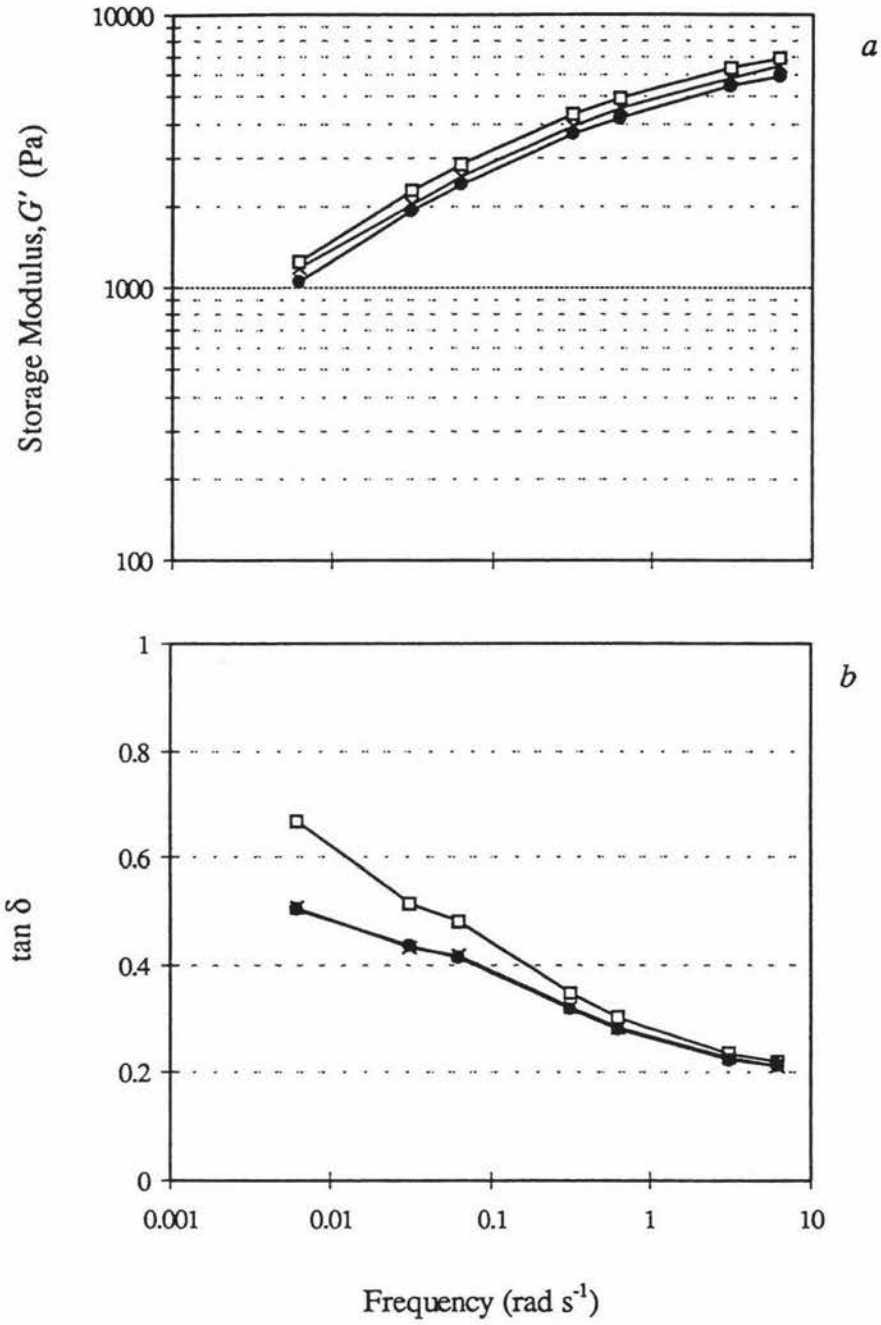


Figure 5.25 *G'* (*a*) and tan δ (*b*) as a function of angular frequency of recombined cheese milks made from low-heat treatment powder that had undergone heat treatment at 70 (□), 78 (×) and 83°C (●) for 3 min after homogenization.

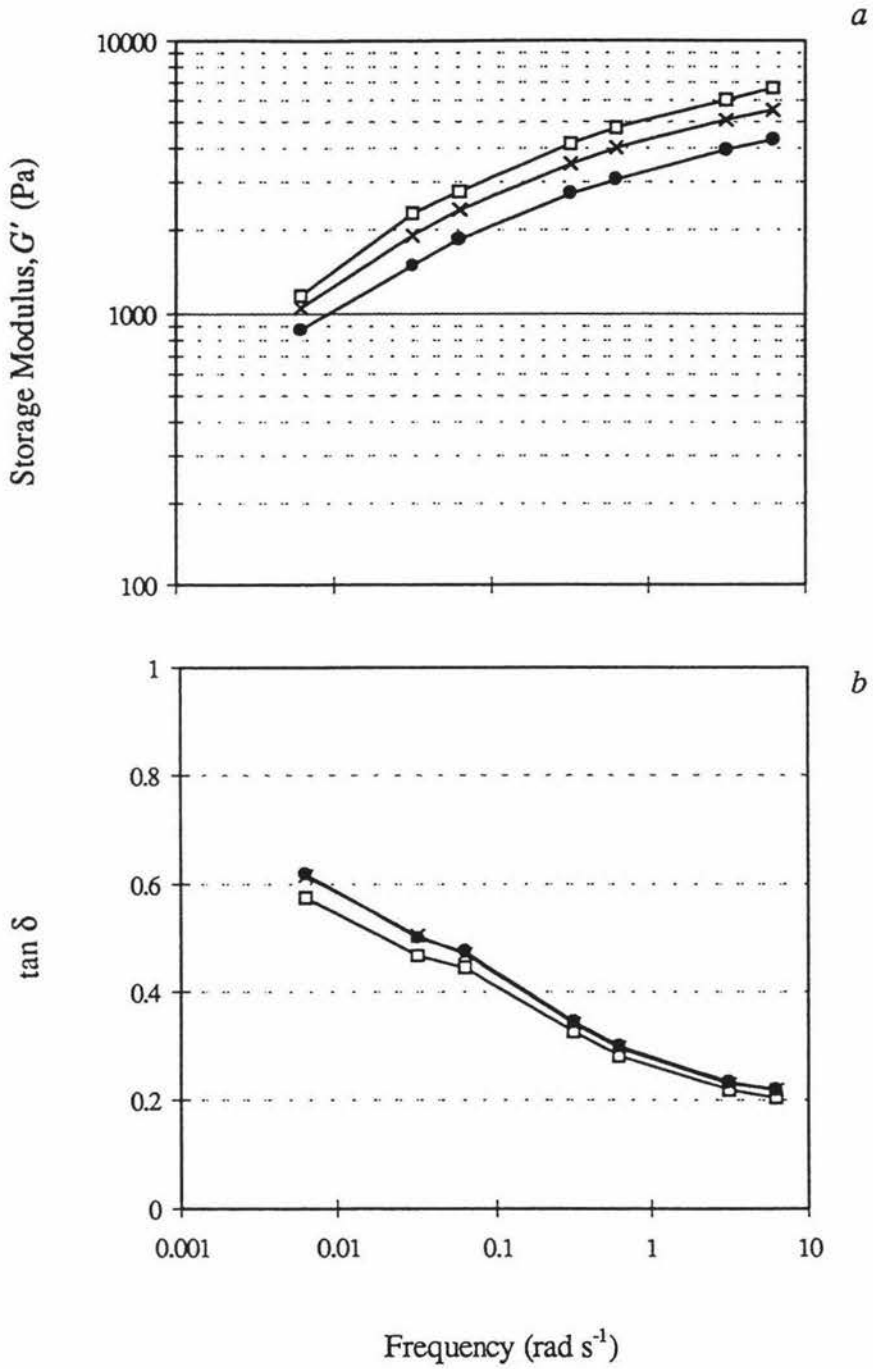


Figure 5.26 *G'* (*a*) and tan δ (*b*) as a function of angular frequency of recombined cheese milks made from commercial MPC-56 powder that had undergone heat treatments at 70 (□), 78 (×) and 83 (●)°C for 3 min after homogenization.

OVERALL DISCUSSION

The results obtained from these experiments demonstrated that heat treatment, both heating time and temperature, influences the physical and rennet coagulation properties of recombined cheese milk made from MPC-56 powder. The effects depend on the severity of heat treatment and the effect is more pronounced in recombined cheese milk made from commercial MPC-56 powder.

The d_{43} and the fat globule size distribution of recombined cheese milk homogenized at 120/50 bar was virtually unchanged by heating temperature and time, however a small increase in particle diameter occurred when severe heat treatment was applied. Heat treatment of the cheese milk after homogenization caused denaturation of whey proteins which may subsequently interact either with casein micelles that adsorb onto the fat globules or with "free" casein micelles, resulting in increased d_{43}. In addition, the recombined cheese milk used in the study contain high total solids, i.e. 20% MPC powder and 20% milk fat, so the effect of heat treatment on the milk components was more pronounced compared to that of normal milk.

It was observed in this study that denaturation of whey proteins had a significant influence on the physical and rennet coagulation properties of recombined cheese milk (Figures 5.6, 5.11, 5.13, 5.18, 5.22 and 5.24) and the effect was dependent on the level of denaturation, which was in agreement with the results reported on normal milk by Singh *et al.* (1988), Law (1995) and Lucey (1995). They reported that significant changes occur in the milk protein system when milk is heated above pasteurization and the denaturation of whey proteins and their interactions with casein micelles alters the functional properties and interferes with the rennet coagulation properties of heated milk. The effect of heat treatment on the rennet coagulation properties of heated milk was recently reviewed by Lucey (1995). Leaver *et al.* (1995) reported that whey protein denaturation begins with an initial swelling of the protein structure when it is exposed to heat, following by unfolding and aggregation through multiple-reaction processes. After denaturation, whey proteins can either interact with themselves or with casein micelles to form aggregates through hydrophobic interactions and disulphide linkages.

Mulder and Walstra (1974) and Sherbon (1988) reported that the viscosity of milk products depends on the temperature and on the amount and state of dispersion of the components. This

means that the casein micelles, whey proteins and milk fat globules and their interactions are the most important factors contributing to the viscosity of the milk. Recombined cheese milks, which contained 20% MPC powder and 20% milk fat, used in this study behave as pseudoplastic material ($n < 1$), i.e. the viscosity decreased and the shear stress increased with increase in shear rate. The n value varied from 0.8424 to 0.5536 in recombined cheese milk made from commercial powder and from 0.9144 to 0.8723 in those made from low-heat treatment powder when different heat treatment were given to the milk. The viscosity, shear stress, n and k values, which represented the flow behaviour of recombined cheese milk changed significantly, i.e the viscosity, shear stress and n value increase, while k values decreased, when recombined cheese milk had undergone heat treatment after homogenization. The effect was more pronounced in recombined cheese milk made from commercial powder.

The increase in viscosity and shear stress in heated milk may be attributed to the denaturation of whey proteins and their association with casein micelles, as suggested by Jeurnink and Kruit (1993) for heated skim milk. Jeurnink and Kruit (1993) also suggested that association of whey proteins may increase micelle size and modify the nature of interactions between micelles. Since homogenized recombined milks were heated in this study, it is likely that some of the denatured whey proteins interacted with the casein micelles or casein subunits that were adsorbed onto the fat globule surfaces during homogenization. This may affect the composition and structure of adsorbed protein around the fat globules, thus influencing the viscosity. The association of whey protein with the casein micelles and/or fat may also affect the viscosity of recombined cheese milk. It appears that over 60% whey protein denaturation is required to influence the viscosity to any significant extent. This can also used to explain why recombined cheese milk made from commercial MPC-56 powder had higher viscosity and shear stress compared to that made from low-heat treatment powder. The recombined cheese milk made from commercial powder had a higher level of whey protein denaturation than that made from low-heat treatment powder.

The rennetability of recombined cheese milk made from low-heat treatment and commercial powder MPC-56 powder was affected by both heating time and temperature, with a larger effect on recombined cheese milk made from commercial powder. The effects of heat treatment were, of course, dependent on the intensity of heat treatment. The GT, rate of aggregation, G'

values and the force required to fracture the renneted gel of heated milk were lower than those of the corresponding unheated milk. A close correlation between the level of denaturation of whey protein and the changes in rennetability was observed. Whey protein denaturation seemed to inhibit the renneting process of recombined cheese milk resulting in a reduced rate of coagulation, lower yield forces required to fracture the gels and also the G' values determined at 1 and 2 h after the addition of rennet were lower.

Denaturation of whey proteins and their interaction with casein micelles may affect the ability of rennet to hydrolysis κ-casein (van Hooydonk *et al.*, 1987; Leaver *et al.*, 1995). Leaver *et al.* (1995) reported that denaturation of about 10% of the whey proteins resulted in partial inhibition of the primary phase. Additional denaturation (up to 60% of the total whey proteins) had no further effect on the reaction until the heating conditions were sufficiently severe to cause chemical changes to the caseins. Their results were confirmed by the report of Reddy and Kinsella (1990) who observed that heating suspensions of casein micelles at high temperature, i.e. at 85°C for 15 min, in the absence of whey proteins had no effect on the rennet hydrolysis and the maximum amount of CMP released.

The rate of aggregation of renneted altered micelles was severely impaired when recombined cheese milk was heated. Leaver *et al.* (1995) and Lucey (1995) studied the effect of heat treatment on rennetability of heated milk and observed that the aggregation phase is severely inhibited. The reduction in aggregation rate may be due to the steric inhibition of enzymatic attack by the attached denatured whey protein on the casein which reduced the κ-casein available as the substrate for renneting enzyme, resulting in the rennet-sensitive Phe-Met bond becoming totally accessibly to the enzyme (Leaver *et al.*, 1995).

Casiraghi *et al.* (1989) reported that the denaturation of β-lactoglobulin and its interaction with κ-casein apparently restricts the access of rennin enzymes to casein micelles surface and retards the clotting reaction. Conversely, the enzyme may hydrolyze κ-casein, but the rennet-altered micelles may be unable to coagulate because of the denatured β-lactoglobulin bound to the surface of micelles (Dalgleish, 1992). Alternatively, the formation of heat-induced colloidal calcium phosphate (CCP) in heated milk might also affect the rate of aggregation of renneted

micelles which is dependent on Ca^{2+} concentration (Walstra & Jenness, 1984; van Hooydonk *et al.*, 1987).

Contrast to that reported for normal and ultrafiltered milk (Wilson & Wheelock, 1972; Damicz & Dziuba, 1975; Casiraghi *et al.*, 1989; Reddy & Kinsella, 1990; McMahon *et al.*, 1993; Waungana, 1995), heat treatment of recombined cheese milk, at the temperature ranging from 70 to 85°C for 3 - 10 min, slightly decreased the gelation time (GT) of recombined cheese milk made from MPC-56 powder (Figure 5.9). This difference might be due to the fact that recombined cheese milk which contained high total solids, 40%, was used in this study. The faster gelation process observed when recombined cheese milk was heated may be attributed to (i) the higher viscosity observed in heated recombined cheese milk, (ii) the preaggregated material that might occur when recombined cheese milk was heated and (iii) probably the reduced hydrolysis of κ-casein required at the point of gelation. Dalgleish (1993) reported that the aggregation phase of ultrafiltered milk commences at a lower degree of proteolysis (approximately 50%) than that observed in unconcentrated milk (80 - 90%).

G' values determined 1 and 2 h after the addition of rennet and the yield force required to fracture rennet-induced milk gels were affected by the severity of heat treatment. With increasing severity of heating, G' and the yield force were progressively decreased. This behaviour was related to the extent of denaturation of whey protein upon heating. Denaturation of whey proteins up to 50% had a much larger effect on G'. Further denaturation had less effect. The denatured whey proteins present in the heated, recombined cheese milk probably interact with the casein micelles, and it is possible that the complexes formed may reduce casein-casein interactions, with the result that they cause changes in the cheese structure resulting in a weaker gel. The presence of whey protein on casein micelles may alter the orientation of casein strands or provide less complete fusion/rearrangement of the gel system making it more susceptible to fracture.

The weaker gel observed when recombined cheese milk was heated was also observed in normal and ultrafiltered milk (Singh *et al.*, 1988; Lucey *et al.*, 1993; McMahon *et al.*, 1993, Waungana, 1995). However, the effect of heat treatment was less pronounced in recombined

cheese milk compared to that of normal milk. McMahon *et al.* (1993) and Waungana (1995) reported that the effect of heat treatment was less severe in ultrafiltered milk (3X) compared to that of corresponding unconcentrated milk. Lucey *et al.* (1995) and McMahon *et al.* (1993) attributed the reduction in gel strength of heated milk to the disruption of the continuity of the gel network by attachment of denatured whey proteins to the casein micelles. The presence of denatured whey protein in the casein network interferes with fusion of casein micelles and slows the rearrangement process which is necessary for an increase in gel strength (G'). Lucey *et al.* (1993) reported that the gel strength of heated milk increases at a slower rate than unheated milk. Thus, the strength of rennet-induced gels made from heated milk, determined at a particular time after renneting, will be lower than that of unheated milk.

GENERAL CONCLUSION

It may be concluded from the results obtained in the present study that heat treatment, both heating temperature and time, have a significant influence on the physical and rennet coagulation properties of recombined cheese milk made from MPC-56 powder. The viscosity, shear stress and level of whey protein denaturation of recombined cheese milk increased as the intensity of heat treatment increased. The rennet coagulation properties were impaired by the heat treatment which the recombined cheese milk had undergone. The gelation time, rate of coagulation, G' and yield force at the yield point decreased as the severity of heat treatment increased. The different in physical and renneting properties may be attributed to the denaturation of whey protein and the interaction between the denatured whey protein and casein micelles. A schematic diagram of the effect of heat treatment on the milk system and rennet coagulation properties is shown in Appendix I, Figure 1.

CHAPTER 6

EFFECT OF PREHEAT TREATMENT DURING MPC POWDER MANUFACTURE ON THE PHYSICAL AND RENNET COAGULATION PROPERTIES OF RECOMBINED CHEESE MILK

Many cheese varieties can be successfully manufactured from recombined milk using low-heat skim milk powder and milk fat (Gilles & Lawrence, 1981). Since it has been well established that heat treatment during milk powder manufacture has a significant influence on its physical and rennet coagulation properties, the skim milk powder used in recombined cheese manufacture is made from skim milk that has not been preheated in excess of 72°C for 15 s or 99°C for 1 s so as to maintain maximum rennetability.

Gilles and Lawrence (1981) reported that the cheesemaking properties of recombined milk are somewhat different from that of the original milk from which the powder is manufactured. Specifically, the rate of coagulation in recombined milk is slower and the coagulum strength is reduced. They attributed this phenomenon to the changes that occurred in milk powder as a results of the powder-making process, especially the heat treatment process. Gilles (1974) also found that the renneting properties of recombined cheese milk are dependent on whey protein denaturation and it has proved difficult to produce recombined cheese from high heat treatment powder. However, it appears attractive to use the high heat treatment powder for recombined cheese due to the increasing cheese yields by incorporation of denatured whey protein. By modification of the manufacturing process, it might be possible to produce the recombined cheese milk from high heat treatment powder that have desired characteristics for the recombined cheese industry.

The present chapter describes the physical and rennet coagulation properties of recombined cheese milk (20% MPC-56 powder and 20% milk fat) and reconstituted skim milk (20% MPC-56 powder without milk fat) made from various MPC-56 powders which were produced using different preheat treatments after the ultrafiltration stage during MPC-56 powder manufacture. The preheating conditions used, percentage of individual whey protein denaturation and whey protein nitrogen index (WPNI) of various MPC-56 powders are shown in Table 6.1. The experimental protocol is followed that shown in Figure 4.1

Table 6.1 The preheat treatment used during powder manufacture, whey protein denaturation and whey protein nitrogen index of MPC-56 powders.

Preheat Treatment (Temp/Time)	WPNI	Whey Protein Denaturation (%)		
		α-Lactalbumin	β-Lactoglobulin A	β-Lactoglobulin B
75°C for 15 s	10.5	0	0	0
85°C for 15 s	9.2	4.77	14.91	21.76
85°C for 60 s	7.4	13.97	34.04	48.60
85°C for 120 s	5.8	21.93	49.29	63.76
85°C for 180 s	4.6	29.76	59.94	66.28
85°C for 240 s	3.9	35.98	69.40	76.53
120°C for 180 s	0.8	77.47	91.75	93.99

All recombined milk samples were homogenized at 120/50 bar, at 60°C. The changes in fat globule diameter ($d_{4,3}$), viscosity and shear stress, whey protein denaturation and rennet coagulation properties were determined. All the experimental results are shown in Appendix II, Table 3.

RESULTS AND DISCUSSION

6.1 Effect on average fat globule diameter ($d_{4,3}$)

The $d_{4,3}$ of recombined cheese milk measured after dispersion of the sample in deionized water and dissociating solution were in the range of 1.10 - 1.14 μm (Figure 6.1). The particle size distributions of recombined cheese milk made from various MPC powders are shown in Figure 6.2. The results show that preheat treatment during MPC-56 powder manufacture did not have significant effect on the $d_{4,3}$ of recombined cheese milk.

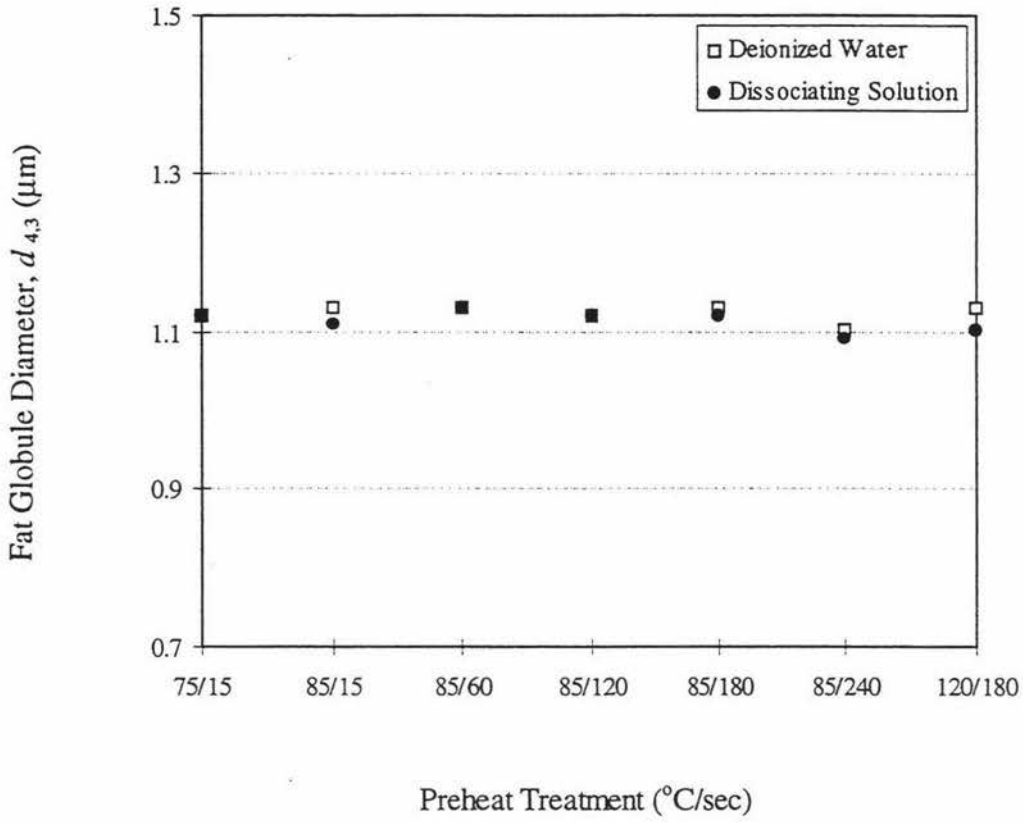


Figure 6.1 Effect of preheat treatment during MPC-56 powder manufacture on average fat globule diameter ($d_{4,3}$) of recombined cheese milk made from various MPC-56 powders with homogenization pressure at 120/50 bar without any additional heat treatment.

In general, the preheat treatment during MPC powder manufacture did not have an significant effect on the particle size distribution. Only when the high heat treatment MPC powder was used to prepared the recombined cheese milk, slight shifting of the particle size distribution toward the smaller particles was observed.

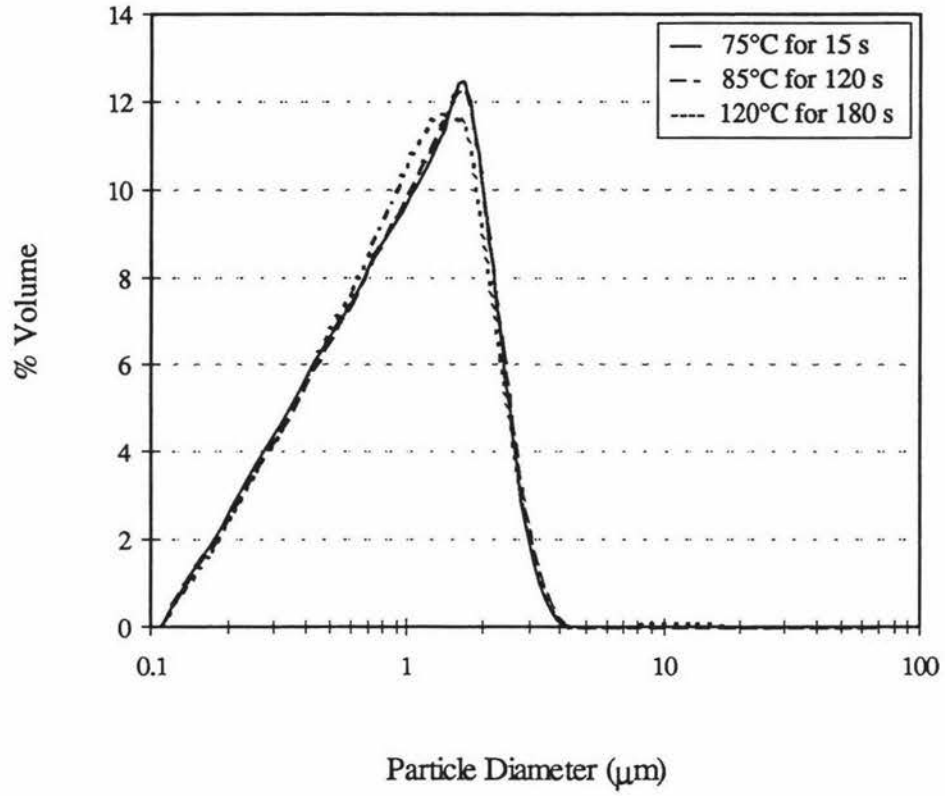


Figure 6.2 The profile of fat globule size distribution in recombined cheese milks made from MPC powders with preheat treatment during MPC powder manufacture at 75°C for 15 s (—), 85°C for 120 s (– –) and 120°C for 180 s (---), homogenized at 120/50 bar without any additional heat treatment.

6.2 Effect on whey protein denaturation

The effects of preheat treatment during MPC-56 powder manufacture on whey protein denaturation in recombined cheese milk and reconstituted skim milk are shown in Figures 6.3 and 6.4. As expected the percentage of whey protein denaturation increased with increasing the severity of preheat treatment.

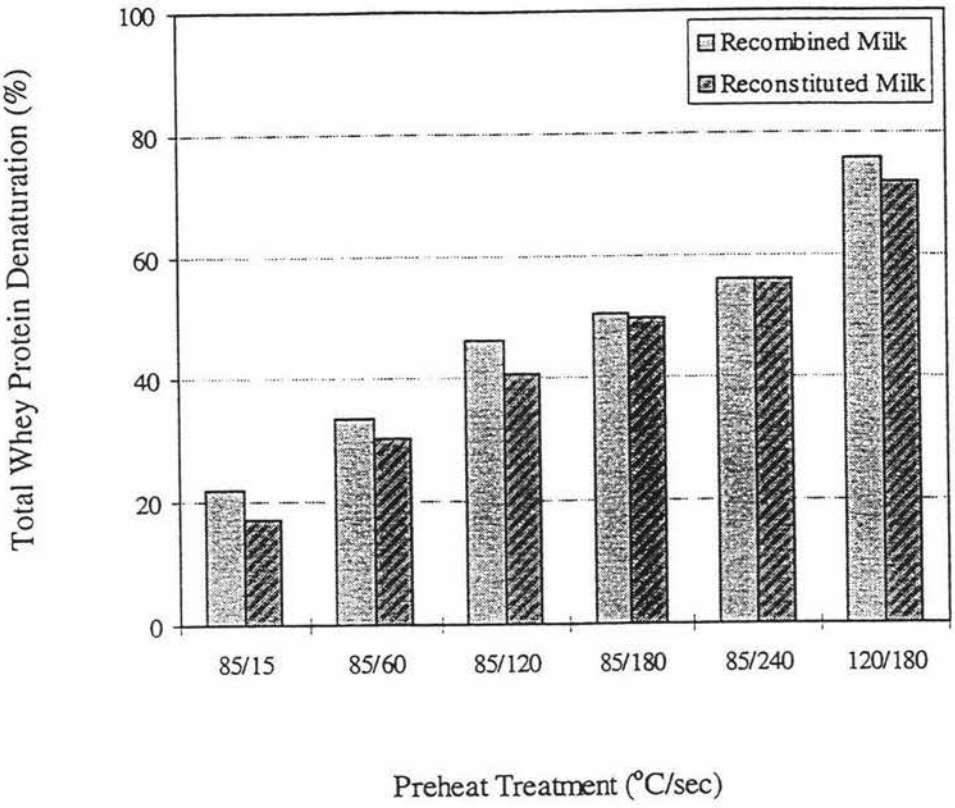


Figure 6.3 Effect of preheat treatment during MPC-56 powder manufacture on percent denaturation of total whey protein in recombined cheese milk and reconstituted skim milk measured by investigated the decreased in soluble whey protein at pH 4.6 using Kjeldahl analysis on the supernatant. All values are relative to those obtained with the milk sample prepared from MPC powder with preheat treatment at 75°C for 15s.

The denaturation of total whey protein increased to approximately 75% when MPC powder had undergone preheat treatment at 120°C for 180 s. Electrophoresis in the absence of denaturing and reducing agents shows that the quantity of native whey protein, β-lactoglobulin (both variant A and B) and α-lactalbumin, decreased with increasing the severity of preheat treatment during MPC powder manufacture.

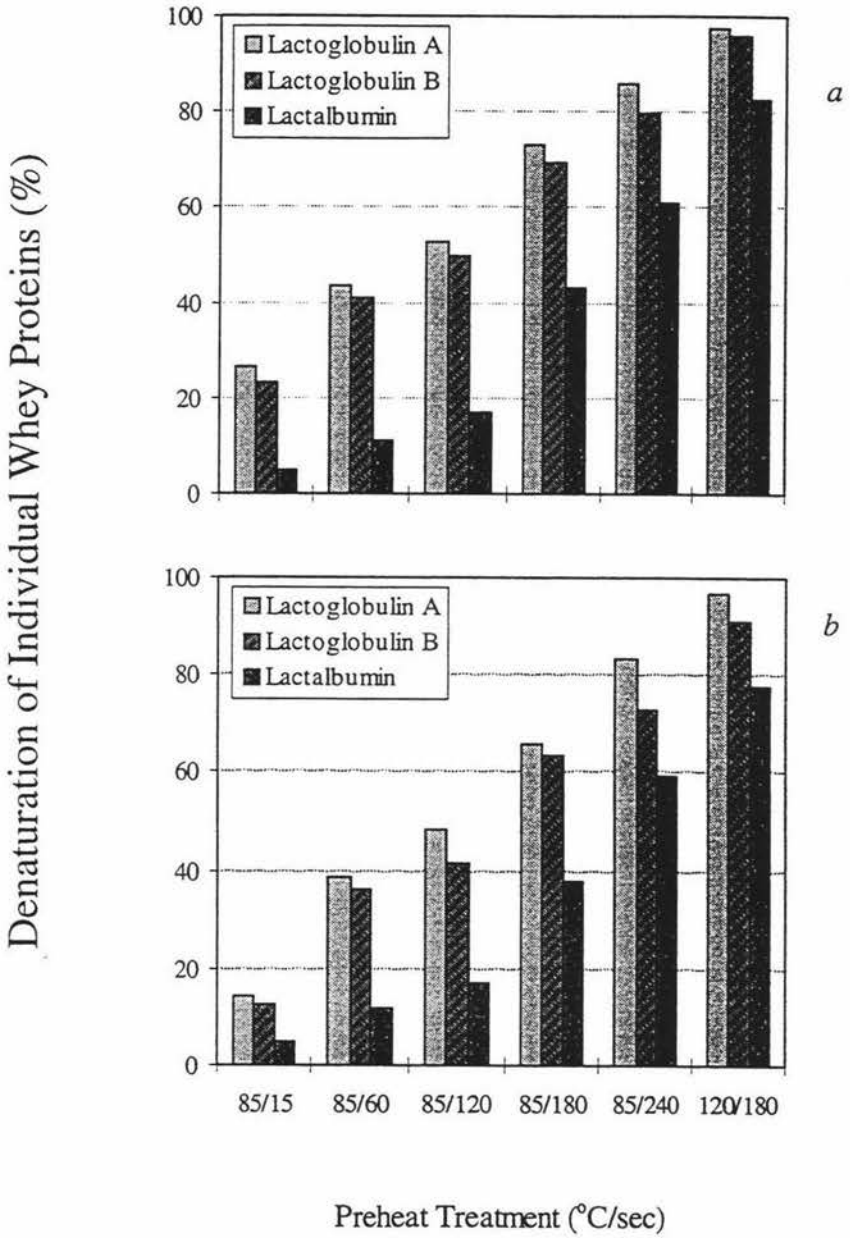


Figure 6.4 Effect of preheat treatment during MPC-56 powder manufacture on percent denaturation of individual whey protein in recombined cheese milk (*a*) and reconstituted milk (*b*) measured using Native-PAGE on the pH 4.6 supernatant. All values are relative to those obtained with the milk sample prepared from MPC powder with preheat treatment at 75°C for 15s.

The results obtained showed clearly that the loss of native whey proteins was faster for β-lactoglobulin A variant than the B variant and α-lactalbumin at all preheating regimes, which is in consistent with the results reported by Dannenberg & Kessler (1988) for skim milk.

However, it was observed that the percentage of whey protein denaturation was somewhat greater in recombined cheese milk (40% total solids) than in reconstituted skim milk (20% total solids) from which it was made. This suggests that when the recombined system is being investigated the effect of fat globules must be taken into account. It has been reported in the literature that caseins and whey proteins, especially β-lactoglobulin, were the major proteins components of the milk fat globule membrane of recombined milk (Henstra & Schmidt, 1970; Mulder & Walstra, 1974; Oortwijn *et al.*, 1979; McPherson *et al.*, 1984; Walstra & Jenness, 1984). Therefore, when the caseins are precipitated at pH 4.6, any whey proteins that are adsorbed at the fat-water interface would be carried with any fat droplets entrapped within the casein precipitated. These whey proteins would not necessarily to be thermally denatured, but have been unfolded by interfacial forces during homogenization (Walstra, 1980). This would explain why more whey proteins were found to have been denatured in recombined cheese milk compared to reconstituted skim milk. These results were in a good agreement with those reported by McPherson *et al.* (1984) and McMahon *et al.* (1993) who found the higher level of whey protein denaturation in homogenized whole milk compared to skim milk produced under the same conditions.

6.3 Effect on viscosity and shear stress of recombined cheese milk

The effect of preheat treatment during MPC-56 powder manufacture on viscosity and shear stress was investigated in recombined cheese milk over the shear rate range of 18.5 to 731 s^{-1} at 34°C and the results are shown in Figure 6.5. It is clear that the preheat treatment of MPC powder had a significant influence on both the viscosity and shear stress of recombined cheese milk.

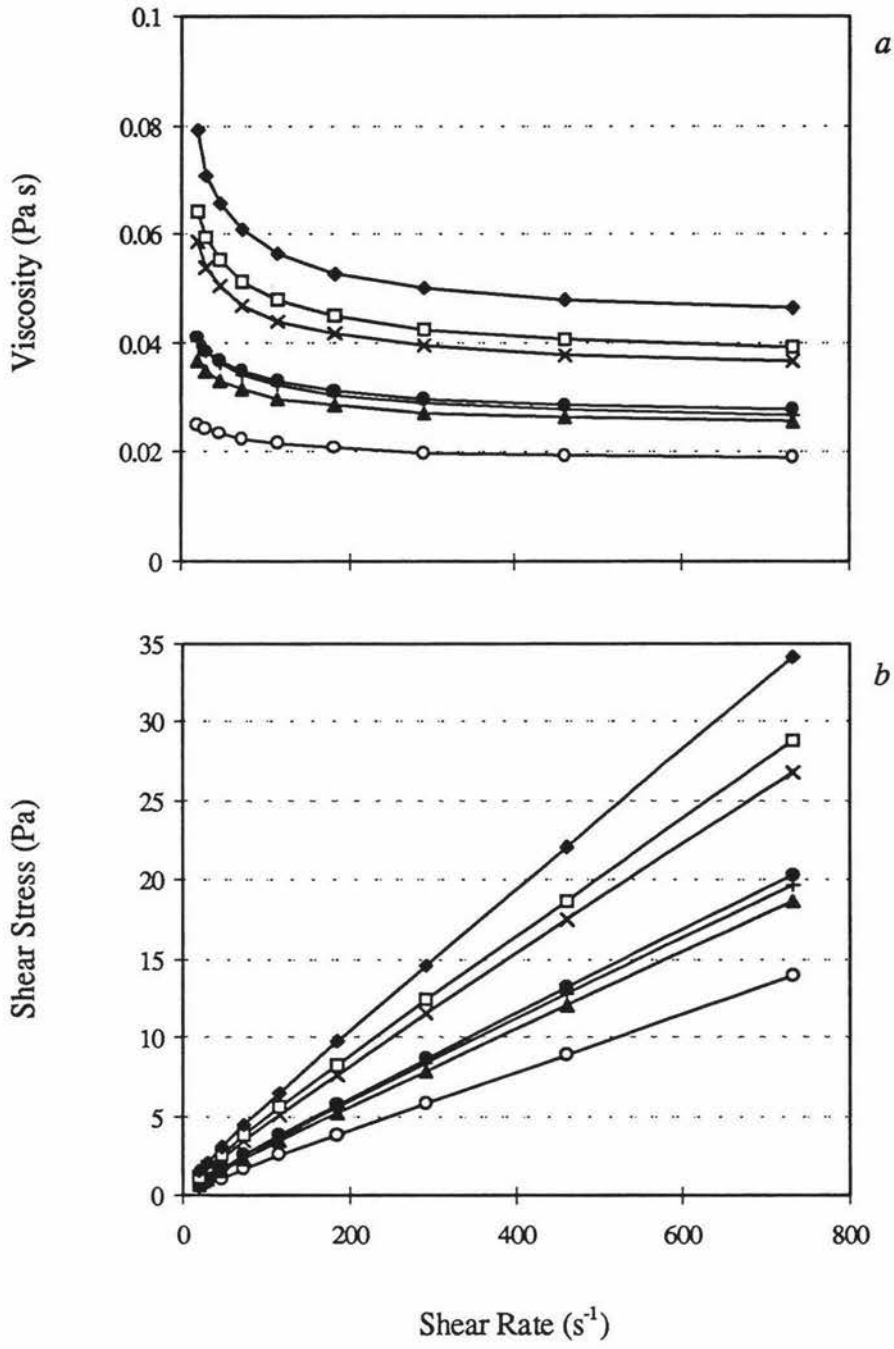


Figure 6.5 Viscosity (*a*) and shear stress (*b*) of recombined cheese milks made from various MPC-56 powders as a function of shear rate at 34°C: MPC-56 powder made using preheat treatment at 75°C for 15 s (○), 85°C for 15 (▲), 60 (+), 120 (●), 180 (×), 240 s (□) and 120°C for 180 s (◆).

Increasing the severity of preheat treatment resulted in increases in viscosity and shear stress of recombined cheese milk. The viscosity of recombined cheese milk determined at 34°C increased about four times when the preheat treatment increased from 75°C for 15 s to 120°C for 180 s. Walstra and Jenness (1984) also found that heating of milk causes some increase in viscosity due to the aggregation of protein and the effect was more pronounced in concentrated milk.

In all cases, the viscosity of recombined cheese milk decreased with increasing shear rate, indicating shear thinning behaviour. Such behaviour implies that the cheese milk does not have a true but an apparent viscosity. The marked shear thinning in recombined cheese milk was observed when high-heat MPC-56 powder (MPC powder with preheat treatment at 120°C for 180s) was used (Figure 6.5), i.e. the slope of the shear stress versus shear rate was very high and the viscosity of the cheese milk decreased significantly with the increase in shear rate. This may be attributed to greater whey protein denaturation, thus more interaction between denatured whey protein and casein and also the more interaction with milk fat globule. The flow behaviour index, n and k values, of recombined cheese milk made from various MPC powders determined at 34°C are shown in Table 6.2.

Table 6.2 The flow behaviour index, determined at 34°C, of recombined cheese milk made from various MPC powders.

Preheat Treatment	Flow Behaviour Index (n)	Consistency Index (k)
75°C for 15 s	0.9222	0.0313
85°C for 15 s	0.8990	0.0487
85°C for 60 s	0.8900	0.0559
85°C for 120 s	0.8849	0.0560
85°C for 180 s	0.8723	0.0825
85°C for 240 s	0.8641	0.0932
120°C for 180 s	0.8557	0.1153

According to the flow behaviour index, recombined cheese milk is a non-Newtonian liquid and its behaviour is of pseudoplastic nature, i.e. the viscosity decreased with increase in shear rate. The n values of recombined cheese milk were in the range of 0.8557 - 0.9222. Increased preheat treatment during MPC powder manufacture resulted in a decrease in n values, but increase in k values. The changes in viscosity, n and k values were related to the degree of whey protein denaturation. Their relationships are demonstrated in Figure 6.6. Up to 40% denaturation of whey proteins, viscosity increased slightly but at higher levels of denaturation, viscosity increased almost in a linear fashion.

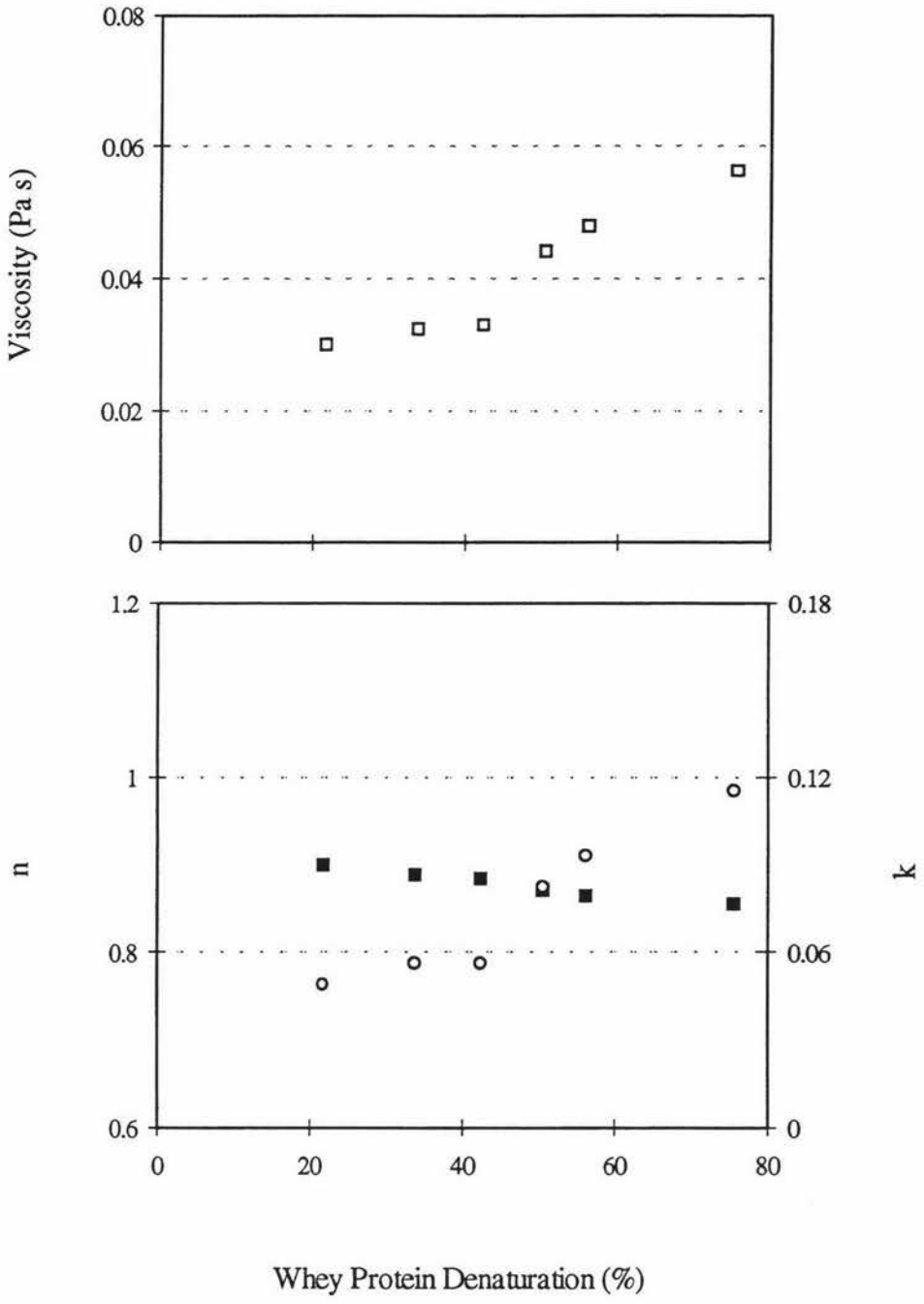


Figure 6.6 The changes in viscosity (□), n (■) and k values (○) of recombined cheese milk as a function of the extent of denaturation of the total whey proteins. The flow behaviour was determined at 34°C at the fixed shear rate of 116 s^{-1}.

6.4 Effect on rennet coagulation properties

Rennet coagulation properties of recombined cheese milk and reconstituted skim milk were investigated and the results are shown in Figure 6.7. Preheat treatment during MPC powder manufacture had a significant effect on rennetability of both recombined cheese milk and reconstituted skim milk, with the effect being more pronounced in the recombined system. In all cases, G' values determined at 1 and 2 h after the rennet addition and the rate of increase in G' decreased with the severity of preheat treatment during MPC powder manufacture.

The results are in a good agreement with those reported by Gilles and Lawrence (1981) for skim milk powders. They reported that the rennetability of milk powder depends on the heat treatment that the milk had undergone during powder manufacture and the milk powder that has the WPNI index less than 6.0 mg/g powder was not suitable for cheese manufacture. It is thought that, to a large extent, the heat sensitive whey proteins are responsible for the different properties of milk powder when different preheat treatments are applied (Gilles & Lawrence, 1981; Singh & Newstead, 1992). However, the cause of the reduced rennetability is still not well understood since complicated interactions take place when milk is heated (Singh & Newstead, 1992).

Heating milk at temperatures greater than pasteurization results in denaturation of whey proteins and the formation of complexes between κ-casein and denatured β-lactoglobulin, and perhaps also some changes in κ-casein. Although, direct interaction between α-lactalbumin and κ-casein is limited, the complexes formed between α-lactalbumin and β-lactoglobulin are also able to interact with κ-casein. The extent of the change is a function of both time and temperature of heat treatment (van Hooydonk *et al.*, 1987; Haque & Kinsella, 1988; Singh *et al.*, 1988). In addition, heat treatment has been reported to has a considerable effect on the distribution of calcium in the milk (Kannan & Jenness, 1961). This inevitably affects the rennet coagulability since ionic calcium is involved in milk coagulation. Mulder and Liska (1972) found that in general low-heat skim milk powders contained more ionic calcium, and resulted in recombined milk with higher curd tensions than high-heat skim milk powder.

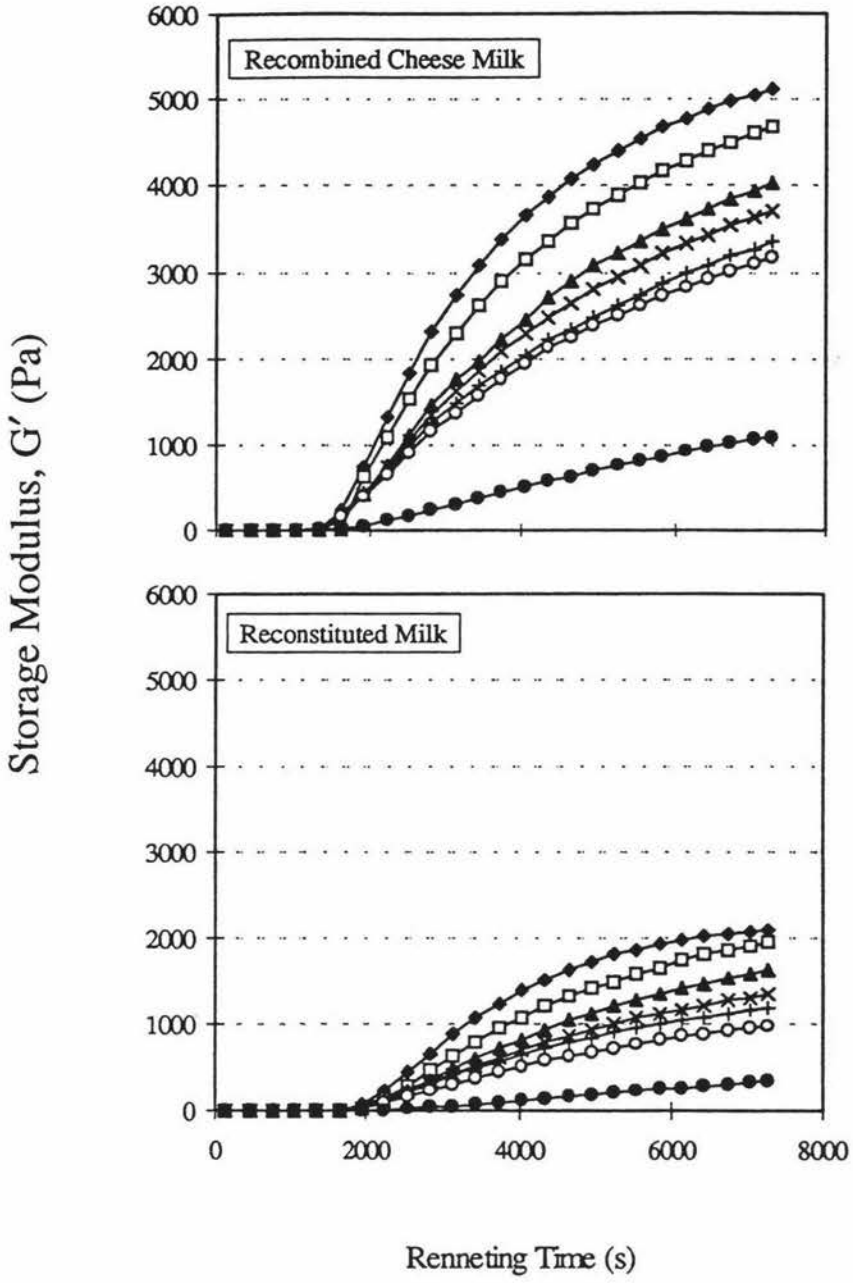


Figure 6.7 Storage modulus (G') of recombined cheese milk and reconstituted skim milk made from various MPC powders as a function of time after rennet addition. Preheat treatment given to skim milk during MPC manufacture at 75°C for 15 s (◆), 85°C for 15 (□), 60 (▲), 120 (×), 180 (+), 240 s (○) and at 120°C for 180 s (●).

Lelievre *et al.* (1990) studied the effect of preheat treatment of milk powder on the properties of halloumi cheese and found that the adverse effect of preheat treatment prevails even with low-heat treatment regimes. The mechanism by which the preheat treatment during milk powder manufacture influences the physical properties of halloumi or mozzarella type cheese is uncertain. Possible mechanisms include whey protein-casein interactions and changes in ionic calcium levels.

In all cases, the G' values determined at any particular time after renneting of recombined cheese milk were twice as much as those of reconstituted skim milk. The GT of reconstituted skim milk was also about twice as that of recombined cheese milk and the rate of increase in G' was slower in reconstituted skim milk. The difference in gelation properties between these two systems may be attributed to the difference in milk composition and total solids content and also the presence of milk fat globule in recombined system. The adsorbed caseins on the surface of milk fat globule in recombined cheese milk may help reinforce the interaction between the casein network resulted in faster gelation and also higher in gel firmness.

Effect on gelation time (GT)

The effects of preheat treatment during MPC-56 powder manufacture on GT of recombined cheese milk and reconstituted skim milk are shown in Figure 6.8. In all cases, the GT of reconstituted skim milk was longer than those of recombined cheese milk. Recombined cheese milk and reconstituted skim milk made from MPC-56 powder that had undergone high heating temperature, i.e. 120°C for 180s, during powder manufacture had slightly longer GT. Preheating under other conditions had no significant effect on GT for both recombined cheese milk and reconstituted skim milk.

The presence of milk fat globules in recombined cheese milk may responsible for the faster gelation process compared to that of reconstituted skim milk. The proteins adsorbed on the surface of milk fat globule may interact with the altered-casein micelles and reinforce the gelation process, resulting in reduction in GT. High total solids in recombined cheese milk may also play an important role in reduced GT. Dalgleish (1993) reported that the GT was dependent

on the concentration of milk. Increased milk concentration resulted in reduced gelation time.

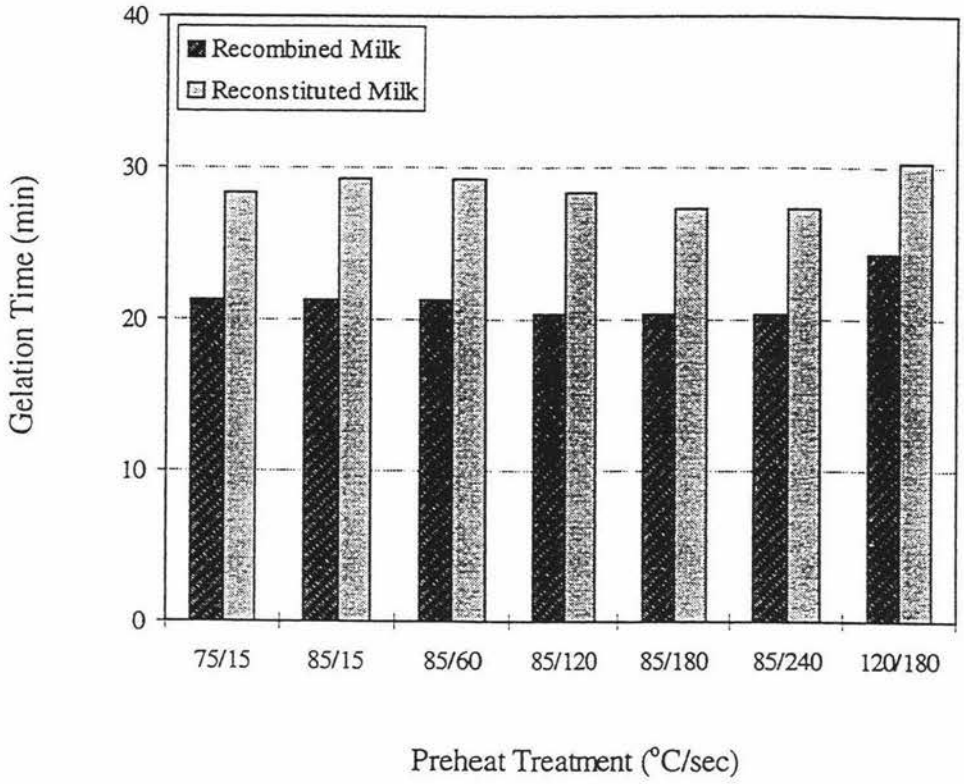


Figure 6.8 Gelation time of recombined cheese milk and reconstituted skim milk prepared from various MPC-56 powders which were subjected to different preheat treatments during manufacture.

Gilles and Lawrence (1981) also found that the recombined milk prepared from milk powder with high heat treatment during its manufacture tended to have a longer gelation time. The longer GT observed in recombined cheese milk and reconstituted skim milk made from high-heat MPC powder (MPC powder with preheat treatment at 120°C for 180s, WPNI 0f 0.8 mg/g powder) may be attributed to the extent of denaturation of β-lactoglobulin and the complex formation between denatured whey proteins and κ-casein. This complex formation may affect

the ability of rennet to hydrolyse κ-casein (van Hoodyonk *et al.*, 1987), resulting in reduced rate of the primary phase.

The difference in GT between recombined cheese milk (40% total solids) and reconstituted skim milk (20% total solids) may be due to (i) the higher concentration of solids in recombined cheese milk (Dalgleish, 1993) and (ii) the presence of the milk fat globules in recombined cheese milk as they act as the crosslink between the altered casein micelles (van Vliet & Dantener-Kikert, 1982).

Effect on storage modulus (G', Pa)

The preheat treatment during MPC powder manufacture considerably reduced G' of renneted gel determined at 1 and 2 h after rennet addition for both recombined cheese milk and reconstituted skim milk as shown in Figure 6.9. The G' was reduced from 5100 Pa in the control sample (MPC powder with preheat treatment at 75°C for 15 s) to 1097 Pa when recombined cheese milk was prepared from high-heat MPC powder (MPC with preheat treatment at 120°C for 180 s). This effect may be attributed to whey protein denaturation and the formation complex with casein. The denatured whey protein may sterically hinder the close approach and contact between the altered casein micelles, resulting in a weaker gel due to reduced crosslinking (van Hooydonk *et al.*, 1987; McMahon *et al.*, 1993; Lucey, 1995).

The effect of preheat treatment on the reconstituted skim system was similar to that of recombined system, but it was less pronounced. In all cases, the G' values of renneted gel made from recombined cheese milk were higher than that made from reconstituted skim milk. This may be due to the effect of milk fat globules that are present in the recombined system. Unlike in normal milk, the milk fat globules of recombined cheese milk composed of casein micelles, sub-micelles and denatured whey protein (Oortwijn *et al.*, 1979; Walstra and Jenness, 1984), which might interact with the casein network and enhance the gel firmness (McMahon *et al.*, 1993). Walstra and Jenness (1984) reported that the presence of fat globules enhanced the development of gel presumably via increasing the rate of crosslinking between the altered casein micelles and membrane around the fat globules.

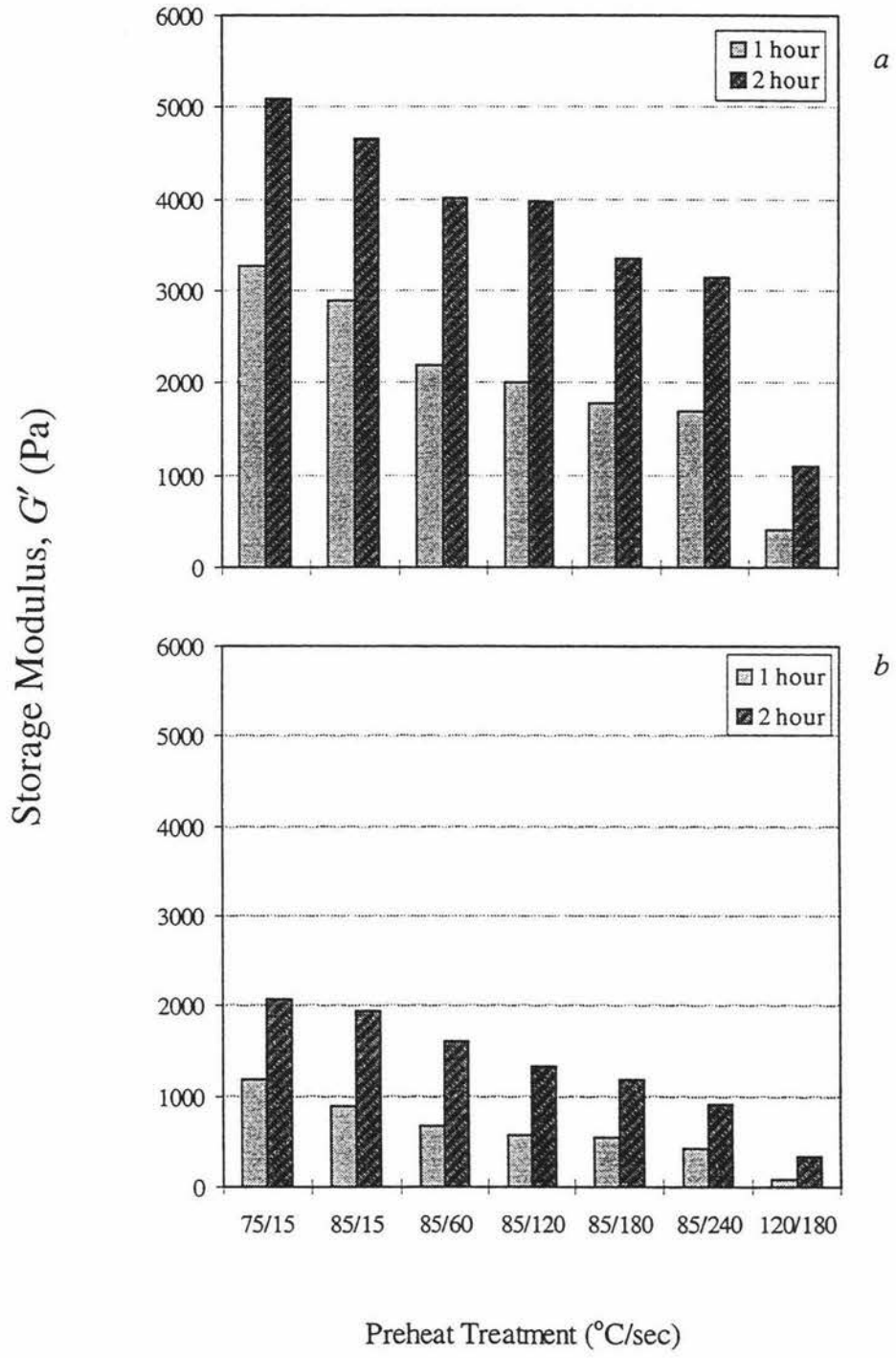


Figure 6.9 Effect of preheat treatment during MPC-56 powder manufacture on storage modulus (G') of recombined cheese milk (*a*) and reconstituted skim milk (*b*), determined at 1 and 2 h after the addition of rennet.

In addition, the recombined cheese milk had higher total solids contents (40%) compared to that of reconstituted skim milk (20%). This in turn may be responsible for the higher firmness of renneted gel made from recombined cheese milk. Dalgleish (1993) also reported that the higher the concentration of milk, the firmness the renneted gels.

It was found that there was a clear relationship between the extent of whey protein denaturation and the reduction in G' values (Figure 6.10). This relationship was linear up to ~ 60% denaturation, but the slope appeared to increase markedly at higher levels of denaturation, especially in recombined cheese milk.

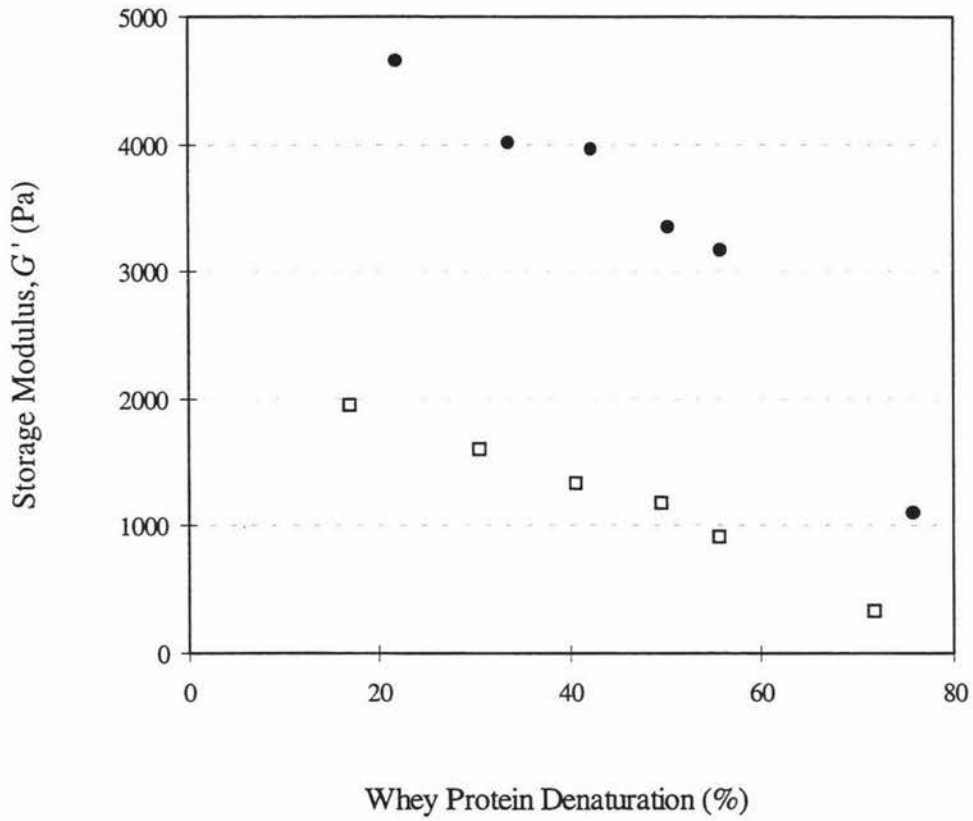


Figure 6.10 The G′ values, determined at 2 h after the addition of rennet, of recombined cheese milk (●) and reconstituted skim milk (□) as a function of the extent of denaturation of total whey protein.

It was evident that the degree of whey protein denaturation is an important factor contributing to the reduction in G′ values observed in recombined cheese milk and reconstituted skim milk. The effect of denaturation appears to be greater in recombined cheese milk than on reconstituted skim milk.

6.5 *Effect on yield force at the yield point*

The effect of preheat treatment on yield force required to fracture renneted gel was determined at 1.3 h after the addition of rennet and the results are shown in Figure 6.11.

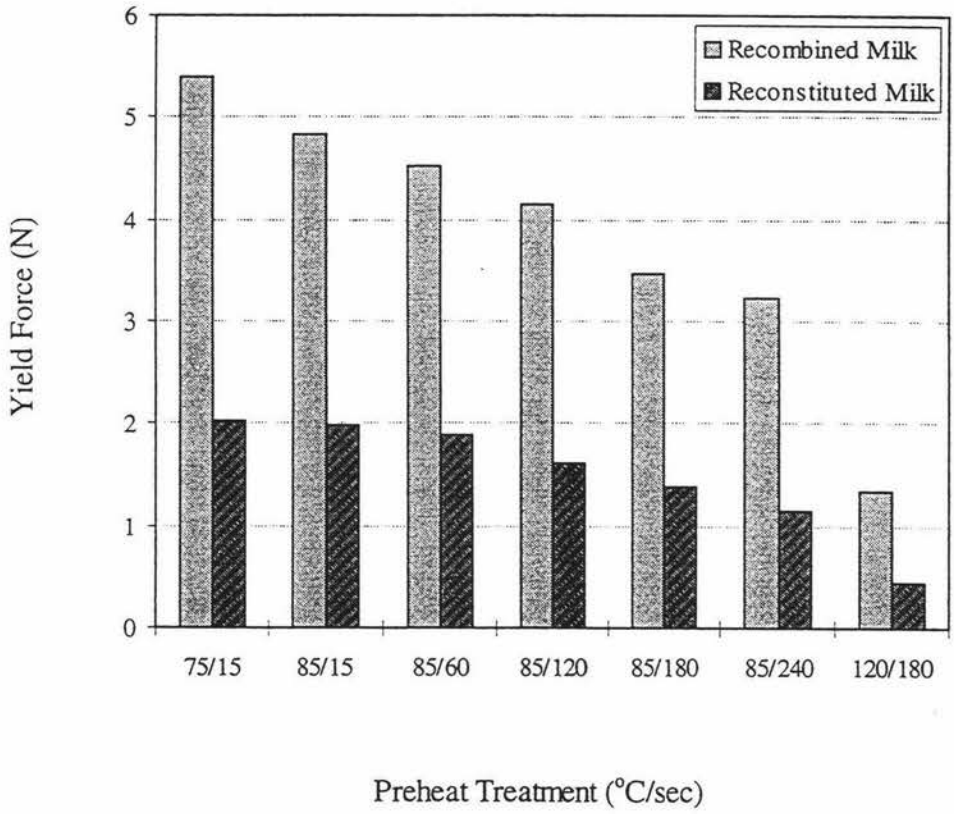


Figure 6.11 Effect of preheat treatment during MPC-56 powder manufacture on yield force at the yield point of recombined cheese milk and reconstituted skim milk determined at 1.3 h after rennet addition.

Similar to the results obtained for the changes in G' values, the force required to fracture renneted gels made from both recombined cheese milk and reconstituted skim milk decreased as the severity of preheat treatment during MPC powder manufacture increased. As expected, the force required for renneted gel made from recombined cheese milk was higher than those made from reconstituted skim milk. The reasons used to explaine the effect of preheat treatment during MPC powder manufacture on G' values can also used to explain the changes in yield force required at the yield point as a function of preheat treatment.

The decrease in the yield force required was related to the denaturation of whey protein as shown in Figure 6.12. When recombined cheese milk or reconstituted skim milk was prepared from high-heated MPC powder, the level of denaturation of whey protein was high and only weak gel was formed.

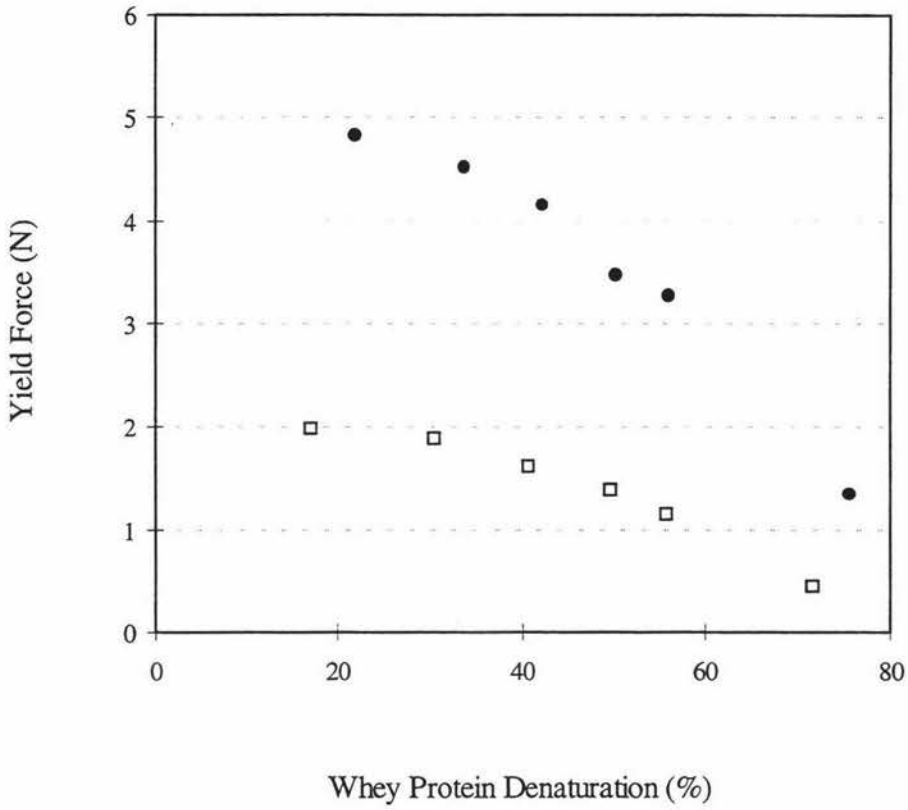


Figure 6.12 The yield force required to fracture the renneted gel, determined at 1.3 h after the addition of rennet, of recombined cheese milk (●) and reconstituted milk (□) as a function of the extent of denaturation of whey protein.

6.6 Frequency sweep

The frequency sweep was carried out on renneted milk gel made from recombined cheese milk and reconstituted skim milk and the results are shown in Figures 6.13 and 6.14, respectively. In all cases, both G' and tan δ were affected by preheat treatment during MPC powder manufacture and angular frequency. G' value increased with increase in angular frequency and the G' was also inversely proportional to the severity of preheat treatment.

The ratio of viscous and elastic characteristic, tan δ, which is related to the time scale of deformation (Walstra & van Vliet, 1982; Zoon *et al.*, 1988; Walstra & Peleg, 1991) slightly increased with heating time at the angular frequencies ranging from 0.00628 to 6.283187 s^{-1}. An increase in tan δ means that a relatively less elastic and more viscous like behaviour. Because the time scale of a dynamic measurement is roughly the inverse of the angular frequency, this implies that with an increase in the severity of preheat treatment during MPC powder manufacture bonds spontaneously break within a shorter time. Zoon *et al.* (1988) reported that a gel with a higher tan δ has more mobile bonds and is more viscous, but less elastic.

The G' values, which are proportional to the numbers of effective bonds or intermolecular crosslinkages within a substance, increased as frequency increased. This behaviour is consistent with the frequency-dependent behaviour of a solid lightly cross-linked viscoelastic polymer (Ferry, 1980). In all cases, the G' of renneted gel made from recombined cheese milk determined at any angular frequency was higher than that made from reconstituted skim milk, whereas, tan δ was lower than that of reconstituted skim milk.

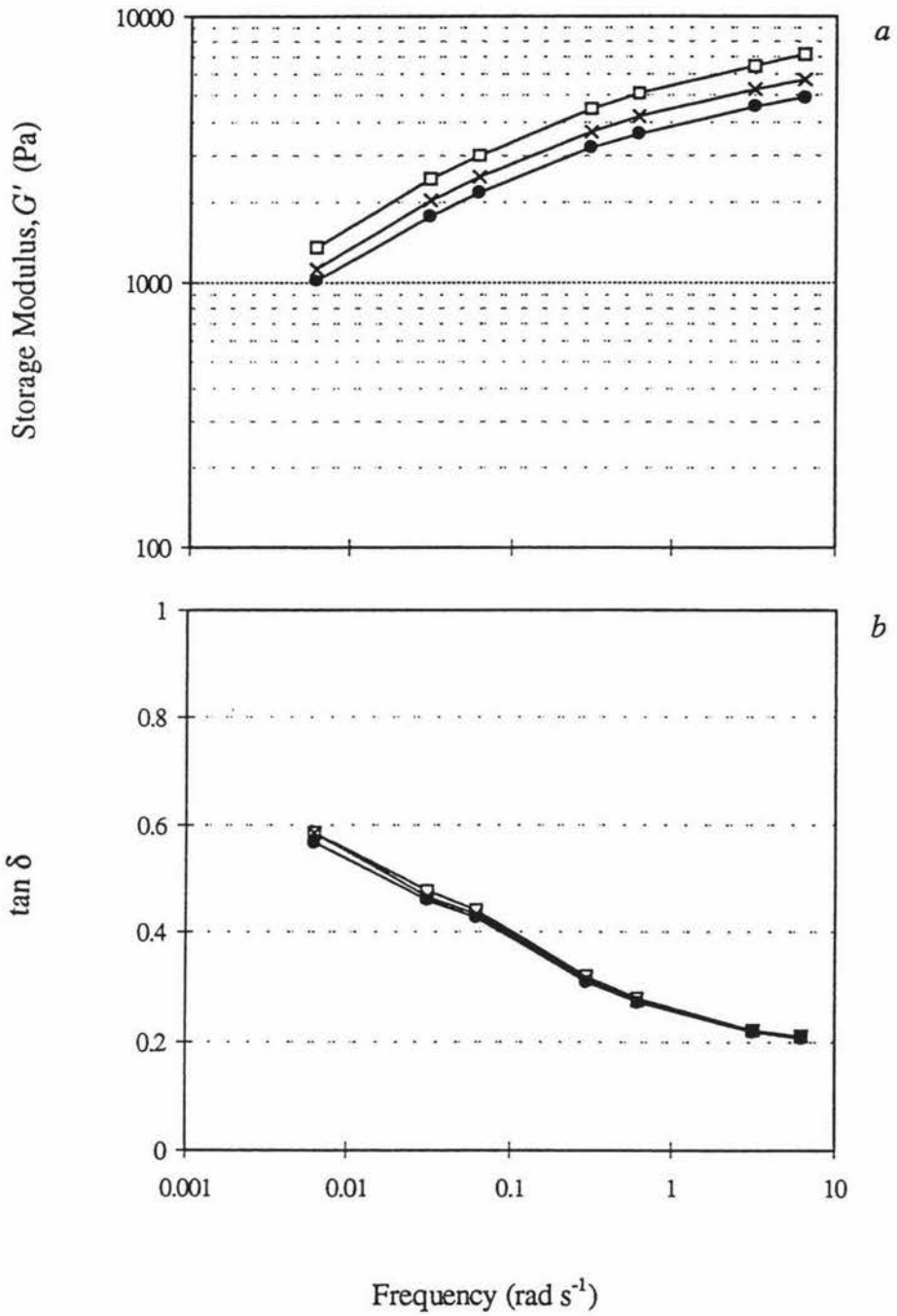


Figure 6.13 *G'* (*a*) and tan δ (*b*) of recombined cheese milk made from various MPC-56 powders as a function of angular frequency determined at 2 h after rennet addition at 34°C.

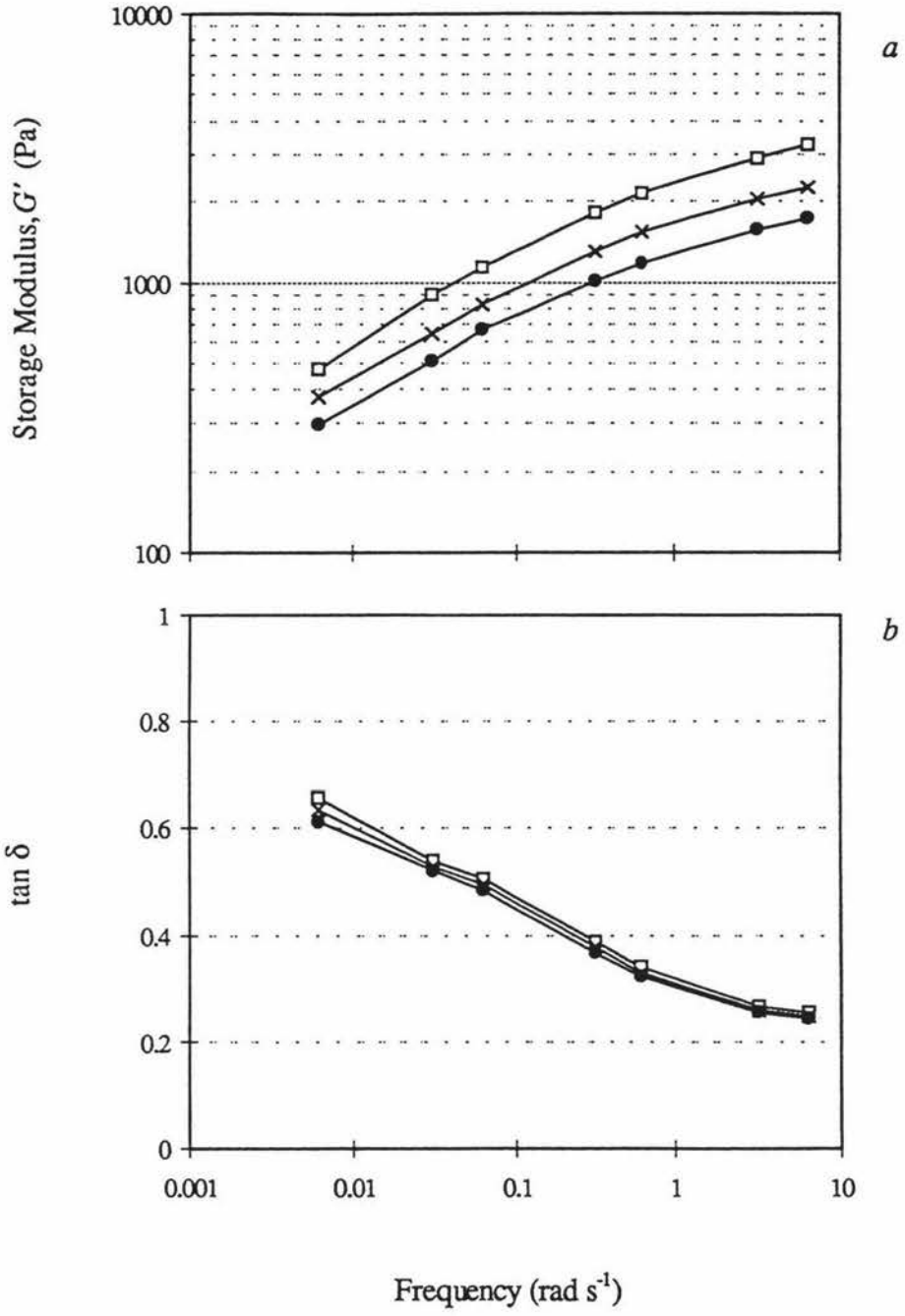


Figure 6.14 G' (*a*) and tan δ (*b*) of reconstituted skim milk made from various MPC-56 powders as a function of angular frequency, determined at 2 h after rennet addition at 34°C.

OVERALL DISCUSSION

Gilles and Lawrence (1981) and Singh and Newstead (1992) reported that many physical and chemical reactions occur when skim milk is preheated prior to concentration and drying. This in turns affect the functional properties of the milk powders. It was found in this experiment that the preheat treatment during MPC-56 powder manufacture significantly influences its physical and rennet coagulation properties and the effect becomes more pronounced as the severity of preheat treatment is increased. However, the preheat treatment during MPC powder manufacture did not have an significant effect on the average fat globule diameter ($d_{4,3}$) and the particle size distribution.

In agreement with the results reported by Novak (1992) and Soo (1994), preheat treatment during MPC powder manufacture had a significant effect on whey protein denaturation. The effect was dependent on both the preheating time and temperature. Denaturation of whey protein in reconstituted skim milk followed the same pattern as that observed in recombined cheese milk, but the degree of whey protein denaturation was less. The presence of milk fat in the recombined system is the factor contributing to this phenomenon. Since it was reported (Mulder & Walstra, 1974; Oortwijn *et al.*, 1979; Walstra & Jenness, 1984) that whey proteins and caseins were adsorbed on the milk fat globule membrane during homogenization. When the recombined cheese milk was adjusted to pH 4.6 to precipitate the caseins, it might be possible that the whey proteins, which was not heat denatured, were entrapped within the casein precipitated, resulted in higher level of whey protein denaturation. The results suggested that the denaturation of whey protein was an important factor contributing to the change in flow behaviour and rennet coagulation properties observed in recombined cheese milk and reconstituted skim milk.

All recombined cheese milks behaved as non-Newtonian liquids. The n values were < 1, indicating that they behave as pseudoplastic material, i.e. the viscosity decreased as increase the shear rate. As expected, preheating during MPC powder manufacture to such an extent that the whey proteins become denatured, led to an increase in viscosity, shear stress and n values of recombined milk. Walstra and Jenness (1984) reported that heating to such a degree that most of the whey protein is heat denatured causes an increase in viscosity by about 10% in skim milk system. Denatured whey proteins may interact with casein and also milk fat globule

membrane material, resulting in increase in viscosity of recombined cheese milk. However, the changes in viscosity observed in recombined cheese milk was more pronounced compared to that of whole milk reported by Walstra and Jenness (1984), which may be attributed the higher total solids content (40%) of recombined cheese milk used in this study.

The rennet coagulation properties of both recombined cheese milk and reconstituted skim milk were interfered by the preheat treatment during MPC-56 powder manufacture. The aggregation rate of altered casein micelles, G' values and yield force were significantly decreased with increase the severity of preheat treatment. While GT was not significant affected by preheat treatment during MPC powder manufacture, unless the high heating regime was used during the powder manufacture. Whey protein denaturation and the complex formation between denatured whey protein and κ-casein may be responsible for this phenomenon. The complex formation may affect the ability of rennet to hydrolysis κ-casein in the enzymatic phase, interfere the aggregation of the alter casein micelles in the secondary-aggregation phase or hinder the close approach and contact between the altered casein micelles in the tertiary phase, resulted in the gel with different characteristics.

GENERAL CONCLUSION

It can be concluded from the results obtained here that both the physical and rennet coagulation properties of recombined (20% MPC powder and 20% milk fat) and reconstituted cheese milk (20% MPC powder without the addition of milk fat) were significantly influenced by the preheat treatment of the MPC powder. The changes in these properties, of course, were dependent on the severity of the preheat treatment during the MPC powder manufacture. The viscosity and shear stress, and percentage denaturation of whey protein increased as the severity of the preheat treatment was increased. The rennet coagulation properties of the milk prepared from MPC powder were significantly impaired with the preheat treatment. The GT was lengthened whereas the gelation rate, storage modulus and yield force at the yield point was reduced as the preheat treatment was increased. The main factor responsible for the change in the functional behaviour of the MPC powder is the denaturation of the whey protein and the complex formation between denatured whey protein, especially β-lactoglobulin, with the κ-casein. Schematic diagrams of the effect of preheat treatment on the milk system and rennet coagulation properties are shown in Appendix I, Figure 1 and 2.

CHAPTER 7

EFFECT OF HOMOGENIZATION ON THE PHYSICAL AND RENNET COAGULATION PROPERTIES OF RECOMBINED CHEESE MILK

Homogenization is an important process used for emulsifying milk fat into reconstituted skim milk during recombination. However, homogenization can interfere with some properties of milk (Mulder & Walstra, 1974; Walstra & Jenness, 1984; McMahon *et al.*, 1993) including its rennet coagulation properties. The present chapter describes the effect of homogenization pressure on physical and rennet coagulation properties of recombined cheese milk (40% total solids) without any heat treatment and with additional heat treatment after homogenization at 75 and 85°C for 3 min. The effects of homogenization pressure were quantified in terms of changes in the average fat globule diameter ($d_{4,3}$), viscosity and shear stress, whey protein denaturation and rennet coagulation properties of recombined cheese milk.

RESULTS AND DISCUSSION

7.1 Effect on fat globule diameter ($d_{4,3}$)

The effects of homogenization pressure on the fat globule size distribution in recombined cheese milk without any heat treatment and with heat treatment at 85°C for 3 min are shown in Figures 7.1 and 7.2. As expected, recombined cheese milk which had undergone homogenization at higher pressures had a narrower particle size distribution as well as smaller average fat globule diameter ($d_{4,3}$). The volume of fat globules with a large diameter was reduced, while the volume of the fat globules with a smaller diameter increased. The $d_{4,3}$ of recombined cheese milk was decreased from approximately 1.20 to 0.58 μm when homogenization pressure increase from 100/50 to 250/50 bar.

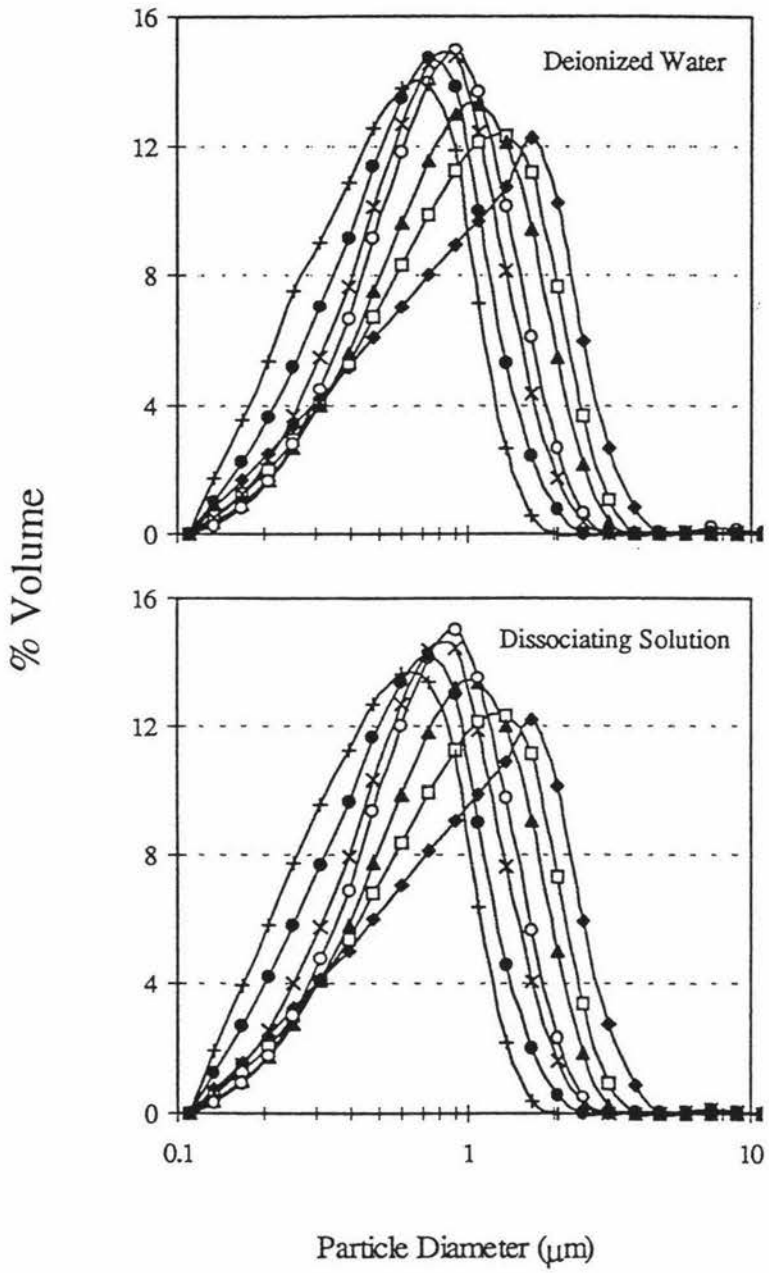


Figure 7.1 The profiles of fat globule size distribution in recombined cheese milks without any heat treatment measured after dilution of sample with deionized water and dissociating solution. Homogenized at 100 (◆), 120 (□), 150 (▲), 180 (○), 200 (×), 220 (●) and 250 (+) for the first stage and 50 bar for the second stage.

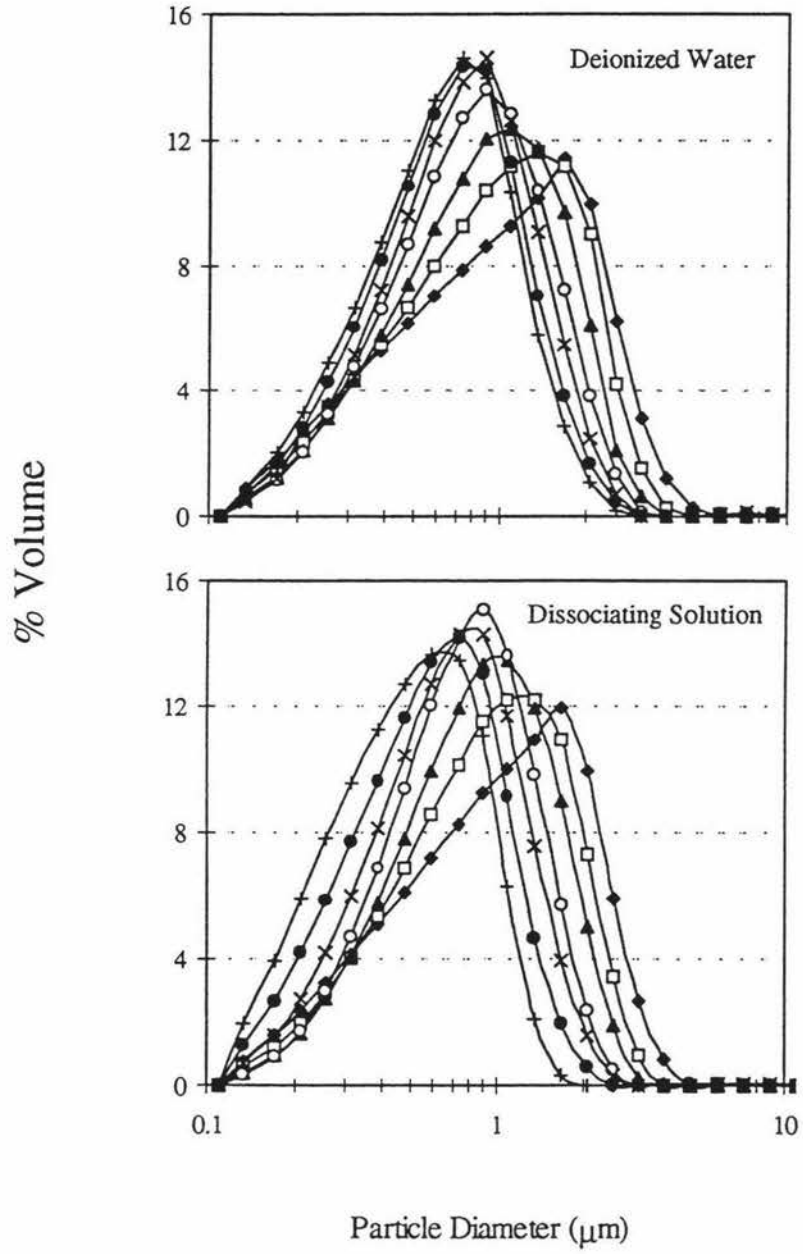


Figure 7.2 The profiles of fat globule size distribution in recombined cheese milks with heat treatment at 85°C for 3 min measured after dilution of sample with deionized water and dissociating solution. Homogenized at 100 (◆), 120 (□), 150 (▲), 180 (○), 200 (×), 220 (●) and 250 (+) for the first stage and 50 bar for the second stage.

The effect of homogenization pressure on the $d_{4,3}$ of recombined cheese milk without any heat treatment and with heat treatment (after homogenization) at 75 and 85°C for 3 min are shown in Figure 7.3. In all cases, the average fat globule diameter measured in dissociating solution was slightly smaller than those measured in deionized water. Dissociating solution dissociated any denatured whey protein and casein micelles that might have adsorbed onto the fat globule membrane, so it allowed evaluation of the $d_{4,3}$ without the contribution of protein aggregation (Dalgleish *et al.*, 1989; Robin & Paquin, 1991). In all cheese milks, $d_{4,3}$ decreased almost linearly with increase in homogenization pressure.

Although the effect of heat treatment after homogenization on $d_{4,3}$ of recombined cheese milk was unclear, heat treatment of high-homogenized milk, i.e homogenization pressure ≥ 180/50 bar, at 85°C for 3 min resulted in slight increase in $d_{4,3}$ measured after dispersion of samples in deionized water. The larger $d_{4,3}$ observed may be attributed to the formation of protein-fat aggregates, which is likely to occur when recombined cheese milk was heated at high temperature due to its high total solids content. This was confirmed when the dissociating solution (SDS-EDTA) was used to measure the fat globule size after dissociating the protein aggregates. In dissociation solution, the $d_{4,3}$ of heated recombined cheese milks were lower than that of unheated corresponding when the milks had undergone high homogenization pressure, i.e. homogenization pressure ≥ 180/50 bar. Sharma and Dalgleish (1993) studied the effect of heating on homogenized whole milk and found that milk that was heated after homogenization showed slightly decreased particle size compared with homogenized milk without heat treatment. However, little is known on the mechanism involves in this phenomenon.

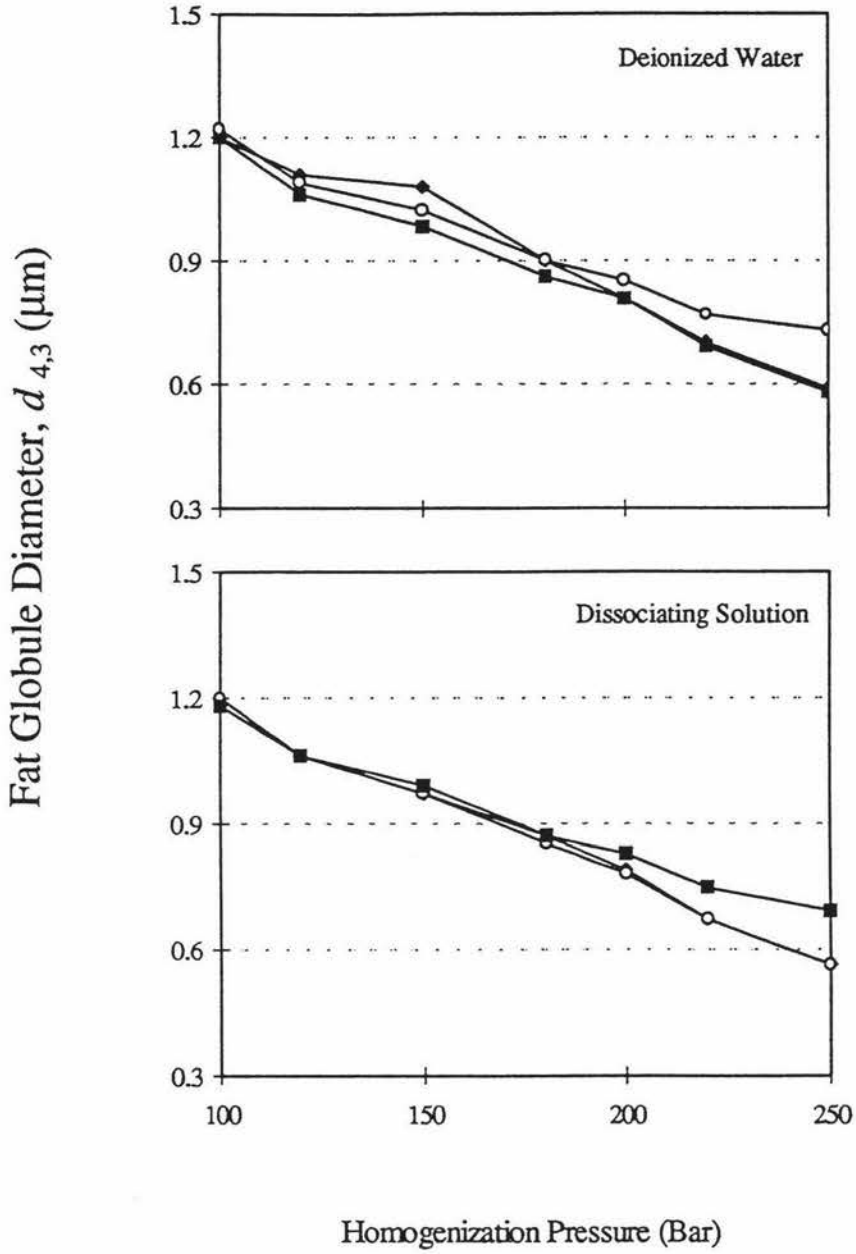


Figure 7.3 Changes in the average fat globule diameter, $d_{4,3}$, of recombined cheese milk as a function of homogenization pressure, measured after dispersion of sample in deionized water and dissociating solution. Recombined cheese milk without any heat treatment (◆) and with heat treatment at 75 (■) and 85°C (○) for 3 min, after homogenization. All samples were subjected to homogenization at 50 bar for the second stage.

7.2 Effect on whey protein denaturation

Effect of homogenization on total and individual whey protein denaturation was investigated in recombined cheese milk without any heat treatment using Kjeldahl analysis and Native-PAGE on pH 4.6 supernatants and the results are shown in Appendix II, Table 4. It was found that homogenization pressure did not have a significant effect on the levels of total and individual whey protein denaturation.

7.3 Effect on viscosity and shear stress of recombined cheese milk

In agreement with the results reported on whole milk system (Mulder & Walstra, 1974; Walstra & Jenness, 1984) homogenization, either with or without heat treatment, had a significant effect on viscosity and shear stress of recombined cheese milk (Figures 7.4, 7.5 and 7.6). Increasing homogenization pressure resulted in an increase in both viscosity and shear stress of recombined cheese milk. Mulder and Walstra (1974) and Desobry-Banon *et al.* (1994) reported that the viscosity of milk varied with particle size distribution; the increase in viscosity was inversely proportional to the size of fat particle.

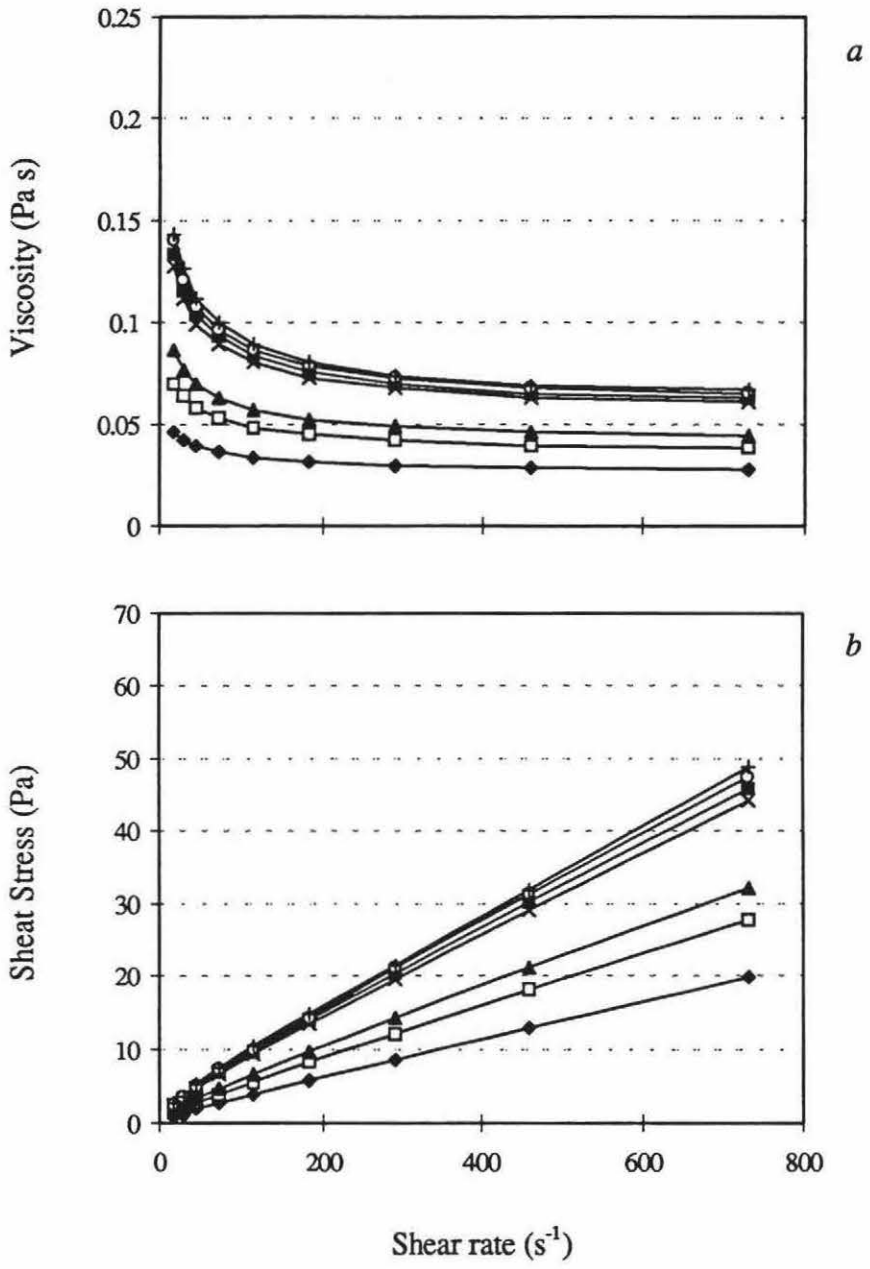


Figure 7.4 Viscosity (*a*) and shear stress (*b*) of recombined cheese milk without any heat treatment after homogenization as a function of different homogenization pressures: 100 (◆), 120 (□), 150 (▲), 180 (×), 200 (■), 220 (○) and 250 (+) bar for the first stage and 50 bar for the second stage.

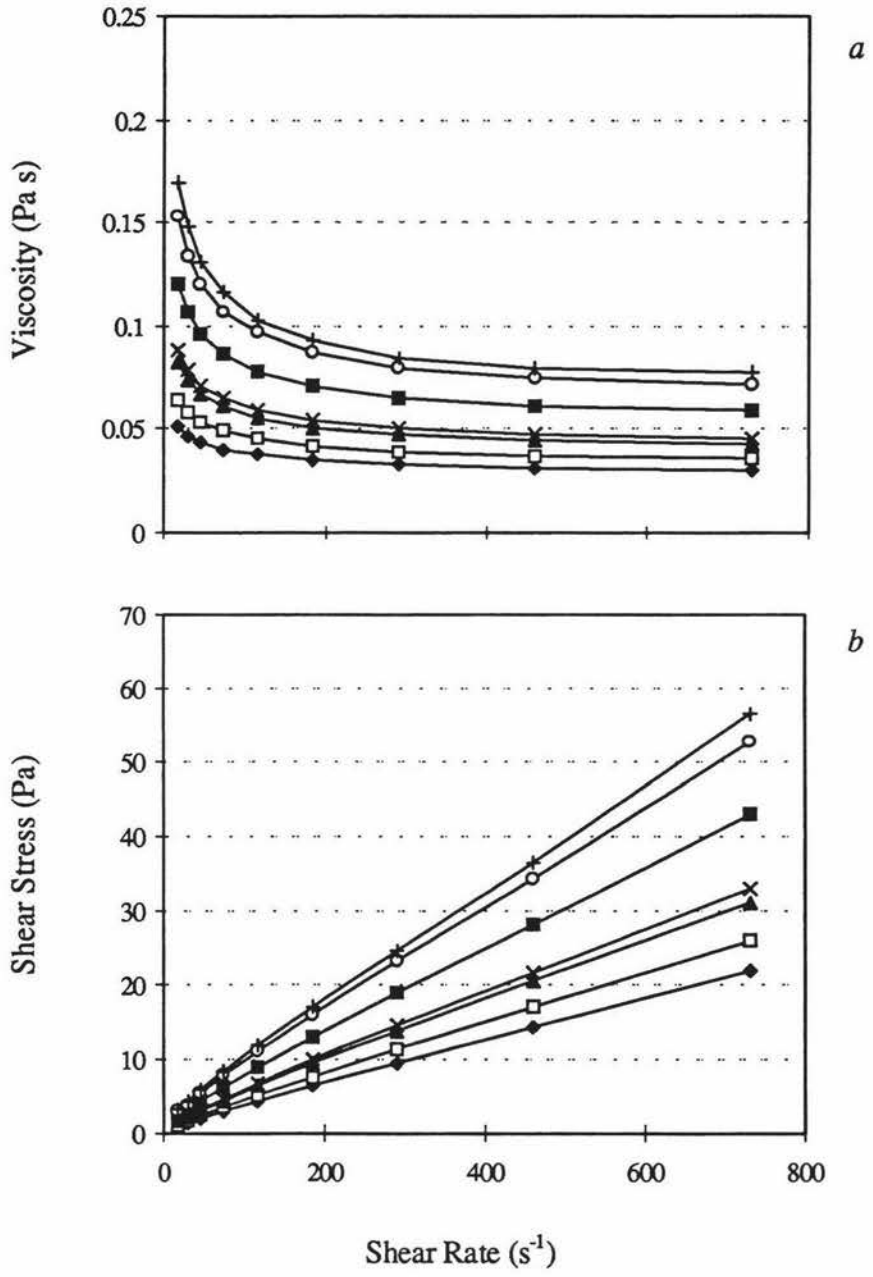


Figure 7.5 Viscosity (*a*) and shear stress (*b*) of recombined cheese milk that was heated at 75°C for 3 min after homogenization as a function of different homogenization pressures: 100 (◆), 120 (□), 150 (▲), 180 (×), 200 (■), 220 (○) and 250 (+) bar for the first stage and 50 bar for the second stage.

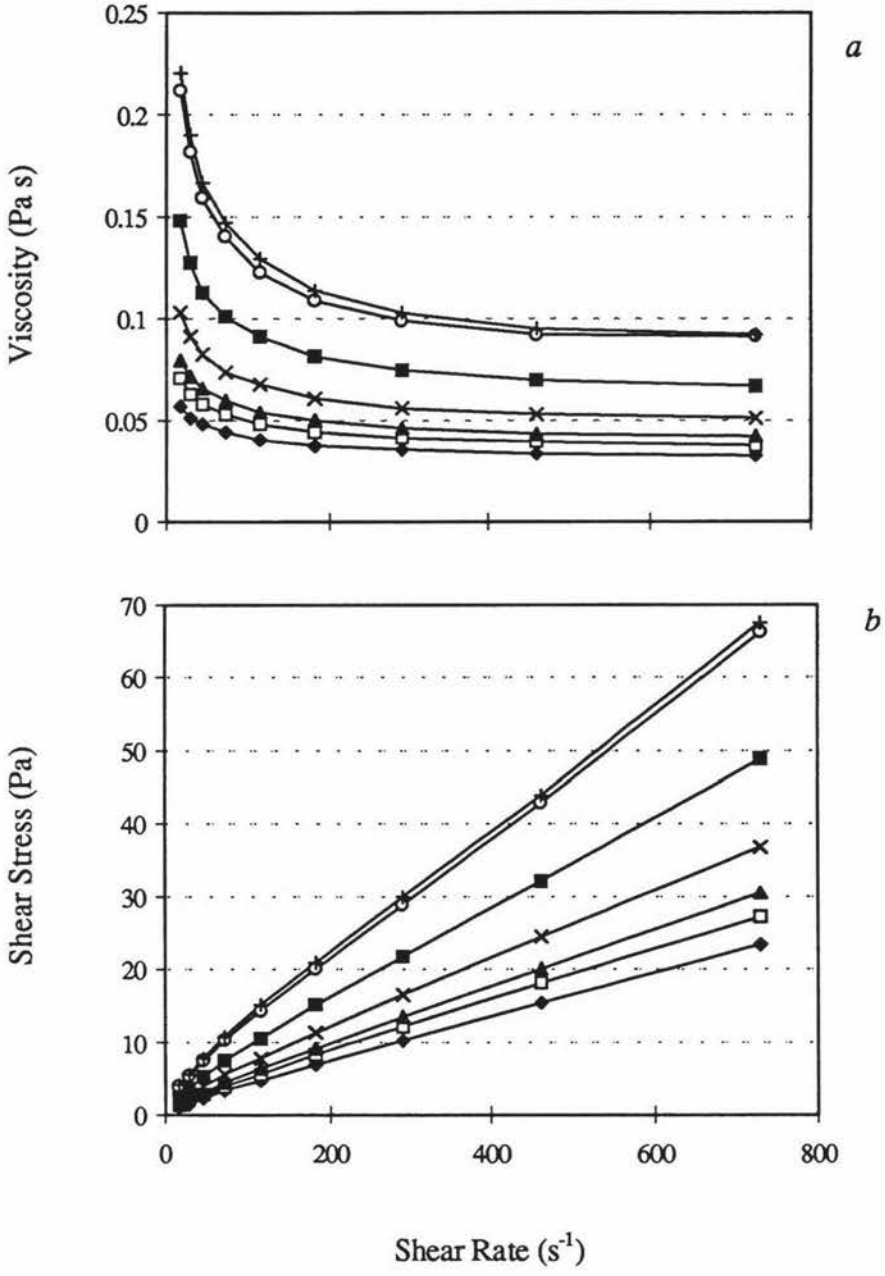


Figure 7.6 Viscosity (*a*) and shear stress (*b*) of recombined cheese milk that was heated at 85°C for 3 min after homogenization as a function of different homogenization pressures: 100 (◆), 120 (□), 150 (▲), 180 (×), 200 (■), 220 (○) and 250 (+) bar for the first stage and 50 bar for the second stage.

The flow behaviour index (n) and consistency index (k) of recombined cheese milk was calculated using the power law as described in Chapter 5 and the results are shown in Figure 7.7. It was found that both n and k values were dependent on the homogenization pressure. Increased homogenization pressure resulted in a decrease in n values, but increased k values. The n and k values of recombined cheese milk were in the range 0.8532 - 0.7546 and 0.0690 - 0.4303, respectively. According to their flow behaviour, recombined cheese milk behave as a non-Newtonian liquid with the characteristics of pseudoplastic material ($n < 1$). This means that the viscosity of recombined cheese milk decreased with increasing the shear rate, which is called shear thinning behaviour.

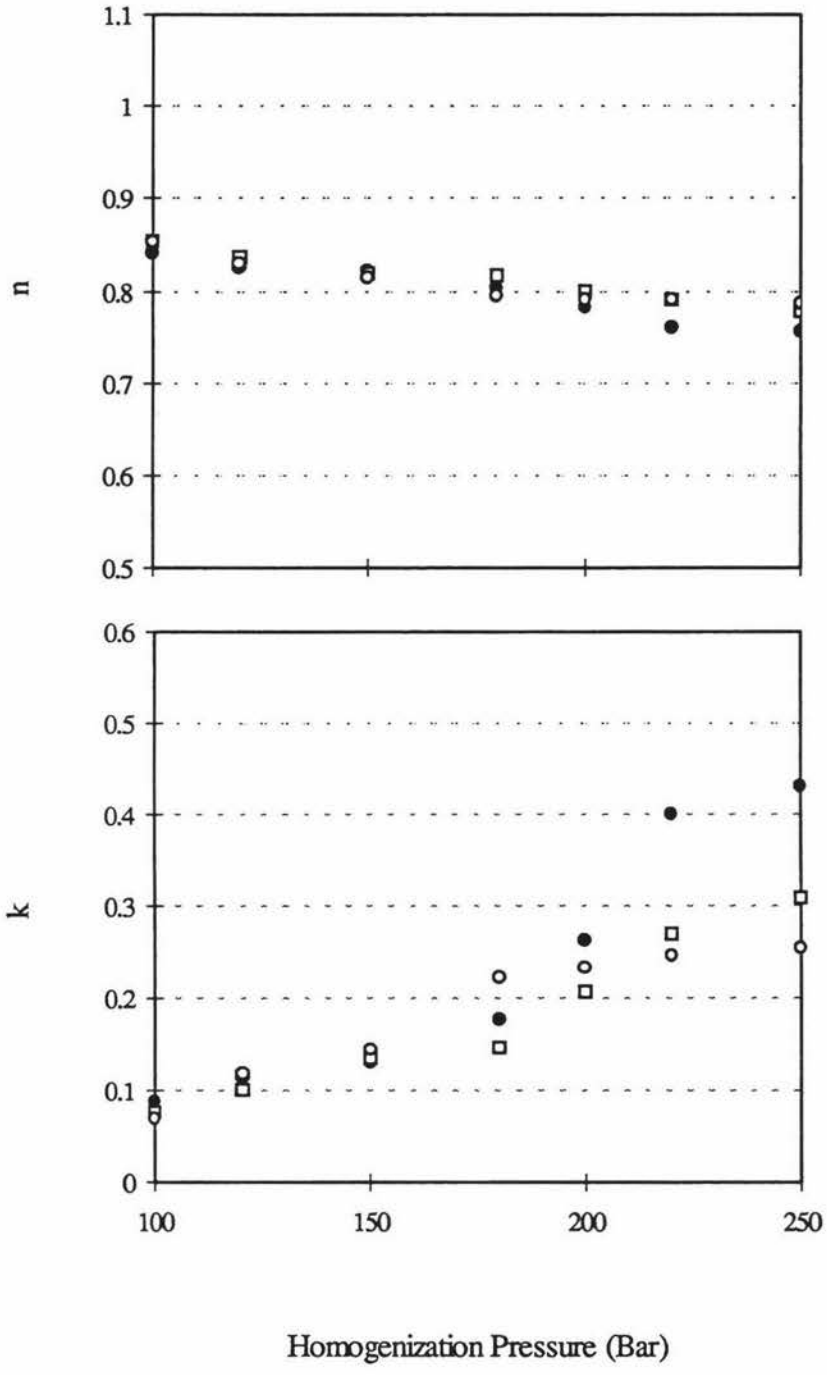


Figure 7.7 Changes in n and k values of recombined cheese milk without any additional heat treatment (●) and with heat treatment at 75(□) and 85°C (○) for 3 min as a function of homogenization pressure. The flow behaviour index were determined at 34°C at the fixed shear rate of 116 s^{-1}.

7.4 Effect on rennet coagulation properties

The influence of homogenization on rennet coagulation properties of recombined cheese milk is shown in Figure 7.8. It was found that homogenization either with or without heat treatment had an effect on the rennet coagulation properties of recombined cheese milk and the effects were more pronounced as homogenization pressure increased. The rheological properties of rennet-induced gels made from recombined cheese milk that was homogenized at high pressures were different from those subjected to low homogenization pressures. The GT and G' values decreased as homogenization pressure increased, indicating that renneted gel became weaker with increasing homogenization pressure. The effect of homogenization was more pronounced when heat treatment was given to recombined cheese milk after homogenization process (Figure 7.8).

This may be attributed to the differences in the composition of milk fat globule membrane. Unlike normal milk, homogenized milk fat globules contain primarily caseins and some serum proteins (if the milk was heated) (Oortwijn & Walstra, 1979) and the amount of adsorbed protein is dependent on the homogenization pressure (Sharma *et al.*, 1994). In a homogenized milk system, it likely that the complexation of fat and protein could have major influence on gel formation and gel strength (Cobos *et al.*, 1995). Adsorption of casein onto the fat globule may affect the accessibility of κ-casein and may, therefore, induce changes in the behaviour during renneting. Ohmiya *et al.* (1987) reported that homogenization at high pressure influenced casein hydrolysis by chymosin (primary enzymatic-phase), resulting in a delay in casein aggregation (secondary phase).

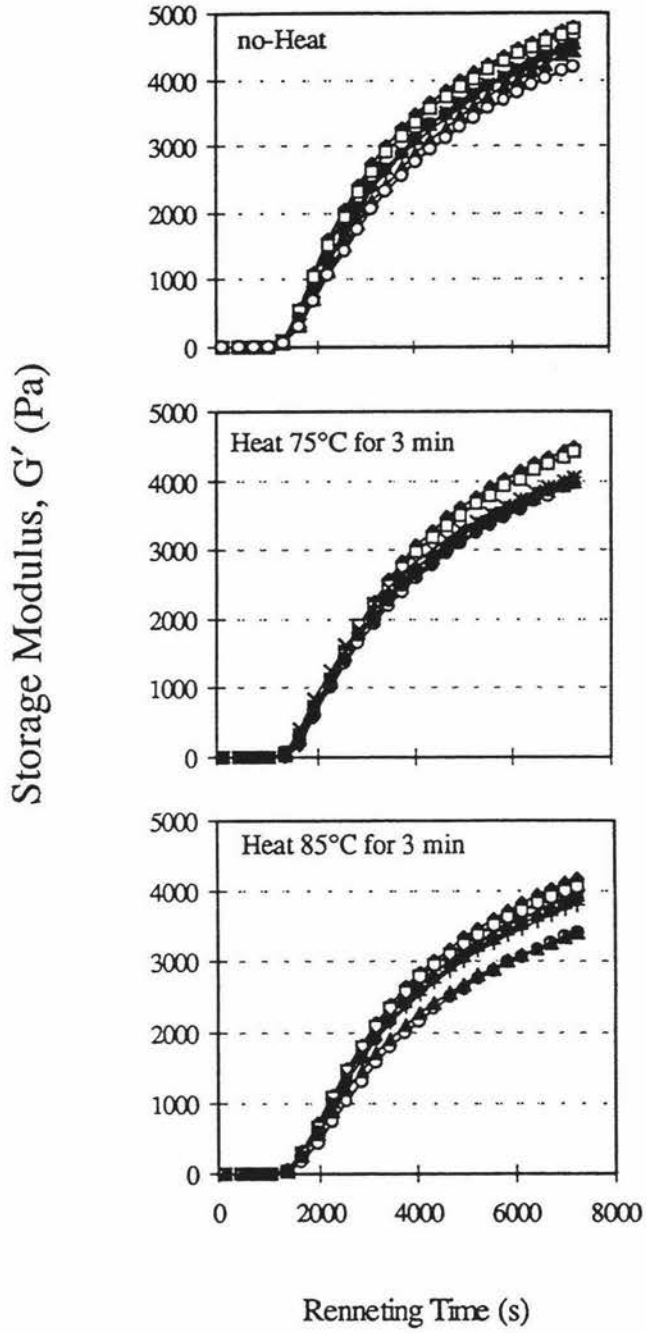


Figure 7.8 Storage modulus, G′, as a function of renneting time of recombined cheese milk without heat treatment and with heat treatment at 75 and 85°C for 3 min: homogenization at 100 (◆), 120 (□), 150 (×), 180 (●), 200 (+), 220 (▲), and 250 bar (○) for the first stage and 50 bar for the second stage.

Effect on gelation time (GT)

The effect of homogenization pressure on the GT of recombined cheese milk with and without heat treatment is shown in Figure 7.9. Although there was no clear relationship between homogenization pressure and the GT of recombined cheese milk, GT shows a slight decreased with increasing homogenization pressure.

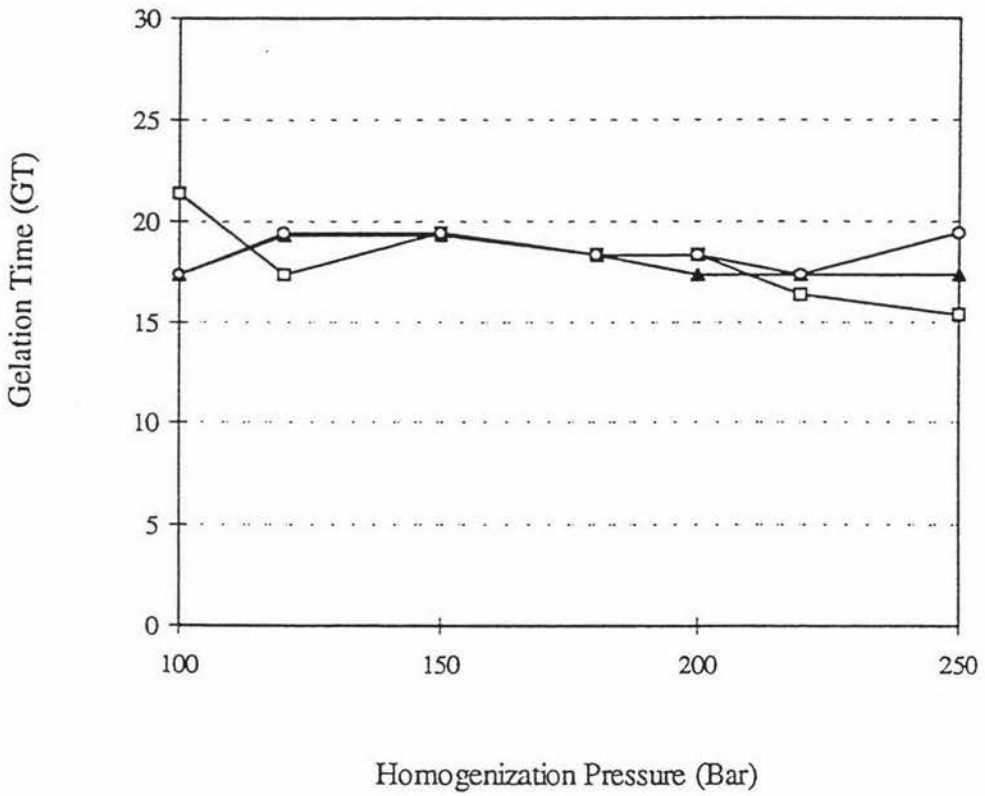


Figure 7.9 Gelation time of recombined cheese milk homogenized at different pressures without any heat treatment (▲) and with heat treatment at 75 (□) and 85°C (○) for 3 min after homogenization. All the samples were homogenized at 50 bar for the second stage.

Green *et al.* (1983), Robson and Dalgleish (1984), McMahon *et al.* (1991), Ghosh *et al.* (1994) found a decrease in GT with an increase in homogenization pressure. They attributed the shorter GT of homogenized milk compared to non-homogenized milk to the structural

differences in the protein surface of homogenized milk. Casein micelles become less stable and more susceptible to coagulation by rennet, as a result of being adsorbed as part of the new membrane around fat globules (Oortwijn *et al.*, 1979). Robson and Dalgleish (1984) and McMahon *et al.* (1991) further reported that a smaller critical extent of hydrolysis is needed to initiate gelation of homogenized milk compared to non-homogenized milk since κ-casein spread more evenly over the surface of homogenized milk fat globule membranes. Ghosh *et al.* (1994) reported that the higher the homogenizing pressure, the greater the amount of κ-casein available for the enzyme action. In addition, the decrease in GT may be attributed to the decreasing particle size because smaller casein particles, i.e. the higher specific area, aggregate faster (Desobry-Banon *et al.*, 1994). Moreover, increasing the number of smaller particles may result in an increased collision probability and, consequently, an enhancement of rennet aggregation.

Effect on storage modulus (G', Pa)

The effect of homogenization pressure on the G' values determined at 1 and 2 h after the addition of rennet is shown Figure 7.10. In all cases, G' values of renneted gel made from unheated or heated recombined cheese milks slightly decreased with increase in homogenization pressure.

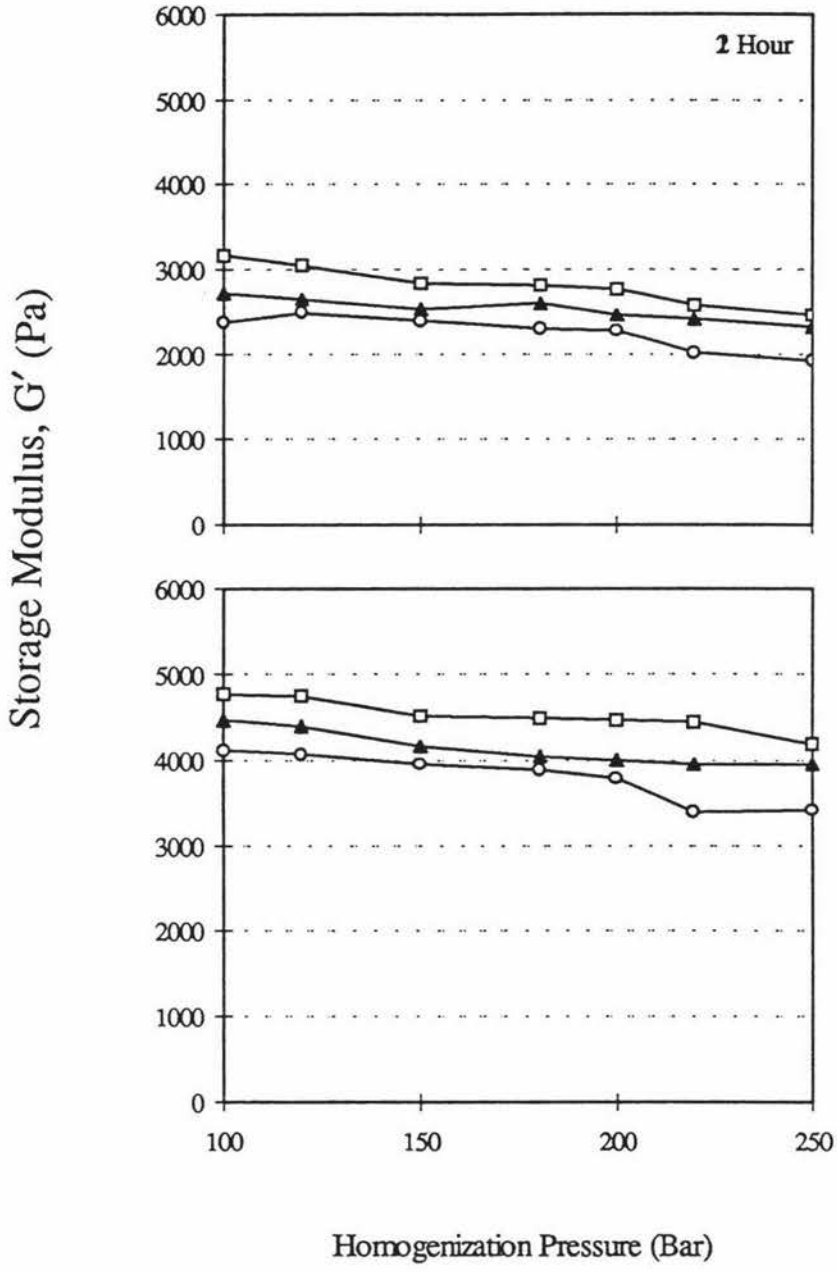


Figure 7.10 Storage modulus (G'), determined at 1 and 2 h after the addition of rennet, as a function of homogenization pressure of recombined cheese milk without any heat treatment (□), and with heat treatment at 75 (▲) and 85°C (○) for 3 min after homogenization. All the samples were homogenized at 50 bar for the second stage.

The slight reduction in G' values observed in recombined cheese milk with high-homogenization pressure compared to those subjected to low-homogenized pressure is probably due to differences in the amount of casein and whey proteins adsorbed onto the new fat globules surfaces formed during the homogenization process. It has been reported that more serum proteins are adsorbed on the milk fat globule membrane when high homogenization pressure is applied to the recombined milk (Walstra & Jenness, 1984; Sharma *et al.*, 1994).

Ghosh *et al.* (1994) reported that homogenization of milk tended to decrease the gel strength of renneted gels, but not markedly. Whereas, McMahon *et al.* (1991) observed no differences in the strength of renneted gels made from non-homogenized and homogenized ultrafiltered milk (3X). They attributed this phenomenon to the increased solids content of concentrated milk which formed a gel network of higher volume density so that any further changes because of incorporation of fat into the network were not significant in increasing gel firmness.

In all cases, rennet gels made from recombined cheese milk that had undergone heat treatment had lower G' values compared to those made from recombined cheese milk without any heat treatment and the effect was more pronounced as the intensity of heat treatment increased (Figure 7.10). The effect of homogenization pressure were similar for both heat treated and non-heat treated recombined cheese milk. Possible reasons for decreased in G' of rennet gels as a consequence of heat treatment have been discussed in Chapter 5.

7.5 Effect on yield force at the yield point

The effect of homogenization on yield force of recombined cheese milk is shown in Figure 7.11. As expected, the yield force of renneted gel made from recombined cheese milk decreased as homogenization pressure increased. At all homogenization pressures, the renneted gel made from heated recombined cheese milk required less force to fracture the gel compared to those prepared from unheated corresponding. The reason used to explain the reduction in G' value as a function of homogenization pressure and heat treatment can also used for the reduction in yield forces required.

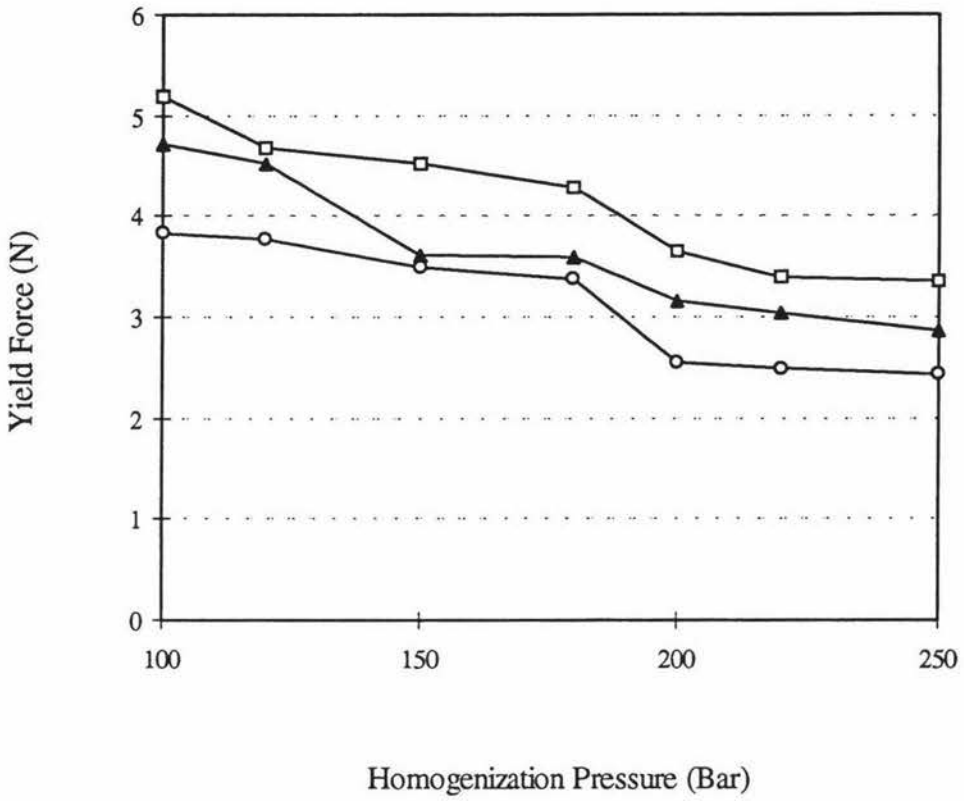


Figure 7.11 Yield force as a function of homogenization pressure of recombined cheese milk without heat treatment (□), and with heat treatment at 75 (▲) and 85°C (○) for 3 min. All the samples were homogenized at 50 bar for the second stage.

7.6 Frequency sweep

Frequency sweeps of renneted-induced gels made from recombined cheese milk with different homogenization pressures are shown in Figure 7.12 and 7.13. In all cases, the G′ values of renneted gels made from recombined cheese milk increased and tan δ decreased as an angular frequency increased. Recombined cheese milk that was homogenized at high homogenization pressure has smaller *G′* values, but high tan δ compared to those subjected to low homogenization pressure. Heat treatment after homogenization slightly reduced the *G′* values determined at any angular frequency.

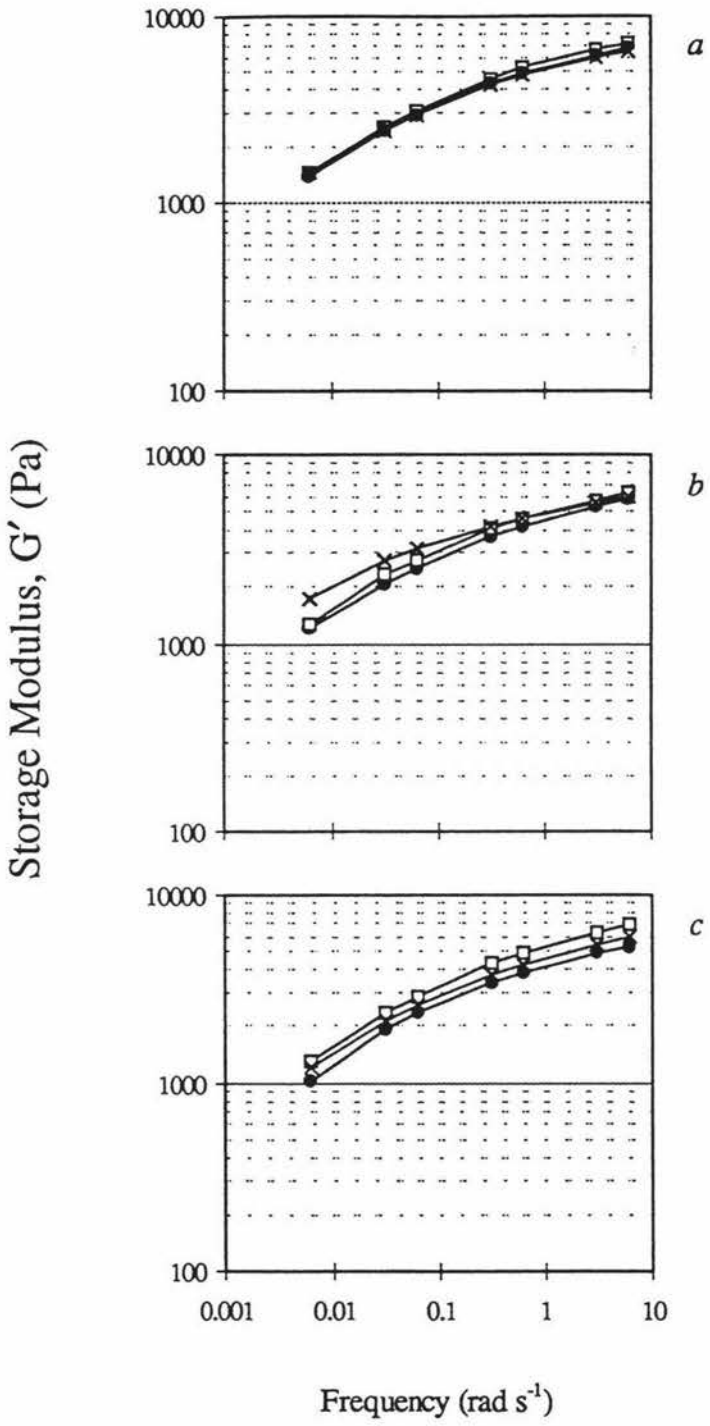


Figure 7.12 Storage modulus as a function of angular frequency of recombined cheese milk without heat treatment (*a*) with heat treatment at 75 (*b*) and 85°C (*c*) for 3 min holding time: homogenization at 100 (□), 180 (×) and 250(●) bar for the first stage and 50 bar for the second stage.

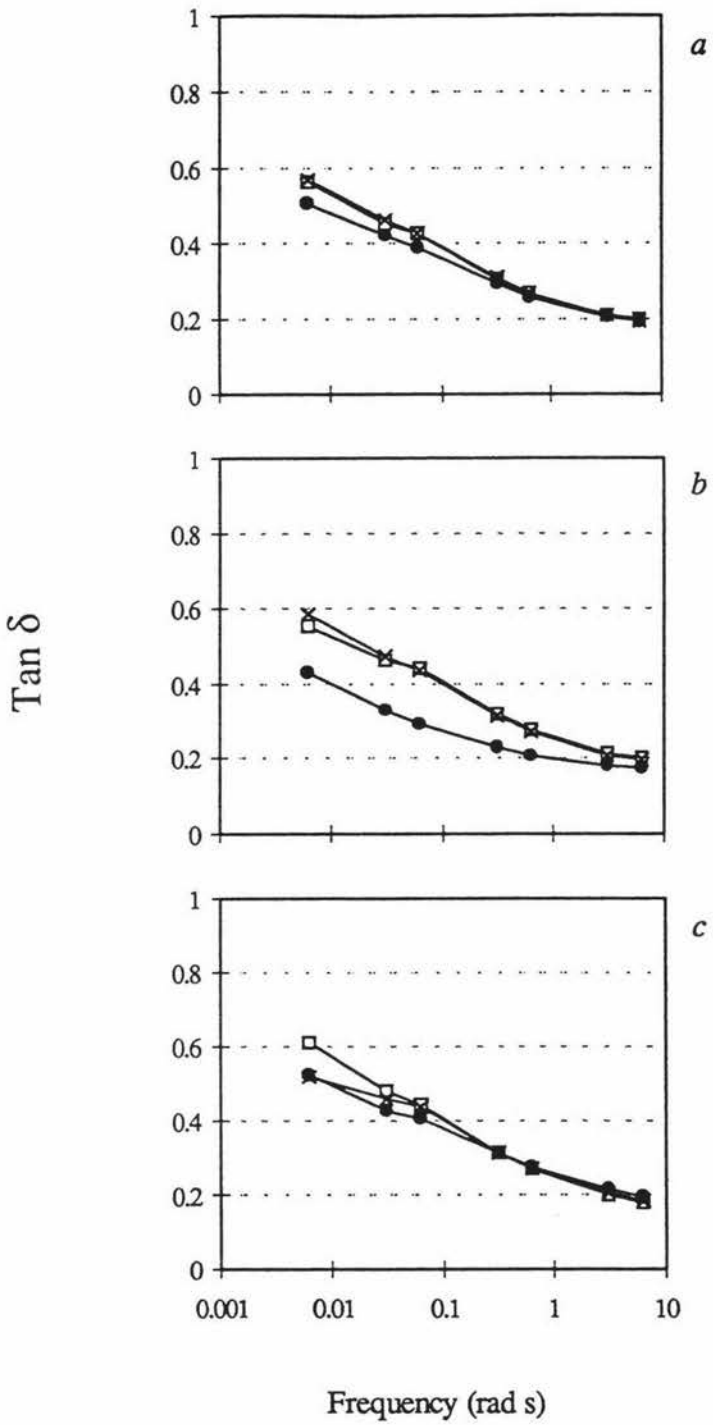


Figure 7.13 Tan δ as a function of angular frequency of recombined cheese milk without heat treatment (a) with heat treatment at 75 (b) and 85°C (c) for 3 min holding time: homogenization at 100 (□), 180 (×) and 250 bar (●) for the first stage and 50 bar for the second stage.

OVERALL DISCUSSION

During homogenization of recombined cheese milk, the fat globules are disrupted into small globules, which in turn increases the number of milk fat globules and the surface area of the milk fat globule membranes. The casein micelles and serum proteins adsorb on to these newly created fat globules (Sharma *et al.*, 1996). This alters the properties of the milk fat globules and the casein micelles, and may be responsible for the changes in physical and renneting properties observed for homogenized recombined cheese milk.

Homogenization of recombined cheese milk did not have an effect on whey protein denaturation, but it had a significant influence on the viscosity and shear stress of recombined cheese milk; the higher homogenization pressure, the higher the viscosity and shear stress (Figures 7.4, 7.5 and 7.6). This phenomenon may be attributed to the change in milk fat globule diameter and also the interaction of fat globules with casein and serum proteins. Smaller fat globules diameter had more adsorbed casein and whey proteins (Sharma *et al.*, 1996), probably resulting in greater increase in viscosity.

The rennet coagulation properties of recombined cheese milk was also affected by homogenization pressure. Homogenization slightly decreased the GT, aggregation rate, G' values and yield force of renneted-induced gels made from recombined cheese milk. The effect of homogenization pressure on recombined cheese milks used in this study is much less marked than that observed for normal milks (Figures 7.10 and 7.11). The observation that homogenized milk produced weaker renneted gels have been attributed to (i) the greater dispersion of fat (Daon, 1954) because of the participation of fat particles coated with casein micelles in the casein gel network (Peters, 1954; Green *et al.*, 1983) or to (ii) the reduced number of free casein particles available to form a strong network (Walstra *et al.*, 1985) as some of the caseins are tied to the surface of the newly formed fat globules. Proteins adsorbed on to fat particles disrupt the continuity of the gel structure and acted as weak centre in the gel (Saito, 1993). This also reduces the ease of movement of network strands and hinders strand rearrangement that help reinforce the network and increase the firmness of the milk gels once it has been formed.

Green *et al.* (1983) and Robson and Dalgleish (1984) reported that the rate of altered-casein micelle aggregation was reduced in homogenized milk compared to that of non-homogenized milk. They attributed this to the reduced surface area of the casein micelles available for mutual interaction due to association with the fat during homogenization. The interactions between fat and casein in homogenized milk cause the fat to become part of the casein network in milk gels, resulting in increases in the volume of the network relative to that of the serum interstices and reducing the ease of movement of the network strands (McMahon *et al.*, 1991), resulting in a weaker rennet gel (Green *et al.*, 1983).

Homogenization appears to have relatively minor effect on physical and rennet coagulation properties of recombined cheese milk when compared to the effect of heat treatment (Chapter 5 and 6). van Boekel and Walstra (1993) reported that heat treatment of milk after homogenization changes both physical and chemical properties of milk fat globule membrane and serum proteins due to the interaction with each other, in which the effect depends on the intensity of heat treatment. The interactions between milk fat globule membranes and milk proteins (caseins and serum proteins) during heating have not yet been fully understood. Dalgleish and Banks (1991) reported that the serum proteins, β-lactoglobulin and α-lactalbumin, were bound to the fat globule membrane during heating and that the amount of these proteins bound to the fat globule increased to a plateau value much more rapidly than the overall denaturation rate of these serum proteins. However, disagreement still exists in the explanation of the mechanism of these interactions. Serum proteins may bind to the proteins (caseins) which are already present in the fat globule membrane and form a layer around the whole fat globule and its membrane system. Dalgleish and Bank (1991) suggested that the interactions between skim milk protein and milk fat globule membrane (MFGM) proteins were based on bond formation, i.e. disulfide linkage. Whereas, Houlihan *et al.* (1992) reported that the loss of MFGM components may facilitate an increase in the concentration of skim milk proteins in the membrane, and β-lactoglobulin and α-lactalbumin, may displace these components during heating. Kim and Jimemez-Flores (1995) proposed that rather than direct disulfide bond formation, milk serum proteins may possibly deposit on the MFGM with the displacement of polypeptides from the membrane.

However, the details of changes in synthetic fat globule membranes when homogenized recombined milk is heated have not been extensively studied and it is assumed that the interactions between synthetic fat globules and serum protein are similar to that occur when fresh milk is heated. No information is available on such interaction in concentrated milk systems.

GENERAL CONCLUSIONS

In general it can be concluded that homogenization with or without heat treatment affected the physical properties and rennetability of recombined cheese milk. Recombined cheese milks that had undergone high homogenization pressure before heat treatment have smaller average fat globule diameter ($d_{4,3}$), higher viscosity and shear stress and different rennet coagulation properties compared to that of low-homogenized milk. The effect was dependent on the homogenization pressure. However, homogenization did not effect the level of whey protein denaturation. The main factors contributing to the alteration in physical and renneting properties of recombined cheese milk are likely to be the fat globule diameter, composition of milk fat globule membranes and also the interaction of fat globules with the casein and whey protein. A schematic diagram of the effect of homogenization on the milk system and rennet coagulation properties is shown in appendix I, Figure 1.

CHAPTER 8

PERMEABILITY AND MICROSTRUCTURE OF RENNET-INDUCED MPC GELS: A PRELIMINARY STUDY

Rennet-induced milk gel are a semi-solid particle-type gel, which has a porous structure, formed mainly by casein network (van Dijk & Walstra, 1986; Zoon *et al.*, 1988). The secondary stage (aggregation) of rennet-altered casein micelles has an important effect on the physical properties of the gel (Green & Grandison, 1987), particularly on its porosity and permeability.

Gel permeability can be defined as the resistance to flow of serum through the gel network, from an applied pressure gradient (van Dijk & Walstra, 1986). The permeability coefficient (B) can be used to characterize the network and it primarily depends on the size and number of large pores (van Dijk & Walstra, 1986). In rennet-induced casein gels permeability increases with time, even in a gel that is constrained so that it cannot undergo overall changes in height.

The objectives of this preliminary study was to obtain information on the rennet-induced gel structure made from MPC powder using the permeability technique and confocal scanning laser microscopy.

RESULTS AND DISCUSSION

8.1 Permeability of rennet-induced MPC gels

The *B* of rennet-induced gels made from reconstituted skim milk (20% MPC without the additional of milk fat) and recombined cheese milk (20% MPC and 20% milk fat) were investigated using the permeability "tube method" as described in Chapter 4. The average values and standard deviations for each experiment are shown in Table 8.1. It was found that the permeability coefficient (*B*) of renneted-induced gel made from both type of milks slightly increased with measuring time. At any particular time the *B* for reconstituted skim milk was higher than that of recombined cheese milk, indicating that the renneted whey can more easily flow through the renneted gel made from reconstituted skim milk. It took approximately 4 h for the whey to rise above the level of renneted gel prepared from reconstituted skim milk (see Figure 4.3), whereas it took almost one day (~ 20 h) for the whey to rise above the recombined cheese milk gel.

Since it was reported in Chapter 6 that renneted gel made from recombined cheese milk had higher G' values and yield force required than that of reconstituted skim milk, it appeared that the gels made from recombined cheese milk were stronger, denser and probably had smaller pore sizes. In addition, milk fat globules, which participated in the casein network, might retard the movement of the whey through recombined cheese milk gels.

Permeability measurements can be used to indicate the likelihood of rearrangements or syneresis in gels. Endogenous syneresis is defined as that occurring in the absence of external pressure. The process of syneresis is well understood for rennet-induced skim milk gels (van Dijk & Walstra, 1986). Initially, the casein particles in the network form only a limited number (2-4) of junctions with others. However, the particles are reactive over their surface, so the forming of bonds is energetically favourable. This is possible because the strands are somewhat flexible and can move to some extent due to heat motion. The formation of new junctions between strands will induce small tensile stresses at other places in the network. If the protein-protein bonds have a relatively short life time, this may lead to yielding of a junction and with that to breaking of a strand (van Vliet *et al.*, 1991). Such a process leads to coarsing of the gel, which can be observed as an increase in *B* with time, which has been observed in rennet-induced skim milk gels ("microsynersis") (van Dijk & Walstra, 1986).

Table 8.1 The permeability coefficient (B) of rennet-induced gel made from reconstituted skim milk and recombined cheese milk determined at 34°C, by the tube method.

Samples	Permeability Coefficient (B x 10^{-13} m^2)					
	Experiment 1			Experiment 2		
	Time (h)	Mean	SD	Time (h)	Mean	SD
Reconstituted Skim Milk Gel	3.6	0.29	0.004	5.8	0.92	0.019
	4.3	0.21	0.011	7.2	0.89	0.049
	7.5	0.62	0.022	8.5	1.78	0.079
	9.2	1.07	0.037	9.5	2.15	0.141
	11.0	1.45	0.059	12.3	5.83	0.390
	13.2	2.84	0.154	13.3	6.06	0.111
Recombined Cheese Milk Gel	22.1	0.30	0.001	23.3	0.74	0.029
	22.9	0.48	0.024	24.3	0.61	0.029
	24.2	0.43	0.029	25.4	0.93	0.031
	26.4	0.32	0.017	27.4	0.38	0.002
	28.1	0.49	0.010	28.3	0.99	0.063
	30.6	0.72	0.011	29.4	0.99	0.049
	32.0	0.71	0.005	31.0	0.51	0.041
	33.3	0.65	0.024	32.2	1.22	0.099
	48.1	1.08	0.020	33.1	1.37	0.171

The B of MPC gels only slightly increased with time and very long measuring times required for MPC gels may allow proteolysis or other factors to change the B. The likelihood of large scale change in the gel structure is small due to its very dense microstructure and very high dynamic moduli.

Comparison of the B of MPC gels with those reported by van Dijk and Walstra (1986) for rennet gels of skim milk (2 x 10^{-13} m^2), suggests that the MPC gels had much smaller pores.

However, it was difficult to determine the permeability coefficient of recombined cheese milk (40% total solids) and reconstituted skim milk (20% total solids) precisely since the gel networks were so stiff, i.e. the firmness of the gel were very high. Instead of passing though the gel matrix, which took a long time, the rennet whey may have flowed along the sides of the glass tube. In addition, the height of renneted MPC gel decreased during the experiment, possibly changing the B. Because the experiments took such a long time, there might be continued proteolysis in the MPC gels due to rennet activity.

8.2 Microstructure of renneted-induced MPC gels

Microstructure of rennet-induced gels made from recombined cheese milk (40% total solids), homogenized at 120/50 bar without any heat treatment was observed using confocal laser scanning microscopy (CSLM). The micrographs in Figure 8.1 shows the microstructure of renneted gels prepared from recombined cheese milk made from commercial powder (preheat treatment at 85°C for 40 s) (Figure 8.1a) and high-heat treatment MPC powder (preheat treatment at 120°C for 180 s) (Figure 8.1b). The black areas in the protein matrix indicates the location of fat globules.

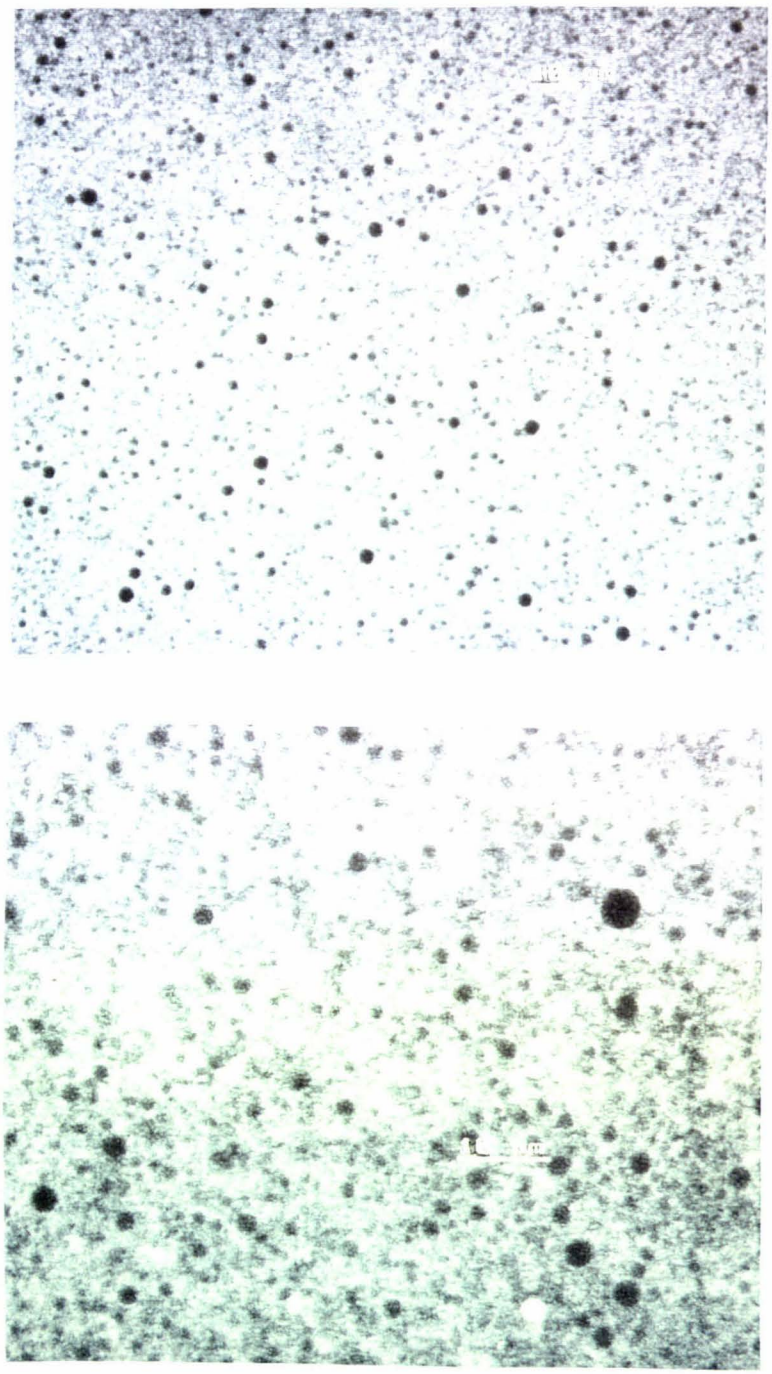


Figure 8.1 Microstructure of rennet-induced gel prepared from recombined cheese milk made from commercial (a) and high-heat treatment MPC powder (b), homogenized at 120/50 bar without any additional heat treatment. Scale bar = 10 μm.

These micrographs show that rennet gels prepared from recombined cheese milk consisted of a strong protein network encapsulating the fat globules. The gel networks were very dense containing very few visible pores, which in contrast to normal rennet gels made from skim milk where one can see pores (> 2 μm) (Bremer, 1992). The fat globules were finely and evenly dispersed throughout the gel network. Homogenization during the preparation of MPC samples caused the fat droplets to be coated with casein micelles and become incorporated into the protein network to become an integral part of the gel structure. The very small pores and the dense matrix were expected since these gels had very low permeability and had very high dynamic moduli. Some differences were observed between the renneted gels prepared from different MPC powders, i.e. the high heat treatment powder gave a "grainy" structure. Electron microscopy would be needed to see the more detailed microstructure of these gels.

GENERAL CONCLUSION

The *B* values of both type of gels were very low, i.e. high resistance to flow, in comparison to those of rennet induced gels made from skim milk. This may be attributed to the very high total solids content, which resulted in gels of very high firmness. The *B* of rennet-induced gels made from recombined cheese milk and reconstituted skim milk increase slightly with time during the measurement. The microstructure of renneted gel made from recombined cheese milk revealed that these gels appeared to have a dense network with milk fat globules incorporated into the network.

REFERENCES

Andrews, A.T. (1983). Proteinases in normal bovine milk and their action on caseins. *J. Dairy Res.* **50**, 45-55.

Aquliera, J.M. & Kessler, H.G. (1988). Physical-chemical and rheological properties of milk fat globules with modified membrane. *Milchwissenschaft* **43**, 411-415.

Bohlin, L., Hegg, P.O. & Ljusberg-Wahren, H. (1984). Viscoelastic properties of coagulating milk. *J. Dairy Sci.* **67**, 729-734.

Banks, J.M. (1988). Elimination of the development of bitter flavour in Cheddar cheese made form milk containing heat-denatured whey protein. *J. Soc. Dairy Technol.* **41**, 37-40.

Blonk, J.C.G. & van Aalst, H. (1993). Confocal scanning light microscopy in food research. Food Res. Technol. 26, 297-311.

Bremmer, L. (1992). Fractal aggregation in relation to formation and properties of particle gels. Ph.D. thesis. Wageningen Agricuktural University, the Netherlands.

Brew, K. & Grobler, J.A. (1992). α-Lactalbumin. In *Advanced Dairy Chemistry-1: Proteins*. P.F. Fox (Ed.), pp. 191-229, Elviser Science Publ., England.

Brooker, B.E. (1995). Imaging food systems by confocal scanning microscopy. In *New physico-chemical techniques for the characterization of complex food systems*. E. Dickinson (Ed.), pp 53-68, Blaxkie Acedemic & Professional, Glasgow.

Brown, R.J. (1988). Milk coagulation and protein denaturation. In *Fundamentals of dairy chemistry.* (3 rd Ed.) N.P. Wong, R. Jenness, M. Keeney, E.M. Marth (Eds.), pp. 583-608, AVI Book, New York, USA.

Burgress, K.J. (1978). Measurement of the firmness of milk coagulum. *Ir. J. Food Sci. Technol.* **21**, 129 -134.

Casiraghi, E., Lucisano, M. & Piazza, L. (1989). Rennet coagulation of milk retentates. 2. The combined effect of heat treatments and protein concentration. *J. Dairy Sci.* **72**, 2457-2463.

Calvo, M.M., Law, A.J.R. & Leaver, J. (1995). Heat-induced interactions between serum albumin, immunoglobulin and κ-casein inhibit the primary phase of renneting. *J. Agric. Food Chem.* **43**, 2823-2827.

Calvo, M.M., Leaver, J., Law, A.J.R. & Banks, J.M. (1992). Changes in casein levels during the ripening of Cheddar-type cheese made from overheated milk. *Milchwissenschaft* **47**, 516-518.

Castle, A.V. & Wheelock, J.V. (1972). Effect of varying enzyme concentration on the action of rennin on whole milk. *J Dairy Sci.* **39**, 15-22.

Chaplin, B. & Green, M.L. (1980). Determination of the proportion of κ-casein hydrolysis by rennet on coagulation of skim milk. *J. Dairy Res.* **47**, 351-358.

Cheryan, M., van Wyk, P.J., Olson, N.F. & Richardson, T. (1974). Secondary phase and mechanism of enzymatic milk coagulation. *J. Dairy Sci.* **58**, 477-481.

Cobos, A., Horne, D.S. & Muir, D.D. (1995). Rheological properties of acid milk gels. I. Effect of composition, process and acidification conditions on products from recombined milks. *Milchwissenchaft* **50**, 444-447.

Creamer, L.K. & Matheson, A.R. (1980). Effect of heat treatment on the proteins of pasteurized skim milk. *NZ. J. Dairy Sci. Technol.* **15**, 37-49.

Culioli, J. & Sherman, P. (1978). Rheological aspects of the renneting of milk concentrated by ultrafiltration. *J. Text. Stud.* **9**, 257-287.

Dalgleish, D.G. (1980). Effect of milk concentration on the rennet coagulation time. *J. Dairy Res.* **47**, 231-235.

Dalgleish, D.G. (1981). Effect of milk concentration on the nature of curd formed during renneting - A theoretical discussion. *J Dairy Res.* **48**, 65-69.

Dalgleish, D.G. (1983). Coagulation of renneted casein micelles: Dependence on temperature, calcium ion concentration and ionic strength. *J. Dairy Res.* **59**, 331-340.

Dalgleish, D.G. (1984). Measurement of electrophoretic mobilities and zeta-potentials of particles from milk using Laser Doppler Electrophoresis. *J Dairy Res.* **51**, 425-438.

Dalgleish, D.G. (1990). The effect of denaturation of β-lactoglobulin on renneting - A quantitative study. *Milchwissenschaft* **45**, 491-494.

Dalgleish, D.G. (1992). The enzymatic coagulation of milk. In *Advanced Dairy Chemistry-1: Proteins.* P.F Fox (Ed.), pp. 579-620, Elsevier Science Publ., England.

Dalgleish, D.G. (1993). The enzymatic coagulation of milk. In *Cheese: Chemistry, physics and microbiology. Volume 1. General aspects.* P.F. Fox (Ed.), pp. 69-100, Chapman & Hall, London.

Dalgleish, D.G. & Banks, J.M. (1991). The formation of complex between serum proteins and fat globules during heating of whole milk. *Milchwissenschaft* **46**, 75-79.

Dalgleish, D.G. & Holt, C. (1988). A geometric model to describe the initial aggregation of partly rennet caseins micelles. *J. Colloid Interf. Sci.* **123**, 80-84.

Dalgleish, D.G., Horne, D.S. & Law, A.J.R. (1989). Size-related differences in bovine casein micelles. *Biochem. Biophys. Acta.* **991**: 383-387.

Damicz, W. & Dziuba, J. (1975). Studies on casein proteolysis. I. Enzymatic phase of casein coagulation as influenced by heat treatment of milk proteins. *Milchwissenschaft* **30**: 399-405.

Dannenberg, F. & Kessler, H. (1988a). Application of reaction kinetics to the denaturation of whey proteins in heated milk. *Milchwissenschaft* **43**, 3-7.

Dannenberg, F. & Kessler, H. (1988b). Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci.* **53**, 258-263.

Darling, D.F. & Butcher, D.W. (1978). Milk-fat globule membrane in homogenized cream. *J. Dairy Res.* **45**, 197-208.

Darling, D.F. & van Hooydonk, A.C.M. (1981). Deviation of a mathematical model for the mechanism of casein micelle coagulation by rennet. *J. Dairy Res.* **48**, 189-200.

Davis, D.T. & Law, A.J.R. (1983). Variation in the protein composition of bovine casein micelles and serum casein in relation to micellar size and milk temperature. *J. Dairy Res.* **50**, 67-75.

Dejmak, P. (1987). Dynamic rheology of rennet curd. *J. Dairy Sci.* **70**, 1325-1330.

Desobry-Banon, S., Richard, F. & Hardy, J. (1994). Study of acid and rennet coagulation of high pressurized milk. *J. Dairy Sci.* **77**, 3267-3274.

Dickinson, E., Whyman, R. & Dalgleish, D.G. (1987). Colloidal properties of model oil-in water emulsions stabilized separated by α_{s1}-casein, β-casein and κ-casein. In *Food Emulsions and Foams.* E. Dickinson (Ed.), pp. 40-51, Royal Society of Chemistry Special Publication No 58.

Doan. F.J. (1954). Physico-chemical characteristics of milk as a result of homogenization. *Amer. Milk Rev.* 54-60.

Eigel, W.N., Butler, J.E., Ernstrom, C.A., Farrel, H.M.Jr., Harwalker, V.R., Jenness, R. & Whitney, R. M. (1984). Nomenclature of proteins of cow's milk. 5th Ed. *J. Dairy Sci.* **67**, 1599-1631.

Eisele, M. & Budny, J. (1964). Possibility of using homogenization in cheesemaking. *Dairy Sci. Abstr.* **27**, 1676.

El-Salam, M.H.A. & El-Shibiny, S. (1982). Manufacture of pickles soft cheese from recombined milk. I. Effect of homogenization pressure. *Eypt. J. Dairy Sci.* **10**, 55-59.

Farell, H.H. (1988). Physical equilibrium: Proteins. In: *Fundamentals of Dairy Chemistry.* N.P. Wong, R. Jenness, M. Keeney, and E.H. Marth (Eds.), pp. 416-450, AVI Book, New York, USA.

Ferry, J.D. (1980). Viscoelastic properties of polymers. John Wiley & Sons, New York.

Fink, A. & Kessler, H.G. (1985). Changes in the fat globule membrane produced by heating. *Milchwissenschaft* **40**, 261-264.

Fox, P.F. (1981). Heat-induced changes in milk proceeding coagulation. *J. Dairy Sci.* **64**, 2127-2137.

Fox, P.F. (1987). Cheese manufacture: Chemical, biochemical and physical aspects. *Dairy Ind. Int.* **52**, 11-13.

Garnot, P. & Corre, C. (1980). Influence of milk protein concentration on the gelling activity of chymosin and bovine pepsin. *J. Dairy Res.* **47**, 103-111.

Garnot, P., Rank, T.C. & Olson, N.F. (1982). Influence of protein and fat contents of ultrafiltered milk on rheological properties of gels formed by chymosin. *J. Dairy Sci.* **65**, 2267-2273.

Gilles, M.L. (1974). Feta cheese manufacture from skimmilk powder and anhydrous milkfat. *XIX International Dairy Congress* **IE**, 782 -783.

Gilles, J. & Lawrence, R.C. (1981). The manufacture of cheese and other fermented products from recombined milk. *NZ. J. Dairy Technol.* **16**, 1-12.

Goddard, S. & Augustin, M. (1995). Formation of acid-heat induced skim milk gels in the pH range 5.0-7.5: Effect of the addition of salts and calcium chelating agents. *J. Dairy Res.* **62**, 491-500.

Ghosh, B.C., Steffl, A., Hinrichs, J. & Kessler, H.G. (1994). Rennetability of whole milk homogenized before and after pasteurization. *Milchwissenschaft* **49**, 363-367.

Green, M. (1990). Cheddar cheesemaking from whole milk concentrated by ultrafiltration heated to 90°C. *J. Dairy Res.* **69**, 559-569.

Green, D.W., Aschaffenburg, R., Cramerman, A. Coppola, J.C., Diamand, R.D., Dunnul, P., Simmons, R.M., Komorowski, E.S., Sawyer, L., Turner, E.M.C. & Woods, K.F. (1979). Structure of bovine β-lactoglobulin at 6°A resolution. *J. Molecular Biology* **131**, 375-397.

Green, M.L., Glover, F.A., Scurlock, E.M.W., Marshall, R.J. & Hatfield, D.S. (1981). Effect of use of milk concentrated by ultrafiltration on the manufacture and ripening of Cheddar cheese. *J. Dairy Res.* **48**, 333-341.

Green, M.L. & Grandison, A.S. (1993). Secondary (non-Enzymatic) phase of rennet coagulation and post-coagulation phenomena. In *Cheese: Chemistry, physics and microbiology. Volume 1. General aspects*. P.F. Fox (Ed.), pp. 101-140, Chapman & Hall, London.

Green, M.L., Hobbs, D.G., Morant, S.V. & Hill, V.A. (1978). Intermicellar relationships in rennet treated separated milk. II. Process of gel assembly. *J. Dairy Res.* **45**, 412-422.

Green, M.L., Marshall, R.J. & Glover, F.A. (1983). Influence of homogenization of concentrated milks on the structure and properties of rennet curds. *J. Dairy Res.* **50**, 341-348.

Green, M.L. & Morant, S.V. (1981). Mechanism of aggregation of casein micelles in rennet-treated milk. *J. Dairy Res.* **48**: 57-63.

Guinee, T.P., O'Brien, N.B. & Frawle, D. (1994). The viscosity of cheese sauces with difference starch systems and cheese powders. *J. Soc Dairy Technol.* **47**, 132-139.

Guinee, T.P., Pudja, P.D., Reville, W.J., Harrington, D., Mulholland, E.O., Cotter, M. & Cogan, T.M. (1995). Composition, microstructure and maturation of semi-hard cheese from high protein ultrafiltered milk retentates with different levels of denatured whey protein. *Int. Dairy J.* **95**, 543-568.

Hambling, S.G., McAlpine, A.S. & Sawyer, L. (1992). β-Lactoglobulin. In *Advanced Dairy Chemistry-1. Proteins*. P.F Fox (Ed.), pp. 191-230, Elsevier Science Publ., England.

Haque, Z. & Kinsella, J.E. (1988). Interaction between κ-casein and β-lactoglobulin: Predominance of hydrophobic interactions in the initial stages of complex formation. *J. Dairy Res.* **55**, 67-80.

Harper, W.J. (1976). Dairy technology and engineering. The AVI Publ. Company, Inc. Westport.

Harper, W.J. (1981). Advances in chemistry of milk. *J. Dairy Sci* **64**, 1028-1037.

Henstra, S. & Schmidt, D.G. (1970). On the structure of the fat-protein complex in homogenized cow's milk. *Neth. Milk Dairy J.* **24**, 45.

Holt, C. (1985). The stability of bovine casein micelles. In *Proceeding of the conference on colloids and science*. E. Wolfram (Ed.), pp. 641-644, Academia, Budapest.

Houlihan, A.V., Goddard, P.A., Kitchen, B.J. & Masters, C.J. (1992). Changes in structure of the bovine milk fat globule membrane on heating whole milk. *J. Dairy Res.* **59**, 321-329.

International Dairy Federation (1990). Recombination of milk and milk products. IDF special Issue 9001, Brussels, Belgium. International Dairy Federation.

Iwaida, M. & Tsugo, T. (1961). The effect of homogenization on nitrogen distribution in skim milk. *Biol. Chem.* **25**, 130.

Jana, A.H. & Upadhyay, K.G. (1992). Homogenization of milk for cheesemaking - A review. *Aust. J. Dairy Technol.* **47**, 72-79.

Jeurnink, T.J.M. & De Kruif, K. (1993). Changes in milk on heating: Viscosity measurements. *J. Dairy Res.* **60**, 139-150.

Jimenez-Flores, R. & Kosikowski, T. (1995). Properties of ultrafiltered skim milk retentate powder. *J. Dairy Sci.* **69**, 329-339.

Kannan, A. & Jenness, P. (1961). Relation of milk serum proteins and milk salts to the effects of heat treatment on rennet clotting. *J. Dairy Sci.* **44**, 808.

Kaw, A.K., McCarthy, O.J. & Singh, H. (1994). Rheological properties of milk concentrates obtained by reverse osmosis. New Zealand Postgraduate Conference for Engineering and Technology Students.

Kebary, K.M.K. & Morris, H.A. (1990). Effect of homogenization of reconstituted nonfat dry milk butter oil mixtures on curd formation and characteristics. *Cultured Dairy Products J.* **25**, 12-18.

Kessler, H.G. & Beyer, H. (1991). Thermal denaturation of whey proteins and its effect in dairy technology. *Int. J. Biol. Macromol.* **13**: 165-172.

Kim, H.H.Y. & Jimenez-Flores, R. (1995). Heat-induced interactions between the proteins of milk fat globule membrane and skim milk. *J. Dairy Sci.* **78**, 24-35.

Lapshina, A.D., Golovchenko, A.A. & Poplavets, P.I. (1978). Effect of homogenization on rennet coagulation of milk. *Dairy Sci. Abstr.* **43**, 6335.

Law, A.J.R. (1995). Heat denaturation of bovine, caprine and ovine whey proteins. *Milchwissenschaft* **50**, 384-389.

Law, A.J.R., Banks, J.M., Horne, D.S., Leaver, J., & West, I.G. (1994). Danaturation of the whey proteins in heated milk and their incorporation into Cheddar cheese. *Milchwissenschaft* **49**, 63-67.

Leaver, J., Law, A.J.R., Horne, D.S. & Banks, J.M. (1995). Influence of heating regime and pH on the primary phase of renneting of whole milk. *Int. Dairy J.* **5**, 129-140.

Lelievre, J.R., Shaker, R. & Taylor, M.W. (1990). The role of homogenization in the manufacture of Halloumi and Mozzarella cheese from recombined milk. *J. Soc Dairy Technol.* **43**, 21-24.

Lucey, J.A. (1995). Effect of heat treatment on the rennet coagulability of milk. *In Heat-Induced Changes in Milk.* 2nd Ed., P.F. Fox (Ed.), pp. 171-187, International Dairy Federation Special Issue 9501.

Lucey, J.A., Gorry, C. & Fox, P.F. (1993). Rennet coagulation properties of heated milk. *Agric. Sci. Finl.* **2**, 361-369.

Lucisano, N., Peri, C. & Donati, E. (1985). Studies on coagulation of milk ultrafiltration retantates. I. Coagulation kinetics. *Milchwissenschaft* **40**, 600-604.

Mackey, K.L., Cooke, P.H., Malin, E.L. & Holsinger, V.H. (1991). Effect of homogenization on casein micelles. *J. Dairy Sci.* **71** (Supp.), 128.

Marshall, R. (1986). Increasing cheese yields by high heat treatment of milk. *J. Dairy Res.* **53**, 313-322.

Masi, P., Aciero, D. & Addeo, F. (1988). Measuring milk gelification by means of an Instron Universal Testing Machine operating in dynamic mode. *J. Text. Stud.* **19**, 161-170.

Maxcy, R.B., Price, W.V. & Irvine, D.M. (1955). Improving curd-forming properties of homogenized milk. *J. Dairy Sci.* **38**, 80-86.

McMahon, D.J. & Brown, R.J. (1984). Enzymatic coagulation of casein micelles: A review. *J. Dairy Sci.* **67**, 919-929.

McMahon, D.J., Savello, P.A., Brown, R.J. & Kalab, M. (1991). Effect of phosphate and citrate on the gelation properties of casein micelles in renneted ultra-high temperature (UHT) sterilized concentrated milk. *Food Struct.* **10**, 27-36.

McMahon, D.J. Yousif, B.H. & Kalab, M. (1993). Effect of whey protein denaturation on structure of casein micelles and their rennetability after ultra-high temperature processing of milk with or without ultrafiltration. *Int. Dairy J.* **3**, 239-256.

McPherson, A.V., Dash, M.C. & Kitchen, B.J. (1984). Isolation and composition of milk fat globule membrane material. II. From homogenized and ultra heat treated milks. *J. Dairy Res.* **51**, 289-297.

Mehaia, M.A. & Cheryan, M. (1983). Coagulation studies of ultrafiltration-concentrated skimmilk. *Milchwissenschaft* **38**, 708-710.

Melsen, I.P. (1989). Dissertation abstracts: The stability of recombined milk fat globules. *Neth. Milk Dairy J.* **43**: 89-95.

Mohammad, K.S. & Fox, P.F. (1987). Heat-induced microstructural changes in casein micelles before and after heat coagulation. *NZ. J. Dairy Sci. Technol.* **22**, 191-203.

Morr, C.V. (1969). Protein aggregation in conventional and ultra-high-temperature heated skim milk. *J. Dairy Sci.* **52**, 1174.

Morrisey, P.A. (1969). The rennet hysteresis of heated milk. *J. Dairy Res.* **36**, 331-341.

Mulder, H. & Walstra, P. (1974). The milk fat globule: Emulsion science as applied to milk products and comparable foods. Wageningen Pudoc, Farnam Royal, Commonwealth Agricultural Bureaux.

Mulvihill, P.M. & Donovan, M. (1987). Whey proteins and their thermal denaturation - A review. *Irish J. Food Sci. Technol.* **11**, 43-75.

Nicholus, L.E. (1947). A non-fat leaking Cheddar cheese. *Dairy Sci Abstr.* **9**, 170C.

Novak, A. (1992). Milk protein concentrate. In *New Applications of Membrane Processes*. P.F. Fox (Ed.), pp. 51-66, International Dairy Federation Special Issue 9201.

Oortwijn, H., Walstra, P. & Mulder, H. (1979). The membranes of recombined fat globules. 1. Electron microscopy. *Neth. Milk Dairy J.* **31**, 134-147.

Payens, T.A. (1984). The relationship between milk concentration and rennet coagulation time. *J. Appl. Biochem.* **6**, 232-239.

Peters, I.I. (1964). Homogenized milk in cheese making. *Dairy Sci. Abstr.* **26**, 457.

Rao, M.A. (1977). Rheology of liquid Foods. A-review. *J. Text. Stud.* **8**, 135-168.

Reddy, I.M. & Kinsella, J.E. (1990). Interaction of β-lactoglobulin with κ-casein in micelles as assessed by chymosin hydrolysis: Effect of temperatures, heating time, β-lactoglobulin concentration and pH. *J. Agric. Food Chem.* **38**, 50-58.

Reuter, H., Hisserich, D. & Prokopek, D. (1981). Study on the formal kinetics of rennet coagulation of milk concentrated by ultrafiltration. *Milchwissenschaft* **36**, 13-18.

Robin, O. & Paquin, P. (1991). Evaluation of the particle size of fat globules in a milk model emulsion by proton correlation spectroscopy. *J. Dairy Sci.* **74**, 2440-2447.

Robson, E.W. & Dalgleish, D.G. (1984). Coagulation of homogenized milk particles by rennet. *J. Dairy Res.* **51**, 417-424.

Roefs, S.P.F.M., de Groot-Mostert, A.E.A. & van Vliet, T. (1990). Structure of acid casein gels. 1. Formation and model of gel network. *Colloids & Surfaces* **50**, 141-159.

Roefs, S.P.F.M., van Vliet, T., van den Bijgaart, H.J.C.M., de Groot-Mostert, A.E.A. & Walstra, P. (1990). Structure of casein gels made by combined acidification and rennet action. *Neth. Milk Dairy J.* **44**, 159-188.

Saito, Z. (1993). Effects of homogenization on the rennet coagulation of milk and cream. In *Protein and fat globule modification by heat treatment, homogenization and other means for high quality dairy products*. P.F. Fox (Ed.), pp. 343-351, International Dairy Federation Special Issue 9303.

Sasaki, R. & Miyasawa, W. (1955). On the influence of homogenization on the properties of milk. I. Coagulation of milk and fat content of whey. *Dairy Sci. Abstr.*, 28.

Schmidt, D.G. (1980). Colloidal aspects of casein. *Neth. Milk Dairy J.* **34,** 42-64.

Schmidt, D.G. (1982). Association of casein and casein micelles structure. In *Developments in Dairy Chemistry*. Vol. 1. P.F. Fox (Ed.), pp. 62-82, Applied Science Publ., England.

Schmidt, D.G., Walstra, P. & Buchheim, W. (1973). The size distribution of casein micelles in cow's milk. *Neth. Milk Dairy J.* **9**, 128-142.

Sharma, S.K. & Dalgleish, D.G. (1993). Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. *J. Agri. Food Chem.* **41**, 1407-1412.

Sharma, S.K., Hill, A.R. & Goff, H.D. (1990). The effect of heat treatment of ultrafiltered milk on its coagulation properties. *Milchwissenschaft* **45**, 432-435.

Sharma, S.K., Hill, A.R. & Goff, H.D. (1994). Effect of milk concentration, pH and temperature on κ-casein hydrolysis at aggregation, coagulation and curd cutting times of ultrafiltered milk. *Milchwissenschaft* **49**, 450-453.

Sharma, R., Singh, H. & Taylor, M.W. (1996). Composition and structure of fat globule surface layers in recombined milk. *J. Food Sci.* **61**, 28-32.

Sherbon, J.W. (1988). Physical properties of milk. In *Fundamentals of Dairy Chemistry*. B. H. Webb, A.H. Johnson and J.A. Alford (Eds.), pp. 402-441, The AVI Publishing Company. Westport, Connecticut.

Singh, H. & Creamer, L.K. (1991). Denaturation, aggregation and heat stability of milk protein during the manufacture of skim milk powder. *J. Dairy Res.* **58**, 269-283.

Singh, H. & Fox, P.F. (1989). Heated-induced changes in casein. *IDF. Bulletin* **238**, 24-30.

Singh, H. & Creamer, L.K. (1992). Heat stability of milk. In *Advanced Dairy Chemistry-1: Proteins*. P.F Fox (Ed.), pp. 621-656, Elsevier Science Publ., England.

Singh, H. & Newstead, D.F. (1992). Aspects of proteins in milk powder manufacture. In *Advanced dairy chemistry Vol. 1: Proteins*. P.F. Fox (Ed.), pp. 656-681, Elsevier Applied Science, England.

Singh, H., Shalabi, S.I., Fox, P.F., Flynn, A. & Barry, A. (1988). Rennet coagulation of heated milk: Influence of pH adjustment before/after heating. *J. Dairy Res.* **55**, 205-215.

Soo. B. (1994). Milk protein concentrates: Recombined cheese unravelled. NZDRI Report. New Zealand Dairy Research Instituted, Palmerston North.

Storry, J.E., Grandison, A.S., Millard, D., Owen, A.J. & Ford, G.D. (1983). Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. *J. Dairy Res.* **50**, 215-219.

Streuper, A. & van Hooydonk, A.C.M. (1986). Heat treatment of whipping cream. II. Effect on cream plug formation. *Milchwissenschaft* **41**, 547-552.

Ustunol, Z. & Brown, R.J. (1985). Effects of heat treatment and post-treatment holding time on rennet clotting of milk. *J. Dairy Sci.* **68**, 526-530.

van Boekel, M.A.J.S. & Folkerts, T. (1991). Effect of heat treatment on the stability of milk fat globules. *Milchwissenchaft* **46**, 758-765.

van Boekel, M.A.J.S. & Walstra, P. (1989). Physical changes in the fat globules in unhomogenized and homogenized milk. *IDF Bulletin* **238**, 13-17.

van Dijk, H.J.M. & Walstra, P. (1986). Syneresis of curd. 2. One-dimensional syneresis of rennet curd in constant conditions. *Neth. Milk Dairy J.* **40**, 3-30.

van Hooydonk, A.C.M., de Koster, P.G. & Boerrigter, I.J. (1987). The renneting properties of heated milk. *Neth. Milk Dairy J.* **41**, 3-18.

van Vliet, T. (1988). Rheological properties of filled gels. Influence of filler matrix interaction. *Colloid. Polym. Sci.* **266**, 518-524.

van Vliet, T. & Dentener-Kikert, A. (1982). Influence of the composition of the milk fat globules membrane on the rheological properties of acid milk gels. *Neth. Milk Dairy J.* **36**, 261-265.

van Vliet, T., Roefs, S.P.E.M., Zoon, P. & Walstra, P. (1991). Rheological properties of casein gels. *J. Dairy Res.* **56**, 529-534.

van Vliet, T., van Dijk, H.J.M., Zoon, P. & Walstra, P. (1991). Relation between syneresis and rheological properties of particles gels. *Colloid Polym. Sci.* **269**, 620-627.

Visser, H. (1992) A new casein micelles model and its consequences for pH and temperature effects on the properties of milk. In Protein Interactions. H. Visser (Eds), pp 139-161, Weinheim, Germany, Newyork.

Walstra, P. (1975). Effect of homogenization on the fat globule size distribution in milk. *Neth Milk Dairy J.* **29**, 279-294.

Walstra, P. (1980). Effect of homogenization on milk plasma. *Neth. Milk Dairy J.* **34**, 181-190.

Walstra, P. (1983). Physical chemistry of milk fat globules. *In Developments in dairy chemistry -2 Lipids*. P.F. Fox (Ed.) Elsevier Applied Science Publ., England.

Walstra, P. (1990). On the stability of casein micelles. *J. Dairy Res.* **73**, 1965-1979.

Walstra, P. & Jenness, R. (1984). Dairy chemistry and physics. John Wiley & Sons, New York.

Walstra, P. & Oortwijn, H. (1982). The membranes of recombined fat globules. 3. Mode of formation. *Neth. Milk Dairy J.* **36**, 103-113.

Walstra, P. & Peleg, M. (1991). Effects of sample size and preparation. *IDF Bulletin* **268**, 26-29.

Walstra, P. & van Vliet, T. (1986). The physical chemistry of curd making. *Neth. Milk Dairy J.* **41**, 241-259.

Waungana, A. (1995). Rennet coagulation properties of heated milk. Master of Technology Thesis, Massey University, Palmerston North, NZ.

Wheelock, J.V. & Kirk, A. (1974). The role of β-lactoglobulin in the primary phase of rennin action on heated casein micelles and heated milk. *J. Dairy Res.* **4**, 367-372.

Whitney, R.M. (1988). Proteins of milk. In *Fundamental of dairy chemistry*, 3rd. Ed., N.P. Wong, R Jenness, M. Keeney & E.H. Marth. (Ed.), pp. 81-169, Van Nostrand Reinhold Company, New York.

Wilson, G.A. & Wheelock, J.V. (1972). Factors affecting the action of rennin in heated milk. *J. Dairy Res.* **39**, 413-419.

Xiong, Y.L & Kinsella, J.E. (1991a). Influence of fat globule membrane composition and fat type on the rheological properties of milk based composite gels. I. Methodology. *Milchwissenschaft* **46**, 150-152.

Xiong, Y.L & Kinsella, J.E. (1991b). Influence of fat globule membrane composition and fat type on the rheological properties of milk based composite gels. II. Results. *Milchwissenschaft* **46,** 203-211.

Zittle, C.A. (1962). κ-Casein-β-lactoglobulin interaction in solution when heated. *J Dairy Res.* **45**, 809.

Zoon, P., van Vliet, T. & Walstra, P. (1988). Rheological properties of rennet-induced skim milk gels. 1. Introduction. *Neth. Milk Dairy J.* **42**, 249-269.

Zwigers, A. (1992). Outline of milk protein concentrate. *IFI NR* **3**, 18-22.

APPENDICES

Appendix I Effect of process variables on milk system and rennet coagulation properties

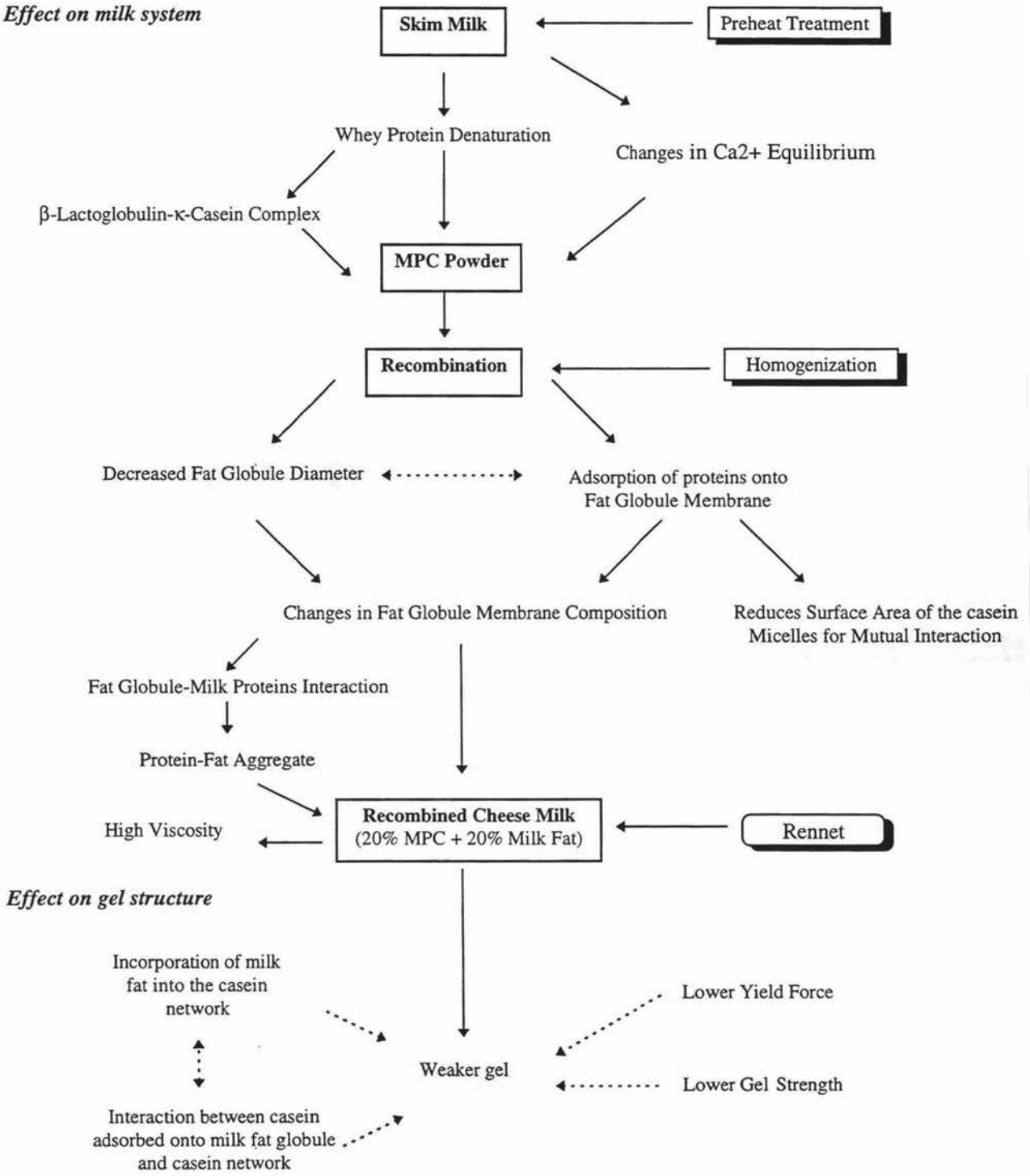


Figure 1 The schematic diagram of the effect of preheat treatment and homogenization on milk system and rennet coagulation properties.

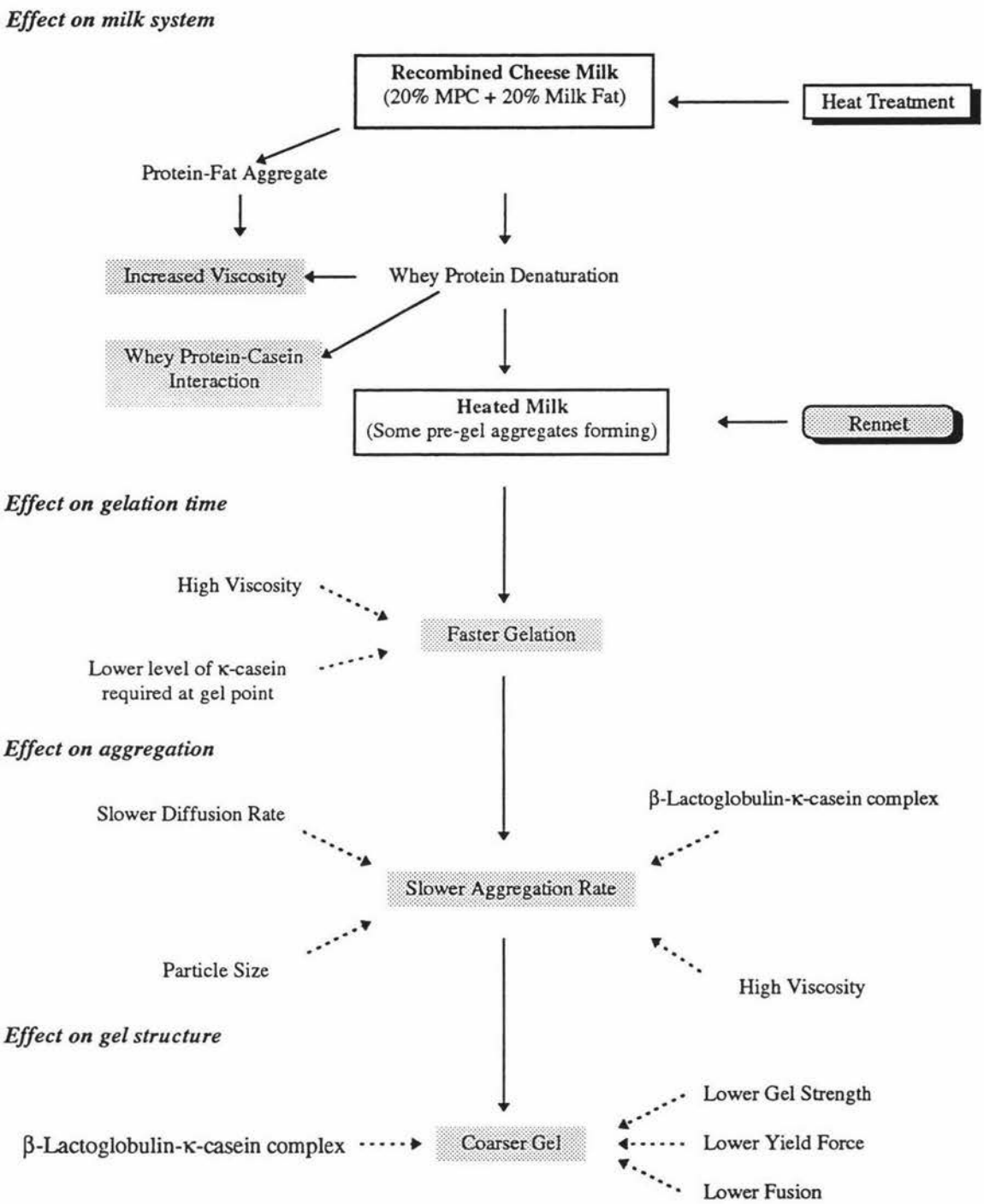


Figure 2 The schematic diagram of the effect of heat treatment on milk system and rennet coagulation properties.

APPENDIX II Experimental Data

Table 1 Effect of heating time on the physical and rennet coagulation properties of recombined cheese milk (20% MPC powder and 20% milk fat).

Heat Treatment	Fat Globule Diameter, d_{43} (μm)		Denaturation of whey protein (%)				Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
	Deionized Water	Dissociating Solution	Total	β-Lactoglobulin A	β-Lactoglobulin B	α-Lactalbumin		1 Hour	2 Hour	
no-Heat	1.11	1.09	0	0	0	0	18.3	3546	5310	5.777
75°C for 3 min	1.11	1.10	7.6	21.4	22.7	16.5	21.3	2888	4733	4.729
75°C for 5 min	1.11	1.10	17.3	28.3	23.9	21.4	19.3	2830	4533	4.335
75°C for 7 min	1.10	1.09	23.5	30.9	28.2	27.3	19.3	2426	4063	4.222
75°C for 10 min	1.13	1.12	26.6	43.7	37.1	35.6	17.3	2372	3940	4.185
80°C for 3 min	1.12	1.11	15.0	30.1	22.8	21.2	19.3	2912	4653	4.243
80°C for 5 min	1.12	1.12	36.8	55.3	50.7	49.5	17.3	2224	3650	3.825
80°C for 7 min	1.12	1.11	59.5	81.8	79.2	78.9	12.3	2230	3437	3.741
80°C for 10 min	1.13	1.12	62.5	85.1	83.6	81.5	12.3	1988	3223	3.664
85°C for 3 min	1.13	1.12	39.2	63.6	57.0	56.5	16.3	2206	3450	3.873
85°C for 5 min	1.11	1.11	47.6	68.1	66.9	58.3	16.3	2106	3257	3.560
85°C for 7 min	1.14	1.13	62.9	94.5	94.2	89.8	6.3	2058	3173	2.796
85°C for 10 min	1.13	1.11	72.1	96.5	96.1	92.3	5.3	1890	3143	2.316

Table 2 Effect of heating temperature for 3 min holding time on the physical and rennet coagulation properties of recombined cheese milk (20% MPC powder and 20% milk fat) made from commercial and low-heat treatment powders.

MPC Powder	Heat Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)		% Whey Protein Denaturation	Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution			1 Hour	2 Hour	
Commercial Powder (85°C for 40 s)	no-Heat	1.09	1.07	18.2	18.3	3110	4637	4.945
	70°C for 3 min	1.10	1.08	24.9	21.3	2670	4337	4.245
	73°C for 3 min	1.10	1.09	31.8	21.3	2624	4217	4.164
	75°C for 3 min	1.10	1.10	38.9	21.3	2414	4033	4.016
	78°C for 3 min	1.08	1.10	40.3	20.3	1990	3390	3.910
	80°C for 3 min	1.09	1.10	44.4	20.3	1954	3347	3.439
	83°C for 3 min	1.11	1.08	62.0	21.3	1812	3173	3.042
Low-heat Powder (75°C for 15 s)	no-Heat	1.09	1.10	0	21.3	3194	4943	5.370
	70°C for 3 min	1.08	1.08	21.2	21.3	2752	4300	4.743
	73°C for 3 min	1.10	1.10	25.2	21.3	2826	4218	4.517
	75°C for 3 min	1.09	1.09	30.8	21.3	2670	4163	4.222
	78°C for 3 min	1.10	1.08	35.6	22.3	2540	4087	3.953
	80°C for 3 min	1.09	1.08	40.4	21.3	2368	3910	3.881
	83°C for 3 min	1.10	1.09	45.1	21.3	2288	3820	3.092

Table 3 Effect of pre heat treatment during MPC powder manufacture on the physical and rennet coagulation properties of reconstituted skim milk (20% MPC powder without an additional milk fat) and recombined cheese milk (20% MPC powder and 20% milk fat)

Milk	Preheat Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)		Denaturation of whey protein (%)				Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution	Total	β-Lactoglobulin A	β-Lactoglobulin B	α-Lactalbumin		1 Hour	2 Hour	
Reconstituted Skim Milk	75°C for 15 s	-	-	0	0	0	0	28.3	1176	2067	2.026
	85°C for 15 s	-	-	17.0	14.1	12.5	4.7	29.3	878	1945	1.987
	85°C for 60 s	-	-	30.5	38.8	36.2	11.6	29.3	683	1610	1.884
	85°C for 120 s	-	-	40.7	48.2	41.6	16.9	28.3	574	1337	1.612
	85°C for 180 s	-	-	49.7	65.9	63.3	37.8	27.3	548	1183	1.376
	85°C for 240 s	-	-	55.8	83.3	72.8	59.0	27.3	416	917	1.154
	120°C for 180 s	-	-	71.8	96.7	91.1	87.7	30.3	87	337	0.446
Recombined Cheese Milk	75°C for 15 s	1.14	1.13	0	0	0	0	21.3	3270	5100	5.402
	85°C for 15 s	1.12	1.12	21.9	26.5	23.2	4.8	21.3	2906	4657	4.830
	85°C for 60 s	1.13	1.12	33.6	43.5	41.1	11.1	21.3	2186	4007	4.521
	85°C for 120 s	1.12	1.12	46.8	52.6	49.7	17.0	20.3	2000	3967	4.153
	85°C for 180 s	1.13	1.12	50.5	72.8	69.4	43.3	20.3	1788	3343	3.467
	85°C for 240 s	1.11	1.11	56.1	85.9	77.6	60.9	20.3	1694	3153	3.240
	120°C for 180 s	1.12	1.11	75.7	97.5	96.0	82.7	24.3	415	1097	1.353

Table 4 Effect of homogenization on the physical and rennet coagulation properties of recombined cheese milk (20% MPC powder and 20% milk fat) without heat treatment and with heat treatment at 75 and 85°C for 3 min holding time.

Heat Treatment	Homogenization Pressure (Bar)	Fat Globule Diameter, d_{43} (μm)		Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution		1 Hour	2 Hour	
no-Heat	100/50	1.12	1.06	17.3	3168	4770	5.183
	120/50	1.11	1.06	19.3	3056	4730	4.686
	150/50	1.08	0.97	19.3	2826	4517	4.529
	180/50	0.90	0.87	18.3	2808	4490	4.287
	200/50	0.81	0.79	17.3	2762	4467	3.642
	220/50	0.75	0.67	17.3	2580	4427	3.404
	250/50	0.59	0.56	17.3	2466	4187	3.349
75°C for 3 min	100/50	1.12	1.20	21.3	2724	4467	4.721
	120/50	1.06	1.06	17.3	2650	4393	4.510
	150/50	0.98	0.97	19.3	2536	4163	3.613
	180/50	0.86	0.85	18.3	2594	4037	3.583
	200/50	0.81	0..78	18.3	2460	3984	3.149
	220/50	0.69	0.67	16.3	2408	3957	3.030
	250/50	0.58	0.56	15.3	2320	3937	2.854
85°C for 3 min	100/50	1.22	1.18	17.3	2366	4103	3.822
	120/50	1.09	1.06	19.3	2474	4057	3.778
	150/50	1.02	0.99	19.3	2388	3937	3.492
	180/50	0.90	0.87	18.3	2292	3870	3.383
	200/50	0.85	0.83	18.3	2266	3793	2.554
	220/50	0.77	0.75	17.3	2026	3390	2.481
	250/50	0.73	0.87	19.3	1912	3400	2.430

Table 5 Effect of processing treatments on viscosity of recombined cheese milk (40% total solids), determined at 34°C over the shear rate of 18.5 - 731 s^{-1}.

Conditions		Viscosity (Pa s)								
		18.5	29.2	46	73	116	184	294	461	731
Heat Treatment Difference Holding Time	no-Heat	0.0531	0.0486	0.0450	0.0416	0.0383	0.0356	0.0332	0.0313	0.0300
	75°C for 3 min	0.0552	0.0496	0.0461	0.0425	0.0390	0.0361	0.0336	0.0321	0.0303
	75°C for 5 min	0.0748	0.0683	0.0611	0.0563	0.0513	0.0471	0.0436	0.0410	0.0390
	75°C for 7 min	0.0985	0.0883	0.0790	0.0709	0.0640	0.0583	0.0537	0.0500	0.0473
	75°C for 10 min	0.1020	0.0913	0.0819	0.0735	0.0663	0.0604	0.0556	0.0519	0.0591
	80°C for 3 min	0.0624	0.0558	0.0525	0.0471	0.0434	0.0399	0.0369	0.0348	0.0332
	80°C for 5 min	0.1270	0.1120	0.0995	0.0885	0.0791	0.0713	0.0651	0.0602	0.0561
	80°C for 7 min	0.2540	0.2190	0.1880	0.1610	0.1400	0.1230	0.1090	0.0975	0.0880
	80°C for 10 min	0.3420	0.2860	0.2420	0.2040	0.1750	0.1510	0.1320	0.1170	0.1050
	85°C for 3 min	0.1090	0.0963	0.0866	0.0776	0.0699	0.0636	0.0583	0.0540	0.0504
	85°C for 5 min	0.1260	0.1110	0.0990	0.0883	0.0793	0.0717	0.0654	0.0603	0.0560
	85°C for 7 min	0.7720	0.6150	0.4960	0.4030	0.3320	0.2760	0.2310	0.1950	0.1650
	85°C for 10 min	0.9940	0.7750	0.6220	0.4990	0.4600	0.3330	0.2750	0.2280	0.1910
Commercial powder Difference Temperature	no-Heat	0.0512	0.0473	0.0438	0.0402	0.0372	0.0344	0.0320	0.0302	0.0289
	70°C for 3 min	0.0582	0.0524	0.0486	0.0448	0.0410	0.0380	0.0354	0.0326	0.0318
	73°C for 3 min	0.0585	0.0527	0.0453	0.0441	0.0405	0.0374	0.0347	0.0330.	0.0311
	75°C for 3 min	0.0590	0.0543	0.0520	0.0468	0.0425	0.0393	0.0367	0.0346	0.0332
	78°C for 3 min	0.0712	0.0652	0.0596	0.0542	0.0497	0.0458	0.0424	0.0398	0.0379
	80°C for 3 min	0.0743	0.0663	0.0608	0.0550	0.0563	0.0463	0.0429	0.0402	0.0381
	83°C for 3 min	0.0938	0.0836	0.0761	0.0686	0.0621	0.0566	0.0520	0.0484	0.0456
Low-heat powder Difference Temperature	no-Heat	0.0248	0.0240	0.0224	0.0215	0.0205	0.0197	0.0190	0.0185	0.0178
	70°C for 3 min	0.0249	0.0242	0.0233	0.0221	0.0211	0.0203	0.0194	0.0187	0.0183
	73°C for 3 min	0.0264	0.0250	0.0234	0.0223	0.0214	0.0206	0.0195	0.0188	0.0182
	75°C for 3 min	0.0274	0.0265	0.0250	0.0236	0.0226	0.0214	0.0204	0.0196	0.0189
	78°C for 3 min	0.0274	0.0268	0.0254	0.0241	0.0229	0.0218	0.0208	0.0200	0.0194
	80°C for 3 min	0.0302	0.0284	0.0270	0.0258	0.0241	0.0229	0.0217	0.0208	0.0200
	83°C for 3 min	0.0332	0.0317	0.0301	0.0281	0.0264	0.0249	0.0235	0.0221	0.0215

Continue...

Continue....

Conditions		Viscosity (Pa.s)								
		18.5	29.2	46	73	116	184	294	461	731
Pre-heat Treatment Difference MPC Powder	75°C for 15 s	0.0250	0.0241	0.0234	0.0224	0.0215	0.0207	0.0199	0.0194	0.0190
	85°C for 15 s	0.0366	0.0349	0.0331	0.0314	0.0298	0.0284	0.0272	0.0262	0.0256
	85°C for 60 s	0.0404	0.0384	0.0361	0.0340	0.0321	0.02304	0.0289	0.0277	0.0269
	85°C for 120 s	0.0411	0.0385	0.0367	0.0348	0.0328	0.0312	0.0297	0.0286	0.0278
	85°C for 180 s	0.0585	0.0537	0.0506	0.0469	0.0441	0.0416	0.0394	0.0378	0.0367
	85°C for 240 s	0.0642	0.0592	0.0553	0.0514	0.0479	0.0450	0.0425	0.0406	0.0393
	120°C for 180 s	0.0792	0.0708	0.0657	0.0607	0.0564	0.0529	0.0501	0.0480	0.0466
Homo- no-Heat Treatment	100/50 Bar	0.0565	0.0513	0.0476	0.0438	0.0404	0.0376	0.0351	0.0332	0.0319
	120/50 Bar	0.0704	0.0626	0.0576	0.0527	0.0483	0.0445	0.0413	0.0389	0.0372
	150/50 Bar	0.0793	0.0715	0.0655	0.0594	0.0541	0.0496	0.0406	0.0434	0.0417
	180/50 Bar	0.1030	0.0908	0.0820	0.0739	0.0672	0.0611	0.0563	0.0527	0.0505
	200/50 Bar	0.1480	0.1270	0.1130	1.1010	0.0908	0.0816	0.0745	0.0696	0.0670
	220/50 Bar	0.2120	0.1810	0.1590	0.1400	0.1230	0.1090	0.0990	0.0925	0.0915
	250/50 Bar	0.2210	0.1900	0.1670	0.1470	0.1290	0.1140	0.1030	0.0954	0.0925
Homo- Heat 75°C for 3 min	100/50 Bar	0.0514	0.0462	0.0432	0.0402	0.0375	0.0348	0.0327	0.0311	0.0299
	120/50 Bar	0.0639	0.0581	0.0537	0.0491	0.0452	0.0418	0.0390	0.0369	0.0355
	150/50 Bar	0.0827	0.0737	0.0666	0.0606	0.0553	0.0508	0.0471	0.0443	0.0425
	180/50 Bar	0.0880	0.0785	0.0711	0.0645	0.0589	0.0540	0.0500	0.0471	0.0452
	200/50 Bar	0.1200	0.1070	0.0957	0.0860	0.0775	0.0740	0.0647	0.0608	0.0587
	220/50 Bar	0.1530	0.1340	0.1200	0.1070	0.0966	0.0872	0.0797	0.0745	0.0721
	250/50 Bar	0.1700	0.1480	0.1310	0.1160	0.1030	0.0970	0.0847	0.0793	0.0772
Homo- Heat 75°C for 3 min	100/50 Bar	0.0462	0.0421	0.0393	0.0362	0.0337	0.0315	0.0296	0.0282	0.0271
	120/50 Bar	0.0694	0.0637	0.0580	0.0527	0.0484	0.0447	0.0417	0.0394	0.0378
	150/50 Bar	0.0864	0.0768	0.0694	0.0628	0.0572	0.0523	0.0486	0.0458	0.0438
	180/50 Bar	0.1270	0.1120	0.0995	0.0891	0.0804	0.0729	0.0672	0.0630	0.0606
	200/50 Bar	0.1330	0.1160	0.1040	0.0931	0.0834	0.0756	0.0694	0.0651	0.0626
	220/50 Bar	0.1400	0.1210	0.1080	0.0965	0.0866	0.0785	0.0723	0.0678	0.0650
	250/50 Bar	0.1430	0.1260	0.1120	0.1000	0.0896	0.0808	0.0738	0.0691	0.0667

Table 6 Effect of processing treatments on shear stress of recombined cheese milk (40% total solids), determined at 34°C over the shear rate of 18.5 - 731 s^{-1}.

Conditions		Shear Stress (Pa)								
		18.5	29.2	46	73	116	184	294	461	731
Heat Treatment Difference Holding Time	no-Heat	0.984	1.420	2.070	3.040	4.440	6.540	9.670	14.500	22.000
	75°C for 3 min	1.020	1.450	2.120	3.110	4.520	6.640	9.760	14.600	22.100
	75°C for 5 min	1.390	1.990	2.860	4.110	5.940	8.650	12.700	18.900	28.500
	75°C for 7 min	1.840	2.560	3.630	5.190	7.430	10.700	15.700	23.200	34.800
	75°C for 10 min	1.890	2.670	3.770	5.370	7.680	11.100	16.200	24.000	35.900
	80°C for 3 min	1.160	1.630	2.410	3.440	5.020	7.340	10.700	16.000	24.300
	80°C for 5 min	2.350	3.270	4.571	6.470	9.160	13.100	19.000	27.800	41.100
	80°C for 7 min	4.700	6.390	8.620	11.800	16.200	22.600	31.700	45.000	64.000
	80°C for 10 min	6.340	8.370	11.100	14.900	20.300	27.800	38.400	54.000	76.700
	85°C for 3 min	1.890	2.710	3.810	5.420	7.760	11.200	16.300	23.900	35.500
	85°C for 5 min	2.330	3.230	4.550	6.450	9.810	13.200	19.000	27.800	40.900
	85°C for 7 min	14.300	18.00	22.800	29.400	38.400	50.700	67.200	89.800	120.000
	85°C for 10 min	18.400	22.600	28.600	36.400	47.000	61.200	80.200	105.000	139.000
Commercial powder Difference Temperature	no-Heat	0.950	1.380	2.010	2.940	4.300	6.830	9.320	13.900	21.100
	70°C for 3 min	1.080	1.540	2.080	3.220	4.690	6.880	10.100	15.100	22.700
	73°C for 3 min	1.080	1.530	2.230	3.270	4.750	6.980	10.300	15.400	23.200
	75°C for 3 min	1.090	1.590	2.320	3.380	4.920	7.230	10.700	16.000	24.300
	78°C for 3 min	1.320	1.900	2.740	3.960	5.750	8.420	12.300	18.400	27.700
	80°C for 3 min	1.380	1.940	2.720	4.010	5.830	8.510	12.500	18.500	27.900
	83°C for 3 min	1.740	2.450	3.500	5.010	7.120	10.400	15.200	22.300	33.300
Low-heat powder Difference Temperature	no-Heat	0.459	0.602	1.030	1.570	2.370	3.620	5.520	8.440	13.000
	70°C for 3 min	0.462	0.707	1.070	1.620	2.440	3.720	5.660	8.460	13.300
	73°C for 3 min	0.490	0.730	1.070	1.680	2.470	3.740	5.680	8.650	13.300
	75°C for 3 min	0.507	0.775	1.150	1.720	2.610	3.940	5.940	9.030	13.800
	78°C for 3 min	0.508	0.783	1.170	1.760	2.650	4.010	6.070	9.250	14.200
	80°C for 3 min	0.560	0.831	1.240	1.850	2.790	4.210	6.330	9.950	14.600
	83°C for 3 min	0.616	0.956	1.390	2.060	3.060	4.580	6.850	10.300	15.700

Continue...

Continue...

Conditions		Shear Stress (Pa)								
		18.5	29.2	46	73	116	184	294	461	731
Pre-heat Treatment Difference MPC Powder	75°C for 15 s	0.463	0.703	1.070	1.640	2.490	3.800	5.790	8.930	13.900
	85°C for 15 s	0.673	1.020	1.520	2.290	3.450	5.220	7.910	12.100	18.700
	85°C for 60 s	0.749	1.120	1.660	2.480	3.710	5.580	8.410	12.800	19.600
	85°C for 120 s	0.762	1.130	1.690	2.540	3.800	5.730	8.560	13.200	20.300
	85°C for 180 s	1.080	1.570	2.330	3.430	5.110	7.640	11.500	17.500	26.800
	85°C for 240 s	1.190	1.730	2.540	3.760	5.550	8.280	12.400	18.700	28.800
	120°C for 180 s	1.470	2.070	3.020	4.440	6.530	9.730	14.600	22.100	34.100
Homo- no-Heat Treatment	100/50 Bar	1.050	1.500	2.190	3.200	4.680	6.900	10.200	15.300	23.300
	120/50 Bar	1.300	1.830	2.660	3.850	5.590	8.180	12.000	18.000	27.200
	150/50 Bar	1.470	2.090	3.010	4.340	6.260	9.120	13.400	20.000	30.500
	180/50 Bar	1.910	2.650	3.770	5.400	7.790	11.200	16.400	24.300	36.900
	200/50 Bar	2.730	3.700	5.210	7.400	10.500	15.000	21.700	32.100	49.000
	220/50 Bar	3.920	5.290	7.320	10.200	14.200	20.100	28.800	42.700	66.200
	250/50 Bar	4.090	5.550	7.680	10.700	17.000	21.000	29.900	44.000	67.600
Homo- Heat 75°C for 3 min	100/50 Bar	0.953	1.350	1.990	2.940	4.340	6.390	9.510	14.400	21.900
	120/50 Bar	1.180	1.700	2.470	3.590	5.230	7.690	11.400	17.000	25.900
	150/50 Bar	1.530	2.150	3.300	4.430	6.400	9.340	13.700	20.500	31.100
	180/50 Bar	1.630	2.290	3.270	4.710	6.820	9.920	14.500	21.700	33.100
	200/50 Bar	2.200	3.120	4.400	6.280	8.970	12.900	18.800	28.100	42.900
	220/50 Bar	2.840	3.900	5.510	7.840	11.200	16.000	23.200	34.400	52.700
	250/50 Bar	3.150	4.310	6.010	8.490	12.000	17.000	24.700	36.600	56.400
Homo- Heat 75°C for 3 min	100/50 Bar	0.856	1.230	12.810	2.640	3.910	5.800	8.620	13.000	19.800
	120/50 Bar	1.290	1.860	2.670	3.850	5.610	8.220	12.100	18.200	27.700
	150/50 Bar	1.600	2.240	3.190	4.590	6.620	9.620	14.200	21.100	32.000
	180/50 Bar	2.360	3.260	4.570	6.510	9.310	13.400	19.600	29.100	44.300
	200/50 Bar	2.470	3.390	4.790	6.800	9.660	13.900	20.200	30.100	45.800
	220/50 Bar	2.600	3.530	4.950	7.050	10.000	14.400	21.100	31.300	47.500
	250/50 Bar	2.640	3.680	5.170	7.300	10.400	14.900	21.500	31.900	48.800

Table 7 Effect of processing treatments on storage modulus, G' (Pa) of renneted gel, determined at 2 h after the addition of rennet, over the frequency ranging from 0.001 to 1 Hz.

Conditions			Storage Modulus, G′ (Pa)						
			0.001	0.005	0.01	0.05	0.1	0.5	1
			0.006	0.031	0.063	0.314	0.628	3.146	6.283
Heat Treatment (Difference Heating Temperature)	Commercial Powder	no-Heat	1265	2280	2830	4210	4825	6190	6770
		70°C for 3 min	1160	2320	2790	4165	4770	6065	6620
		78°C for 3 min	1046	1900	2375	3520	4045	2095	5560
		83°C for 3 min	861	1495	1845	2715	3090	3940	4300
	Low-heat Treatment powder	70°C for 3 min	1180	2035	2545	4210	4935	6345	6960
		78°C for 3 min	1245	2265	2815	3910	4510	5840	6410
		83°C for 3 min	1052	1920	2400	3650	4205	5440	5970
Homogenization	no-Heat Treatment	Homo 100/50 Bar	1435	2515	3090	4600	5235	6595	7170
		Homo 180/50 Bar	1450	2440	2965	4365	4940	6200	6730
		Homo 250/50 Bar	1390	2435	2950	4205	4755	5925	6420
	Heat 70°C for 3 min	Homo 100/50 Bar	1740	2750	3200	4155	4580	5760	6250
		Homo 180/50 Bar	1255	2310	2735	4030	4565	5315	5970
		Homo 250/50 Bar	1195	2040	2490	3685	4210	5520	5780
	Heat 85°C for 3 min	Homo 100/50 Bar	1305	2350	2860	4285	4880	6265	6870
		Homo 180/50 Bar	1195	2115	2565	3745	4255	5425	5940
		Homo 250/50 Bar	1015	1940	2350	3415	3855	4845	5300
Preheat Treatment	Recombined Cheese Milk	Preheat 85°C/15 s	1350	2430	2990	4480	5110	6505	7100
		Preheat 85°C/120 s	1135	2015	2475	3670	4170	5300	5780
		Preheat 85°C/240 s	1020	1780	2165	3170	3595	4560	4970
	Reconstituted Skim Milk	Preheat 85°C/15 s	471	895	1125	1825	2150	2900	3230
		Preheat 85°C/120 s	373	646	820	1295	1530	2050	2270
		Preheat 85°C/240 s	297	509	666	1004	1170	1560	1730

Table 8 Effect of processing treatments on tan δ of renneted gel, determined at 2 h after the addition of rennet, over the frequency ranging from 0.001 to 1 Hz.

Conditions			tan δ						
			0.001	0.005	0.01	0.05	0.1	0.5	1
			0.006	0.031	0.063	0.314	0.628	3.146	6.283
Heat Treatment (Difference Heating Temperature)	Commercial Powder	no-Heat	0.554	0.456	0.427	0.315	0.271	0.199	0.180
		70°C for 3 min	0.575	0.467	0.443	0.325	0.281	0.218	0.205
		78°C for 3 min	0.615	0.503	0.472	0.340	0.295	0.231	0.218
		83°C for 3 min	0.619	0.508	0.474	0.345	0.301	0.235	0.220
	Low-heat Treatment powder	70°C for 3 min	0.504	0.434	0.415	0.317	0.302	0.235	0.212
		78°C for 3 min	0.506	0.436	0.417	0.321	0.284	0.223	0.213
		83°C for 3 min	0.623	0.514	0.480	0.348	0.280	0.226	0.220
Homogenization	no-Heat Treatment	Homo 100/50 Bar	0.506	0.421	0.392	0.297	0.260	0.204	0.193
		Homo 180/50 Bar	0.561	0.455	0.427	0.37	0.266	0.209	0.196
		Homo 250/50 Bar	0.570	0.461	0.429	0.310	0.269	0.209	0.200
	Heat 70°C for 3 min	Homo 100/50 Bar	0.435	0.329	0.291	0.231	0.210	0.182	0.176
		Homo 180/50 Bar	0.553	0.465	0.437	0.312	0.271	0.210	0.198
		Homo 250/50 Bar	0.586	0.474	0.439	0.321	0.275	0.214	0.202
	Heat 85°C for 3 min	Homo 100/50 Bar	0.517	0.428	0.428	0.312	0.271	0.198	0.180
		Homo 180/50 Bar	0.525	0.459	0.436	0.314	0.273	0.204	0.185
		Homo 250/50 Bar	0.609	0.483	0.442	0.315	0.274	0.215	0.196
Preheat Treatment	Recombined Cheese Milk	Preheat 85°C/15 s	0.584	0.459	0.428	0.308	0.271	0.218	0.207
		Preheat 85°C/120 s	0.586	0.465	0.432	0.313	0.275	0.219	0.209
		Preheat 85°C/240 s	0.567	0.475	0.440	0.317	0.277	0.222	0.211
	Reconstituted Skim Milk	Preheat 85°C/15 s	0.577	0.520	0.484	0.367	0.320	0.256	0.242
		Preheat 85°C/120 s	0.612	0.518	0.493	0.380	0.327	0.259	0.246
		Preheat 85°C/240 s	0.634	0.528	0.516	0.386	0.339	0.266	0.253

Appendix III ***Replicated results***

Effect of processing treatments on the physical and rennet coagulation properties of reconstituted skim milk (20% MPC powder without an additional milk fat) and recombined cheese milk (20% MPC powder and 20% milk fat)

Conditions	Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)		Denaturation of whey protein (%)				Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution	Total	β-Lactoglobulin A	β-Lactoglobulin B	α-Lactalbumin		1 Hour	2 Hour	
Difference Heating Time	no-Heat	1.11	1.09	0	0	0	0	19.3	3290	5130	-
	75°C for 3 min	1.11	1.10	21.2	17.0	16.3	12.7	21.3	2610	4747	-
	75°C for 5 min	1.10	1.11	36.4	19.8	17.1	18.6	20.3	2728	4325	-
	75°C for 7 min	1.12	1.11	40.6	26.5	22.6	21.0	18.3	2482	4250	-
	75°C for 10 min	1.12	1.11	65.0	35.5	32.6	33.4	21.3	2210	4100	-
	85°C for 3 min	1.13	1.10	69.2	94.5	95.8	78.8	8.3	2168	3747	-
	85°C for 5 min	1.12	1.10	76.7	97.8	97.6	97.6	9.3	2244	3710	-
	85°C for 7 min	1.12	1.11	84.7	98.9	98.6	97.8	8.3	2162	3697	-
	85°C for 10 min	1.11	1.12	95.6	99.2	99.3	98.8	8.3	2064	3177	-

Continue...

Continue...

Conditions	Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)		Denaturation of whey protein (%)				Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution	Total	β-Lactoglobulin A	β-Lactoglobulin B	α-Lactalbumin		1 Hour	2 Hour	
Commercial MPC powder	no-Heat	1.09	1.09	0	-	-	-	22.3	2002	4617	3.266
	70°C for 3 min	1.10	1.08	19.7	-	-	-	18.3	1790	3057	2.941
	73°C for 3 min	1.10	1.10	21.4	-	-	-	20.3	1634	2710	2.318
	75°C for 3 min	1.10	1.10	23.3	-	-	-	22.3	1294	2380	1.878
	78°C for 3 min	1.08	1.11	25.1	-	-	-	21.3	1242	2233	1.866
	80°C for 3 min	1.09	1.08	45.6	-	-	-	23.3	920	1750	1.633
	83°C for 3 min	1.11	1.10	56.5	-	-	-	22.3	775	1483	1.401
	85°C for 3 min	1.11	1.09	60.5	-	-	-	22.3	754	1388	1.350
	88°C for 3 min	1.12	1.11	66.7	-	-	-	22.3	624	1233	1.015
Low-heat Treatment MPC Powder	no-Heat	1.09	1.10	0	-	-	-	17.3	3740	5143	5.010
	70°C for 3 min	1.08	1.08	11.0	-	-	-	18.3	2358	3557	2.974
	73°C for 3 min	1.12	1.11	19.8	-	-	-	19.3	2218	3190	2.897
	75°C for 3 min	1.09	1.09	22.5	-	-	-	19.3	2030	2917	2.756
	78°C for 3 min	1.10	1.10	32.9	-	-	-	19.3	1718	2830	2.308
	80°C for 3 min	1.09	1.10	38.0	-	-	-	19.3	1444	2320	1.947
	83°C for 3 min	1.10	1.10	59.1	-	-	-	18.3	1210	1950	1.688
	85°C for 3 min	1.12	1.09	60.6	-	-	-	18.3	1084	1887	1.558
	88°C for 3 min	1.11	1.10	65.8	-	-	-	19.3	869	1437	1.470

Continue...

Continue...

Conditions	Preheat Treatment	Fat Globule Diameter, $d_{4,3}$ (μm)		Denaturation of whey protein (%)				Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution	Total	β-Lactoglobulin A	β-Lactoglobulin B	α-Lactalbumin		1 Hour	2 Hour	
Recombined Cheese Milk	75°C for 15 s	1.10	1.10	0	-	-	-	21.3	2774	4083	3.279
	85°C for 15 s	1.09	1.08	8.4	-	-	-	22.3	2438	3956	3.248
	85°C for 60 s	1.11	1.10	28.4	-	-	-	22.3	2036	3630	3.225
	85°C for 120 s	1.10	1.10	34.1	-	-	-	24.3	1390	2770	3.175
	85°C for 180 s	1.12	1.11	42.1	-	-	-	22.3	1440	2730	3.069
	85°C for 240 s	1.10	1.10	45.3	-	-	-	22.3	1198	2287	1.618
	120°C for 180 s	1.09	1.10	63.8	-	-	-	22.3	326	859	1.068
Reconstituted Skim Milk	75°C for 15 s	-	-	0	-	-	-	-	-	-	-
	85°C for 15 s	-	-	14.3	-	-	-	25.3	1068	1960	2.119
	85°C for 60 s	-	-	28.3	-	-	-	24.3	770	1573	1.859
	85°C for 120 s	-	-	38.9	-	-	-	26.3	583	1307	1.544
	85°C for 180 s	-	-	41.7	-	-	-	27.3	548	1183	1.376
	85°C for 240 s	-	-	46.9	-	-	-	24.3	457	1007	1.297
	120°C for 180 s	-	-	60.2	-	-	-	30.3	87	337	0.446

Continue...

Continue...

Heat Treatment	Homogenization Pressure (Bar)	Fat Globule Diameter, $d_{4,3}$ (μm)		Gelation Time (min)	Storage Modulus G' (Pa)		Yield Force (N)
		Deionized Water	Dissociating Solution		1 Hour	2 Hour	
no-Heat	100/50	1.13	1.10	17.3	3586	5323	4.453
	120/50	1.09	1.07	17.3	3350	5147	4.328
	150/50	1.07	1.07	23.3	2360	4723	3.678
	180/50	0.95	0.90	18.3	2966	4720	3.411
	200/50	0.84	0.82	18.3	2708	4590	3.386
	220/50	0.73	0.71	16.3	2596	4580	3.014

Appendix IV

The definition of the important values illustrated in the output of Malvern MasterSizer

Volume distribution = Distribution expressing the proportion on a volume basis of particles below a particular size

Observation = The measure of the laser light observed by the sample (%)

= 1 - [Light intensity with sample/Light intensity without sample]

Residual = The degree to which the scattering light calculated for the size distributionmatches measured light scattering (%)

$$= \frac{100}{S} \sum_{j=1} n \quad \text{[light calculated - Light measured]}$$

Where S = The total light measured

Span = The width of the distribution

$D_{(v,0.1)}$ = Percentage points of undersize volume distribution

$D_{(4,3)}$ = The volume-moment average diameter (μm)

$= \sum N_i d_i^4 / \sum N_i d_i^3$

Where N_i = Number of particles

d_i = Diameters of particles

$D_{(3,2)}$ = The volume/surface average diameter (μm)

$= \sum N_i d_i^3 / \sum N_i d_i^2$

Specific surface area calculated = The total surface area per unit volume of the particle from the distribution

= SUMA x 3/SUMV

Where	SUMA	=	$\sum W_i [R_i]^2$
	SUMV	=	$\sum W_i [R_i]^3$
	R_i	=	The measure size in each class
	W_i	=	The Proportion in each class

The formation was obtained from Malvern IM 100 Issue 2, Manual.

NEW ZEALAND DAIRY RESEARCH INSTITUTE
PRIVATE BAG ■ PALMERSTON NORTH ■ NEW ZEALAND
Telephone: 0-6-350 4649 Fax: 0-6-350 1476

Version 1.1 Fri, Sep 29, 1995 9:24AM

Recombined MPC 56 :Run Number 1

Homogenization 120/50 bar
no-Heat Treatment

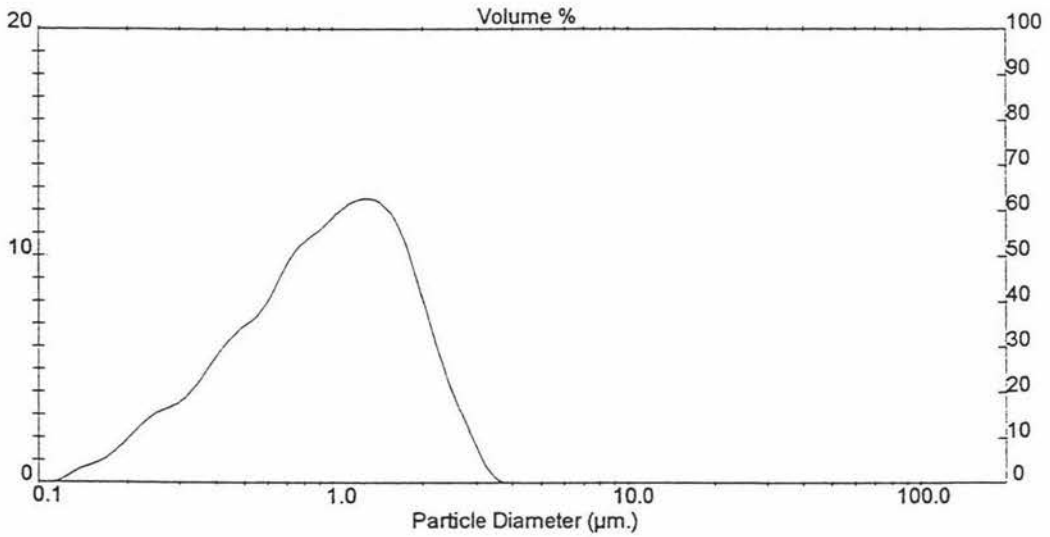
Sample File Name: 28SEP , Record: 2 Source: Analysed
Measured on: Fri, Sep 29, 1995 9:23AM Last saved on: Fri, Sep 29, 1995 9:23AM

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

Residual = 0.412 % Concentration = 0.009 % Obscuration = 20.11 %
d (0.5) = 0.96 µm d (0.1) = 0.34 µm d (0.9) = 1.97 µm
D [4, 3] = 1.08 µm Span = 1.69 Standard Deviation = 0.64 µm
Sauter Mean (D[3,2]) = 0.69 µm Mode = 1.30 µm
Specific Surface Area = 8.7112 sq. m. / gm Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.10	0.00	0.12	0.00
0.12	0.57	0.15	0.57
0.15	1.25	0.19	1.82
0.19	2.01	0.23	3.83
0.23	2.92	0.28	6.75
0.28	4.00	0.35	10.75
0.35	5.27	0.43	16.01
0.43	6.71	0.53	22.72
0.53	8.30	0.66	31.02
0.66	9.87	0.81	40.89
0.81	11.23	1.00	52.11
1.00	12.08	1.23	64.19
1.23	12.27	1.51	76.46
1.51	11.17	1.86	87.63
1.86	7.61	2.30	95.24
2.30	3.68	2.83	98.92

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
2.83	1.08	3.49	100.00
3.49	0.00	4.30	100.00
4.30	0.00	5.29	100.00
5.29	0.00	6.52	100.00
6.52	0.00	8.04	100.00
8.04	0.00	9.91	100.00
9.91	0.00	12.21	100.00
12.21	0.00	15.04	100.00
15.04	0.00	18.54	100.00
18.54	0.00	22.84	100.00
22.84	0.00	28.15	100.00
28.15	0.00	34.69	100.00
34.69	0.00	42.75	100.00
42.75	0.00	52.68	100.00
52.68	0.00	64.92	100.00
64.92	0.00	80.00	100.00



Malvern Instruments Ltd.
Malvern, U.K.

MasterSizer E Ver. 1.1
Serial No. 7204

p. 1
29 Sep 95 09:24

Figure 1 The typical Malvern MS20 MasterSizer output