INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH
MASSEY UNIVERSITY, PALMERSTON NORTH
NEW ZEALAND

INFLUENCE OF THE STRUCTURE OF RENNET–INDUCED GELS ON THE CHEESEMAKING PROCESS AND CHEESE COMPOSITION

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

MEI-LI CHIANG
2002

ABSTRACT

The purpose of this study was to investigate the effects of some selected processing conditions on the formation, structure and properties of rennet-induced milk gels and the impact of these conditions on the cheesemaking process and final cheese composition.

The effect of pH, temperature, calcium chloride addition, rennet concentration and protein concentration on rennet gels were determined using rheology, permeability measurement and confocal microscopy. In rennet gels formed at pH 5.8, 6.2 and 6.5, the storage modulus (G') increased with a decrease in pH while the gelation time (GT) increased with pH increase. The permeability coefficient (B) increased with a decrease in pH. Rennet gels made at 25, 32 and 40°C showed that the G' values increased while gelation time decreased with an increase in temperature, with a maximum G' at 32°C. The B values increased with an increase in temperature. The rennet gels made with zero, 0.01% and 0.02% $CaCl_2$ addition showed a slight increase in G' with $CaCl_2$ addition, but no significant differences in B values. For gels made with 40, 80 and 120μl rennet addition/l, the G' values increased slightly with an increase in rennet concentration, but there were no significant differences in B values. For rennet gels with different protein contents (range from 3.45 to 5.10%), the G' values increased whereas B values decreased with increasing protein content. All the confocal micrographs corresponded well with the results from the permeability and rheological analyses.

Two rennet gel systems were developed in this study. "High syneresis" gel systems were made using low pH, high temperature and normal protein concentration and "low syneresis" systems with high pH, low temperature and high protein concentration. These two systems were used in cheese manufacture in a pilot plant. It was expected that 'high syneresis' gel system would expel more whey and result in low moisture cheese, while the 'low syneresis' system would yield cheese with higher moisture content. It was found that the cheese produced from high syneresis system

had higher moisture content than the cheeses made from low syneresis system. It was opposite to what was expected. The set to cut time and acid production (starter levels) are thought to be the reasons for the outcome of the first pilot plant trial. The process conditions were redesigned to investigate the effect of gel firmness and pH on the composition of cheese. Similar results were found in the second pilot plant trial.

The factors involved in the cheesemaking process are much more complex than what has been investigated in the present study. Therefore, further investigations on the other factors affecting cheese moisture content are recommended (e.g. syneretic power).

ACKNOWLEDGMENTS

I would like to gratefully acknowledge my chief supervisor, Professor Harjinder Singh, for his excellent supervision, constant help and encouragement throughout my study especially for guiding me through writing my thesis, experimental works and discussion.

My gratitude and appreciation are extended to my supervisors Dr. Keith Johnston and Dr. Lawrie Creamer for their help during the experimental works and guided me through difficulties encountered during the experimental works and contribution of their expertise in the area. I am also appreciated that the New Zealand Dairy Institute funded this project.

Most warm thanks to Ms. Nicky White (NZDRI) for her assistance with the rheometer and other equipment. Her warm caring and sharing of knowledge are greatly appreciated.

Special thanks to Mr. Graham Holdaway and Mr. Gary Taekema (NZDRI) for helping me with the pilot plant trials and analysis of the results. I am very grateful for their warm encouragement and loving heart during my time of sickness.

I also appreciated the help from Mrs. Liz Nickless for her assistance on the confocal microscope and helpful advice on the sample preparation.

I am very grateful to all the Massey staff Mrs. Michelle Tamehana, Mr. Steve Glasgow, Mr. Garry Radford, Mrs. Geedha Reid, Mr. Byron McKillop, Dr. Yacine Hemar and Mrs. Karen Pickering for their excellent technical assistance, providing materials for this research and constant encouragement.

Thanks to my fellow post-graduate students and friends Maya Sugiarto, Aiqian Ye, Carol Ma, Kelvin Goh, and Hong Ping Gao for their friendship, constant encouragement, helpful advice for my study and life. Their loving hearts toward me are greatly appreciated.

Lastly, I would like to thank God for getting me through my study and without you I would not be able to do this. I am very grateful that I have such supportive, loving parents without them nothing can be accomplished. I can not thank them enough. I also appreciated the support and love from my sister and brothers. Final thanks to people who helped me along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

It has long been known that the conditions (prior heat treatment, protein concentration, protein type, pH, temperature, calcium chloride addition, coagulant concentration) at the time of coagulation during cheesemaking have a significant influence on the gelation time, the rate of firming, structure and rheological properties (Mellema, 2000). A number of papers by various authors have been published (Zoon *et al.*, 1988; Roefs *et al.*, 1985; van Dijk and Walstra, 1986). Most notable are van Hooydonk & van den Berg's (1988) "Control and determination of the curd-setting during cheesemaking", the series of five papers published by Zoon *et al.* (1988) titled "Rheological properties of rennet-induced skim milk gels" and Guinee *et al.'s* (1994) paper on the 'Effect of milk protein standardization, by ultrafiltration, on the manufacture, composition and maturation of Cheddar cheese".

van Hooydonk and van den Berg (1988) studied several factors affecting the renneting properties of the milk gels and the suitability of an instrument to evaluate the process of curd firming. Zoon *et al.* (1988) studied the structure and mechanical properties of rennet-induced milk gels that were determined by the dynamic moduli G′ and G″ under different conditions. The conditions they had investigated were: the effect of protein concentration, rennet concentration, temperature, pH and NaCl, calcium and phosphate and large deformation. They found that concentrating the milk by ultrafiltration resulted in an increase in G′ and G″. A higher rennet concentration resulted in an increase in the rate of gelation and an increase in the values of G′ and G″ after a long ageing time. Increasing temperature during gel formation and ageing mainly resulted in an increase of the firming rate. The G′ and G″ values decreased with increasing temperature. A minimum amount of calcium was needed for gelation to occur and there was no significant influence on the G′ and G″ values with the calcium range studied. Decreasing the pH in the range from pH 6.7 to 5.7 resulted in a maximum of the G′ near pH 6.15. The effect of NaCl addition appeared to be dependent of the experimental conditions. For large deformation, it appeared that

with decreasing time up to fracture, with decreasing measuring temperature and increasing ageing time, the stress needed to break a gel increased.

In the study by Guinee *et al.* (1994), the milk was concentrated by ultrafiltration to determine if this affect the cheddar cheese manufacture, composition and maturation. They found that increasing milk protein level resulted in reduced gelation time, increased curd firming rates and a decrease in set-to-cut time when cutting at equal firmness values (i.e. G′ ~ 16Pa). They also found that while increasing milk protein level in the range 30 – 70 g/l had little effect on cheese composition, it resulted in slower proteolysis and maturation.

From the studies mentioned above, few if any past investigations give reliable information or models on how the way in which the gel is formed impacts on the remaining parts of the cheesemaking process (particularly the syneretic properties of the curd), end cheese composition, cheese microstructure, maturation (particularly s syneresis on storage). Clearly, an understanding of the effects of the rennet gel structure during the early stages of cheesemaking would be of considerable benefit in understanding how to manipulate the final cheese properties. Therefore, the major objective of this study was to determine the impact of the cheese coagulum structure on the cheesemaking process (particularly, the syneretic properties of the curd) and cheese composition.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk

The main components of milk are water (~ 87.3%), lactose (~4.6%), fat (~4%), protein (~ 3.3%) and minerals (~ 0.6%). These contribute to about 14% total solids content in milk (Walstra and Jenness, 1984). Milk 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 major dairy products and its manufacture is dependent on the milk proteins, especially the caseins and to a lesser extent on the milk salts (Waungana, 1995).

The protein content of milk is about 3.3% (w/w) which can be divided into two major groups, namely the caseins and the whey proteins (Walstra and Jenness, 1984, Dalgleish, 1992). Of the two, caseins are the most important for the manufacture of cheese. Caseins are more abundant in milk and comprise about 80% of the total protein when precipitated at 20°C from milk adjusted to pH 4.6 (Swaisgood, 1992). The remaining proteins that remain soluble at pH 4.6 are called the whey proteins. The whey proteins are of little relevance in normal cheese manufacture, but may become important if cheese milk is ultrafiltered or heated to temperatures greater than 60°C. In such circumstances, whey proteins are denatured and can be incorporated into the cheese curd (Banks, 1988).

2.1.1 Casein micelles

Several researchers have reviewed the structure and properties of casein and casein micelles (Walstra, 1990; Holt, 1992; Swaisgood, 1992; Horne, 1998). Caseins are not homogenous and can be fractionated into four distinct groups; α_{s1}-, α_{s2}-, β-, and κ-casein. 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 (Walstra and Jenness, 1984). The fact that casein in milk is not present in solution but in the micelles has important consequences for the properties of milk. To a large extent, the

casein micelles determine the physical stability of milk products during heat treatment, concentration and drying. Their behaviour is essential in the first stages of cheesemaking. The micelles largely determine the physical properties of sour and concentrated milk products (Walstra *et al.*, 1999).

The micelle is composed of casein protein (92%) and inorganic salts (8%) largely in the form of calcium phosphate (Swaisgood, 1992). Casein micelles are roughly spherical particles, mostly 80-300 nm in diameter. The micelle structure is rather porous, indicated by the high values for voluminosity and hydration that have been found (Rollema, 1992).

The precise structure of the casein micelle is a matter of considerable debate at the present time. A number of models have been proposed over the past 40 years, but none can describe completely all aspects of casein micelle (Singh and Bennett, 2002). One model is believed to be composed of a number of sub-micelles or sub-units linked together by colloidal calcium phosphate (CCP), with hydrophobic and hydrogen bonds contributing to the relatively stable structure. The structure shown in Figure 2.1 was developed by Walstra and Jenness (1984) which include the concept of steric stabilisation of the micelle by κ-casein. They consider this model to be in best agreement with the numerous observations on properties and stability of the casein micelles, including renneting.

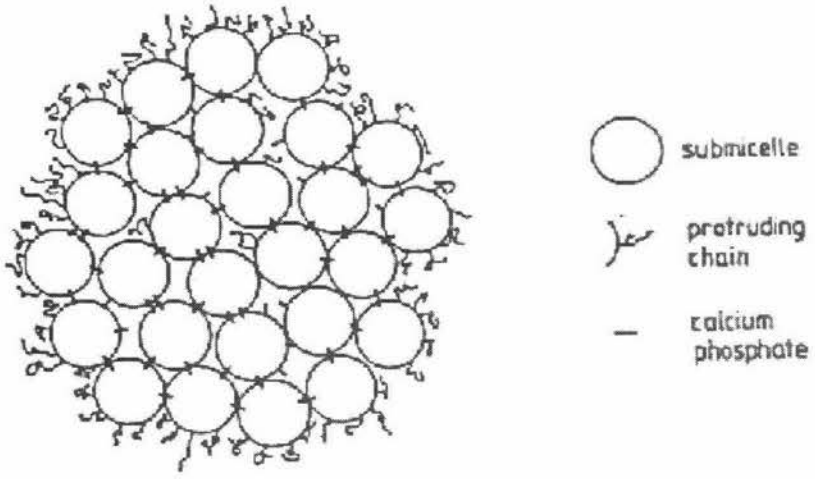


Figure 2.1 Structure of the casein micelle (from Walstra and Jenness, 1984)

The other model considered the micelle as a mineralised, crosslinked protein gel, the colloidal calcium phosphate nanoclusters are the agents responsible for crosslinking the proteins and holding the network together (Holt, 1992). A recent model proposed by Horne (1998) assumes that assembly of the casein micelle is controlled by a balance of electrostatic and hydrophobic interactions between casein molecules. Two or more hydrophobic regions from different molecules within the caseins form a bonded cluster. Growth of these polymers is inhibited by the protein charged residues whose repulsion pushes up the interaction free energy. Neutralisation of the phosphoserine clusters by incorporation into the colloidal calcium phosphate diminishes that free energy as well as producing the second type of cross-linking bridges. κ-Casein acts as a terminator for both types of growth, because it contains no phosphoserine cluster linkage via CCP and no other hydrophobic anchor point to extend the chain (Horne, 1998).

The common factor in all models is that the different caseins, α_{s1}-, α_{s2}-, β-, 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 stabilising effect upon the native micelles and prevent them from coagulation. This stability effect arises because of κ-casein and the hydrophilic macropeptide or glycomacropeptide (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 stabilise the micelles (Walstra, 1990; Dalgleish, 1992).

The casein micelles are considered to be a very stable system, however, the proteins and calcium phosphate are not covalently bound, therefore, renneting, acidification, and ethanol can destabilise the system. Renneting involves proteolytic enzyme (i.e. chymosin) splits a macropeptide off κ-casein, followed by aggregation of the formed paracasein micelles. Renneting occurs faster at lower pH which is due to the enzymic reaction proceeding faster. Moreover, the casein micelles greatly change in properties upon lowering the pH, mostly due to dissolution of colloidal phosphate; at lower pH,

increased formation of salt bridges predominates. Ethanol stability decreases with decreasing pH, hence decreasing charge; Ca^{2+} activity and ionic strength also play a part. Addition of ethanol lowers the dielectric constant which reduces electrostatic repulsion and lowers the solvent quality for the macropeptide part of κ-casein, resulting in collapse of hairs and aggregation.

2.1.2 Whey proteins

Whey proteins are those milk proteins remaining in the whey after precipitation of the caseins at pH 4.6 (20°C). Raw bovine milk contains about 0.7% whey protein. There are four major groups of whey proteins which include β-lactoglobulin (β-Lg), bovine serum albumin (BSA), α-lactalbumin (α-La), immunoglobulins and small molecular weight peptides, derived by proteolysis of some of the caseins (Walstra and Jenness, 1984; Swaisgood, 1992; Eigel *et al.*, 1984).

2.1.3 Milk salts

Milk salts consist mainly of chlorides, phosphates, citrates and bicarbonates of sodium, potassium, calcium and magnesium (Pyne, 1962). Their practical importance arises largely from their marked influence on the condition and stability of the milk proteins, particularly caseins. Some of the milk salts (i.e. the chlorides, sulphates and compounds of sodium and potassium) are soluble and are present almost entirely as ions dissolved in milk whey. Others, calcium and phosphate in particular, are much less soluble and at the normal pH of milk exist partly in dissolved and partly in insoluble (i.e. colloidal) form, in close association with the caseins. The partition of calcium phosphate between the whey and casein micelles is very important since it significantly influences the properties of milk.

2.2 Cheese manufacture

Due to the wide variety of ways for cheese manufacturing, here cheddar cheese manufacture is described briefly as an example. The information was gathered from several sources, including, Lawrence *et al.* (1993), Ramkumar (1997) and Scott (1998).

(1) Preparation of cheese milk: To compensate for seasonal variation in the compositional quality of raw milk to produce a consistent raw material, simple

process of adding skim milk or skimming some cream off the milk is carried out. The minimum thermal treatment to avoid the presence of pathogenic bacteria in the cheese milk is at 71.7°C for 15 seconds. It also alters the numbers of other microorganisms and enzymes (i.e. lipases) present in the milk. Excess fat in the milk decreases moisture loss from the curd and increases the moisture in the non-fat substances and high fat cheeses tend to have a weak body. The standardised milk is normally cooled to 30°C and pumped into the cheese vat.

(2) Starter addition: The bulk starter is added according to the recipe in quantities ranging from 0.05 to 4% or even 5%. Mesophilic starters are used for Cheddar cheese production, which have growth optimum around 30°C. The quantity of starter used, the strain types and the strain ratios determine the rate and extent of lactic acid production during cheesemaking. The starter strains are selected on the basis of temperature sensitivity, phase resistance and acid-producing ability.

(3) Additives: Any chemicals (sodium nitrate or calcium chloride) are added to the milk while the temperature is raised to renneting temperature. Chemicals must be added in solution and quantities are usually specified by the recipe.

(4) Rennet addition: Calf rennet (which contains the enzyme chymosin) is the coagulating agent traditionally used which causes the conversion of milk into a semi-solid gel through the action of the added coagulation enzyme. The proportion of rennet added to the cheese milk at 32°C should be the minimum necessary to give a firm gel in 30-40 minutes and is typically 10-12ml/100 l milk.

Following the addition of rennet and thorough mixing, the milk is left undisturbed during coagulation. After about 30 minutes, the gel is sufficiently firm and ready to be cut. Normally the firmness of the gel is determined by the subjective

judgement of the cheesemakers; they feel the gel by their fingers to decide the firmness.

(5) Cutting: The objective of cutting is to allow moisture loss or syneresis. The size of the curds after cutting is defined in the recipe. The coagulum should be cut at the desired firmness. If it is too weak or too firm, excess fines are generated which would be lost in the whey, reducing the yield; therefore, it is seldom to cut the curd into smaller than 6 mm cubes. The finer the cut the greater the surface area and the greater the potential for fat and moisture loss.

(6) Stirring/cooking: The mixture of the curd and whey is stirred to prevent the curd particles matting or fusing together. Cooking promotes contraction of the protein matrix, causing the curd to shrink and expel more whey. The increase in temperature also speeds up the metabolism of bacteria which causes an increase in lactic acid production. Consequently, the pH declines which assists in shrinking the particles to expel whey. The rate at which heat is applied is important. If the rate is too fast, "case hardening" or the development of a "skin" on the surface of the curd particle occurs, preventing moisture expulsion. The combination of particle size, stirring rate and cooking regime controls the moisture content of the curd.

The rate and extent of acid development are dependent on the cooking temperature. The optimum growth temperature for starter bacteria is 29-30°C. Cooking temperature thus helps to control both the rate of acid production in the vat and the number of viable organisms in the final cheese. The pH drop in the vat encourages syneresis, inhibits the growth of undesirable organisms and results in a concomitant loss of colloidal calcium phosphate from the casein micelles.

(6) Draining: During the draining process, the whey is separated from the curd. This is achieved by draining the whey through a screen to retain curd. The whey pH at this stage is typically 6.15.

(7) Curd drying: The curd is dry stirred or agitated (by hand) for few times to remove excess whey. The pH at the end of this stage is about 6.0.

(8) Cheddaring: A holding period is used to allow the curd to fuse or mat together. A series of operations consisting of packing, turning, piling and re-piling the slabs of matted curd constitutes the cheddaring process. The influence of physical forces – pressure and flow – results in the development of a fibrous texture in the mass of curd. The pH continues to drop and is about 5.35 at the end of cheddaring.

(9) Milling: The milling operation consists of mechanically cutting the cheddared curd in small pieces. This increases the surface area to assist uniform salt distribution into the curd, and to encourage whey drainage. The cheddared blocks are cut into chips. After milling, the curd is usually left for 2-3 minutes to allow for some loss of moisture so that the surface becomes wet and improves the adhesion of granular salt.

(10) Salting: The aim is to produce a salt content of 1.5 – 2.5%. The presence of significant quantities of salt will inhibit the metabolism of the starter bacteria. The salting stage also stops the acid production, so the pH of the curd does not decrease further after salting. The proportion of moisture in the curd, the pH of the curd and the amount of salt added all affect the final salt content of the curd, which influences the final pH and the overall flavour and texture of the cheese and controls the growth of microorganisms.

(11) Pressing: The aim of the cheese pressing is to form the loose curd particles into a shape which is compact enough to be handled and to expel any free whey. Pressing should be gradual because sudden high pressure compresses the surface layer of the cheese creating an impermeable layer which lead to moisture entrapment. In close-textured cheeses such as Cheddar, the curd is often vacuum pressed. This allows the curd particles to fuse together into a solid block of cheese. Free whey or moisture is also expelled during pressing.

(12) Packaging: Recently Cheddar cheese has been produced in block shapes because of consumer demands and the use of impermeable film prevents

mould growth and cheese mite damage. The cheese blocks are then placed in cardboard cartons, which protect the plastic bag and also the cheese.

(13) Rapid cooling: Rapid cooling of the cheese from 30 to 18°C within the next 24 hours is critical in reducing the growth of non-starter lactic acid bacteria which can cause off-flavour in the cheese. During this process, the cheese also "firms" considerably, and the surface becomes smooth and shiny as the curd particles knit together.

(14) Ripening: The cartons of cheese are stacked on to pallets and transferred into ripening or curing rooms. The cheese is initially stored at 10°C until the initial evaluation at about 30 days after manufacture. After this, the cheese goes into coolstores and is held at temperatures in the range of 2-10°C for a period of 6 months or longer depending on the maturation required. Considerable changes in texture occur during ripening as a consequence of proteolysis. The rubbery texture of fresh cheese changes rapidly to a more brittle cheese. The biochemical changes during cheese ripening due to the activity of microorganisms and enzymes also lead to the development of the typical Cheddar cheese flavour.

Because the rennet-induced coagulation of milk is a very important cheesemaking step, more information on rennet coagulation properties of milk is discussed in the following sections.

2.3 Rennet coagulation of milk

Before milk is renneted, the casein micelles in milk show no tendency to aggregate. After milk has been treated with rennet, there is a stage during which apparently little happens, followed, after some time, by a rapid coagulation of milk. This phenomenon, which is the first step of cheese making, results from two processes. The first is the attack on κ-casein, which stabilises the casein micelles, by the proteolytic enzymes (chymosin, pepsin or microbial proteinases) contained in the rennet; and the second is the subsequent clotting of the micelles which have been

destabilised by the enzymatic attack. These processes have been described as the primary and secondary stages of the renneting reaction (Dalgleish, 1992).

Coagulation is initiated by the action of proteolytic enzymes called rennets. These rennets consist of the enzymes, chymosin and pepsin, and are traditionally prepared from the stomachs of calves, kids, lambs or other mammals in which rennins are the principal proteinases. Increased production of cheese worldwide, coupled with a trend to slaughter calves at an older age, has led to a search for rennet substitutes. The most successful of these substitutes over the past two decades have been bovine, porcine and chicken pepsins as well as the acid proteinases from microbial sources, such as *Mucor pucillus, Mucor meihei* and *Endiothia parasitica*. The gene for chymosin has been cloned and expressed in microorganisms (e.g. yeasts and *E. Coli*) to allow production of rennets containing chymosin only (Foltmann, 1987). The products of cloning are now becoming available and used commercially with promising results (Hicks *et al.*, 1988; Bines *et al.*, 1989; Pszczola, 1989). General and molecular aspects of rennets have been reviewed by Foltmann (1987).

2.3.1 Mechanism of coagulation

The rennet coagulation process may be divided into three phases.

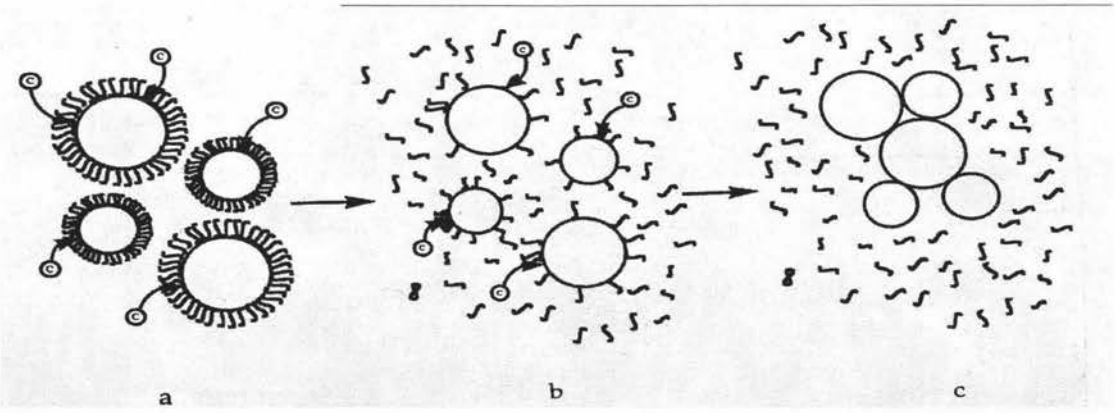


Figure 2.2 Schematic representation of the rennet coagulation of milk. (a) Casein micelles with intact κ-casein layer being attacked by the chymosin; (b) micelles partially denuded of κ-casein; (c) extensively denuded micelles in the process of aggregation (from Fox *et al*, 2000).

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 Phe105-Met106 bond, to yield two peptides with markedly different properties, caseinmacropeptide and para-κ-casein (Castle and Wheelock, 1972). The macropeptide moiety is hydrophilic and soluble, so it diffuses away from the micelles. Micelles begin to coagulate once sufficient κ-casein has been hydrolysed (Cheryan *et al.*, 1974; Culioli and 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 structure, 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 formation of calcium "bridges" between aggregating micelles and reducing the net negative charge on casein micelles.

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 homogenisation and heat treatment (Mehaia and 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 rennet-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.

2.4 Factors affecting rennet coagulation

Several factors are known to influence the rennet coagulation of milk. These effects may be on the primary, secondary or both phases.

2.4.1 Influence of pH

It has been suggested that the optimum pH for the attack of chymosin on κ-casein is in the range 5.0-5.5 (Humme, 1972), but in milk it appears that this optimum pH is approximately pH 6.0 (van Hooydonk *et al.*, 1986). The isoelectric point of κ-casein is about pH 4.5 and so measurements of the true optimum pH are complicated by substrate precipitation below pH 5. For all proteases, hydrolysis of κ-casein is increased steadily by reducing the pH to between pH 6.7 and 5.0.

The state of the colloidal calcium phosphate (CCP) is an important factor requiring consideration in discussing the effect of pH on rennet coagulation. It is well established that a decrease in pH leads to a decrease in rennet coagulation time (RCT) (van Hooydonk *et al.*, 1986) but most of this is probably caused by the increase in enzyme activity as the pH is lowered (Humme, 1972; van Hooydonk *et al.*, 1986). However, it has also been shown that the pH does exert some effect on the rate of coagulation of the renneted micelles (Kowalchyk and Oslon, 1977). The rate of aggregation increases as the pH decreases (Kim and Kinsella, 1989) and the extent of proteolysis required for aggregation decreases markedly (van Hooydonk *et al.*, 1986). These results, however, may depend on the method of acidification, how long the acidified milk is stored and the effect of the buffer into which the milk is diluted, since the composition of the micelles changes markedly with pH (Snoeren *et al.*, 1984; Dalgleish and Law, 1989). The increase in rate of aggregation has been suggested to arise from the increase in the activity of Ca^{2+} as the pH is lowered (Pearce, 1976), but an alternative explanation may be found by considering the effect of pH on the micellar calcium phosphate (Shalabi and Fox, 1982). It has been suggested that dissolution of CCP may in fact lead to a decrease in the efficiency with

which micelles coagulate. As the pH is lowered more calcium phosphate dissolves, but in milk this only serves to increase the concentration of Ca^{2+}. The effects therefore tend to cancel out to give only a small pH-dependence of the aggregation.

2.4.2 Influence of temperature

The activity of rennet increases with temperature in the range 28-36°C (Phelan, 1975). However, κ-casein hydrolysis has been shown to proceed at temperatures as low as 2°C. The secondary phase, however, is extremely dependent on temperature and Q_{10} (Q_{10} describes the temperature dependence of a reaction as a factor by which the reaction rate is changed when the temperature is changed by 10°C) ranging from 13 to 16 have been reported between 20°C and 50°C (Berridge, 1942; Ernstrom and Wong, 1974). The secondary phase is markedly retarded at temperature < 18°C.

2.4.3 Influence of ionic strength

The ionic strength of the renneting medium is important in defining the activity of the proteinase. This may be because the enzyme and substrate are both negatively charged and tend to repel each other: an effect which can be overcome by increasing the ionic strength. If the ionic strength is increased too much, it will interfere with specific charge interactions which are essential for enzyme activity and consequently the activity will decrease (Payens and Both, 1980; Payens and Visser, 1981).

2.4.4 Influence of calcium concentration

There is still some debate on the effect of Ca^{2+} on the primary phase. Some researchers (Mehaia and Cheryan, 1983; van Hooydonk *et al.*, 1986) accept that Ca^{2+} ions have no effect on the rate of conversion of κ-casein. However, others have found an increased rate of proteolysis after addition of calcium and other multivalent cationic additives (Green and Marshall, 1977). Yet other researchers have claimed that concentrations of Ca^{2+} above 8mM decrease the enzymatic activity (Bringe and Kinsella, 1986). According to Dalgleish (1992) these different observations possibly relate to the types of experiments that were attempted, since in some experiments, milk was diluted into a buffer solution containing Ca^{2+} (Bringe and Kinsella, 1986) while in others the solution of Ca^{2+} was added to milk (van Hooydonk *et al.*, 1986).

The secondary phase is completely dependent on a critical Ca^{2+} concentration and above this level the RCT of milk decreases with increase in the amount of calcium (Walstra and Jenness, 1984). The mechanism of the effects of calcium on the renneting process is not fully understood. Green (1982) suggested that the rate of aggregation is increased by adsorbed cations shielding the negatively charged groups of the casein and this increases the hydrophobicity of the rennet-converted micelles and promotes aggregation. Dalgeish (1993) attributed the accelerating effect of calcium to a specific interaction of unknown nature and not to a simple charge neutralisation of renneted micelles. Reduction of CCP by about 20% prevents coagulation unless the Ca^{2+} concentration is increased (Pyne and McGann, 1960) presumably because removal of CCP, which is considered to be attached through organic casein phosphate groups, increase micellar charge (Pearce, 1976).

2.5 Gel formation in renneted milk

It has been shown that the structure of the gel determines its properties and the retention of fat and moisture on which depend cheese yield and composition (Green and Grandison, 1993). During renneting of milk, rennet split off the caseinomacropeptide part of the κ-casein molecules, thereby diminishing steric and electrostatic repulsion (Zoon *et al.*, 1988); then the para-casein micelles aggregate at first into loose floccules, often of a thread-like configuration. Flocculation may lead either to a coagulum consisting of separate, compact flocs, which usually sediment and hence form a precipitate or to a gel in which the flocculated particles form a network.

The network then extends and becomes more differentiated, with the chains of micelles aligning together. During this time, the linkages between the micelles also appear to strengthen because of breaking and reforming of strands at other places. Initially, many micelles are jointed by bridges and eventually causing partial fusion. These changes result in a coarsening of the spatial distribution of the casein network and an increase in stiffness of the strands (Zoon *et al.*, 1988). The network consists of strands of para-casein micelles of variable thickness, and the space between them contains whey and fat globules. The casein micelles of milk usually form a gel on flocculation if the liquid is kept at rest, as in renneting, but when the liquid is stirred

coagulum forms (Walstra and Jenness, 1984). The gel changes considerably with time even under constant conditions (Walstra and van Dijk, 1983) and with time the increase in gel firmness is due to increases in both the number and strength of linkage between micelles.

2.6 Gel structure and properties

2.6.1 Rheological properties

The structure of the gel is determined somewhat by the mode and conditions of its formation. A gel is a semirigid precipitate that retains its original dispersing medium. It is a system consisting of two continuous phases, a solid and a liquid phase. The most characteristic properties of gels are rigidity and elastic response to distortion. This implies that a structure exists between the macromolecules or particles of one of the phases of the system. The other phase is the liquid, which fills the spaces in and around the structural members of the gel.

The gel formed is made up of linked particles and behaves differently from one consisting of cross-linked, randomly coiled molecules. It shows very low yield stress and low breaking stress (brittleness). Most gels are visco-elastic materials and show time-dependent rheological behaviour. These are characterised by two parameters. The storage modulus, G', is a measure of the true elastic property of the gel, and the loss modulus, G", is the measure of the viscous property. Both the storage modulus (G'), and the loss modulus (G"), increase with time, reflecting an increase in firmness. The moduli depend on the number, strength and relaxtion time of the bonds between the particles in the network. Probably, there is first an increase in the number of junctions between particles and then an increase in the number of bonds per junction (Green and Grandison, 1993).

The modulus of the gel increases strongly after it is formed. Presumably, the increase is due to two phenomena. One is that additional junctions are formed between casein particles, partly because there are strands of particles that are attached to the gel at only one end, partly because additional casein particles and small clusters thereof become incorporated into the gel. The latter situation will always occur to some extent during formation of a particle gel, but more strongly during normal renneting,

since at the moment of gel formation, not all casein micelles have been fully transformed into para-casein micelles. The other phenomenon is that any junction, which is a contact region between two original micelles, must contain several bonds, and the number of bonds per junction increases on ageing. One may say that the micelles more or less fuse and after some hours the original particles making up the gel can no longer be distinguished. If no starter is added and the proteolytic enzymes of milk have been inactivated, the increase in the number of bonds does not lead to a significant change in the loss tangent (Green and Grandison, 1993).

The structure of the gel is related to its process of formation. There is a tendency for faster firming rates to lead to gels with coarser network structure. For instance, calcium depletion in the milk reduced the firming rate and the gel network was finer, while higher temperatures increased the firming rate and gave coarser gel networks (Green and Grandison, 1993).

2.6.2 Syneresis

Structure of the gel is important in determining the ability of the gel to synerese. Syneresis means the loss of whey from the curd particles. This is not accompanied by a change in casein hydration; it is not a simple physical process. In fact, it involves rearrangement of the casein network in the curd, with strands being broken and reformed to form a highly cross-linked, more compact structure.

The interactions between casein micelles leading to syneresis are incompletely understood. Although some are probably non-specific hydrophobic interactions, others are very specific. They may not be identical to the interactions occurring during curd formation, although considerable overlap would be expected. There are few factors affecting curd formation and syneresis greatly i.e. increasing acidity, temperature and modification of casein amino groups. Fat content, added $CaCl_2$, cold storage, psychrotroph growth and homogenisation tend to have less effect on syneresis (Green and Grandison, 1993).

Increasing the fat content of the milk probably also decreases the rate of whey loss by physical means. As well as limiting casein aggregation, fat globules may acts as

'plugs', blocking the flow of whey through channels in the curd (Green and Grandison, 1993).

It has generally been observed that syneresis rate is increased as the pH is lowered, possibly as a result of a reduction in net micelle charge and hence of the electrostatic repulsion between micelles. Syneresis is very temperature-dependent (Patel *et al.*, 1972). The rate of syneresis accelerates as temperature increases; however, reports vary as to the extent of the effect of temperature (Pearse and MacKinlay, 1989). Lowering of the pH and increase of temperature induce shrinkage of casein micelles and these factors are usually changed during cheesemaking. These changes as such may cause additional syneresis (Walstra and van Dijk, 1983). The ease with which the moisture can be removed depends strongly on pH and temperature (Walstra *et al.*, 1985; van Dijk and Walstra, 1986; Roefs *et al.*, 1990); it is closely related to the endogenous syneresis behaviour and the effect of pressure on it.

Lucey *et al.* (1997) found that syneresis at 30°C was very small, and it was concluded that the endogenous syneresis pressure would be about zero. Little syneresis occurs at 20°C, but at 40°C it can be considerable (van Vliet *et al.*, 1997). At 40°C, a finite endogenous syneresis pressure may exist. Endogenous syneresis can occur if rearrangement of the newly formed network occurred. This may happen when strands in the network breaks. At 30°C, the strands are fairly thick and would not break readily, while at 40°C the breaking stress of the gel was only 0.3 times that at 30°C (Lucey *et al.*, 1997).

Similar disagreement exists in the literature regarding the effect on syneresis of $CaCl_2$ addition. According to Marshall (1982), addition of 2mM $CaCl_2$ increased syneresis and 4 mM $CaCl_2$ gave an additional increase in syneresis only at shorter cutting times (2 to 3X rennet coagulation time). Kaytanli *et al.* (1994) also found that addition of 10mM $CaCl_2$ had a stimulating effect on drainage. However, excessive addition of $CaCl_2$ (> 50mM) was found to reduce drainage rate due to its effect caused by the increasing ionic strength. Cheeseman (1962) found that syneresis was inhibited at all concentrations of $CaCl_2$ tested (10, 50 and 100mM). It appears that the addition of

very low levels of calcium enhances syneresis, possibly as a result of charge neutralisation whereas high levels of $CaCl_2$, as with NaCl and KCl addition, inhibit syneresis by ionic effects or possibly as the result of Ca^{2+} occupying sites on the caseins that otherwise would participate in syneresis.

Addition of calcium chloride to the milk prior to renneting not only increases the calcium concentration but also decreases the pH of the milk. Both factors accelerate the renneting process. The acceleration of the coagulation reaction is probably not so much due to the slight increase in the calcium ion activity but more likely to the amount of calcium interacting with the casein micelles.

A study by Kaytanli *et al.* (1994) found that increase in rennet concentration significantly increase the drainage rate which is in agreement with Marshall (1982). This was due to a more rapid aggregation at higher enzyme concentrations which led to a coarser network with fewer junctions causing increased amount of whey drainage.

Daviau *et al.* (2000) found that the rennet gel firmness increased with the increase in the concentration of casein and increasing the concentration of casein also led to a decrease in syneresis. The increase in concentration of casein led to an increase in the solids content of the drained curd, which explains the increase in the firmness of the curd with the higher concentration of casein.

Walstra *et al.* (1985) found the increase in the concentration of casein increased the rate of drainage. However, in the study of Daviau *et al.* (2000), they found no effect of the concentration in casein on the rate of drainage.

2.6.3 Permeability of rennet gels

After the gel is formed, the surfaces of the para-casein micelles that are not in contact with each other are still reactive. By Brownian motion or deformation these surfaces may locally come close together and stick, thus causing a stress in the strands. This resulting endogenous pressure causes the matrix to shrink or synerese. Syneresis is thus a function of the pressure and the resistance against flow through the matrix, expressed in the permeability (van Dijk and Walstra, 1986).

Gel permeability can be defined as the whey transfer capacity resulting from an applied pressure and/or gel network contraction and it gives information about the inhomogenities at the level of the gel network i.e. size and number of the largest pores (van Dijk and Walstra, 1986).

The permeability of a porous medium particularly depends on its porosity and on the continuity of pores or gaps. If these gaps are closed without connections between them, permeability could be nil, regardless of their size. In milk gel, the gaps were surrounded by 'virtual' walls, i.e. open or discontinuous walls, and the continuity of the aqueous phase is normal (Lagoueyte *et al.*, 1994).

It was found in most of the studies that gel permeability increased rapidly with temperature (Lagoueyte *et al.*, 1994; van Dijk, 1982; Roefs *et al.*, 1986; van Dijk and Walstra, 1986; Zoon *et al.*, 1988). The reasons for this increase might be that the network gaps that were filled with whey increased in size; gap walls became more discontinuous; gaps increased in size and walls became more discontinuous at the same time (Lagoueyte *et al.*, 1994).

2.7 Factors affecting gel formation, structure and properties

2.7.1 Effect of protein concentration

Ultrafiltration is commonly used to concentrate proteins in the milk system. During this process, protein and fat are retained virtually completely in the concentrate. Minerals such as calcium, magnesium, phosphorous, and citrate are present partly bound to protein in milk and partly in solution (Green *et al.*, 1984).

Studies by Garnot and Corre (1980) and Mehaia and Cheryan (1983) showed that concentration of milk results in a decrease in the degree of κ-casein proteolysis during the enzymic phase. The degree of proteolysis at coagulation is dependent of pH and the type of enzyme used. It is known that the modulus of rennet milk gels increases more than proportionally with increasing protein concentration (Tokita *et al.*, 1985). In the study of Zoon *et al.* (1988), the dependence of the dynamic moduli on the casein concentration was measured as a function of time after rennet addition. The casein concentration factor c_f, which is the ratio of casein concentration in the sample

and the casein concentration in unconcentrated milk, was varied from 1 to 2.21. They found that the clotting time increased slightly with increasing casein concentration; the difference between the sample with the lowest and with the highest casein concentration was only about one minute. This was about 8% increase in clotting time. Waungana *et al.* (1998) found that the gel strength increased with increased protein concentration and in all cases G' tended to approach a constant value after ~ 90 min (Figure 2.3). The final value of G' increased with VCR (volume concentration ratio) at both natural pH and at pH 6.5.

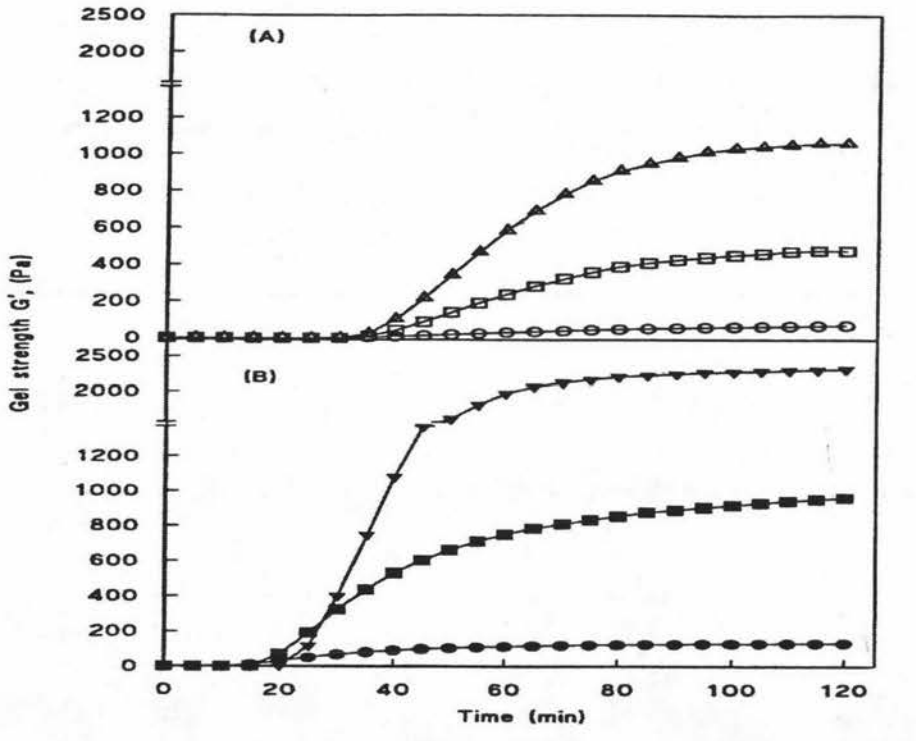


Figure 2.3 The changes in storage modulus (G') with time during renneting at (A) natural pH of normal skim milk (o), 2X UF concentrate (□) or 3X UF concentrate (Δ) and (B) at pH 6.5 of normal skim milk (•), 2X UF concentrate (■) or 3X UF concentrate (σ) (from Waungana, 1995).

The gels made with concentrated milk differ from normal rennet milk gels in their structure. Gels prepared from concentrates become progressively coarser as the

protein concentration of the milk increases; the less developed structure of the curd tends to make it fragile (Lelievre and Lawrence, 1988).

2.7.2 Effect of rennet concentration

A number of studies (Hossain, 1976; Garnot *et al*, 1982; McMahon *et al.*, 1984) reported that a higher enzyme concentration causes not only a shorter clotting time but also a higher rate of curd firming and gel strength appeared to increase with decreasing rennet concentration. However, van Hooydonk & van den Berg (1988) found an increase in gel strength with increasing rennet concentration. These conflicting results may be due to differences in the accuracy of and the maximum strain occurring in the measuring equipment used (Zoon *et al.*, 1988).

A study by Zoon *et al.* (1988) shows clearly that at higher rennet concentration a gel is formed sooner after rennet addition and that the increase of G' as a function of time is faster (Figure 2.4). Even after an ageing time of more than 8 hours, the G' values were higher for milks containing higher rennet concentrations. The reason for this might be that at a higher rennet concentration more rapid aggregation takes place, leading to a coarser network with fewer junctions but with more bonds per junction.

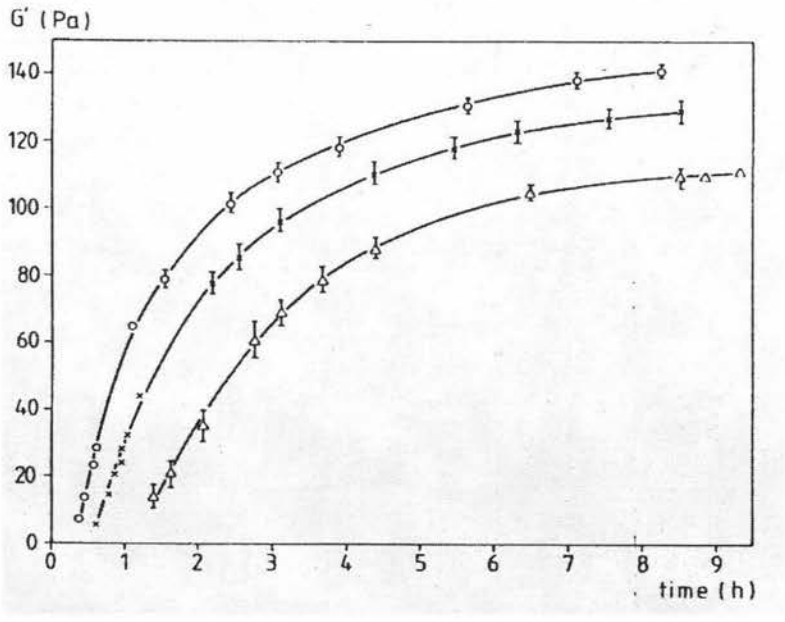


Figure 2.4 Storage modulus G' as a function of time for reconstituted skim milk gels. Rennet concentration was varied: 0.5% (o), 0.025% (x), 0.01% (Δ). Angular frequency was 1 rad/s. T = 30°C, pH = 6.65 (from Zoon *et al.*, 1988).

2.7.3 Effect of temperature

Temperature is a very important variable during cheesemaking. It affects the rate of renneting, gel formation and syneresis. It has a little effect on enzymic reaction, but strongly affects the aggregation reaction. The rate of aggregation becomes almost zero below 15°C and syneresis virtually stops below 18°C (Walstra *et al.*, 1985). van Hooydonk & van den Berg (1988) and Hossain (1976) found a steady increase in rate of moduli up to 35°C. Tokita *et al.* (1982) found increase in rate of moduli up to 40°C. Scott-Blair & Burnett (1959) found maximum at 32°C for gelation. Zoon *et al.* (1988) found that increasing temperature during gel formation and ageing mainly resulted in an increase of the firming rate. Figure 2.5 shows that at 20°C, G' values increased very slowly with time. Between 20 and 30°C, a strong temperature effect on the increase of G' values with time was found while above 30°C the initial rates were similar to that at 30°C. At 30°C, G' values reached a plateau value after about 9 hours, but G' values decreased again after 9 hours. At 35 and 40°C, the plateau value as well as the subsequent decrease in G' values reached sooner than at 30°C. They concluded that G' values measured after a long ageing time decreased with increasing temperature and gels formed and aged at 20, 30, 35°C all gave similar results for G' values at 30°C.

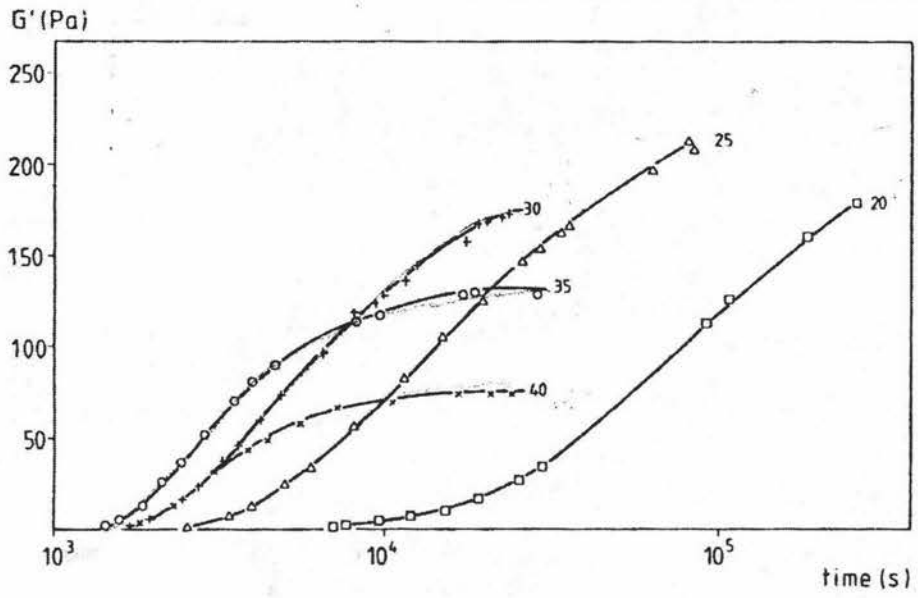


Figure 2.5 Storage modulus G' as a function of time after rennet addition for milk gels made and measured at the temperature as indicated. Angular frequency was 1 rad/s (from Zoon *et al.*, 1988).

2.7.4 Effect of calcium and phosphate

The influence of calcium on the aggregation reaction is very pronounced. A minimum calcium concentration is needed to initiate gelation of rennet-converted casein micelles. Above this minimum level, the rennet coagulation time of milk decreases with the calcium concentration (van Hooydonk *et al.*, 1986). At very high concentrations of added $CaCl_2$ (>50mM) the coagulation time decreases if the pH is kept constant (McMahon *et al.*, 1984; McGann & Pyne, 1960). If small amounts of $CaCl_2$ are added to the milk at constant pH the gel strength after a long ageing time increases. McMahon *et al.* (1984) found a maximum in the gel strength for milks with 50mM $CaCl_2$ addition.

Zoon *et al.* (1988) found that a minimum amount of calcium was needed for clotting to occur. For CCP concentration higher than in milk the moduli tended to decrease. A slight decrease of the CCP did not affect tan δ, but if CCP was lower than half the concentration in milk, higher values of tan δ were found. For example, Figure 2.6 shows that after a long ageing time the G' value of the samples with added $CaCl_2$

(6mM) was about 10% higher than of those samples without $CaCl_2$. The values of tan δ were not affected by the calcium addition. At low CCP concentration enhanced visible syneresis occurred. The calcium ion activity influenced the clotting time and the moduli, but not the tan δ.

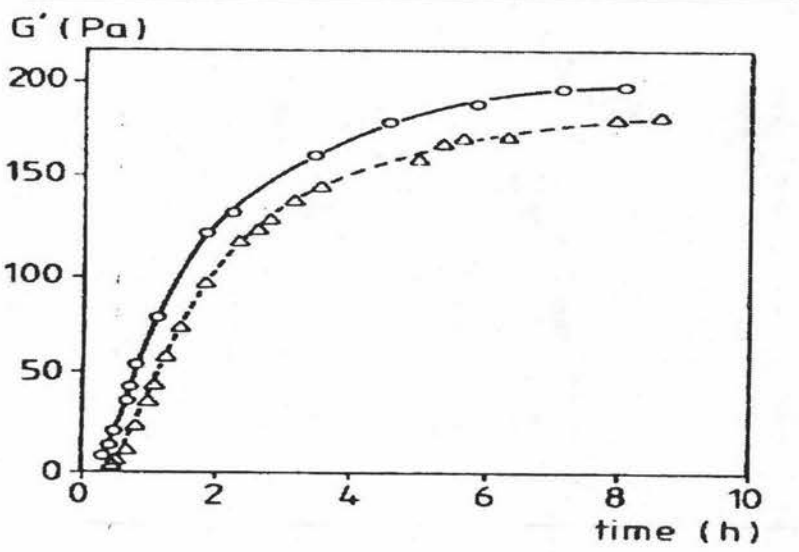


Figure 2.6 Storage modulus G' as a function of time after rennet addition for skim milk gels; (O) 6mM $CaCl_2$ added, (Δ) no $CaCl_2$ added; pH = 6.6, T = 30°C, angular frequency = 1 rad/s (from Zoon *et al.*, 1988).

2.7.5 Effect of pH and NaCl

The pH is an important factor in the manufacture of several types of dairy products, such as yoghurt, quarg and cheese (Zoon *et al.*, 1988). Hossain (1976) found in the pH range from 6.36 to 6.7, the ultimate curd firmness increased with decreasing pH. This implies that since the minimum storage modulus was found at pH 5.2, and the curd firmness decreased with increased pH between pH 6.36 to 6.7; these two findings suggest that a maximum in the curd firmness must be somewhere between pH 5.2 – 6.36. In the study by Zoon *et al.* (1988), the rheological properties of rennet-induced skim milk gels were investigated in the pH range of 5.7 to 6.7. The shape of the ageing curves was dependent on pH (Figure 2.7). For a lower pH, the initial slope tended to be steeper and a constant value and subsequent decrease of the moduli were reached sooner after rennet addition. This was especially clear for the gel made at pH 5.72. They also found that decreasing the pH in the range from pH 6.7 to 5.7 resulted

in a maximum of the storage modulus G' near pH 6.15. Above pH 6.0, no significant influence was found of pH on tan δ, whereas at pH 5.7 a higher tan δ was observed at all frequencies between 10^{-3} to 4.5 rad/s. A lower pH possibly goes along with a faster rearrangement of strands and fusion of micelles, resulting in a fast increase of a plateau value of the modulus.

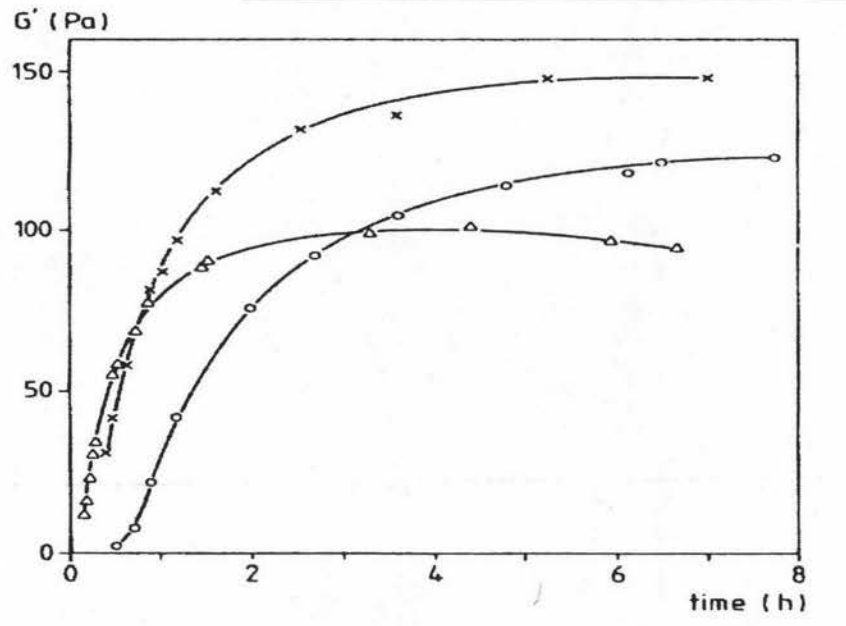


Figure 2.7 Storage modulus G' as a function of time after rennet addition for skim milk gels. (O) pH 6.65, (×) pH 6.25, (Δ) pH 5.72. T = 30°C, ω = 1 rad/s, 0.025% rennet added (from Zoon *et al.*, 1988).

Another effect of the acidification of milk is the increase in the ionic strength of milk. Roefs *et al.* (1986) found that the ionic strength had a marked effect on the formation of acid casein gels. A minimum salt concentration of about 100 mM NaCl was required to make a gel from sodium caseinate solutions by acidifying in the cold and subsequent heating. However, adding more than 240 mM NaCl prevented gel formation even at high temperatures. For acid skim milk gels, addition of NaCl always resulted in a lower storage modulus in dynamic measurements, especially if more than 50 mM was added. The upper limit for gel formation at 30°C seemed to be around 150 mM of added NaCl (Zoon *et al.*, 1988).

Some conflicting results exist on the influence of NaCl addition on the modulus or the 'curd tension' of rennet-induced milk gels. Grufferty and Fox (1985) observed that the curd tension, after 2.5 times the clotting time, was not affected by added NaCl up to 500 mM. Gouda *et al.* (1985) reported a decrease in the curd firmness with increasing NaCl addition at high concentrations of added NaCl (500 – 1500 mM). Jen and Ashworth (1970) reported an increase of curd tension with the addition of NaCl up to 100 mM, but a decrease at high concentrations.

Zoon *et al.* (1988) investigated the effect of addition of sodium chloride, in the range of 50-300 mM added NaCl, at pH 6.65 and 6.25 by performing dynamic and stress-relaxation measurements. The samples at pH 6.65 with a constant rennet concentration, addition of 50 and 100 mM NaCl did not change the initial increase of G' with time, but the increase continued longer, eventually resulting in higher G' values. If more than 100 mM NaCl was added, the onset of gelation was clearly retarded and also the increase of G' was less than at lower concentrations of added NaCl. The G' values after a long ageing time were about the same as that of the sample to which 50 mM NaCl was added. They also showed that NaCl addition did not affect tan δ. Its influence on gel formation and ageing appeared to be dependent on the experimental conditions.

2.7.6 Behaviour of rennet milk gels at large deformation

Large deformation and fracture of milk gels occur during cheesemaking, for instance during cutting and pressing of the curd. Some studies have been done on the large deformation of milk gels. Roefs *et al.* (1986) reported that the stress that had to be applied to fracture the gel within a certain time was higher for acid milk gels than for rennet-induced milk gels, while the deformation at fracture was smaller.

Zoon *et al.* (1988) have studied the rheological properties at large deformation of rennet-induced milk gels in constant stress (creep) experiments. The effects of ageing time and the pH of the gels, the measuring temperature and the applied stress were investigated. They found with decreasing applied stress time, with decreasing measuring temperature and increasing ageing time, the stress needed to break a gel increased. The strain at fracture increased with increasing measuring temperature,

due to increased plastic flow, whereas it decreased with increasing ageing time, due to a decrease in the instantaneous deformation in a plastic flow. For gels differing in pH (6.65, 6.25 and 5.75), no significant differences in strain as a function of time were observed. The strain was depicted as a function of t_{re} [ageing time (t) / time after stress application at which fracture of the gel occurred (t_f)] of gels aged for 0.5, 1 and 3.5 hours for stresses causing about t_f. It was seen that the instantaneous strain decreased with increasing ageing time. With increasing ageing time, the deformation due to plastic flow decreased. The stress-strain curves are depicted for the gels with different ageing times for stress being applied during about 30s; increasing the ageing time increased the energy to fracture a gel within a certain time.

2.8 Drainage of whey (syneresis)

Syneresis of curd formed by the action of rennet on milk comprises shrinkage with the expulsion of whey. It is an essential step in reducing the moisture of the curd, which is an important factor in determining cheese quality (Lawrence and Gilles, 1980). The rate and extent of drainage of whey from renneted milk gels (syneresis) is influenced by chemical factors, such as rennet concentration and Ca^{2+} concentration (Ernstrom & Wong, 1974) and also by mechanical factors, such as cutting and stirring (Marshall, 1982). The separation of curds and whey is normally measured at a fixed time after rennet addition in laboratory experiments, and at this stage gels may not be of equal strength (Lelievre, 1977); therefore, the gels are expected to undergo different patterns of syneresis.

Effect of pH on drainage rate

Drainage rate is accelerated by acidification. The increased rate of drainage was probably due to the reduction of overall charge at reduced pH values. Reducing pH resulted in increasing closer contact between the micelles, leading to fusion and separation of whey. Kaytanli *et al.* (1994) found that the rate of drainage process increased considerably with a decrease in pH which also agreed with the previous studies of Walstra *et al.* (1985) and Marshall (1982).

Effect of coagulation temperature

Temperature greatly affects the syneresis rate of rennet curd. The rate of drainage was increased by increase in temperature. The maximum drainage was obtained at 40°C (Kaytanli *et al.*, 1994). It also found by Marshall (1982) that increasing the temperature accelerates the initial rate of syneresis.

Effect of $CaCl_2$ concentration

In cheesemaking $CaCl_2$ is mostly added to enhance the renneting properties of the reaction medium so that a firmer coagulum is obtained. Walstra *et al.* (1985) reported that the addition of 10 mM $CaCl_2$ had a stimulating effect on drainage. However, addition of excessive amount of $CaCl_2$ (> 50mM) was generally found to reduce drainage rate due to its simultaneous effect observed by the reduction of pH. This is in full agreement with the study carried out by Kaytanli *et al.*, (1994).

Effect of enzyme concentration

Kaytanli *et al.* (1994) and Marshall (1982) found that the effect of rennet concentration on drainage rate was significant. They found that the rate of drainage process was enhanced by increased rennet concentration. The reason for the effect was that a more rapid aggregation at higher enzyme concentrations that led to a coarser network with fewer junctions causing more whey drainage.

CHAPTER 3

OBJECTIVES

The overall aim of this project was to determine the impact of the cheese coagulum structure (determined by coagulation conditions) on the cheesemaking process (particularly the syneretic properties of the curd) and moisture content of cheese.

The specific objectives are as follow:

(1) To develop and establish methods for measuring microstructure, permeability and rheological properties of rennet-induced skim milk gels.

(2) To determine the influence of extreme conditions of pH, temperature, calcium addition, rennet concentration and protein concentration on the properties and microstructure of rennet gels. The conditions that have been called 'extreme' are close to the normal limits of a range of conditions selected in cheesemaking. However, some combinations of the 'extreme' conditions would not be used in normal cheesemaking.

(3) To investigate the influence of combined extreme conditions on the cheesemaking process and cheese composition.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Milk source

Low heat skim milk powder was obtained from New Zealand Dairy Board, Wellington. The powder contained 38% protein, 0.5% fat and 4.2% moisture; the other components being lactose and salts.

Bulk raw milk was obtained from the New Zealand Dairy Research Institute or Massey University Dairy Farm. Town milk was used since this shows less variation in composition than the seasonal manufacturing milk throughout the year.

4.1.2 Rennet source

Australian double-strength calf rennet, which contains 80 RU/ml, was obtained from the Dairy Meats NZ Limited enzyme division (Eltham, New Zealand). This rennet was freshly diluted 1:10 with Milli-Q water (Millipore Corporation, Bedford, USA) and used at the rate of 60 µl/l, 80 µl/l or 120 µl/l milk (undiluted).

4.1.3 Starter source

Mesophiles type of starter, produced from *Lactococcus lactis* subsp. *cremoris*, was obtained from the New Zealand Dairy Research Institute Starter Production Unit (Palmerston North, New Zealand).

4.1.4 Sodium azide (NaN_3)

An antibacterial agent, sodium azide (BDH Laboratory Supplies, Poole, BH15 1TD, England), was added to all the milk samples at the rate of 0.02% (w/v) before the samples were stored at 4°C.

4.2 Processing methods

4.2.1 Preparation of reconstituted skim milk

Reconstituted skim milk was prepared by dissolving 10 grams of skim milk powder in 100 grams of water. Reconstituted skim milk was then stirred for 2 hours and left in 4°C overnight before further use.

4.2.2 Preparation of high protein skim milk

Skim milk was warmed to 50°C and then concentrated to volume concentration ratio (VCR) of 1.1 to 1.5X (the VCR is defined as the initial volume of milk divided by the retentate volume) using the Protosep III KOCH-UF pilot plant. A HFM 100 membrane of area 0.28 m^2 and molecular weight cut off 30,000 Da was used.

The operating conditions used were as follows: the product flow rate was approximately 1400 litres per hour, the operating temperature was 50°C, the inlet pressure was 210 kPa and outlet pressure was 160 kPa.

4.3 Analytical methods

4.3.1 Protein content

The concentration of protein was estimated by determining the total nitrogen content by the Kjeldahl method, using a Kjeltec 1026 system (Tecator, Sweden). The protein content was calculated from the nitrogen percentage by multiplying by an empirical factor of 6.38. Duplicate samples were analysed.

4.3.2 Moisture content

The moisture contents of the experimental cheeses were determined by the oven drying method. A sample of known weight (approximately 7g) was weighed into an aluminum dish and heated for 16 hours in an air oven at 102°C. After cooling in the desiccator for 1 hour, the dry samples were weighed, and the % moisture content was calculated by subtracting the dry weight from the original sample weight. Duplicate samples were analysed.

4.4 Experimental procedures

4.4.1 Rheological properties

Renneting

A sample of reconstituted skim milk was adjusted to pH 5.8, 6.2 or 6.5, using 1M HCl or 1M NaOH, and equilibrated for at least 30 minutes. Diluted rennet (refer to section 4.1.2) (1:10) at concentrations, ranging from 60 µl/l milk to 120 µl/l milk, was then added to the reconstituted milk at a defined temperature.

Dynamic low amplitude oscillation test

(a) Theoretical background

The viscoelastic properties of the renneted milks were determined by sinusoidal oscillation in a Paar Rheometer (USD-200, Physika Messtechnik GmbH and Co. KG Stuttgart, Germany) using low amplitude oscillation as described by Bohlin *et al.* (1984). The Paar Rheometer is a computer-controlled instrument.

The measuring system consisted of a coaxial cylinder (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 the time in s and cos ω is a simple harmonic function. The applied shear strain results in a 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 is 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 change 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 viscoelatic gels are subjected to a sinusoidally oscillating strain, the stress is neither completely in-phase nor completely out-of-phase.

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 solution to an infinitely large network (gel) (Zoon *et al.*, 1988). The gelation time, GT, which marks the transition of milk from a solution to a gel, is 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 sample (~7.5ml) was carefully placed into the measuring system consisting a DG 25 (double gap) cup and a bob. The bob was then slowly lowered into the cup until the milk solution just covered the top of the bob.

Measurements were started approximately 120s after the addition of rennet and were continued every 30s for 2 hours. Table 4.1 shows the parameters selected to follow the rheological characteristics of rennet-induced gels.

Table 4.1 The parameters used for rheological measurements

Measuring system	DG 25 (double gap)
Temperature	25°C, 32°C or 38°C
Frequency	1Hz
Amplitude tau	0.0053 Pa

4.4.2 Permeability measurements

Preparation of rennet whey

Rennet whey was used for the permeability tests. Skim milk powder (500g), dissolved in 5 litres deionised water and left overnight, was warmed to 32°C for 30 minutes and then diluted rennet solution (1/10 dilution, 10μl/l of milk) was added. The renneted milk was incubated at 32°C overnight to form a gel. The gel was cut to expel the whey. The whey was then drained on cheese cloth (muslin) overnight. The whey was centrifuged at 200 rpm for 20 minutes and then filtered through Whatman # 1 and Whatman # 42 filter papers. 0.02% NaN_3 was added as a preservative.

Measurement technique

The *tube method* for measuring gel permeability was originally developed by 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 the desired temperatures. The length of the gel was normally 11-12 cm. After 2 hours of incubation, the gels were formed inside the tubes. The tubes were withdrawn, cleaned from the outside and then placed vertically in a vessel filled with whey, with the top of the gels below the whey surface. The pressure difference over the gel gave rise to an increase in the whey liquid height on top of the gel. The level of the whey on top of each gel was read at regular intervals by means of a "level meter". A diagram

representative of the system is shown in Figure 4.1. The temperature of the whey bath was the same as the temperature at which the milk was incubated (25°C, 32°C or 40°C).

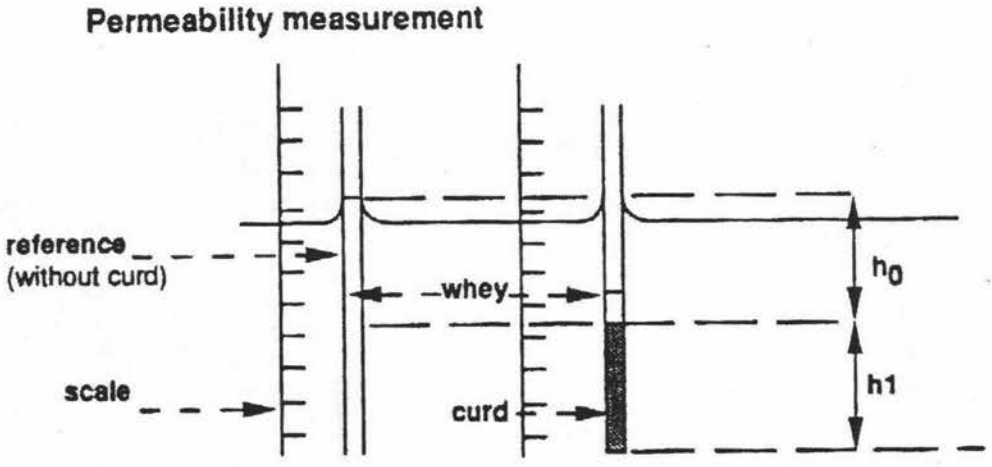


Figure 4.1: Schematic diagram of permeability measurement (from Lagoueyte *et al.*, 1994).

The level of the whey in the tubes was measured over a period of time (usually up to 4 hours) and for each tube the permeability coefficient (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_0 as in Fig. 4.1)
$h_{(t)}$	=	height of serum level in the gel tube (m) at time t
H	=	length of the gel (m) (h_1 as in Fig. 4.1)
g	=	gravitational acceleration (ms^{-2})

η = viscosity of the serum (Pa.s)

ρ = density of the serum (kg/m^3)

t_1 = start time of the measurement

t_2 = end time of each measurement

4.4.3 Determination of microstructure

A Leica (Heidelburg, Germany) confocal scanning laser microscope with a 100 mm oil immersion objective lens and an Ar/Kr laser with an excitation wavelength of 488 nm was used to determine the microstructure of rennet gels.

Reconstituted skim milks were warmed to the desired temperature (25, 32 or 40°C) for 30 minutes. A small quantity of 1% aqueous solution of fast green FCF (BDH Chemical) fluorescent protein dye was added to 2 ml skim milk. An appropriate amount of rennet was then added and a few drops of this mixture were placed on the special microscope slides with a concave hollow. A cover slide was placed over the milk sample and microscope slides were placed in a box (with water-wetted tissues in it to ensure the gels would not dry off) which was placed in an incubator at the renneting temperature (i.e. 32°C). Approximately 1 hour after the addition of rennet, the microstructure of the rennet-induced gels was examined by the Leica Confocal Microscope.

Duplicate or triplicate samples were prepared. Several different places on each slide were examined.

Sometimes the gel had very large pores or had lost contact with the glass surface. If this happened, the images were discarded.

4.4.4 Syneresis measurements

Reconstituted skim milk or cheese milk was set at the desired conditions with five levels of starter (0.25, 0.5, 1.0, 1.5, 2.0%). An appropriate amount of rennet was added to a large amount of milk, which was then divided into 25 ml samples for incubation. Samples were incubated at the desired temperature (32°C or 38°C) for 1 hour for the gels to reach appropriate firmness.

The firm gels were placed in the container shown in Figure 4.2 above the mesh screen (approximate pore size ~ 0.07 cm). The gels were given two cuts (one horizontal cut and one vertical cut) to ensure maximum syneresis (the curd was cut into a size of ~ 13mm^2 cubes). The whey solutions expelled and drained through the mesh screen. The weight of the whey solution was measured every 20 minutes for 3 hours. The position of the gels was adjusted to ensure the gels would not attach to the surface of the container which could affect syneresis. Duplicate samples were tested for each experiment.

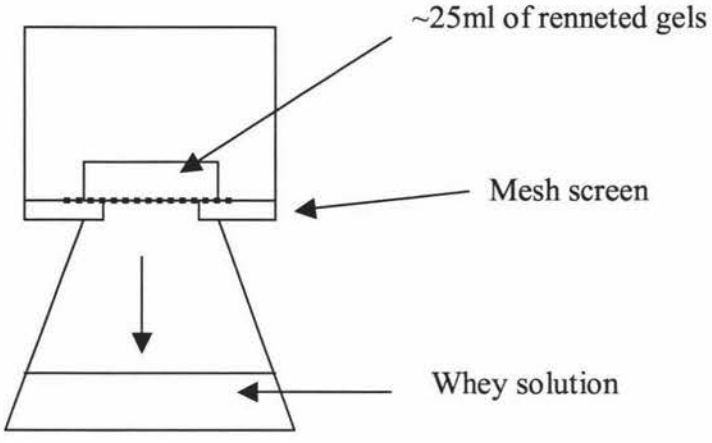


Figure 4.2 The schematic diagram of the syneresis system

4.4.5 Cheese manufacture–pilot plant trials

Figure 4.3 shows the schematic diagram of the cheesemaking process used for the pilot plant trials carried out at the New Zealand Dairy Research Institute. 375 kg of whole milk (protein to fat ratio was 0.8) was used for each batch. The differences in conditions for each batch are shown in Table 4.2. For more detailed information, refer to Table 6.1 and 6.4 in Chapter 6.

Table 4.2 The processing conditions used for different cheese production batches

Processing conditions	**Levels**
Protein concentration (VCR)	None or 1.24X
Temperature (°C)	32 or 38
pH	6.20 or 6.50
Rennet concentration (μl/l)	60, 90, 120
Addition of $CaCl_2$ (g/100ml)	None, 0.01%, 0.02%
Starter levels	1 or 2%

Each batch was processed as described in Figure 4.3. The final cheeses were analysed for moisture, fat, calcium, pH and salt at the New Zealand Dairy Research Institute. Duplicate samples were used for each analysis.

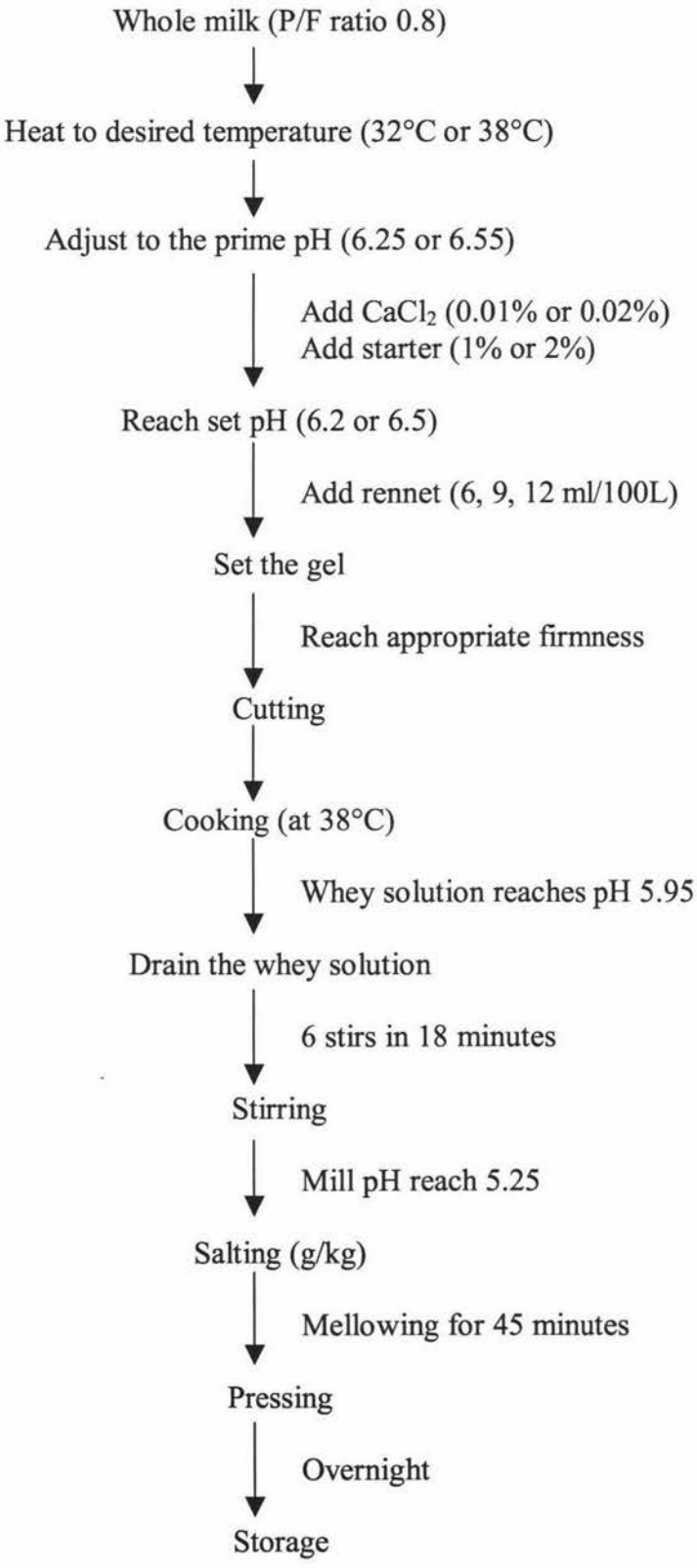


Figure 4.3 Process flow chart for production of experimental cheeses at the New Zealand Dairy Research Institute.

CHAPTER 5

INFLUENCE OF SOME PROCESSING AND COMPOSITIONAL FACTORS ON THE FORMATION AND PROPERTIES OF RENNET-INDUCED SKIM MILK GELS

5.1 Introduction

The gelation of milk by rennet action is an important step in the production of many cheese products. During cheesemaking, rennet is normally added just after the starter, and it contains proteolytic enzymes, which hydrolyse a specific peptide bond of κ-casein. The casein micelles lose their colloidal stability and a gel is formed (Roefs *et al.*, 1990; Walstra, 1990; Dalgleish, 1998). Gels formed by rennet action show viscoelastic behaviour, which is a response to a stress or strain and is partly viscous and partly elastic. However, in rennet milk gels, the viscous component is much more important, especially at higher gelation temperature and for longer time scale (Zoon *et al.*, 1988).

Extensive studies on the structure and mechanical properties of milk gels have been performed by van Dijk and Walstra (1986), Roefs *et al.* (1985), Tokita *et al.* (1982) and Zoon *et al.* (1988). van Dijk and Walstra (1986) has paid attention to the structure and syneresis behaviour of rennet milk gels made at natural pH of milk. The structure and rheological properties of casein gels formed by cold acidification and subsequent heating were studies by Roefs *et al.*, (1985). Zoon *et al.* (1988) and Tokita *et al.* (1982) investigated the rheological properties of rennet-induced skim milk gels made at natural pH of milk. Electron microscopy was performed by Kalab (1979) on the milk gels. Very few studies have been reported on how the structure and properties the rennet-induced milk gels vary with important variables, such as pH, temperature, $CaCl_2$ addition, rennet and protein concentration and what impact they have on the cheesemaking process and final cheese composition.

Therefore, in this study the effects of these factors on the rennet-induced skim milk gel properties were investigated. The extreme levels of conditions were used for these

factors; however, the "extreme" was not overly exceeding the conditions for normal cheesemaking process. In this way, the conditions can be applied to normal cheesemaking. The gels were examined using permeability measurements, which was found to be very useful in an earlier study e.g. by Verheul and Roefs, (1998) and is, in a practical sense, related to the water-holding capacity of gels, rheological measurements and confocal microscopy.

Table 5.1 The target conditions used for the formation of rennet gels.

Factors	Low conditions	Medium conditions	High conditions
pH	5.8	**6.2**	6.5
Temperature (°C)	25	**32**	40
$CaCl_2$ addition (g/100ml)	**No addition**	0.01%	0.02%
Protein concentration	**1X** (normal skim milk)	1.2X	1.5X
Rennet concentration (μl/100ml of milk)	40	**80**	120

Note:

(1) Protein concentration was increased by ultrafiltration and is represented as volume concentration ratios (VCR).

(2) Rennet concentration unit = μl of (1:10) rennet / 100ml of milk.

(3) Only one factor was varied during gel formation and other factors were fixed at the standard conditions which are pH 6.2, temperature 32°C and rennet concentration 80μl/L of milk (refer to the bold text in the table).

5.2 Results and discussion

5.2.1 Effect of pH on rennet-induced milk gels

Three pH values (5.8, 6.2 and 6.5) were chosen for this experiment. The values chosen were according to the normal cheesemaking process. To ensure that the chosen pH was constant during the measurement, sufficient time (2 hours) was taken for adjustment.

5.2.1.1 Rheology

The changes in dynamic rheological values of G′, G″ and tan δ were measured, as a function of time after rennet addition, and represent the development of a rennet gel structure. During the gelation process, G′ and G″ increased with renneting time; G′ was greater than G″ in all cases. Approximately 1000 seconds after rennet addition, 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, but thereafter there was a slower rate of increase in G′ values. There was no sign of reaching a plateau G′ value during the two hour course of measurement. After gel formation, tan δ remained almost constant; therefore, both G′ and G″ continued to increase. The increase in G′ is because of the formation of the network structure (Zoon *et al.*, 1988; Pomprasirt, 1996).

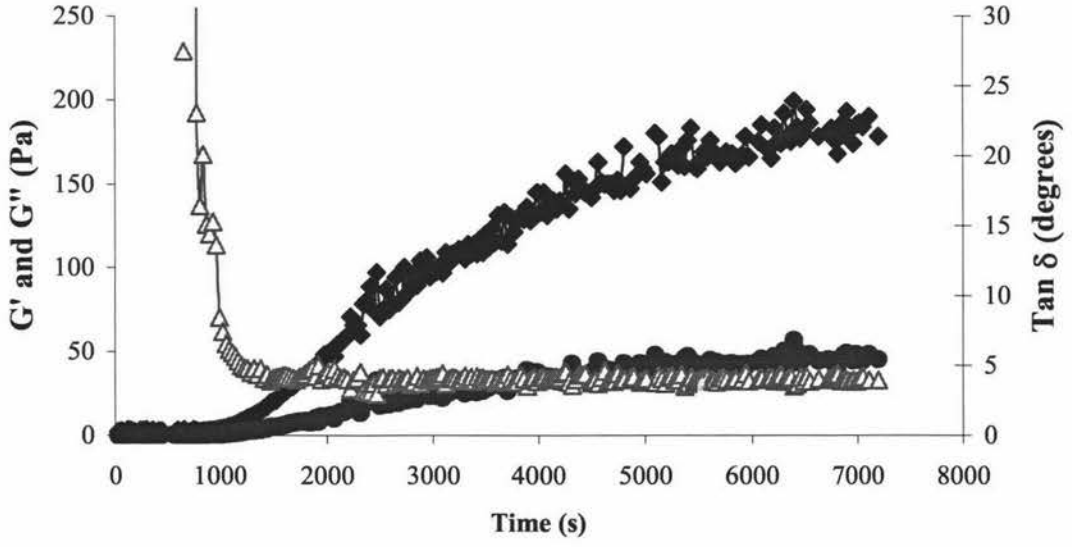


Figure 5.1 Rheological properties which develop during the rennet coagulation of skim milk at 32°C, pH 6.2 and 80μl/l rennet addition. Storage modulus, G′ (♦), loss modulus, G″ (●) and tan δ, (△).

Over the pH range, gel formation occurred at different times after rennet addition (Figure 5.2). The gelation times were 6, 10 and 21 minutes for pH 5.8, 6.2 and 6.5 samples, respectively. The gelation times here referred as the time when G′ exceeded

greater than 10 Pa. The shape of the G′ versus time curves was dependent on pH (Figure 5.2). For the pH 5.8 gel, the initial slope tended to be steeper, a plateau was reached sooner than at other pHs, followed by slight decrease in G′. Similarly, pH 6.2 sample showed the same trend, but without the slight decrease. For the pH 6.5 sample, G′ tended to increase gradually without reaching a plateau during the 2 hours of measurement. However, the G′ values for different pH samples tended to reach similar G′ values (~180 Pa) toward the end of the measurement time i.e.120 minutes.

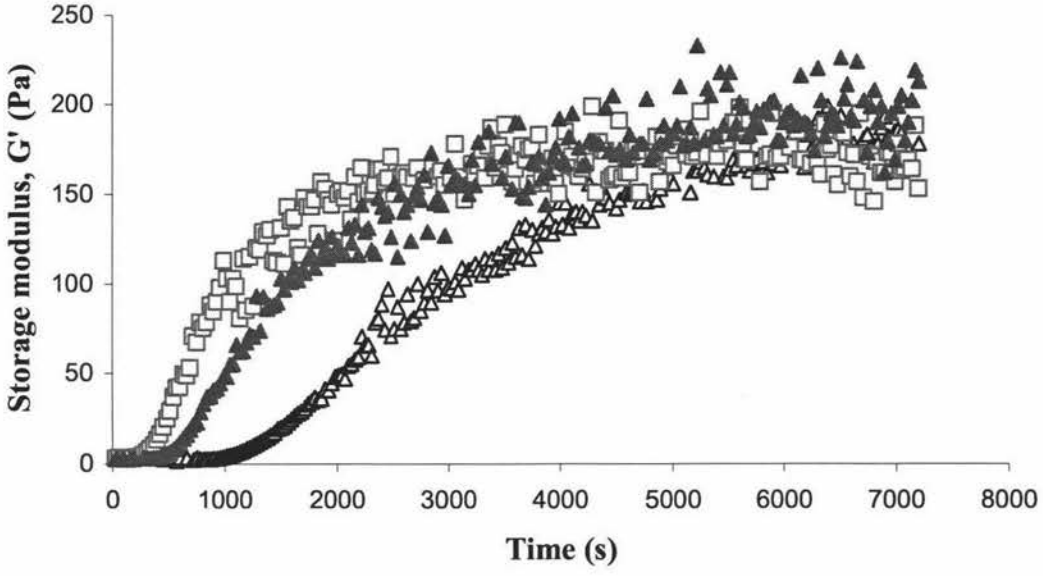


Figure 5.2 Storage modulus, G′, as a function of time, for skim milk samples adjusted to pH 5.8 (□), pH 6.2 (▲), and pH 6.5 (△) at 32°C and then 80μl/l of rennet was added.

The increase in G′ values after gelation was attributed to an increase in the number of bonds caused by network formation, which included the rearrangement of the strands and fusion of the micelles (Zoon *et al.*, 1988). van Hooydonk *et al.* (1986) found that the rennet coagulation time of milk increased with pH in the range from 5.2 to 7.0. Above pH 7.0 para-κ-casein micelles became progressively more stable and did not aggregate, and below pH 5.2 the measurement of the renneting process in milk was complicated by pH-induced aggregation of the casein micelles. They also found that the rate of aggregation and gel formation increased with decreasing pH, which is in

agreement with this study (Figure 5.2). The reason for this effect is that lowering the pH from the physiological level approximately 6.65 to ~5.8 increases the relative importance of electrostatic interactions.

5.2.1.2 Permeability coefficient

Rennet-induced milk gels are semi-solid, particle-type gels, which have porous structures (van Dijk and 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, 1993), particularly on its porosity and permeability.

Gel permeability can be defined as the ease of flow of serum through the gel network, from an applied pressure gradient (van Dijk & Walstra, 1986). The permeability coefficient (B) can be used to characterise the network and can give information on the size of the pores in the gel. The equation for permeability coefficient is shown below (refer to section 4.4.2 for more details).

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

Figure 5.3 shows the permeability coefficient (B) of rennet-induced milk gels made at different pHs as a function of time. The permeability increased as the pH of the milk gels decreased to pH 5.8; this was an indication of rearrangements taking place within the gels that caused the gel pores to become larger. The increase in pore size may be due to the casein micelles coming together into larger clusters and, in later stages, due to breaking of strands (Mellema, 2000). It was apparent that the gels made at pH 5.8 had the greatest permeability. There was no significant difference in the permeability coefficient between pH 6.2 and pH 6.5 samples. The permeability coefficient increased much more rapidly with time for pH 5.8 sample compared to pH 6.2 and 6.5 samples (Figure 5.3). The milk gels collapsed faster than those formed at pH 6.2 or 6.5. Towards to the end of measurement, the gels tended to 'collapse' which means the gels started to shrink, crack or falling off the tubes. When this happened, the

measurements were stopped. The reason for collapse of gel is possibly the rearrangement of network which makes the pore size larger, as discussed below.

Since the B values for pH 6.2 and 6.5 were similar, it appears that the overall pore size (or at least the number and sizes of the largest pores) did not differ greatly.

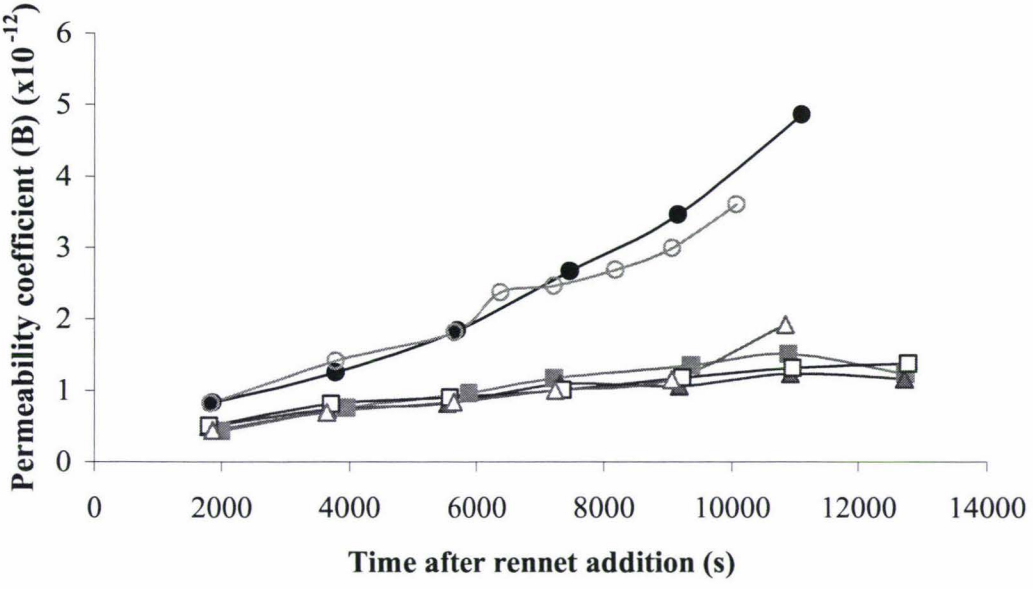


Figure 5.3 Permeability coefficients as a function of time of the rennet skim milk gels made at pH 5.8 (● ○), pH 6.2 (■ □), and pH 6.5 (▲ △).

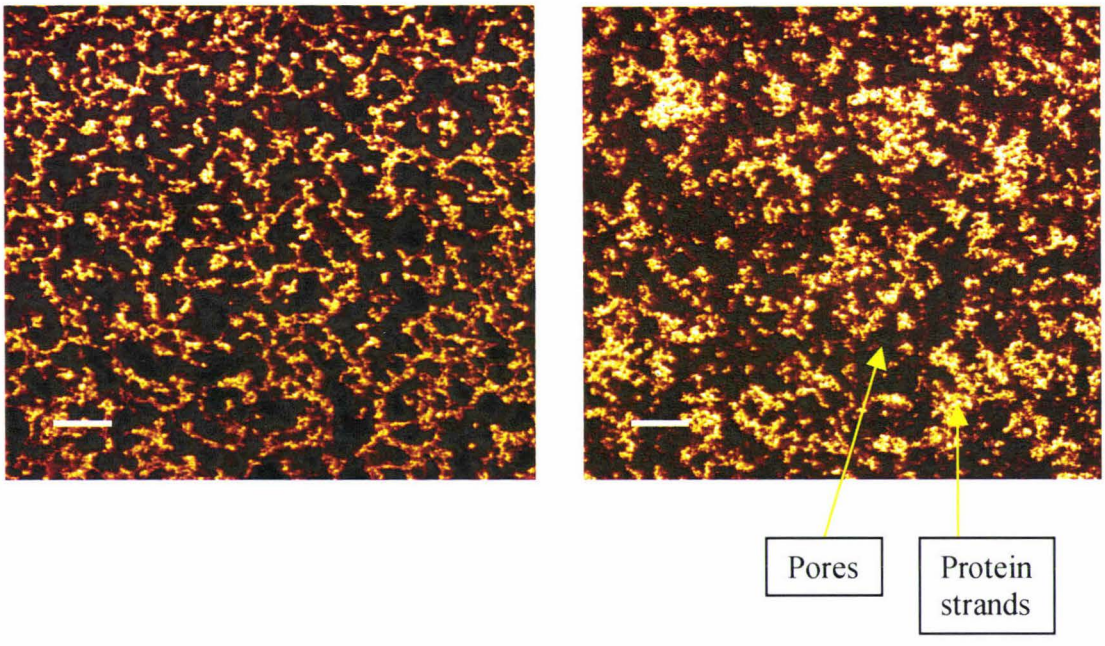
The permeability coefficient (B) changed with time, suggesting that the strands in the network rearrange, or change in diameter or both. The fact that B increases implies that the matrix becomes less homogeneous and that the diameter of the strands or the micelles is reduced. The latter holds for casein micelles and rennet curd if the pH falls, but the pH was kept constant during the experiments of present study. Therefore, it possibly implies that new cross-links are formed. As a result of the newly formed cross-links the strands will be under stress. The stress can relax by syneresis. The broken strands that have more freedom to move therefore enhance permeability, as the pores will enlarge (van Dijk and Walstra, 1986).

5.2.1.3 Confocal microscopy

The confocal micrographs (Figure 5.4) show the microstructure of the milk gels made at different pHs. The protein matrices appear in yellow or orange colour, while the dark areas correspond to other components in the gel. The micrograph for the milk gel made at pH 5.8 showed larger pore size, a more open structure with thinner strands as compared with the samples at pH 6.2 and 6.5. In contrast, pH 6.2 and 6.5 samples showed denser structure and smaller pore size. Milk gels made at 6.2 and 6.5 showed similar structures, although pH 6.2 sample had somewhat larger clusters compared with the pH 6.5 sample. These results compared very well with the permeability results (Fig 5.3).

(a) pH 5.8

(b) pH 6.2



(c) pH 6.5

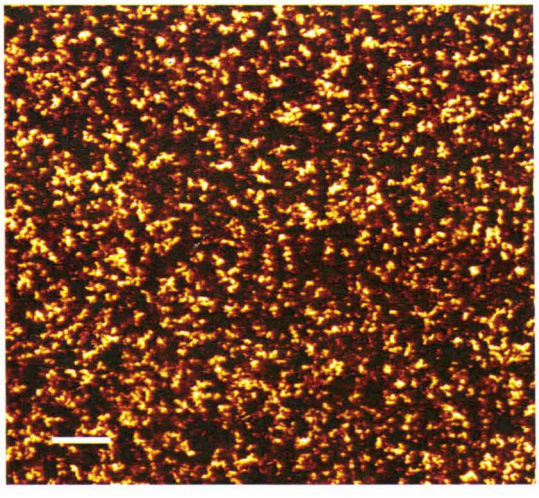


Figure 5.4 Confocal laser scanning micrographs of standard skim milk gels made at 32°C with rennet concentration of 80μl/l and pH 5.8 (a), 6.2 (b) or 6.5 (c). Samples stained with Fast Green FCF, and the magnification is 400X. The scale bar represents 10 μm.

5.2.2 Effect of temperature on the rennet milk gels

5.2.2.1 Rheology

Figure 5.5 shows the G′ versus renneting time profile of milk gels made at different temperatures. The gelation time (GT) increased with a decrease in renneting temperatures (GT values were 57, 21 and 16.5 minutes for gels formed at incubation temperatures 25, 32 and 40°C respectively). The gels made at 25°C had very long gelation time and they were very weak. The G′ values increased very slowly with time for these gels. Even after 2 hours, G′ continued to increase and did not seem to reach a plateau value. It seems impossible that G′ values at 25°C would be higher than those at 32°C and 40°C after very long ageing time. Between 25°C and 32°C, a strong temperature effect on the increase of the G′ values with time was found. The gels made at 32°C had not reached a plateau value after two hours (7200 s), whereas in the gels made at 40°C, a plateau value was reached approximately after one hour (3600 s). The gels made at 32°C proved to be strongest after one hour.

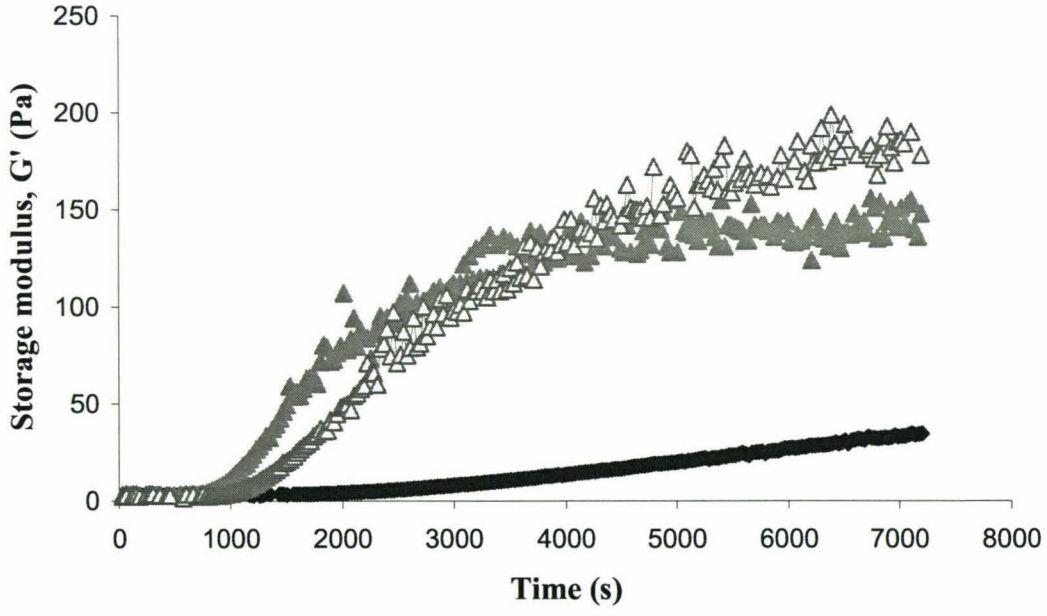


Figure 5.5 Storage modulus, G′, as a function of time after rennet addition for skim milk samples incubated at 25°C (■), 32°C (△), and 40°C (▲) with pH of 6.2.

For a higher temperature, the renneting reaction is faster (e.g. κ-casein hydrolysis), resulting in a measurable value of the G′ value soon after rennet addition. The fusion of micelles within the strands proceeds faster. Initially this results in a higher G′ value, because more bonds would be formed, but the fusion would also be completed earlier, so a plateau value is reached sooner. These factors imply a more rapid coarsening of the network and this would result in lower final G′ values (Zoon *et al.*, 1988).

It is known that the strength of hydrophobic interactions decreases with decreasing temperature below about 45°C. It is thought that hydrophobic interactions mainly occur between casein molecules within the micelles, and a decrease in hydrophobic interactions may lead to a swelling of the micelles. This would mean that the aggregated particles could form large junction zones. Moreover, rennet gelation is slow at low temperatures, which would allow time for large junction zone to form. A large junction means a strong junction, since many individual protein-protein bonds will exist in one junction. Therefore, the gels formed at low temperature have higher G′ values. Large strong junctions, formed at an early stage, would also prevent rearrangement of newly formed junctions. At higher temperature, the micelles are not swollen, gelation proceeds faster and the G′ value was low. Thus, at a high temperature, smaller and weaker junctions are formed, which have a greater susceptibility for localised rearrangement (Lucey *et al.*, 1997). Another explanation given by Zoon *et al.* (1988) is that at low temperatures due to the swelling, the micelles in the strands have a large contact area between them. Therefore, the close contact of the micelles probably induce a rearrangement of the bonds between them and results in an increased interaction between the micelles, leading to a higher G′ at a lower temperature i.e. at 32°C compared with 40°C. These two explanations on the effect of temperature change lead to the same conclusion.

The observed decrease in the plateau value of the G′ with increasing temperature (Fig. 5.5) was in agreement with the results of Zoon *et al.*, (1988). Although Tokita *et al.* (1982, 1983) found the opposite results. Tokita *et al.* (1982, 1983) measured the moduli of the gels during ageing and extrapolated the results to a plateau value. Their

G′ values were very low compared to the results of present study and from Zoon *et al.* (1988). The G′ values found in the present experiments were similar to that of Zoon *et al.* (1988).

5.2.2.2 Permeability coefficient

Figure 5.6 shows the permeability coefficient (B) results for gels made at different temperatures. It was found that the gels made at 40°C had the highest permeability coefficient values. The gels made at 32°C had slightly higher B values than those made at 25°C. For 25°C and 32°C samples, there were slow increases in permeability and the gels collapsed later than the gels made at 40°C. The gels made at 40°C showed higher permeability that increased rapidly with time and the gel collapsed after 2 hours. Even in the beginning of the measurement, gels made at 40°C showed the highest permeability, which suggests that these gels had a more open structure than those made at other temperatures.

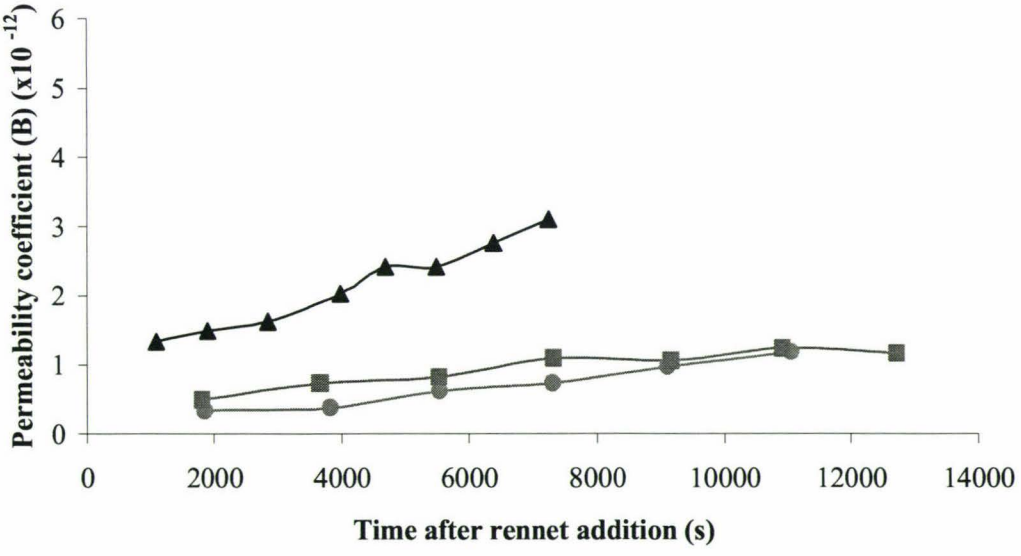


Figure 5.6 Permeability coefficients of the rennet skim milk gels made at 25°C (●), 32°C (■), and 40°C (▲).

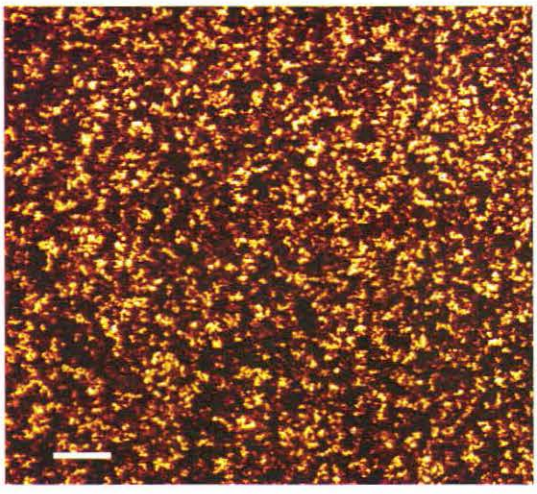
The present results which showed that B increased rapidly with temperature confirm those of other researchers (van Dijk, 1982; Roefs *et al.*, 1986; van Dijk and Walstra, 1986; Zoon *et al.*, 1988; Lagoueyte *et al.*, 1994). It is likely that permeability increased because (1) the network gaps that were filled with whey increased in size; (2) gap walls became more discontinuous; (3) gaps increased in size and walls became more discontinuous; so that micelle strands and clusters which form the gap walls became denser and thicker (Lagoueyte *et al.*, 1994). In addition, at high temperature, internal hydrophobic interactions are stronger and the micelles are smaller. The increased hydrophobic interactions could be a driving force for rearrangements within the network (Mellema, 2000).

5.2.2.3 Confocal microscopy

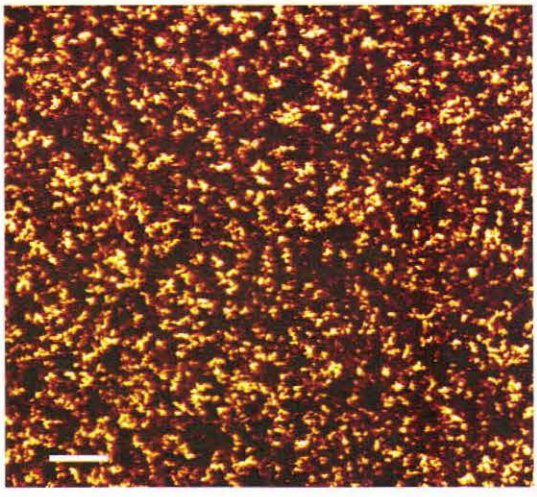
The confocal micrographs of skim milk gels made at various temperatures are shown in Figure 5.7. At 25°C, the gel appeared to be fairly homogeneous, with small pores and denser structure. At 40°C, the gel was much coarser, with very large pores. At 32°C, the structure was intermediate between that observed for the 25 and 40°C samples.

Overall, it appears that increasing temperature speeds up the gel ageing or rearrangement process. Especially in Figure 5.7 (c) the rearrangements have reached a very advanced stage, because the matrix has "collapsed". At this point the system is about to show syneresis (Mellema, 2000). This is probably why cheesemaking is done near 30°C than heated to higher temperature. The results from these micrographs corresponded well with the results from the rheological and permeability measurements.

(a) 25°C



(b) 32°C



(c) 40°C

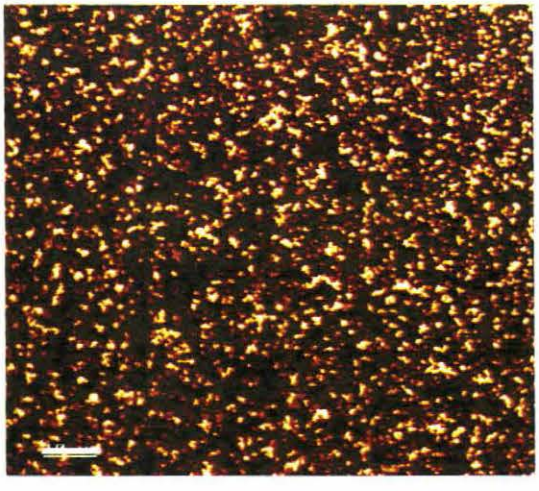


Figure 5.7 Confocal laser scanning micrographs of standard rennet skim milk gels made at pH 6.2 with rennet concentration of 80μl/L and at 25°C (a), 32°C (b) or 40°C (c). The gels were stained with Fast Green FCF, and the magnification is 400X. The scale bar represents 10 μm.

5.2.3 Effect of calcium chloride addition on the rennet milk gels

5.2.3.1 Rheology

The effect of calcium chloride addition on the storage modulus versus time plot is shown in Figure 5.8. These plots were very similar for milks with 0.01% and 0.02% $CaCl_2$ addition; the gelation times for these milks were similar (26.5 and 27 minutes for milks with 0.01% and 0.02% $CaCl_2$ addition respectively). The milk gel, without any $CaCl_2$ addition, showed the lowest G′ values during renneting for 120 minutes. The gelation time of this sample was longer (40 minutes) compared with those with added $CaCl_2$. None of the samples reached a plateau value for G′ after two hours of measurement.

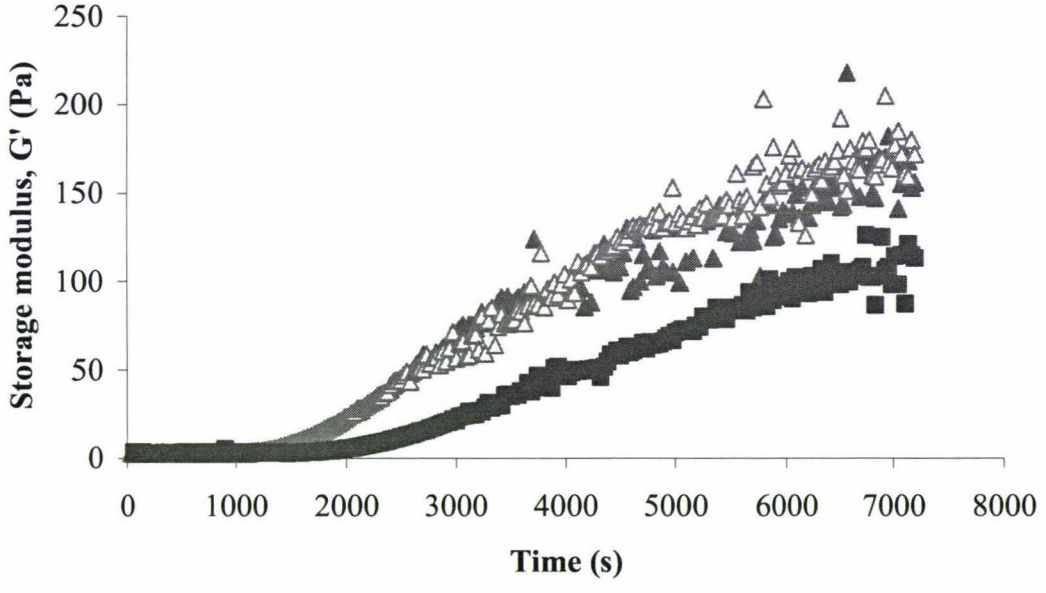


Figure 5.8 Storage modulus, G′, as a function of time for skim milk with 0% (■), 0.01% (▲), or 0.02% (△) added calcium chloride and then renneted at 32°C and pH 6.2.

The effects of calcium chloride on the rennet coagulation of milk are well documented (Mehaia and Cheryan, 1983; van Hooydonk *et al.*, 1986; Singh *et al.*, 1988) and $CaCl_2$ is frequently added to cheesemilk to enhance rennet coagulation during commercial cheese manufacture (Waungana, 1995). Addition of Ca^{2+} reduces the rennet coagulation time of milk and also increases gel firmness.

It is well established that the secondary phase is completely dependent on a critical Ca^{2+} concentration. Therefore, the decrease in gelation time and increase in G′ observed with the milk samples containing added $CaCl_2$ was probably due to increased aggregation of renneted micelles caused by the increased [Ca^{2+}]. The exact mechanism of calcium action is, however, still unclear. It may be related to the charge reduction on casein micelles by binding of Ca^{2+} ions, thus reducing electrostatic resistance to aggregation of casein micelles (Green, 1982).

G′ increased with added calcium up to a certain concentration, suggesting that there may be a limit to the degree of 'cross-linking', which can occur at a given concentration of calcium (Solorza and Bell, 1998). Lucey and Fox (1993) showed that addition of up to 10mM (~0.1%) Ca^{++} to milk increased the strength of rennet gels, but further addition cause a decrease in gel strength.

5.2.3.2 Permeability coefficient

A plot of permeability coefficient of milk gels containing 0, 0.01% and 0.02% $CaCl_2$ as a function of time is shown in Figure 5.9. The permeability decreased slightly with increase in calcium chloride concentration. The permeability increased slightly with time for milk gels made with or without added $CaCl_2$.

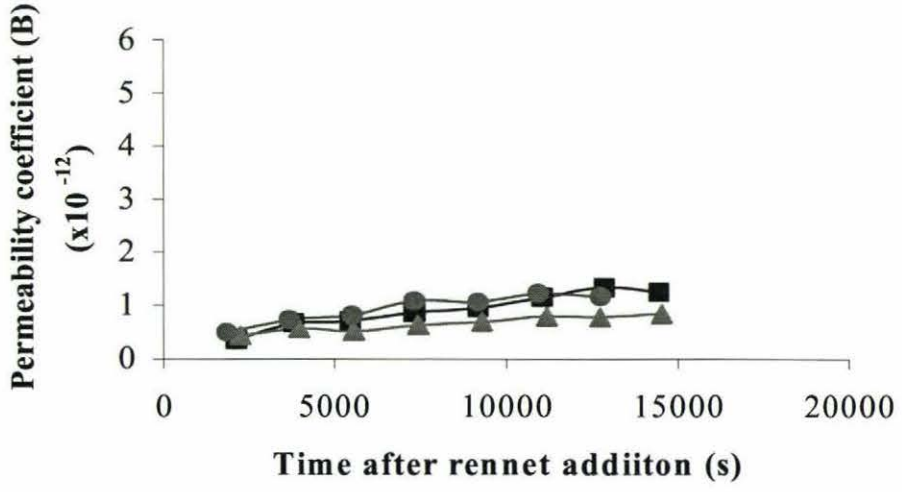


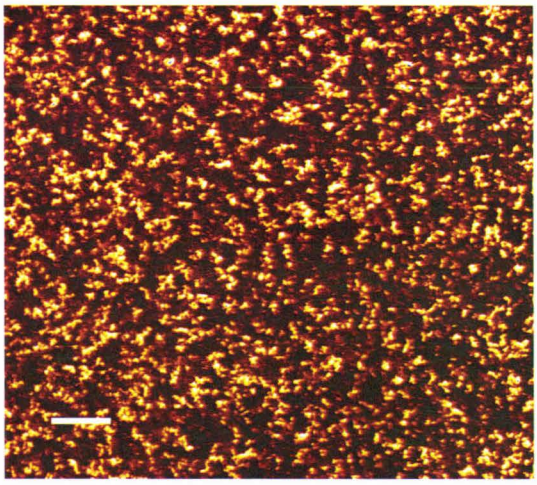
Figure 5.9 Permeability coefficients (B) of the rennet skim milk gels containing 0% (●), 0.01% (■), and 0.02% (▲) calcium chloride.

van den Bijgaart (1989) found that the permeability and the change of permeability with time in rennet gels were not significantly affected by addition of 4.5mM (0.045%) $CaCl_2$, if the pH was kept constant. The syneresis rate was slightly higher and the maximum rate of syneresis was reached sooner after rennet addition in $CaCl_2$ containing systems; this was ascribed to faster aggregation of casein micelles.

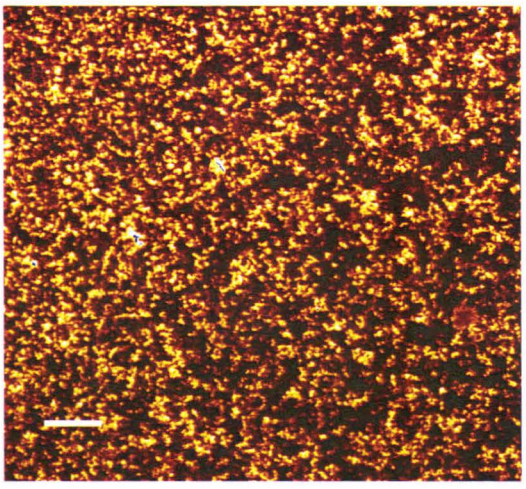
5.2.3.3 Confocal microscopy

The micrographs of the rennet milk gels with and without $CaCl_2$ addition are shown in Figure 5.10. There were no clear differences between the three samples. The gel structures were quite dense for all samples and the pores were small.

(a) 0% $CaCl_2$ addition



(b) 0.01% $CaCl_2$ addition



(c) 0.02% $CaCl_2$ addition

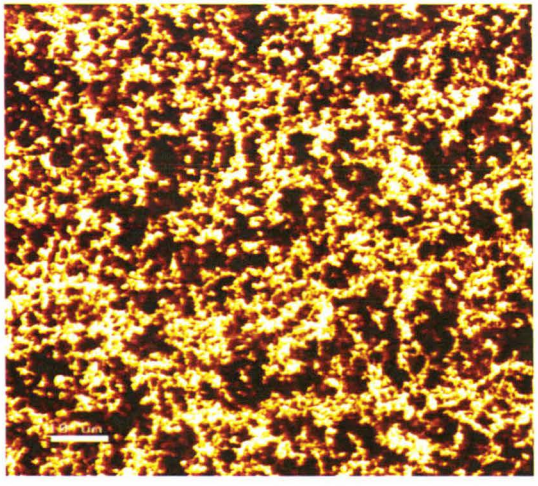


Figure 5.10 Confocal laser scanning micrographs of rennet skim milk gels made at 32°C with rennet concentration of 80µl/l of milk, pH 6.2 and $CaCl_2$ addition of 0% (a), 0.01% (b) or 0.02% (c). These gels were stained with Fast Green FCF, and the magnification is 400X. The scale bar represents 10 µm.

5.2.4 Effect of rennet concentration on the rennet milk gels

5.2.4.1 Rheology

Figure 5.11 shows the G′ versus renneting time profile of milk gels made using different rennet concentrations. The G′ values increased with increase in rennet concentration, but the difference between rennet concentration 80 μl/l and 120 μl/l was small. The gelation time was slightly longer for samples containing rennet concentration 80 μl/l than those containing 120 μl/l (21 minutes versus 15 minutes). For samples containing the lowest rennet concentration of 40 μl/l, the gelation time was much longer (approximately 46 minutes) and the G′ values were lower than the other two samples.

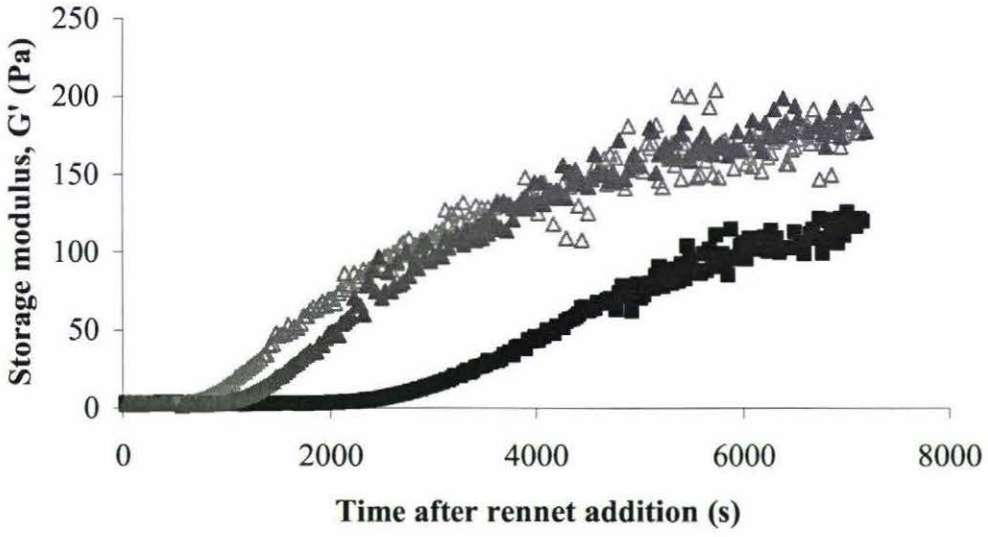


Figure 5.11 Storage modulus, G′, as a function of time, for skim milk gels made using 40 μl/l (■), 80 μl/l (▲), and 120 μl/l (△) rennet at 32°C.

It is well known that the rate of the renneting reaction increases when the amount of rennet is increased. An increase in rennet concentration leads to an increased rate of proteolysis of κ-casein and thus a faster coagulation and a higher rate of gel firming (Lomholt and Qvist, 1999). van Hooydonk and van den Berg (1988) stated that the increase in renneting action was partly because the percentage of GMP released at the onset of gelation increased with increasing rennet concentration. They also suggest

that at a higher rennet concentration more rapid aggregation of casein micelles takes place, leading to a coarser network with fewer junctions but with more bonds per junction.

5.2.4.2 Permeability coefficient

The permeability plot for different rennet concentrations is shown in Figure 5.12. The permeability coefficient increased for the samples made with less rennet. Gels made with rennet concentration of 40 µl/l showed the highest permeability, but the increase in permeability with time was slowest. For gels made with a rennet concentration 80 µl/l, the permeability was higher than the gels made with 120 µl/l in the beginning; however, they reached the same permeability towards the end of measurement time (> 3 hours). This means that in the gels made with a rennet concentration of 120 µl/l, the permeability coefficient increased faster as a function of time compared with gels made with skim milk containing 80 µl/l of rennet.

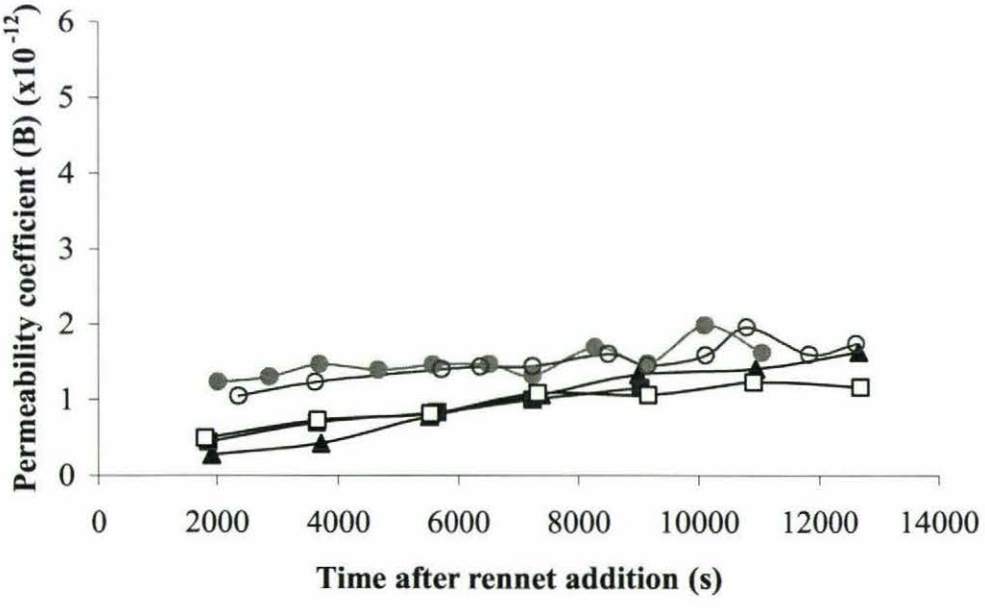
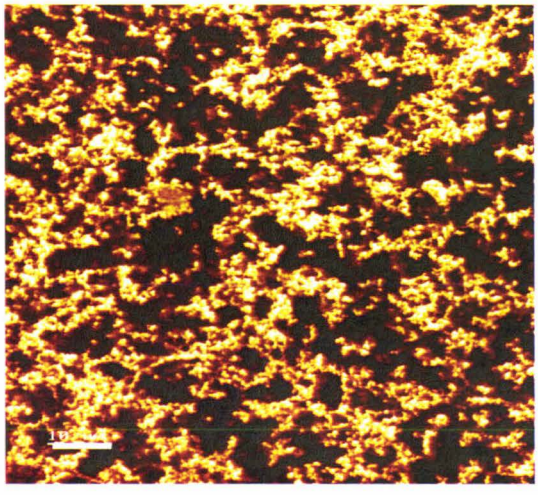


Figure 5.12 Permeability coefficients (B) as a function of time, of the skim milk gels made using 40 µl/l (● ○), 80 µl/l (■ □), and 120 µl/l (▲) rennet.

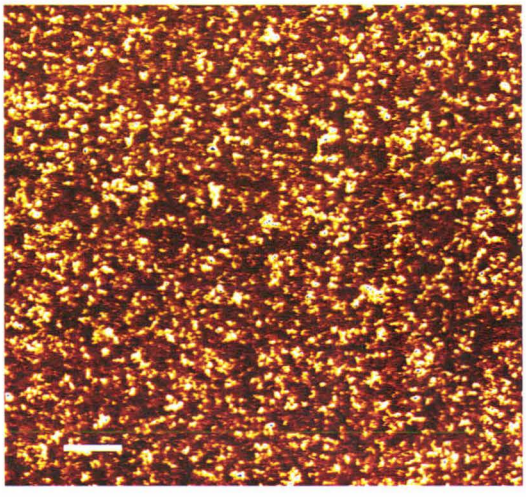
5.2.4.3 Confocal microscopy

Figure 5.13 shows the confocal micrographs of milk gels made using different rennet concentrations. The difference between the samples made with 80 μl/l and 120 μl/l rennet on the micrographs was small. Gels made with rennet concentrations of 80 μl/l and 120 μl/l showed a denser structure than the gels made with 40 μl/l.

(a) 40μl/l rennet concentration



(b) 80μl/l rennet concentration



(c) 120μl/l rennet concentration

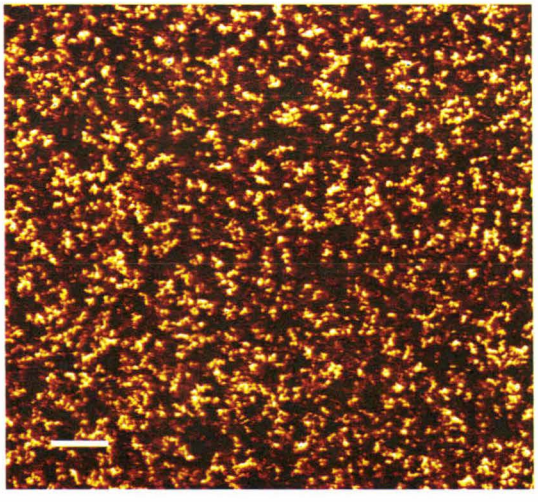


Figure 5.13 Confocal laser scanning micrographs of rennet skim milk gels made at 32°C and pH 6.2 using rennet concentration 40μl/l of milk (a), 80μl/l (b) or 120μl/l (c). The gels were stained with Fast Green FCF and the magnification is 400X. The scale bar represents 10 μm.

5.2.5 Effect of protein concentration on the rennet milk gels

5.2.5.1 Rheology

Skim milk was concentrated by ultrafiltration to volume concentrations ratios (VCRs) of 1.04X, 1.13X, 1.21X, 1.37X, and 1.48X instead of what was mentioned in Table 5.1 for clearer effect of different level of protein concentration and analysed for total protein concentration as described previously (chapter 4). The protein content of these samples were 3.44%, 3.80%, 3.89%, 4.16%, 4.71% and 5.10% for VCR 1X, 1.04X, 1.13X, 1.21X, 1.37X, and 1.48X, respectively.

Figure 5.14 shows G′ as a function of time for milk gels containing different protein concentrations. The G′ values increased when the protein concentration was higher. The higher the protein concentration, the faster the increase in G′ values. The gelation time increased with a decrease in protein concentration; gelation time were 40, 23.5, 21.5, 21, 25, and 20 minutes for 3.44%, 3.80%, 3.89%, 4.16%, 4.71% and 5.10% protein content, respectively. In addition, the higher the protein concentration the sooner the G′ versus time curve attained a state of steady increase with time.

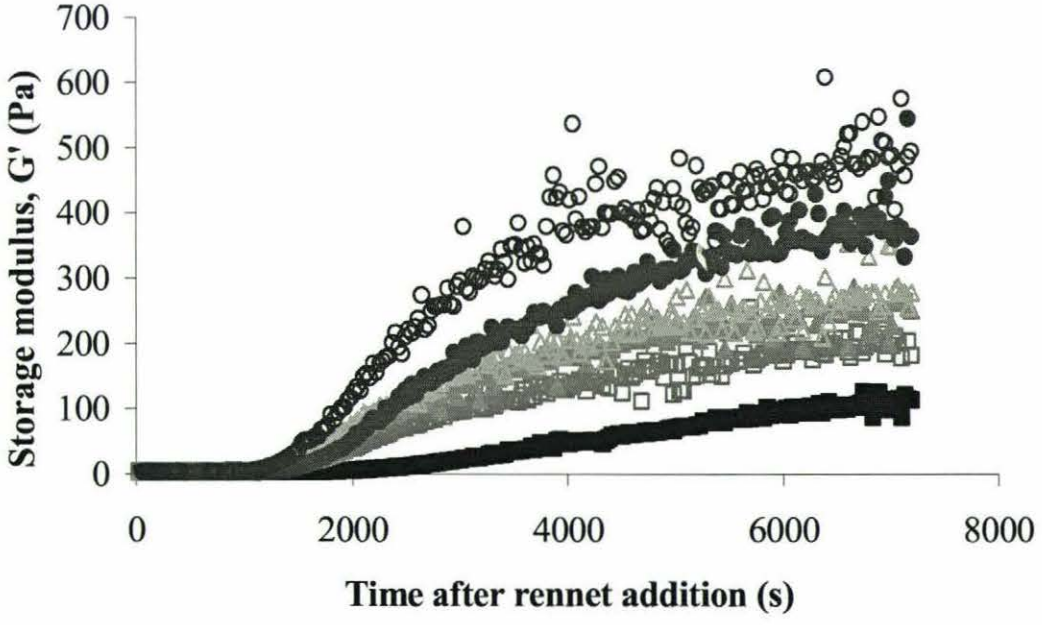


Figure 5.14 Storage modulus, G′, as a function of time, for skim milk containing 3.44% (■), 3.80% (□), 3.89% (▲), 4.16% (△), 4.71% (●), and 5.10% (○) protein and been renneted at 32°C and pH 6.2.

Waungana (1995) found that gelation time showed a linear decrease with increasing VCR, when skim milk samples were renneted at pH 6.5. The result for G′ reported in this study were essentially in agreement with Culioli and Sherman (1978), Reuter *et al.*, (1981), Green *et al.*, (1981), Green (1990) and Waungana (1995), who demonstrated that the rates of firming of curds formed from UF milk increased in proportion to the extent of concentration of the milk used in cheese manufacture. The increase in G′ could be attributed to the higher casein micelle concentration in these concentrates which, once renneted, aggregate at a faster rate and form stronger links due to their closer proximately (Waungana, 1995).

5.2.5.2 Permeability coefficient

The permeability plot of renneted milk gels containing different protein concentrations is shown in Figure 5.15. Only three concentrations were measured for permeability. There was no difference between samples containing 3.89% and 4.16% protein. The permeability was relatively higher for milk gels containing 3.44% protein compared to those containing 3.89% and 4.71% protein. The increase in permeability with time was faster for 3.44% protein sample than 3.89% and 4.71% protein samples.

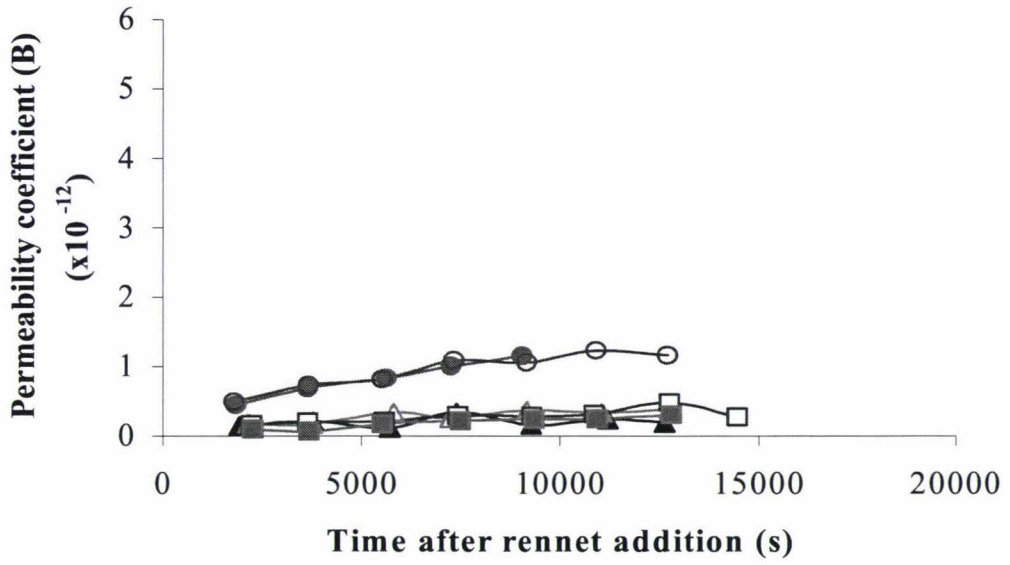
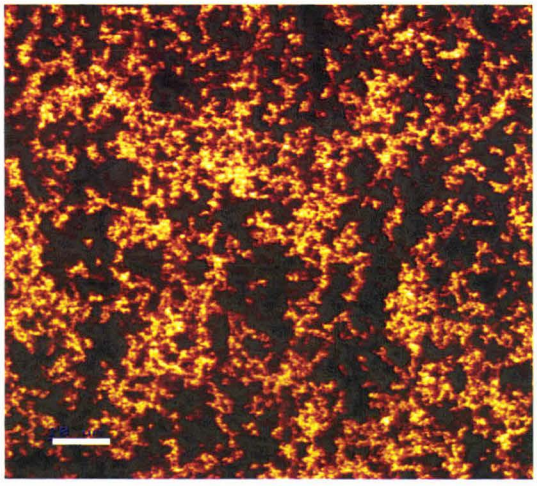


Figure 5.15 Permeability coefficients (B) as a function of time of the rennet skim milk gels containing 3.44% (● ○), 3.89% (■ □), and 4.71% (▲ △) of protein.

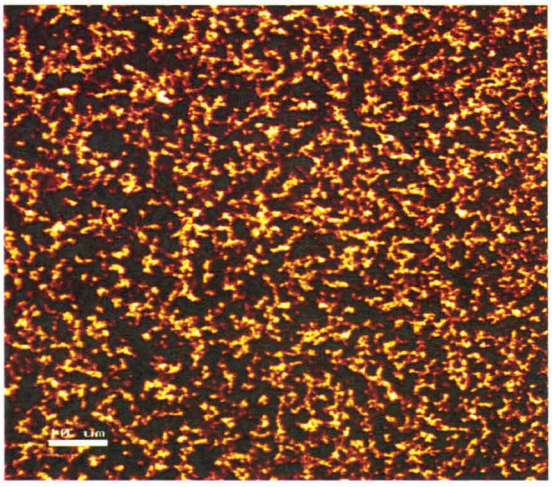
5.2.5.3 Confocal microscopy

The confocal micrographs of rennetted skim milk gels containing different protein concentrations are shown in Figure 5.16 (a) and (b). It appears that the higher the protein concentration the denser the structure. For the standard skim milk gel (a), the pores were quite open and large. By comparison, the structure was very dense and the pores were very small for 5.10% protein sample [Figure 5.16 (f)]. In general, the gel structures become more open as the protein content was decreased.

(a) Protein content 3.44%



(b) Protein content 3.80%



(c) Protein content 3.89%

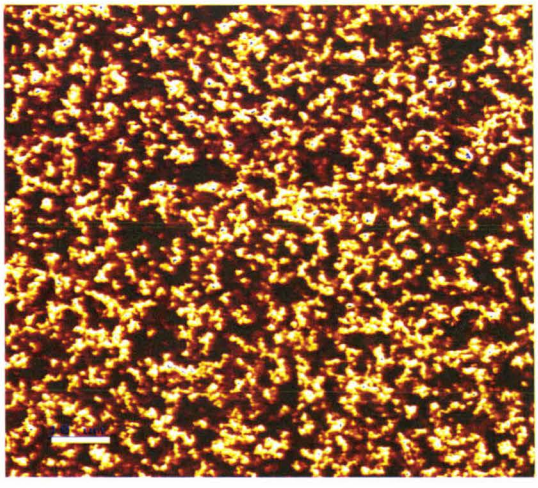
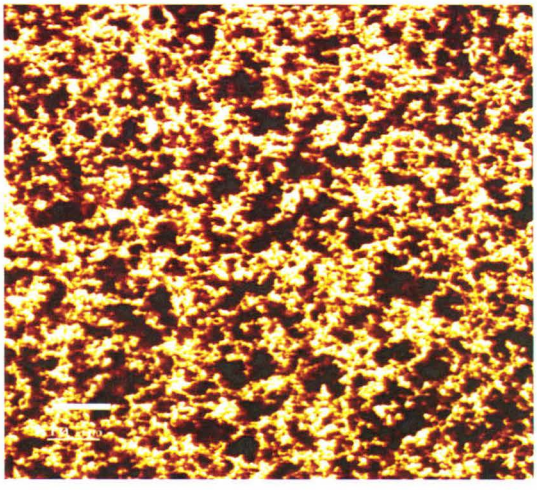
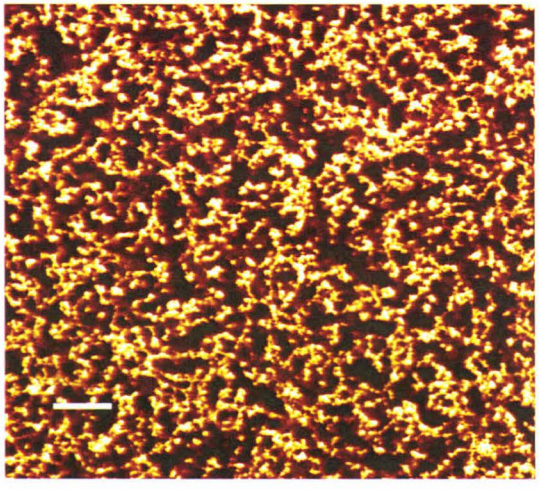


Figure 5.16(a) Confocal laser scanning micrographs of rennet skim milk gels made at 32°C with rennet concentration of 80μl/l of milk, pH 6.2 and protein content of 3.44%(a), 3.80% (b) or 3.89% (c). The gels were stained with Fast Green FCF, and the magnification is 400X. The scale bar represents 10 μm.

(d) Protein content 4.16%



(e) Protein content 4.71%



(f) Protein content 5.10%

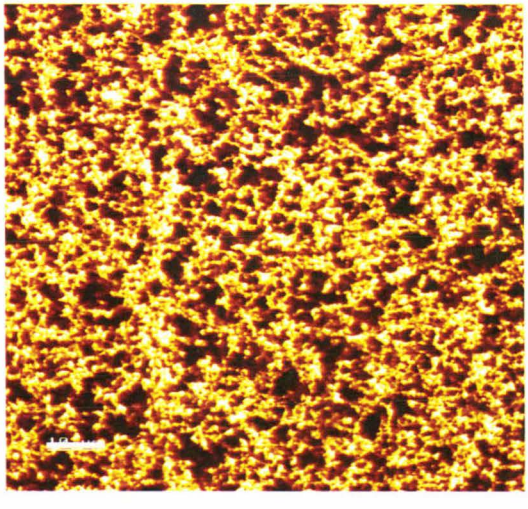


Figure 5.16(b) Confocal laser scanning micrographs of rennet skim milk gels made at 32°C with rennet concentration of 80μl/l of milk, pH 6.2 and protein content of 4.16% (d), 4.71% (e) or 5.10% (f). The gels were stained with Fast Green FCF, and the magnification is 400X. The scale bar represents 10 μm.

5.3 Effect of a combination of different conditions on the rennet milk gels

Having found the significant effects of renneting conditions on permeability and gel strength in section 5.2, an attempt was made to use more than one factor to achieve the benefit of these factors but not the drawbacks of any factor. If faster syneresis was desired, then high permeability is needed. However, high protein concentration and high pH would give low syneresis, hence it was decided to do the following comparison of cheesemaking conditions trials.

Table 5.2 shows the conditions that should produce rennet gels with high and low syneresis, based on the two extreme conditions required to produce different rennet milk gel structures. These conditions were determined from the results presented in section 5.2. It was assumed that if the gel had high permeability and low G′, the whey can travel through the gel matrix easier, hence the gel should expel more whey, resulting in low moisture cheese curd i.e. high syneresis – low pH and high temperature.

Table 5.2 The conditions used for making rennet gels with high and low level of syneresis

Conditions	**Condition 1 (High syneresis)**	**Condition 2 (Low syneresis)**
pH	6.2	6.5
Set Temperature (°C)	38	32
Protein (VCR)	No standardisation	1.2X
$CaCl_2$ addition (g/100ml)	0	0.02
*Rennet concentration (µl/100ml)	40	120

*Rennet concentration unit = µl of (1:10) diluted rennet / 100ml of milk

The rennet milk gels, made under the conditions shown in Table 5.2, were analysed for permeability and by confocal scanning microscopy. The purpose of these preliminary experiments was to test the hypothesis that if the combined conditions

would give the results expected from the effect of individual conditions indicated in section 5.2.

As expected, the milk gel made using condition 1 (high syneresis) had higher permeability than the gels made using condition 2 (low syneresis) (Figure 5.17). The permeability coefficients increased significantly as the time increased for condition 1 milk gels. As for condition 2, the permeability did not change much during the course of measurement. It also showed that the milk gels made using condition 1 collapsed much faster than those made using condition 2.

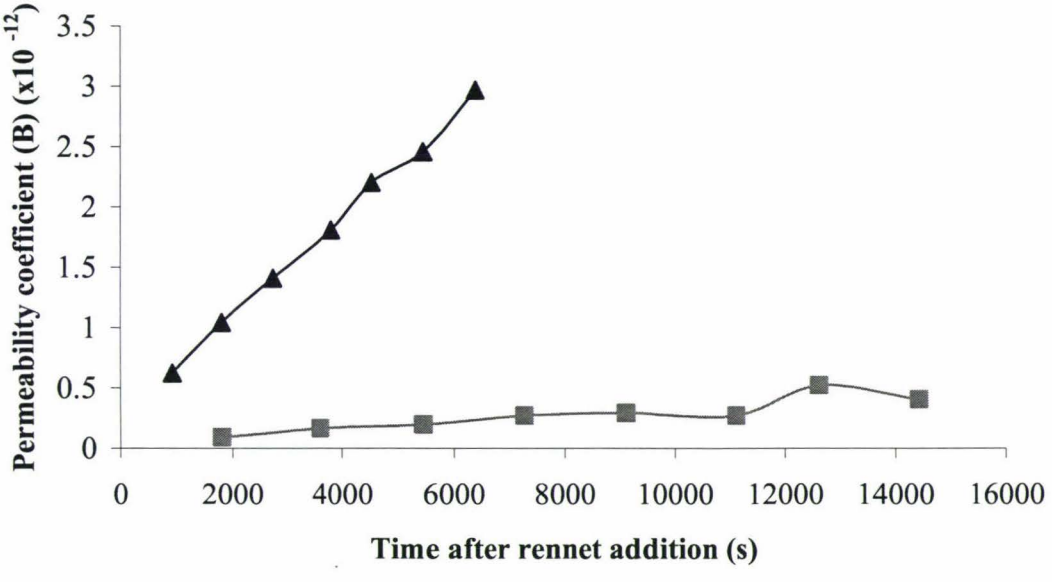


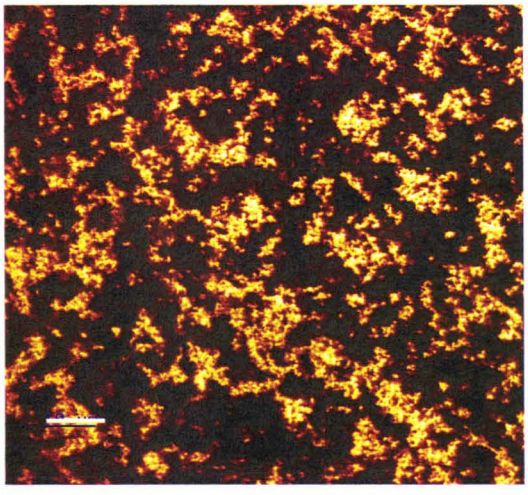
Figure 5.17 Permeability coefficients of renneted milk gels made using the conditions shown in Table 5.2 for high syneresis (▲) and low syneresis (■) systems.

It has been shown in section 5.2 that pH, temperature and protein concentration had significant effect on gel structure which has also been proven in this experiment. A combination of these factors also produces the expected effect as individual factors. Temperature and pH are most likely to have the greatest effect. These factors are believed to override the effect of other factors on the gel properties due to their stronger effects.

The confocal scanning micrographs of the high and low syneresis milk gels are shown in Figure 5.18. The micrographs showed a clear difference between the high and low syneresis system. The high syneresis system showed coarse network and large pores, which means a weak structure. The low syneresis system showed dense and small pore size structure which indicates a strong gel structure. These micrographs corresponded well with the permeability results. It was expected that gels that show high permeability will have coarser network and gels that show low permeability have the opposite, and the coarser network structure would expel more whey (moisture) out of the gel system.

From the results for permeability and confocal microscopy the combined conditions were proven to produce gels that have open, coarse structure or close, dense structure with different properties, as expected.

(a) High syneresis system



(b) Low syneresis system

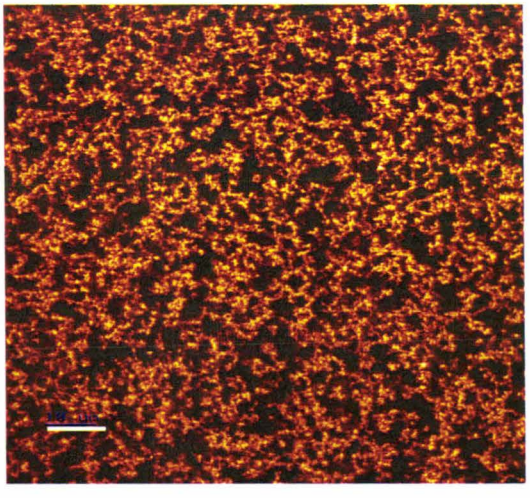


Figure 5.18 Confocal laser scanning micrographs of standard skim milk gels made using conditions (as shown in Table 5.2) for high syneresis system (a) and low syneresis system (b). The gels were stained with Fast Green FCF, and the magnification was 400X. The scale bar represents 10 μm.

5.4 Conclusions

The following general conclusions may be drawn from the results obtained in this chapter and the effect of various processing conditions on the properties of rennet gel are summarised in Table 5.3:

(1) The permeability coefficient is a useful parameter for describing the ability of a gel to allow whey to pass through it. The results showed that among the various factors studied, pH and temperature have the greatest effect on permeability. The permeability of skim milk gels increases with a decrease in pH and an increase in temperature. It was also found that gels made at 40°C, pH 6.2 and at 32°C, pH 5.8, the gels were permeable but collapsed after some time.

(2) Change in protein concentration has the greatest effect on G' values compared to the other factors investigated. The G' values increased with increases in protein concentration while the permeability decreased.

(3) Calcium chloride addition has a small effect on permeability of the milk gels. The permeability decreases slightly with $CaCl_2$ addition whereas the G' values increased with $CaCl_2$ addition.

(4) Rennet concentration had a small effect on permeability. Generally, the G' values increased with increased rennet concentration.

(5) The confocal microscopic observations are an effective examination method for supporting the results determined by other methods in this study. The confocal micrographs corresponded well with the results from permeability and rheological measurements.

(6) As independent factors, temperature, pH and protein concentration had the greatest effects on the gel structure and properties.

(7) A combination of conditions i.e. low pH, and high temperature produced open network structure with high permeability. These systems are expected to show high syneresis.

(8) A combination of conditions i.e. high pH, low temperature and 1.2X concentrated milk (protein content ~ 3.65%) produced dense network structure with low permeability. These systems are expected to show low syneresis.

Table 5.3 Summary of storage modulus (G′), gelation time (GT) and permeability coefficient (B) results.

Conditions	**Storage modulus (G′) (Pa) (after 120 mins)**	**Gelation time (GT) (mins)**	**Permeability coefficient (B) (X 10^{-12}) (at time ~ 7000s)**
pH – 5.8	173	6	2.67
6.2	202	10	1.17
6.5	184	21	1.09
Temperature – 25°C	344	57	0.73
32°C	148	21	1.09
40°C	184	16.5	3.1
Calcium concentration			
0%	113	40	1.09
0.01%	172	26.5	0.861
0.02%	180	27	0.63
Rennet concentration			
40 µl/l	120	46	1.09
80 µl/l	184	21	0.25
120 µl/l	181	15	0.29
Protein concentration			
3.44% (1X)	113	40.0	1.45
3.80% (1.04X)	204	23.5	
3.89% (1.13X)	264	21.5	1.09
4.16% (1.21X)	278	21.0	
4.71% (1.37X)	380	25.0	1.07
5.10% (1.48X)	495	20.0	

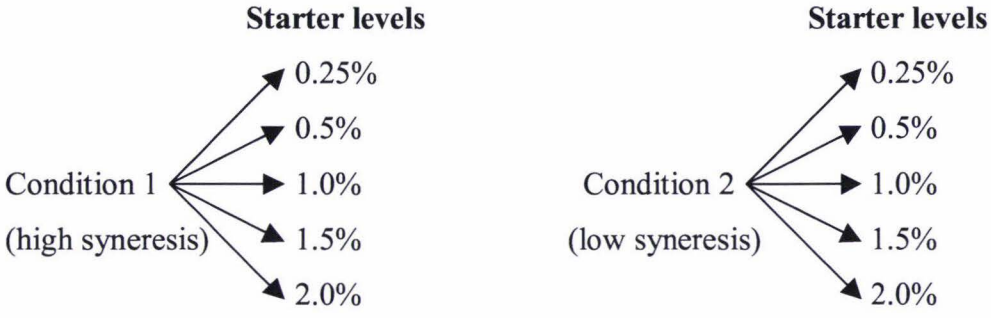
CHAPTER 6

MANUFACTURE OF CHEESE FROM "HIGH SYNERESIS" AND "LOW SYNERESIS" GELLING SYSTEMS

6.1 Introduction to syneresis tests

The gel characteristics of rennet curd, such as water-holding capacity and gel-strength, are important parameters in the cheesemaking process and affect characteristics such as yield, moisture content, and textural attributes. Gels formed from milk by renneting or acidification under quiescent conditions show syneresis i.e. expel whey because the gel contracts. Under quiescent conditions, the gel may lose two-thirds of its volume and up to nine-tenths if external pressure is applied. (Walstra *et al.*, 1985). Syneresis of rennet curd is affected by a number of variables such as pH, temperature, rate of heating, physical handling, addition of calcium chloride, amount of rennet, surface to volume ratio of curd and addition of various salts (Patel *et al.*, 1971). Therefore, a preliminary trial on the effect of a combination of the conditions found in Chapter 5 (Table 5.2) on the syneretic properties was investigated. Syneresis properties are important in determining the final moisture of the cheese produced and also the cheese yield.

Since the addition of starter is part of the cheesemaking process, 5 levels of starter addition were chosen for the present experiment as shown in Figure 6.1. A drainage test as outlined in section 4.4.4 was used to determine the rate of syneresis, as the conditions used reflects more on what happens during the cheesemaking process than measuring shrinkage while immersed in whey.



Note: Starter level % = ml of starter / 100ml of milk

Figure 6.1 The experimental design for determining the effect of combined conditions with starter addition on syneresis.

The drainage rate of high and low syneresis systems with different levels of starter addition are shown in Figures 6.2 and 6.3 respectively. It was found that the amount of drained whey increased with increased starter levels for both high and low syneresis systems, although, the difference in whey expelled between the starter levels was small. The milk gels expelled faster and more whey in the early stages and less and slower in the later stages which means that the whey released from gels increases with time to a limiting value. Not all the whey will be expelled during syneresis. For the high syneresis system, the gels expelled nearly 80% of their weight. As for the low syneresis system, the gels expelled about 70% of the total weight. As expected, the high syneresis system would expel more whey because of the coarser network structure. This is in an agreement with the results reported by Lelievre (1977).

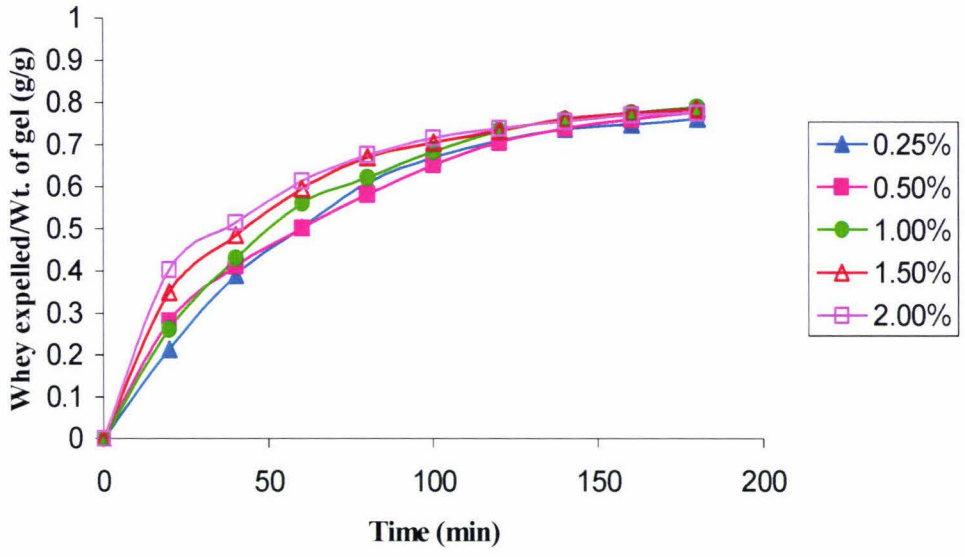


Figure 6.2 The amount of whey expelled per gram of rennet milk gel, as a function of time, for the high syneresis system, at starter levels of 0.25% (σ), 0.5% (ν), 1.0% (●), 1.5% (△) and 2.0% (□).

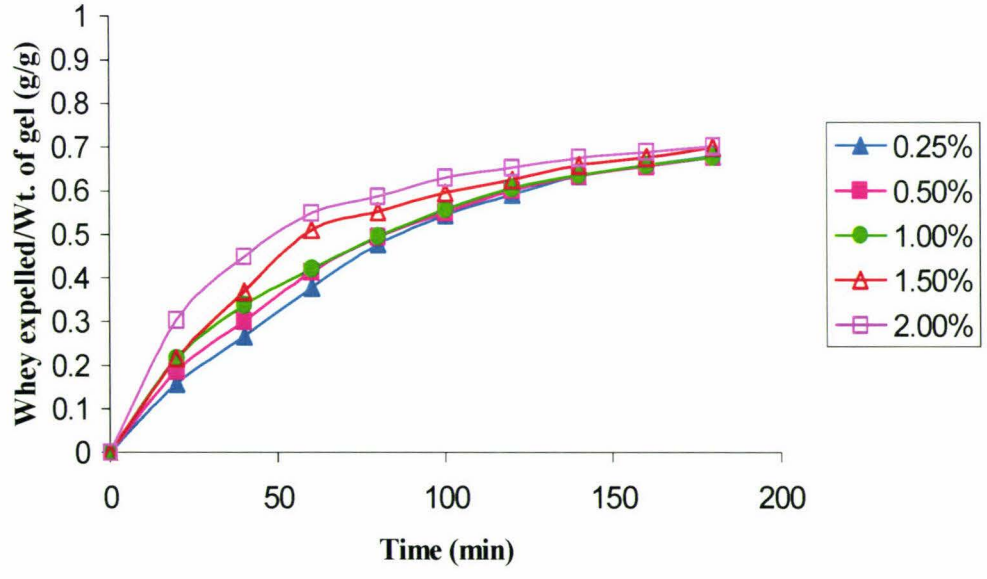


Figure 6.3 The amount of whey expelled per gram of rennet milk gel as a function of time, for the low syneresis system at starter levels of 0.25% (▲), 0.5% (ν), 1.0% (●), 1.5% (△) and 2.0% (□).

Generally, a good correlation was found between the rate of change in B (permeability) (refer to Figure 5.17) and the rate of syneresis (Figures 6.2 and 6.3). The rate of syneresis is determined by the balance of the syneresis pressure and the flow resistance of the expelled whey through the network (i.e. permeability). The process is complicated, since both pressure and permeability change with time and with gel shrinkage (Walstra and van Dijk, 1983). At a relatively low gel strength, whey drainage tends to increase while at high gel strength, the opposite effect is observed (Lelievre, 1977).

The conditions under which the syneresis experiments are performed may strongly affect the results and they may vary widely. Important variables are pH, temperature, size of curd pieces, ionic strength, degree of agitation, extent of cutting of the curd, the volume of whey surrounding the curd and application of external pressure (Walstra *et al.*, 1985; Pearse and MacKinlay, 1989). Disturbance of the gel during setting may considerably enhance syneresis rate because enlarges the free surface area.

In the present experiments, the free surface was kept constant for maximum syneresis (i.e. the curds were cut into same shapes). As a result, the effect of free surface would not be a variable. In the present study, the curds were not surrounded by the whey solution during the drainage process. As a result, the pH measured for the whey solution did not reach the desired pH as expected. Because the whey and the curd were separated, the starter may not be active because of the oxygen. Therefore, the pH did not decrease to the desired value. As the desired pH was not reached during the process, HCl was added to lower the pH of the curd to the desired pH (i.e. 5.95). However, sufficient time might not have been provided for the curd to attain the new pH. For this reason the effect of the starter level on the drainage process might not have been very accurate, it was expected to have a greater effect, as lower pH enhances syneresis.

The finding in this whey drainage test agreed with the hypothesis, which was that the high syneresis system would expel more whey since the structure of the rennet milk gels was coarser. It can be concluded from the results obtained from the experiments

in this section that the amount of whey expelled increased with higher starter addition levels for both high and low syneresis systems. However, the increase was small. From these findings, the cheesemaking conditions could be selected to make cheeses with desired properties, e.g. low in moisture content.

6.2 Pilot plant trial - I

Cheesemaking essentially involves the removal of most of the water in the form of whey from the remaining milk constituents. Consequently, careful control of moisture content is needed to obtain the desired characteristics in the final product. On the other hand, an improper gel structure of rennet curd could result in a higher loss of fat, starter and curd fines. It is important in cheesemaking to cut the curd at an appropriate firmness so that the whey drains properly while the loss of milk solids is minimised (Green, 1977). Variations in curd firmness at the time of cutting may result in losses of milk solids and reduce the cheese yield (McMahon and Brown, 1984)

In the previous chapter, conditions for producing high and low permeability rennet gels and hence 'high' or 'low' syneresis rennet gel systems were established. The objective of this work was to investigate how these gel systems behaved during the cheesemaking process and what impact this has on the cheese moisture content. It was expected that 'high syneresis' gel system would expel more whey and result in low moisture cheese, while the 'low syneresis' system would yield cheese with higher moisture content. A control sample was used in the present study for comparison. The standard processing conditions was used for this control sample. The permeability, rheological properties and microstructure were determined for all the cheese milk samples and the composition of all the final cheeses was also determined.

Table 6.1 shows the conditions used for the manufacture of various cheeses in the pilot plant. This trial was carried out over two consecutive days. Vat 1, 3, 4 were repeated in the second day of the trial.

Table 6.1 Pilot plant trial I: The processing conditions used in the manufacture of various cheeses (high and low syneresis systems).

	Control	High syneresis	High syneresis	Low syneresis	Low syneresis
Vat	1	2	3	4	5
Vat volume (kg)	375	375	375	375	375
P/F ratio	0.8	0.8	0.8	0.8	0.8
Protein standardisation	**none**	**none**	**none**	**1.24X**	**1.24X**
Past C/Sec	Standard	Standard	Standard	Standard	Standard
Temp milk to vat (°C)	**32.0**	**38.0**	**38.0**	**32.0**	**32.0**
Added $CaCl_2$ (%)	**0.01**	**none**	**none**	**0.02**	**0.02**
Prime pH	**15min**	**6.20**	**6.20**	**6.50**	**6.50**
Starter type	Mesophiles	Mesophiles	Mesophiles	Mesophiles	Mesophiles
Starter %	**2.0**	**1.0**	**2.0**	**1.0**	**2.0**
Set pH	15min	6.20	6.20	6.50	6.50
Set (°C)	32.0	38.0	38.0	32.0	32.0
Coagulant	Calf (ADS)	Calf (ADS)	Calf (ADS)	Calf (ADS)	Calf (ADS)
ml/100L	**9.0**	**6.0**	**6.0**	**12.0**	**12.0**
Cook (°C)	38	38	38	38	38
Drain pH (whey)	5.95	5.95	5.95	5.95	5.95
Mill pH	5.25	5.25	5.25	5.25	5.25
Salt addition rate (g/Kg)	26	26	26	26	26
Mellowing time (min)	45	45	45	45	45
Pressing	overnight	overnight	overnight	overnight	overnight

6.2.1 Rheology

Samples of cheese milk were taken from the vats, the required amount of rennet and starter added and the samples were then transferred to the rheometer. Figure 6.4 illustrates how storage modulus (G′) developed with time after rennet addition with different processing conditions. The increase of G′ with time for the high syneresis and control vats showed almost the same G′ values throughout the course of measurement and even the gelation times were very similar. A rapid increase in G′ occurred during the first 34 minutes, and then G′ reached a plateau value of about 150 Pa. The gelation times for the low syneresis vats were longer than the times of the control or high syneresis system. The increase in G′ for the low syneresis system was gradual and no plateau value was reached even after two hours. It is obvious that the G′ value of the low syneresis sample was lower than the high syneresis sample up to a renneting time of 50 minutes, but it was higher at longer renneting time. The G′ was about 300 Pa for the low syneresis sample after approximately 2 hours. The vats with 1% starter had slightly higher G′ than those with 2% starter for low syneresis sample.

The gelation times were about 9, 11, 12.5, 22.5, and 27 minutes for control (vat 1), high syneresis 1% (vat 2), high syneresis 2% (vat 3), low syneresis 1% (vat 4), and low syneresis 2% (vat 5) samples, respectively. Here, the gelation time is defined as the time when G′ exceeds 10 Pa.

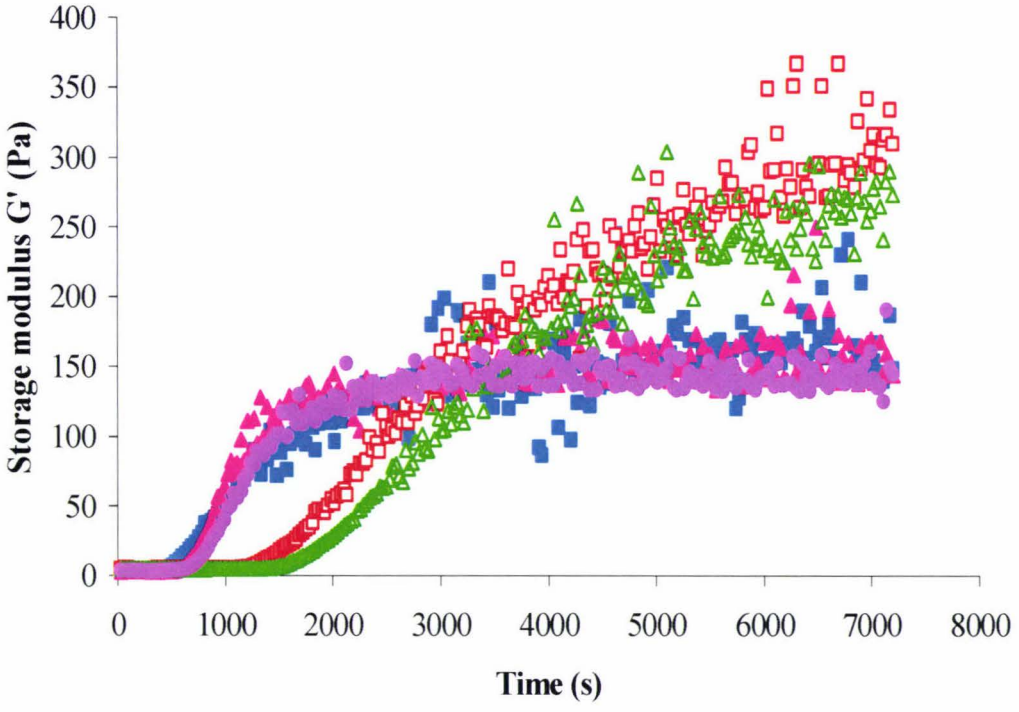


Figure 6.4 Storage modulus, G′, after rennet addition, as a function of time for control sample vat 1 (▽), high syneresis 1% starter vat 2 (▲), high syneresis 2% starter vat 3 (●), low syneresis 1% starter vat 4 (□) and low syneresis 2% starter vat 5 (△).

6.2.2 Permeability coefficient

The permeability coefficients of various samples, as a function of time are shown in Figure 6.5. The pH was adjusted to the desired pH (refer to Table 6.1); the pH should decrease during the measurements since starter would be active to reduce the pH of the curds. Permeability coefficients were markedly higher for the high syneresis samples compared with the low syneresis and the control samples. The permeability of the high syneresis sample increased rapidly in a short time for the high syneresis sample with 2% starter. The sample with 1% starter was slightly slower. The high syneresis gels 'collapsed' faster than the low syneresis and the control samples.

The permeability coefficients of the low syneresis samples with 1% and 2% starter were similar and they were both lower than the control sample. These results generally corresponded well with the storage modulus results which showed that high storage modulus after 2 hours may be linked with low permeability coefficients. They indicate strong and impermeable gels after two hours.

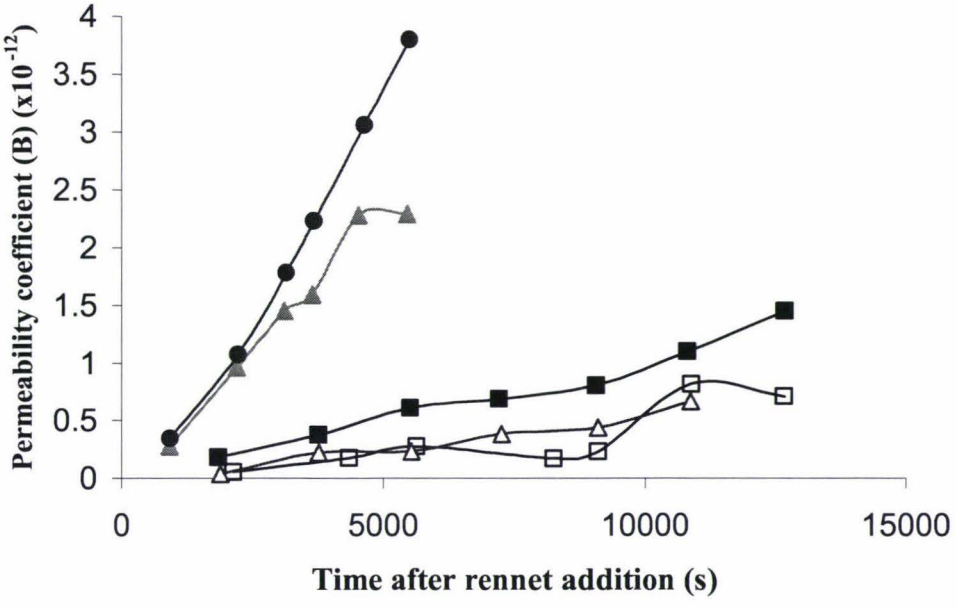


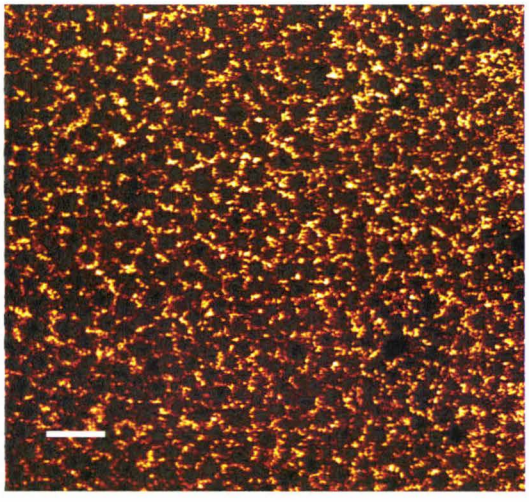
Figure 6.5 Permeability coefficients as a function of time of renneted cheese milk gels for control vat 1 (v), high syneresis 1% starter vat 2 (▲), high syneresis 2 % starter vat 3 (●), low syneresis 1% starter vat 4 (□), low syneresis 2% starter vat 5 (△).

6.2.3 Confocal microscopy

The effect of different processing conditions (refer to Table 6.1) on the microstructure of renneted whole milk gels made at 32 and 38°C, after one hour is shown in Figure 6.6. In the micrographs, the protein matrices appear bright yellow or orange, while the dark round structures immersed in them correspond to fat globules and entrapped air bubbles. The milk was not stained for examining fat globules and only the protein matrix was stained; therefore the fat globules appeared to be black round holes in the images.

Gels made from the control conditions (vat 1) appeared to have a network of dense clusters. High syneresis (vat 2 and 3) samples appeared to have coarser network and larger pores. The difference between vat 2 and 3 was not obvious. Low syneresis (vat 4 and 5) samples showed denser network and smaller gel pores; the fat globules were more evenly distributed than in other vats. It was difficult to see any differences between the microstructure of samples from vat 4 and 5.

(a) Vat 1



(b) Vat 2

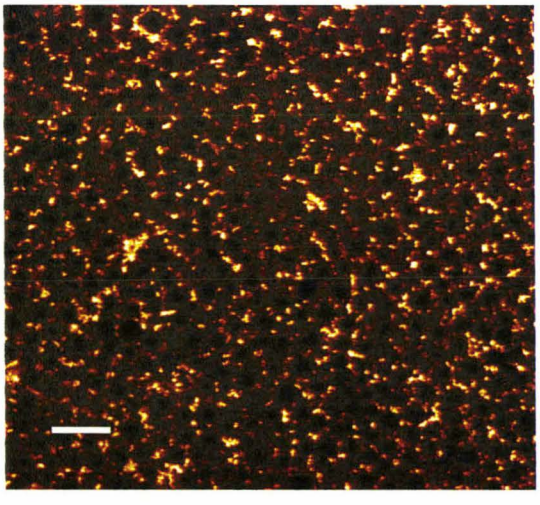
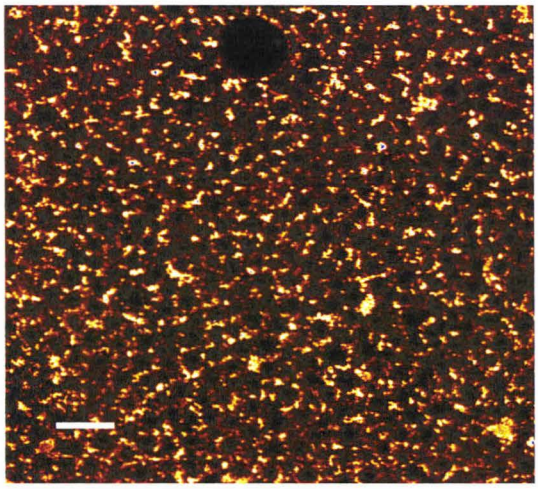
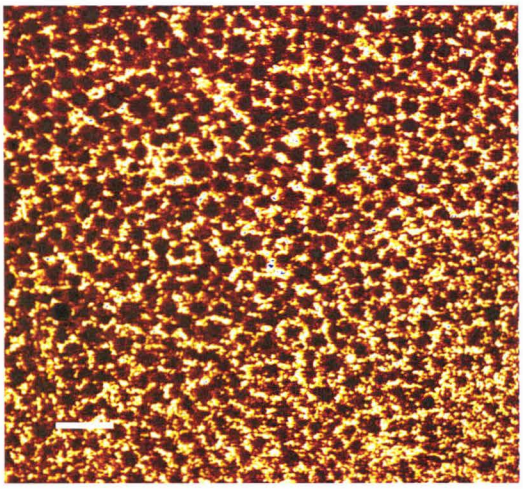


Figure 6.6(a) Confocal laser scanning micrographs of cheese milk gels made using processing conditions shown in Table 6.1 for vat 1 (a) and vat 2 (b) stained with Fast Green FCF, obtained with a 40X oil immersion objective (magnification 400X). The scale bar represents 10 μm.

(c) Vat 3



(d) Vat 4



(e) Vat 5

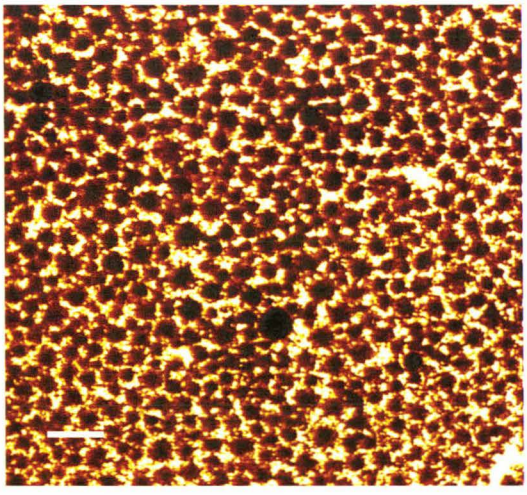


Figure 6.6(b) Confocal laser scanning micrographs of cheese milk gels made using processing conditions shown in Table 6.1 for vat 3 (c), vat 4 (d) and vat 5 (e) stained with Fast Green FCF, obtained with a 40X oil immersion objective (magnification 400X). The scale bar represents 10 μm.

The effect of the processing conditions on the syneresis of the whole milk gels is shown in Figure 6.7. As expected, the amount of whey expelled was highest for the gel made under high syneresis conditions with 2% starter i.e. vat 3, which expelled about 75% of the moisture. The amount of whey expelled for the high syneresis sample with 1% starter (i.e. vat 2) was similar to the control sample (i.e. vat 1). In the beginning, the amount of whey expelled was slightly higher for high syneresis sample with 2% starter. However, at the end of the measurement period (i.e. 200 minutes), the quantity of whey expelled was about the same for the high syneresis sample with 1% or 2% starter and the control sample. For the low syneresis samples, the 1% starter (i.e. vat 4) sample had a much lower rate of syneresis than the rest of the samples and the rate for the 2% starter sample was higher than that for the 1% starter (vat 5) sample. The amount of whey expelled was about 68% and 58% of the moisture for low syneresis samples made with 2% starter and 1% starter, respectively.

It appears that the amount of starter made a significant difference to the amount of whey expelled from the milk gel. For the gels made under low syneresis conditions, the 1 and 2% starter gels appeared to be distinctly different. The starter lowered the pH of the curd which probably caused contraction of the curd, hence more whey was expelled.

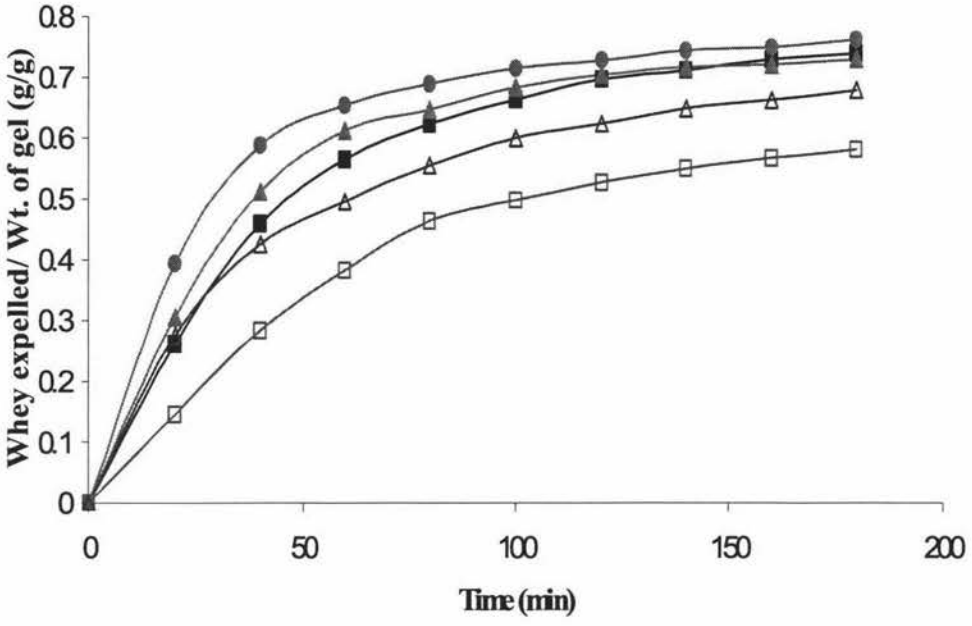


Figure 6.7 The amount of whey expelled per gram of milk gels as a function of time for control sample vat 1 (v), high syneresis 1% starter vat 2 (▲), high syneresis 2% starter vat 3 (●), low syneresis 1% starter vat 4 (□) and low syneresis 2% starter vat 5 (△).

The compositions of the final cheeses produced from vat 1 to 5 are listed in Table 6.2. The difference between the vats was processing conditions used as shown in Table 6.1. Also, the setting time for all cheese samples are shown in Table 6.3. The set to cut time was determined by the cheesemaker after examining the curd firmness.

Table 6.2 The compositional analysis of the final cheeses.

Sample	Fat content % w/w	Protein content % w/w (N* 6.38)	**Moisture content % w/w**	pH
Control (Vat 1)	38.0	25.07	**33.55**	5.34
High syneresis (Vat 2)	37.0	24.37	**34.50**	5.46
High syneresis (Vat 3)	37.0	25.01	**33.80**	5.36
Low syneresis (Vat 4)	38.5	25.07	**32.95**	5.36
Low Syneresis (Vat 5)	37.5	25.33	**32.75**	5.28

Table 6.3 The set to cut time in the cheesemaking processing.

Samples	Set to cut time (mins)
Control (vat 1)	33
High syneresis (vat 2)	21
High syneresis (vat 3)	21
Low syneresis (vat 4)	23
Low syneresis (vat 5)	23

The moisture contents of the final cheeses showed that the cheeses made with 'low syneresis' conditions had lower moisture contents (Table 6.2) than the cheeses made with 'high syneresis' conditions. The moisture content of the control sample was intermediate between low and high syneresis samples. The cheeses made with the higher starter level had lower moisture contents in both cases for the high and low syneresis conditions. These results were opposite of what was expected. The reason could be the different cutting times and also the time the curd spent in the whey before drainage. Table 6.3 showed that the times to cut the curds were different. Commonly, in the cheesemaking process, cheesemakers select the appropriate cutting time using their own subjective judgement. This was the case in this experiment.

Therefore, it was thought that the cutting time might have an effect on cheese moisture, since the milk gels were left for different times to attain the same (subjective strength) at cutting. The curds were cut at the same strength by subjective judgement; however, the curds were in fact having different gel firmness. Therefore, the different firmness at cutting affects the amount of whey expelled during the process. The firmer curds expel less whey and soft curds expel more, as expected from experiments carried out earlier (section 6.1).

During the cheesemaking, the desired pH at draining time and milling was not achieved for vat 4 (low syneresis). This might be due to the smaller amount of starter and the lower setting temperature. Therefore, the curd was left in the vat for relatively longer than the other vats. This might also be the reason for the different final cheese moisture.

The effect of pH at coagulation on moisture content of curd may be significant. Patel *et al.* (1971) found that moisture content increased as the pH at coagulation increased. This effect might be caused by decreased hydration of casein micelles as the isoelectric pH was approached. The effect of cooking temperature on cheese moisture was also found to be significant (Patel *et al.*, 1971); they found increases in cooking temperature significantly reduced the moisture content.

6.3 Pilot plant trial - II

The choice of the most appropriate time for cutting the rennet coagulum has long been regarded as an important decision in cheesemaking practice. Cutting too early, i.e. when the coagulum is too soft is widely claimed to affect cheese yield adversely due to increased loss of fat and curd fines into the whey. Cutting too late, i.e. when the coagulum is too firm, is claimed to retard whey release and lead to high moisture cheese (Mayes and Sutherland, 1984).

As the different cutting times used in the first pilot plant trial may have affected the moisture content in the final cheese, in the second pilot plant trial two cutting times were chosen to determine the effect of cutting time on the moisture content of the

final cheese. These two cutting times were decided based on the G′ versus time curves shown in Figure 6.4 (section 6.2); 33 minutes lies in the early stage of gel formation and 66 minutes lies in the later stage of gel formation. The gel structure would be relatively different when the curd was cut at these two different times.

Because in the first trial, the desired pH value was not attained, it was not certain whether or not the effect on the amount of whey released was affected by acid development. Therefore, in this pilot plant trial the same process was used as the first pilot plant trial for high and low syneresis systems. However, to determine the effect of the acid development one of the samples from both high and low syneresis systems did not have starter addition, hence there was no acid development i.e. the pH was constant throughout the whole process. Consequently, the pH effect can be determined throughout the cheesemaking process.

The moisture contents were determined at different stages; 50 minutes after cutting, after draining, before salting, at pressing and the final cheese. In this way, the moisture changes could be followed during processing.

Table 6.4 Pilot plant trial II: The processing conditions used for cheese production with or without starter.

	High syneresis	High syneresis	Low syneresis	Low syneresis
Vat	1	2	3	4
Vat volume (kg)	10	10	10	10
P/F ratio	0.8	0.8	0.8	0.8
Protein standardisation	none	none	1.24X	1.24X
Past C/Sec	Standard	Standard	Standard	Standard
Temp milk to vat (°C)	**38.0**	**38.0**	**32.0**	**32.0**
Added $CaCl_2$ (%)	**none**	**none**	**0.02**	**0.02**
Prime pH	**6.25**	**6.25**	**6.55**	**6.55**
Starter type	Mesophiles		Mesophiles	
Starter %	**2.0**	**none**	**2.0**	**none**
Set pH	6.20		6.50	
Set (°C)	**38.0**	**38.0**	**32.0**	**32.0**
Coagulant	Calf (ADS)	Calf (ADS)	Calf (ADS)	Calf (ADS)
ml/100L	**6.0**	**6.0**	**12.0**	**12.0**
Cook (°C)	38	38	38	38
Drain pH	5.95		5.95	
Mill pH	5.25		5.25	
Salt addition rate (g/Kg)	26	26	26	26
Mellowing time (min)	45	45	45	45
Pressing	overnight	overnight	overnight	overnight

Table 6.5 The composition of cheeses with different set to cut times

Set to cut time	Sample	Moisture content % w/w	Std. Dev.	pH
33 minutes	High syneresis (Vat 1)	38.05	0.404	5.38
	High syneresis (Vat 2)	41.98	0.050	6.24
	Low syneresis (Vat 3)	35.63	0.126	5.38
	Low syneresis (Vat 4)	42.50	0.346	6.53
66 minutes	High syneresis (Vat 1)	38.30	0.535	5.35
	High syneresis (Vat 2)	43.08	0.050	6.31
	Low syneresis (Vat 3)	36.58	0.222	5.43
	Low syneresis (Vat 4)	43.73	0.096	6.53

The moisture content of the final cheeses made with different cutting times are shown in Table 6.5. All the samples cut at 33 minutes had less moisture content compared with the samples cut at 66 minutes. The results showed that the cheeses made without the starter (vats 2 and 4) had higher moisture content. All the high pH and no starter cheese had significant higher moisture contents. No obvious differences between 'high syneresis' and 'low syneresis' systems for the cheese made without the starter additions were observed. When the cheeses were made with the starter (vats 1 and 3), the 'low syneresis' samples tend to be lower in moisture than the 'high syneresis' samples.

Figures 6.8 and 6.9 show that moisture content of cheese curds at different cutting time during the different stages of processing. The moisture decreased as the process went on. For curds cut at 33 minutes, the curds made without starter had higher moisture for both high and low syneresis systems (9 out of 10 cases). There was no obvious difference between the curds made with starter for high and low syneresis systems. For the curds made without starter, the low and high syneresis system showed no significant difference in moisture content during process and in the final cheese. Same trends also observed in Figure 6.9 which the curds were cut at 66 minutes setting time. There was no obvious difference in moisture content between two cutting time.

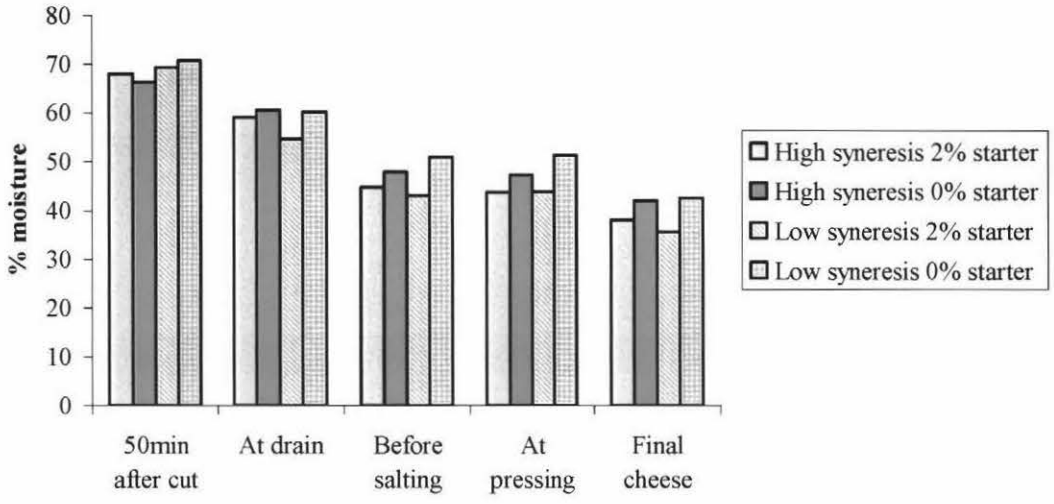


Figure 6.8 Percentage moisture for cheese curds cut at 33 minutes after rennet addition at different stages of the process for high syneresis system with 2% and 0% starter levels and low syneresis system with 2% and 0% starter levels.

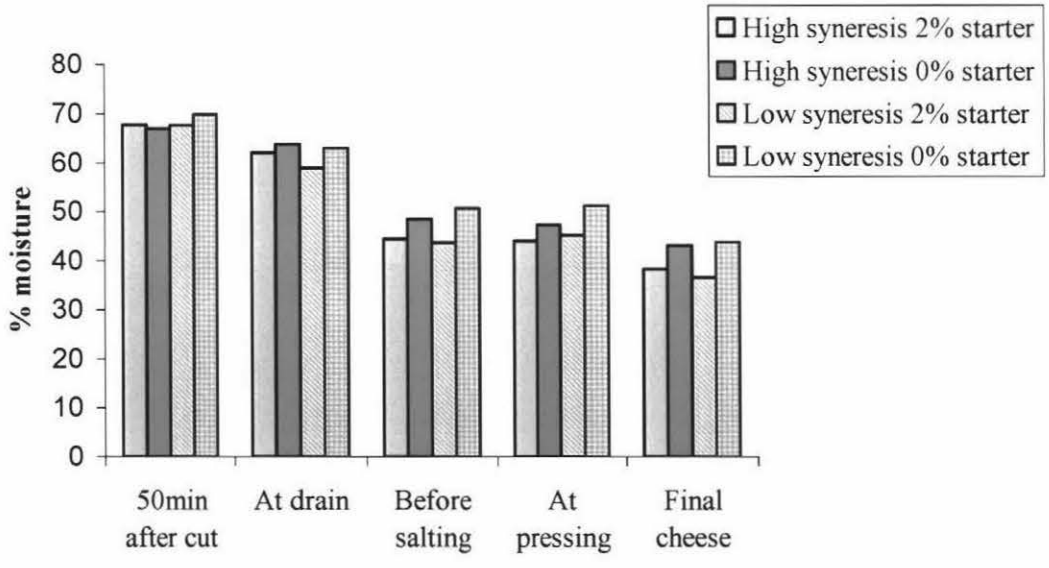


Figure 6.9 Percentage moisture for cheese curds cut at 66 minutes after rennet addition at different stages of the process for high syneresis system with 2% and 0% starter levels and low syneresis system with 2% and 0% starter levels.

It is obvious from the Table 6.5 and Figures 6.8 and 6.9, that pH had an effect on the moisture content of the cheese produced, since the cheeses made with the starter had lower moisture content. Therefore, lowering the pH caused the gel structure to rearrange and contract and therefore allow more moisture out of the matrix.

From Table 6.5, the low syneresis system with starter addition (e.g. vat 3) produced a lower moisture content cheese which was not expected since it was expected that high permeability gels would produce cheese with lower moisture content. One most important reason might be the 'syneretic power' of the gel which is the 'ability for the gel to synerese'. The permeability coefficient results were used as the basis for the syneresis properties of the gel which means that the more permeable the gels are, the more moisture should be lost. From the results of the cheesemaking, the cheeses did not show that. Therefore, there is an another more dominating factor that controls the moisture content of the final cheeses and it was not shown as the permeability of the gel. If the structure of the gel was established sooner in the process, then the synergetic power of the gel would be weak and the gel would not be able to expel whey out of the system, therefore, less moisture would be lost independent of permeability. The gels can be very permeable, but the structure of the gel may be so crosslinked that there is no room for moisture expulsion.

The other possible explanation for this is that the 'low syneresis curd' spent a considerably longer time in the whey compared to the 'high syneresis' curd (e.g. vat 1) (2 hour 36 minutes versus 1 hour 52 minutes). This effect seems to be accentuated in the final cheese i.e. lower moisture where the curd spent a longer time in the whey and more stirring after cutting. Walstra *et al.* (1985) measured the water content of curd cut 30 minutes after renneting and then the curd was taken out of the whey and put into a cheese mould for 2 and 6 hours with and without added starter. They found that the percentage of water content was ~ 50 and 10% for curd with added starter that was taken out at 2 and 6 hours respectively. Moreover, for curd without added starter, the % water content was ~55% and ~52% for curd that was taken out at 2 and 6 hours respectively. Therefore taking curd out of the whey at different times induces considerable differences in the moisture content.

Aleandri *et al.* (1989) found that an increase in curd firmness at cutting correlated with an increased cheese yield (increased moisture) but with a lower recovery of fat. Mayes and Sutherland (1984) found a small increase in moisture content with increased setting time (i.e. 16% of control). In addition, doubling of the setting time resulted in cheese with significantly higher moisture content. The observed relationship between setting time and cheese moisture content is similar to that observed by Gilles (1976) and has been explained by Green, Marshall and Glover (1983). They suggested that all of the protein structural changes during cheesemaking represent a continuation of the rennet-induced aggregation process and are governed by the same forces. Thus, syneresis is viewed as a consequence of the continued joining of casein strands into larger aggregates, with the aqueous phase being physically squeezed out. If this hypothesis is accepted, it is reasonable to predict that excessive delay in cutting of the coagulum would allow some of the syneresis potential of the protein matrix to be dissipated in the formation of a more extensively cross-linked and hence more rigid gel, thus leading to higher moisture content in the resultant cheese.

Recently, Johnson *et al.* (2001) found that increasing the coagulum firmness at cutting significantly increased cheese moisture and cheese yield. These results were attributed to the rigidity and structure of the network (Lagoueyte *et al.*, 1994). In their experiment, they used the milled curd method for making Cheddar cheese. In the stirred curd method of making Cheddar cheese, the differences in moisture between the cheeses may not have been as noticeable. The difference between the results reported by Johnson *et al.* (2001), other results and the present study might be the difference in manufacturing practices after the curd is cut. The expected difference in moisture content in cheese with different curd firmness could be less in a full fat cheddar cheese (present study) than in a lower fat cheddar cheese (Johnson's study).

There are inconsistent results on the effect of gel firmness at cutting on the cheese yield and moisture content (Bynum and Olson, 1982; Banks and Muir, 1984). Bynum and Olson (1982) reported that there was no correlation between cutting time and moisture of the cheese. The results of present study also found no significant difference in moisture content with increased curd firmness at cutting.

Johnson *et al.* (2001) stated that there would be a limit to the amount of increase in moisture obtained through increasing the rennet coagulation time. There will also be a point at which increasing coagulum firmness at cutting will not result in an increase in moisture in cheese. In addition, unless other manufacturing steps are carefully controlled, the increase in moisture obtained through cutting at a firmer coagulum stages will be negated (Johnson *et al*, 2001).

6.4 Conclusions

(1) Analysis of the permeability, rheological properties and confocal microscope examination showed that the high syneresis system had high permeability, lower G′ values and more open structure. Low syneresis system showed the opposite. This confirmed with the results from the previous chapter.

(2) The moisture content of the final cheese showed the low syneresis system had lower moisture content than the high syneresis system. This was unexpected from the results obtained for structural properties.

(3) The cheese made without starter or lower starter level had higher moisture content.

(4) The pH of the curd and whey did make a difference in determining the cheese moisture. The addition of starter lowers the pH of the curd and the whey which resulted in contraction of the curd, resulting in more whey being pushed out, therefore, lower moisture content.

(5) The different cutting time did not have significant effect on the moisture content of cheeses.

(6) The reason for the unexpected results for the moisture content might be the syneretic power of the gels and the time the curds spent in the whey solution before drainage.

CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

The initial stage of manufacturing most varieties of cheese involves the coagulation of milk by the addition of rennet enzyme. The structural changes occurring in milk during this stage have implications for the development of the physical properties of the finished cheese (Everett and Olson, 2000). During rennet coagulation, the rennet enzyme (mainly chymosin) hydrolyses a specific peptide bond of κ-casein. As a result, the casein particles lose their colloidal stability and a gel is formed under quiescent conditions. Several factors are considered to have significant effect on the gelation time and firming rate after the rennet addition. These factors include protein concentration, protein type, pH, temperature, calcium chloride addition, rennet concentration and prior heat treatment.

Extensive studies have been carried out on the structure and rheological properties of milk gels (Zoon *et al.*, 1988; Roefs *et al.*, 1985; van Dijk and Walstra, 1986). However, the information on how the structure of the gels impacts on the cheesemaking process is limited. Therefore, the objective of this research was to investigate the effects of the milk gel structures formed with different coagulation conditions and their impact on the cheesemaking process, especially on the curd syneresis properties and end cheese moisture content.

The present study consisted of two parts. The first part investigated the effect of varying several factors (temperature, pH, rennet concentration, protein concentration and addition of calcium chloride) on the rheological and structural properties of the skim milk rennet gels. The second part attempted to translate the findings of part one to control water flow during cheesemaking and to control water content in the final cheeses.

7.2 Effect of different conditions on the structure and physical properties of skim milk rennet gel

The effects of temperature, pH, rennet concentration, protein concentration and calcium chloride addition on the rheological properties, permeability, and microstructure of rennet gels were determined.

Studies of the effects of different pHs (5.8, 6.2, and 6.5) on the skim milk gels showed that the G′ value increased with a decrease in pH and the gelation time increased with pH. Permeability coefficients increased with a decrease in pH. The microstructures corresponded well with the permeability results. The higher the permeability coefficients the coarser the network structure. For temperatures (25, 32 and 40°C), the G′ values increased while gelation time decreased with an increase in temperature; G′ showed a maximum at 32°C. The permeability coefficients increased with increased temperature. The microstructure showed a coarser network structure for gels made at higher temperatures. For calcium chloride addition (no addition, 0.01% and 0.02%), there was a slight increase in storage modulus (G′) with $CaCl_2$ addition. However, there was no significant difference between the levels of addition for the permeability coefficients and the microstructures. For rennet concentration (40 μl/l, 80 μl/l and 120 μl/l), there was no significant difference between the different concentrations for the permeability coefficients and the microstructures observed. The G′ had increased slightly with increase in rennet concentrations. For different protein concentrations (3.45%, 3.80%, 3.89%, 4.16%, 4.71% and 5.10%), the permeability coefficient decreased with increase in protein content and the G′ increased with protein concentration. All the confocal micrographs corresponded well with the results from permeability and rheological analyses.

From the above study, three factors, e.g. temperature, pH and protein concentration were found to have a greater effect on the gel structure and properties. From these results, it was hypothesised that low pH, high temperature and normal protein concentration would produce coarser, open milk gels and therefore a high degree of syneresis. These systems are referred to as 'high syneresis' systems. Conversely, high pH, low temperature and high protein content would produce more rigid gels

with a lower ability to syneresis; this is referred to as 'low syneresis' system. Analysis of high and low syneresis systems showed that the high syneresis systems expelled more whey compared with a low syneresis system. The quantity of whey expelled was affected by the starter level. The higher the starter level the greater the amount of whey expelled during syneresis indicating that pH had a great effect on whey expulsion.

7.3 Cheesemaking process and cheese composition

It was assumed that the processing conditions used for making high syneresis rennet skim milk gels would produce a low-moisture cheese since the gels made under these conditions had more open and coarser structure and caused the gels to expel more moisture during syneresis. However, the final cheese composition showed the opposite results. The high syneresis conditions produced a higher final moisture content cheese, and the low syneresis conditions produced a lower moisture content cheese.

After reviewing the process, it was found that the curd was cut at different firmness according to the time at cutting as shown in Figure 6.4 (Chapter 6). Also the final pH of some of the vats was not achieved, due to the smaller amount of starter used. For these reasons, the processing conditions were redesigned to investigate the effect of gel firmness and pH on the moisture content of cheese.

Unfortunately, the results were still inconclusive. It is certain that addition of starter lowers the moisture in the final cheese and 'low syneresis' conditions tended to give cheese higher in moisture than that made using 'high syneresis' conditions. One possible reason might be that the 'syneretic power' of the gel which is the ability of the gel to synerese significantly affects the water retention within the gel matrix. The permeability does not represent the water flow in the gel. If the structure of the gel was established early then there would be less opportunity for the water/whey to be squeezed out of the gel. The syneretic power appeared to be dominant in determining the final cheese moisture. The other reason is that during cooking, the low syneresis curds spent longer time in the whey than the high syneresis curd.

It was mentioned in the study of Pearse and MacKinlay (1989) that if whey is physically squeezed from the curd in a similar way as water is squeezed from a sponge, then changing the structure of the curd would be expected to affect syneresis properties. However, this effect was not observed in the study of Pearse *et al.* (1986). Similarly, curd strength at cutting would be expected to affect syneresis with the prediction being that weaker gels should be favorable for syneresis, because they would be compressed more easily during agitation. In a number of investigations however, the rate of syneresis was independent of cut time and therefore of curd firmness (Pearse *et al.*, 1984; Storry and Ford, 1982). Therefore, the syneresis process appears to be more than just a physical or chemical process. It is a combination of a range of mechanical processes and the interaction of several chemical processes.

7.4 Recommendations

Production of cheese involves several important factors and changing the conditions should produce different kind of cheeses. Much more remains to be investigated to have a clear understanding of factors that influence the properties of the rennet-induced milk gels and their impact on cheese production. Based on the findings of this project, the following areas are recommended for further study.

Process conditions

(1) As mentioned, during the cheesemaking process, there are a number of factors that need to be monitored and conditions usually change, for instance pH, temperature and pressure acting on the curd, during the manufacturing process. Moreover, several factors are interrelated, for instance, temperature affects the rate of acidification (Walstra *et al.*, 1985). During the pilot plant trial, a number of factors were changed at the same time. Consequently, the effect of individual factors could not be determined. For future study, altering a limited number of factors would be desirable to determine the influence of the important variables and also limit the effect of interactions between the factors.

(2) To observe the effect of gel structure on the cheese syneretic properties and microstructure, the standardisation of the mechanical parameters (cutting time, cooking time, whey surrounding and so on) would be important. All mechanical parameters should be controlled to make the conditions as similar as possible to eliminate any effect on the final cheese. From the results obtained from this project, the mechanical variables probably had significant effects on the cheese properties. Hence, investigation of the influence of mechanical variables (cutting time, cooking time and temperature, stirring, pressing) would facilitate the understanding of the changes that the curds undergo during the process, since all these factors significantly influence the moisture content of the cheese.

(3) There are few methods that have been suggested for reducing moisture content of cheese. Johnson *et al.* (2001) mentioned that to decrease the cheese moisture the following factors are important: softer coagulum at cutting, higher pH at rennet addition, smaller particle size at cutting, more stir-out after cutting, increase acid development during stir-out, more stir-out time after whey and curd separation, and higher cooking temperatures. Washing the curd i.e. adding water after part of the whey has been removed, was reported to enhance syneresis and to give slightly lower water content (van den Berg and de Vries, 1975). These factors could be taken into account if further moisture content reduction is desired in the cheese production.

(4) Careful control of moisture content is desired since improper moisture content of the cheese would result in poor body and textural properties and at the same time would influence the ripening characteristics of cheese. Optimising the conditions to produce desired cheese moisture is reasonable, however other resulting effects should also be noted i.e. variations in curd firmness at cutting may result in greater losses of milk components and reduced cheese yield (Bynum and Olson, 1982). The flavour, texture, melt and firmness all could be changed with the altering of the process conditions. Sensory evaluation would be the sensible future step to determine if the cheeses produced are acceptable.

REFERENCES

Aleandri, R., Schneider, J.C. and Buttazzoni, L.G. (1989). Evaluation of milk for cheese production based on formagraph measures. *Journal of Dairy Science*, **72**: 1967-1975.

Banks, J.M. and Muir, D.D. (1984). Coagulum strength and cheese yield. *Dairy Industries International*, **39**: 17, 19, 21, 36.

Banks, J.M. (1988). Elimination of the development of bitter flavour in Cheddar cheese made from milk containing heat-denatured whey protein. *Journal of the Society of Dairy Technology*, **40**: 37-41.

Berridge, N.J. (1942). The second phase of rennet coagulation. *Nature*, **149**: 194-197.

Bines, V.E., Young, P. and Law, B.A. (1989). Comparison of Cheddar cheese made with recombinant calf chymosin and with standard calf rennet. *Journal of Dairy Research*, **56**: 657-664.

Bringe, N.A. and Kinsella, J.E. (1986). Influence of calcium chloride on the chymosin-initiated coagulation of casein micelles. *Journal of Dairy Research*, **53**: 371-379.

Bynum, D.G. and Olson, N.F. (1982). Influence of curd firmness at cutting on Cheddar cheese yield and recovery of milk constituents. *Journal of Dairy Science*, **65**: 2281-2290.

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

Cheeseman, G.C. (1962). Syneresis of milk and caseinate solutions. 16th International Dairy Congress, B: 465-472.

Cheryan, M., van Wyk, P.J., Olson, N.F. and Richardson, T. (1974). Secondary phase and mechanism of enzymic milk coagulation. *Journal of Dairy Science*, **58(4)**: 477-481.

Culioli, J. and Sherman, P. (1978). Rheological aspects of the renneting of milk concentrated by ultrafiltration. *Journal of Texture Studies*, **9**: 257-281.

Dalgleish, D.G. (1983). Coagulation of renneted bovine casein micelles: dependence on temperature, calcium ion concentration and ionic strength. *Journal of Dairy Research*, **50**: 331-340.

Dalgleish, D.G. and Law, A.J.R. (1989). pH-induced changes of bovine casein micelles. II. Dissociation of calcium and phosphate. *Journal of Dairy Research*, **56**: 727-735.

Dalgleish, D.G. (1992). The enzymatic coagulation of milk. In *Advanced Dairy Chemistry Volume 1: Protein.* P.F. Fox (Ed). Elsevier Science Publishers Ltd. Essex, London. pp 529-620.

Dalgleish, D.G. (1993). The enzymatic coagulation of milk. In *Cheese: Chemistry, Physical and Microbiology. Vol 1. General Aspects.* P.F. Fox (Ed). Chapman & Hall, London, England, pp 69-100.

Dalgleish, D.G. (1998). Casein Micelles as colloids: surface structures and stabilities. *Journal of Dairy Science*, **81**: 3013-3018.

Daviau, C., Famelart, M.H, Goudédranche, Pierre, A., D., Garnier, M. and Maubois, J.L. (2000). Rennet coagulation of skim milk and curd drainage: effect of pH, casein concentrtration, ionice strength and heat treatment. *Lait*, **80(4)**: 397-415.

Eigel, W.N., Butler, J.E., Ernstrom, C.A., Farrel, H.M.Jr., Harwalker, V.R., Jenness, R. and Whitney, R. McL (1984). Nomenclature of proteins of cow's milk. Fifth revision. *Journal of Dairy Science*, **67**: 1599-1631.

Ernstrom, C.A. and Wong, N.P. (1974). Rennin action and cheese chemistry. In *Fundamental of Dairy Chemistry*. B.H. Webb, A.H. Johnson and J.A. Alford (Ed.). AVI Publishing Co., Westport, CT. pp 662.

Everett, D.W. and Olson, N.F. (2000). Dynamic rheology of renneted milk gels containing fat globules stabilized with different surfactants. *Journal of Dairy Science*, **83**: 1203-1209.

Foltmann, B. (1987). General and molecular aspects of rennets. In *Cheese: Chemistry, Physics and Microbiology, volume 1*. P.F. Fox (Ed.). Elvisier Applied Science, London. pp 33-61.

Fox, P.F. (1987). Cheese manufacture: chemical, biochemical and physical aspects. *Dairy Industries International*, **52**: 11-13.

Fox, P.F., Guinee, T.P., Cogan, T.M. and McSweeney, P.L.H. (2000). *Fundamentals of cheese science*. Aspen Publishers Inc. Gaithersburg, Maryland. pp 104

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

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

Gilles, J. (1976). Control of salt in moisture levels in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology*, **11**: 219-221.

Gouda, A. El-Zayat, A. I. El-Shabrawy, S. A. (1985). Partition of some milk salts and curd properties as affected by adding sodium chloride to the milk. *Deutsche Lebensmittel-Rundschau,* **81(7)**: 216-218.

Green, M.L. (1977). Review of the progress of dairy science: milk coagulants. *Journal of Dairy Research*, **44**: 159-188.

Green, M.L. and Marshall, R.J. (1977). The acceleration by cationic materials of the coagulation of casein micelles by rennet. *Journal of Dairy Research*, **44**: 521-531.

Green, M.L., Hobbs, D.G., Morant, S.V. and Hill, V.A. (1978). Intermicellar relationships in rennet-treated separated milk. *Journal of Dairy Research*, **45**: 413-422.

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

Green, M.L. (1982). Effect on the composition and properties of casein micelles of interaction with ionic materials. *Journal of Dairy Research*, **49**: 87-98.

Green, M. L. Marshall, R. J. and Glover, F. A. (1983). Influence of homogenization of concentrated milks on the structure and properties of rennet curds. *Journal of Dairy Research*, **50(3)**: 341-348.

Green, M.L., Scott, K.J., Anderson, M., Griffin, M.C.A and Glover, F.A. (1984). Chemical characterisation of milk concentrated by ultrafiltration. *Journal of Dairy Research*, **51**: 267-278.

Green, M.L. (1990). Cheddar cheesemaking from whole milk concentrated by ultrafiltration heated to 90°C. *Journal of Dairy Research*, **69**:559-569.

Green, M.L. and 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). Chapman & Hall Inc. London. pp109-111.

Grufferty, M.B. and Fox, P.F. (1985). Effect of added NaCl on some physicochemical properties of milk. *Irish Journal of Food Science & Technology,* **9(1)**, 1-9.

Guinee, T.P., Pudja, P.D. and Mulholland, E.O. (1994). Effect of milk protein standardization, by ultrafiltration, on the manufacture, composition and maturation of Cheddar cheese. *Journal of Dairy Research*, **61**: 117-131.

Hicks, C.L., O'Leary, J. and Bucey, J. (1988). Use of recombinant chymosin in the manufacture of Cheddar and Colby cheese. *Journal of Dairy Science*, **71**: 1127-1131.

Hossain, M. A. (1976). The influence of protein fractions on the coagulation of milk and firmness of the curd. *Kieler Milchwirtschaftliche Forschungsberichte*, **28(1)**: 43-58.

Holt, C. (1992). Structure and stability of the bovine casein micelles. In *Advances in Protein Chemistry*. C.B. Anfinsen, J.D. Edsall, F.K. Richards and D.S. Eisenberg (Eds), **43**, 63-151.

Horne, D.S. (1998). Casein interactions: casting ling on the Black Boxes, the structure in dairy products. *International Dairy Journal*, **8**: 171-177.

Humme, H.E. (1972). The optimum pH for the limited specific proteolysis of χ-casein by rennie (primary phase of milk clotting). *Netherlands Milk and Dairy Journal*, **26**: 180-185.

Jen, J.J. and Ashworth, U.S. (1970). Factors influencing the curd tension of rennet coagulated milk – Salt balance, *Journal of Dairy Science*, **53(9)**: 1201-1206.

Johnson, M.E., Chen, C.M. and Jaeggi, J.J. (2001). Effect of rennet coagulation time on composition, yield, and quality of reduced-fat Cheddar cheese. *Journal of Dairy Science*, **84**: 1027-1033.

Kalab, M. (1979). Scanning electron microscopy of dairy products: an overview. *Scanning Electron Microscopy*, **3**: 261-271.

Kaytanli, M., Erdem, Y.K. and Tamer, I.M. (1994). Factors affecting whey drainage rate of renneted skim milk gels: kinetic approach. *Milchwissenschaft*, **49(4)**: 197-200.

Kim, B.Y. and Kinsella, J.E. (1989). Effect of temperature and pH on the coagulation of casein. *Milchwissenschaft*, **44(10)**: 622-625.

Kowalchyk, A.W. and Olson, N.F. (1977). Effect of pH and temperature on the secondary phase of enzymatic milk clotting by rennet. *Journal of Dairy Science*, **60(8)**: 1256-1259.

Lagoueyte, N., Lablee, J., Lagaude, A. and Tarodo De La Fuente, B. (1994). Temperature affects microstructure of renneted milk gel. *Journal of Food Science*, **59(5)**: 956-959.

Lawrence, R.C. and Gilles, J. (1980). The assessment of the potential quality of young Cheddar cheese. *New Zealand Journal of Dairy Science and Technology*, **15**: 1-12.

Lawrence, R.C., Gilles, J. and Creamer, L.K. (1993). Cheddar cheese and related dry-salted cheese varieties. In *Cheese: Chemistry, Physics and Microbiology. Vol 2. Major Cheese Groups*. .P.F. Fox (Ed.). Chapman & Hall, London, England, pp 1-38.

Lelievre, J. (1977). Rigidity modulus as a factor influencing the syneresis of renneted milk gels. *Journal of Dairy Research*, **44**: 611-614.

Lelievre, J. and Lawrence, R.C. (1988). Review: Manufacture of cheese from milk concentrated by ultrafiltration. *Journal of Dairy Research*, **55**: 465-478.

Lomholt, S.B. and Qvist, K.B. (1999). Gel firming rate of rennet curd as a function of rennet concentration. *International Dairy Journal*, **9**: 417-418.

Lucey, J.A. and Fox, P.F. (1993). Importance of calcium and phosphate in cheese manufacture: a review. *Journal of Dairy Science*, **76**: 1714-1724..

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.). Brussels: International Dairy Federation.

Lucey, J.A., van Vliet, T., Grolle, K., Geurts, T. and Wlastra, P. (1997). Properties of acid casein gels made by acidification with glucono-δ-lactone. 2. Syneresis, permeability and microstructural properties. *International Diary Journal*, **7**: 389-397.

Marshall, R.J. (1982). An improved method for measurement of the syneresis of curd formed by rennet action on milk. *Journal of Dairy Research*, **49**: 329-336.

Mayes, J.J. and Sutherland, B.J. (1984). Coagulum firmness and yield in Cheddar cheese manufacture – the role of the curd firmness instrument in determining cutting time. *The Australian Journal of Dairy Technology*, June: 69-73.

McGann, T.C.A. and Pyne, G.T. (1960). The colloidal phosphate of milk. III. Nature of its association with casein. *Journal of Dairy Research*, **27**: 403-417.

McMahon, D.J., Brown, R. J., Richardson, G. H., Ernstrom, C. A. (1984). Effects of calcium, phosphate and bulk culture media on milk coagulation properties. *Journal of Dairy Science*, **67(5)**: 930-938.

McMahon, D.J. and Brown, R.J. (1984). Enzymic coagulation of casein micelles: a review. *Journal of Dairy Science*, **67**: 919-929.

Mehaia, M.A. and Cheryan, M. (1983). Coagulation studies of ultrafiltration-concentrated skim milk. *Milchwissenschaft*, **38**: 708-710.

Mellema, M. (2000). Scaling relations between structure and rheology of ageing casein particle gels. Universal Press, Veenendaal. pp2-9, 37-61, 91-118.

Patel, M.C., Lund, D.B. and Olson, N.F. (1971). Factors affecting syneresis of rennetd milk gels. *Journal of Dairy Science*, **55(7)**: 913-918.

Patel, M. C., Lund, D. B. and Olson, N. F. (1972). Factors affecting syneresis of renneted milk gels. *Journal of Dairy Science*, **55(7)**: 913-918.

Payens, T. A. J. and Both, P. (1980). Enzymatic clotting processes. IV. The chymosin-triggered clotting of p- kappa -casein. *Advances in Chemistry Series,* **188**: 129-141.

Payens, T.A.J. and Visser, S. (1981). What determines the specificity of chymosin towards χ-casein? *Netherlands Milk and Dairy Journal*, **35**: 387-389.

Pearce, K.N. (1976). Moving boundary electrophoresis of native and rennet-treated casein micelles. *Journal of Dairy Research*, **43**: 27-36.

Pearse, M. J., Mackinlay, A. G., Hall, R. J., Linklater, P. M. (1984). A microassay for the syneresis of cheese curd. *Journal of Dairy Research*, **51(1)**: 131-139.

Pearse, M.J., Linklater, P.M., Hall, R.J. and Mackinlay, A.G. (1986). Effect of casein micelle composition and casein dephosphorylation on coagulation and syneresis. *Journal of Dairy Research*, **53(3)**: 381-390.

Pearse, M.J. and Mackinlay, A.J. (1989). Biochemical aspects of syneresis: A review. *Journal of Dairy Science*, **72**: 1401-1407.

Phelan, J.A. (1975). Cheese. Incorporation of denatured whey protein into cheese. An *Foras Taluntais: Animal Production Research Report*: 129-130.

Pomprasirt, V. (1996). Physical and rennet coagulation properties of recombined cheese milk made from milk protein concentration (MPC-56) – A Master Thesis. Massey University, Palmerston North, New Zealand.

Pszczola, D.E. (1989). Rennet containing 100% chymosin increases cheese quality and yield. *Food Technology*, **43**: 84, 88-89.

Pyne, G.T. and McGann, T.C.A. (1960). The colloidal phosphate of milk. III. Nature of its association with casein. *Journal of Dairy Research*, **27**: 403-417.

Pyne, G.T. (1962). Review of the progress of dairy science: section C. Dairy chemistry – some aspects of the physical chemistry of the salts of milk. *Journal of Dairy Research*, **29**: 101-130.

Ramkumar, C. (1997). The effect of pH shift on early cheese maturation – A PhD Thesis. Massey University, Palmerston North, New Zealand.

Reuter, H., Hisserich, D. and Prokopek, D. (1981). Study on the formal kinetics of rennet coagulation of milk concentrated by ultrafiltration. *Milchwissenschaft*, **36(1)**: 13-18.

Roefs, S.P.F.M., Walstra, P, Dalgleish, D.G. and Horne, D.S. (1985). Preliminary note on the change in casein micelles caused by acidification. *Netherlands Milk and Dairy Journal*, **39**: 119-122.

Roefs, S. P. F. M., Walstra, P., Dalgleish, D. G. and Horne, D. S. (1986). Preliminary note on the change in casein micelles caused by acidification. *Netherlands Milk & Dairy Journal*, **39(2)**: 119-122.

Roefs, S.P.F.M., van Vilet, T., van den Bijgaart, H.J.C.M., de Groot-Mostert and Walstra, P. (1990). Structure of casein gels made by combined acedification and rennt action. *Netherlands Milk and Dairy Journal*, **44**: 159-188.

Rollema, H.S. (1992). Casein association and micelle formation. In *Advanced Dairy Chemistry Volume 1: Protein*. P.F. Fox (Ed.). Elsevier Science Publishers Ltd. Essex, London. pp 111-140.

Scott, R. (1998). Cheesemaking Practice. R.K. Robinson and R.A.Wilbey (Ed.). Aspen Publishers, Inc. Gaithersburg. pp 165-192.

Scott Blair, G.W. and Burnett, J. (1959). Physical changes in milk caused by the action of rennet. III. Effects of varying rennet concentration and temperature. *Journal of Dairy Research*, **26**: 58-62.

Shalabi, S.I. and Fox, P.F. (1982). Influence of pH on the rennet coagulation of milk. *Journal of Dairy Research*, **49**:153-157.

Singh, H., Shalabi, S.L., Fox, P.F., Flynn, A. and Barry, A. (1988). Rennet coagulation of heated milk: influence of pH adjustment before or after heating. *Journal of Dairy Research*, **55**: 205-215.

Singh, H and Bennett, R.J. (2002). Milk and milk processing. In *Dairy Microbiology Handbook (3rd Ed.)*. R.K. Robinson (Ed). Wiley-Interscience, Inc. New York. (Not yet published).

Snoeren, T. H. M. Klok, H. J. Hooydonk, A. C. M. van. and Damman, A. J. (1984). The voluminosity of casein micelles. *Milchwissenschaft, 39(8), 461-463.*

Solorza, F.J. and Bell, A.E. (1998). The effect of calcium addition on the rheological properties of a soft cheese at various stages of manufacture. *International Journal of Dairy Technology*, **51(1)**: 23-29.

Storry, J.E. and Ford, G.D. (1982). Development of coagulum firmness in renneted milk - a two-phase process. *Journal of Dairy Research*, **49(2)**: 343-346.

Swaisgood, H.E. (1992). Chemistry of the caseins. In *Advanced Dairy Chemistry Volume 1: Protein*. P.F. Fox (Ed). Elsevier Science Publishers Ltd. Essex, London. pp 63-110.

Tokita, M. Hikichi, K. Niki, R. and Arima, S. (1982). Dynamic viscoelastic studies on the mechanism of milk clotting process. *Biorheology,* **19(1 / 2)**, 209-219.

Tokita, M., Futakuchi, H., Niki, R., Arima, S. and Hikichi, K. (1983). Dynamic mechanical properties of milk and milk gel. *Biorheology*, **20**: 1-10.

Tokita, M., Niki, R. and Hikichi, K. (1985). Critical-behaviour of modulus of gel. *Journal of Chemical Physics*, **83(5)**: 2583-2586.

van den Bijgaart, H.J.C.M. (1989). Syneresis of rennet-induced milk gels as influenced by cheesemaking parameters. *Netherlands Milk and Dairy Journal*, **43**: 92-94.

van den Berg, G. and de Vries, E. (1975). Whey composition during the course of cheese manufacture, as affected by the amount of starter and curd washing water. *Netherlands Milk & Dairy Journal,* **29(2/3)**: 181-197.

van Dijk, H.J.M. (1982). Syneresis of curd – A PhD Thesis. Wageningen Agricultural University, Wageningen, Netherlands.

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

van Hooydonk, A.C.M., Boerrigter, I.J. and Hagedoorn, H.G. (1986). pH-induced physico-chemical changes of casein micelles in milk and their effect of renneting. 2. Effect of pH on renneting of milk. *Netherlands Milk and Dairy Journal*, **40**: 297-313.

van Hooydonk, A.C.M. and van den Berg, G. (1988). Control and determination of the curd-setting during cheesemaking. *Bulletin of the International dairy Federation*, **225**: 2-10.

van Vliet, T., van Dijk, H.J.M, Zoon, P. and Walstra, P. (1991). Relation between syneresis and rheological properties of particle gels. *Colloid and Polymer Science*, **269**: 620-627.

van Vliet, H.J.M., Lucey, J.A., Grolle, K. and Walstra, P. (1997). Rearrangements in acid-induced casein gels during and after gel formation. In *Food Colloids: Protein, Lipids and Polysaccharides*, E. Dickinson and B. Bergenstahl (Ed.). Royal Society of Chemistry, Cambridge, pp 335-345.

Verheul, M. and Roefs, S.P.F.M. (1998). Structure of particulate whey protein gels: Effect of NaCl concentration, pH, heating temperature, and protein composition. *Journal of Agricultural and Food Chemistry*, **46**: 4909-4916.

Walstra, P. and Jenness, R. (1984). Dairy Chemistry and Physics. A Wiley-Interscience Publication, John Wiley & Sons, New York. pp 1-7, 229-253.

Walstra, P., van Dijk, H.J.M. and Geurts, T.J. (1985). The synereis of curd. 1. General considerations and literature review. *Netherlands Milk and Dairy Journal*, **39**: 209-246.

Walstra, P. (1990). On the stability by casein micelles. *Journal of Dairy Science*, **73**: 1965-1979.

Walstra, P. 1993. The syneresis of curd. In *Cheese: chemistry, physics and microbiology Volume 1: General aspects.* P.F. Fox (Ed). Chapman & Hall Inc. London. pp141-191.

Walstra, P. (1999). Casein sub-micelles: do they exist? *International Diary Journal*, **9**: 189-192.

Walstra, P., Geurts, T.J., Noomen, A., Jellema, A. and van Boekel, M.A.J.S. (1999). Dairy Technology – Principles of milk properties and processes. Marcel Dekker, Inc. New York. pp 539-

Walstra, P. and van Dijk, H.J.M. (1983). Gel formation and syneresis. *Netherlands Milk and Dairy Journal*, **37**: 92-93.

Waungana, A. (1995). Rennet coagulation properties of heated milk – A Master Thesis. Massey University, Palmerston North, New Zealand.

Waungana, A., Singh, H. and Bennett, R.J. (1998). Rennet coagulation properties of skim milk concentrated by ultrafiltration: effect of heat treatment and pH adjustment. *Food Research International*, **31**: 645-651.

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

Zoon, P., van Vliet, T. and Walstra, P. (1988). Rheological properties of rennet-induced skim milk gels 2. The effect of temperature. *Netherlands Milk and Dairy Journal*, **42**: 271-294.

Zoon, P., van Vliet, T. and Walstra, P. (1988). Rheological properties of rennet-induced skim milk gels 3. The effect of calcium and phosphate. *Netherlands Milk and Dairy Journal*, **42**: 295-312.

Zoon, P., van Vliet, T. and Walstra, P. (1989). Rheological properties of rennet-induced skim milk gels 4. The effect of pH and NaCl. *Netherlands Milk and Dairy Journal*, **43**: 17-34.

Zoon, P., van Vliet, T. and Walstra, P. (1989). Rheological properties of rennet-induced skim milk gels 5. Behaviour at large deformation. *Netherlands Milk and Dairy Journal*, **43**: 35-52.