EFFECT OF BIOPOLYMER ADDITION ON THE PROPERTIES OF RENNET-INDUCED SKIM MILK GELS

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Abstract

The main objectives of this study were to determine the effect of adding different biopolymers (κ-Carrageenan, xanthan gum, guar gum, high-methoxyl pectin and gelatin) on the properties of rennet skim milk gels. A collection of techniques, namely strain-controlled rheometery, spontaneous whey separation measurements, confocal laser scanning microscopy and diffusing wave spectroscopy, were used.

The effects of these biopolymers were investigated for rennet skim milk gels made under model system and cheesemaking conditions. However, only rheological measurements were performed for samples made under cheesemaking conditions.

For samples made under model system conditions, the concentration of the biopolymer was varied from 0 wt% to 0.1 wt%. Experimental conditions, such as renneting temperature (30°C), total milk-solids (10 wt% reconstituted skim milk), pH 6.7 and rennet concentration (200 μL per 100 g sample) were kept constant.

The rheological behaviour of these samples was affected by the addition of κ-carrageenan, xanthan, guar, high-methoxyl (HM) pectin and gelatin. Both rheology and diffusing-wave spectroscopy (DWS) showed that the aggregation and gelation time and the gel strength was affected by the addition of these biopolymers. It was also shown that the syneresis behaviour, as well as the microstructure of rennet gels as imaged by confocal laser scanning microscopy (CLSM), was altered upon adding these biopolymers.

The rheological and microstructural properties of model renneted skim milk systems improved by adding small amounts (0.025 wt%) of κ-carrageenan, guar, HM pectin and gelatin, but not xanthan. Renneted skim milk containing HM pectin and gelatin had higher G^*, decreased aggregation time and gelation time and lower syneresis values as the concentration of biopolymer was increased.

On the other hand, lower G^* and higher syneresis values were obtained for samples containing higher concentrations (> 0.025 wt%) of κ-carrageenan, xanthan and guar gum. Higher syneresis index was a consequence of the presence of larger pores in these samples, as shown from the CLSM micrographs.

The effects caused by the addition of κ-carrageenan, xanthan and guar gum were believed to be due to phase separation in rennet skim milk gels containing polysaccharide, and was explained in term of a depletion-flocculation mechanism.

For rennet gels made under cheesemaking conditions (pH 6.2 with addition of 0.68 mM $CaCl_2$), it was found that the addition of xanthan, guar, HM pectin and gelatin had similar effect to that when added to samples made under model system conditions. This was due to the fact that the differences in pH and salt were known to not affect the properties of the biopolymers. However, the addition of κ-carrageenan, which was very sensitive to ions such as calcium, improved the viscoelastic properties of rennet skim milk gels made under cheesemaking conditions.

Overall, this work provides useful information on the effects of adding κ-carrageenan, xanthan, guar, high-methoxyl pectin and gelatin on the properties of rennet-induced gels.

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1 Introduction

1.1 Background

Despite the introduction and development of numerous new food products, milk and dairy products continue to play an important role in the nutrition of people in all parts of the world. Milk is a perishable food because of its high water content and an almost neutral pH. Like any other perishable foods, unless it is destined for immediate consumption, it needs to be processed into various products such as milk powders and cheese.

Traditionally, cheese was made as a way of preserving the nutrients of milk. Defined simply, cheese is the fresh or ripened product obtained after coagulation and whey separation of milk, cream or partly skimmed milk, buttermilk or a mixture of these products. Cheese is obtained by the addition of rennet to milk, which causes the milk proteins to aggregate and ultimately transform fluid milk to a semi-firm gel. World trade atlas (2002) reported that the New Zealand dairy industry exported 289,000 tonnes of cheese between June 2001 and 2002. Cheese emerged as the second highest exported dairy produce after milk powder for the NZ dairy industry.

Many processed and formulated foods are multi-component systems, containing protein/polysaccharides/fat mixtures. In order to achieve desirable functional properties in such foods, the use of various additives has been widely practised. Of particular interest in this regard, because of their ability to bind water, improve viscosity and gelation, are water-soluble, food-grade polysaccharides. Polysaccharides are already extensively used in a variety of manufactured dairy products as stabilizers and thickening or gelling agents. The current trend towards new dairy products with lower fat and lower total solids content has created a need for the use of polysaccharides.

Extensive research work has been carried out on cheesemaking, especially on understanding the physical chemistry of milk gelation, its processing conditions as well as properties. A number of papers by various authors (Dalgleish, 1979, 1981 and 1983; van Hooydonk, 1984, 1986, 1987 and 1988; Zoon *et al.*, 1988a, b, and c and 1989a and b; Walstra, 1983, 1986, 1990 and 1993) accumulated over the years have established

information on the effects of milk renneting conditions like pH, temperature, ionic strength, calcium concentration, casein concentration and the temperature history of the milk on the final property of the (cheese) product. The availability of this information had no doubt led to further research as well as application in commercial cheesemaking processes. However, there still remains a great deal to be understood in the complex interactions of proteins, fat and minerals during cheesemaking. In addition, the effect of the incorporation of polysaccharides in cheesemaking is practically unknown.

The main aim of this thesis was to study the effect of adding polysaccharides and gelatin on the gelation of skim milk by the addition of rennet, which is an important step in cheesemaking. Hence, an investigation of the effect of kappa (κ-) carrageenan, xanthan gum, guar gum, high-methoxyl (HM) pectin and gelatin, on the properties of rennet-induced skim milk gels will be presented.

1.2 Thesis Outline

This thesis seeks to provide an understanding on the effects of adding biopolymers like κ-carrageenan, xanthan, guar, HM pectin and gelatin on the properties of rennet-induced skim milk gels.

Chapter 2 reviews the literature and summarises the knowledge relevant to rennet-induced milk gels and milk protein/polysaccharides interactions. The scope of the project limits the review primarily to rennet-induced skim milk gels as well as understanding properties of the biopolymers used in this research.

Chapter 3 describes the analytical methods used in this research work. Brief background information on the methods used is included before detailing the experimental conditions.

Chapter 4 discusses the effect of polysaccharide as well as gelatin addition on the rheological properties of rennet-induced skim milk gels using a stress-controlled rheometer.

Chapter 5 reveals the microstructure of renneted skim milk containing polysaccharides and gelatin, and supports the findings through syneresis measurements.

Chapter 6 displays results of one recent analytical technique, Diffusing wave spectroscopy (DWS). The effect of adding polysaccharides and gelatin on the extent of aggregation and gelation time on rennet-induced skim milk was examined.

Rheological properties of renneted skim milk containing polysaccharides and gelatin in a commercial cheesemaking conditions are presented in Chapter 7.

Chapter 8 gives a general discussion highlighting the interactions involving renneted casein micelles and the biopolymers used.

Finally, the thesis closes with the final chapter summarising the major conclusions and recommendations.

2 Literature Review

2.1 Milk

All dairy products originate from milk, thus it is important to understand the properties of milk as fully as possible. Milk is a complex biological fluid consisting mainly of water (~ 86%), fat (~ 4.8%), carbohydrate (~ 4.7%), protein (~ 3.4%), minerals (~ 0.8%) and vitamins (trace). Casein micelles, whey proteins and milk fat globules are the most important components in most milk products, where they contribute to about 14% total solids content in milk.

2.1.1 Milk Proteins

General and more detailed information about milk proteins has been well documented by several authors (Whitney, 1977; Morr, 1979; Kinsella, 1984; Swaisgood, 1992; Holt and Roginski, 2001). There are several proteins present in bovine milk at a total protein concentration of 30-35 g/litre. Milk proteins (~ 3.4% w/w) are often subdivided into two major groups: *caseins* (2.7% w/w) and *whey proteins* (0.7% w/w).

Figure 2.1 illustrates the distribution of fractions and proteins in bovine milk (Swaisgood, 1992). As shown, about 80% of these proteins are presented in casein micelles, which are large spherical complexes, containing 92% protein and 8% inorganic salts, principally calcium phosphate (Schmidt, 1980; Swaisgood, 1985 and Whitney, 1988). The composition of whey proteins in bovine milk is 20%, which is composed of beta (β-) lactoglobulin (54%), alpha (α-) lactalbumin (21%), and lesser amounts of serum albumin, immunoglobulins, and proteose peptones (Kinsella, 1984).

2.2 Milk Gels: Formation, Structure and Properties

2.2.1 Casein Micelle

The basic element of milk involved in the transformation into curd and cheese is the casein micelle. The principal casein fractions, alpha (α_{s1}- and α_{s2}-) caseins, beta (β-) casein, and kappa (κ-) casein, which account for 80% of milk proteins, exist as spongy spherical micellar aggregates (average diameter size of 120nm) containing from 2 to 3 g of water per gram of protein and a charge of - 18 mV (Kinsella, 1984). The α_{s1}-, α_{s2}-, β- and κ- casein account for ~ 38, ~ 10, ~ 36 and ~ 13% of milk caseins, respectively (Swaisgood, 1982).

For the past few decades, several authors (Shimmin and Hill, 1964; Morr, 1967; Slattery, 1976; Schmidt and Payens, 1976; Schmidt, 1982) have extensively reviewed the structure and models of the casein micelle. In the last decade, several authors (Walstra, 1990; Rollema, 1992; Holt, 1992; and Holt and Horne, 1996) conducted comprehensive reviews of the casein micelle structure, particularly on the existence of a sub-micellar structure.

Several workers have assumed that the core of the micelle is built of sub-micelles: roughly spherical units of about 14 nm diameter that were fairly tightly aggregated. It was originally assumed that the sub-micelles were held together in the micelle by bridges consisting of calcium phosphate. In addition to numerous reviews on submicellar units, Walstra (1999) revealed a latest version of the subunit model. Instead of subunits linked by calcium phosphate, as was the case in some earlier models, calcium phosphate was located as diserete packages within the sub units. Recently, De Kruif and Holt (2001) reported a more detailed account of the nanocluster model of casein micelle substructure.

Horne (1998) put forward a non-subunit model (Figure 2.2) that featured individual caseins able to bind to each other through hydrophobic bonding and to the calcium phosphate through their phosphate centers.

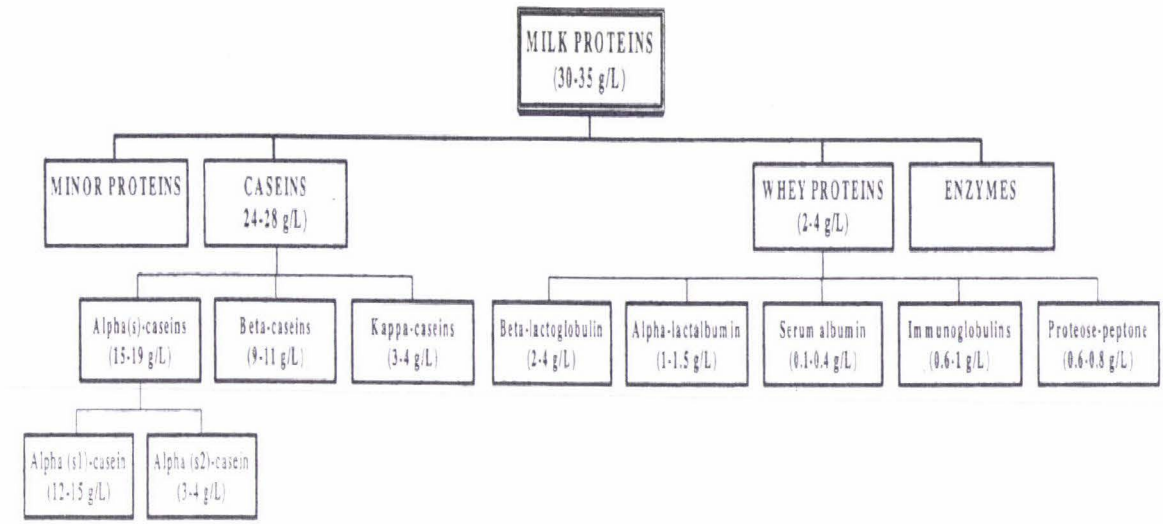

Figure 2.1 Distribution of fractions and proteins in bovine milk (Swaisgood, 1992).

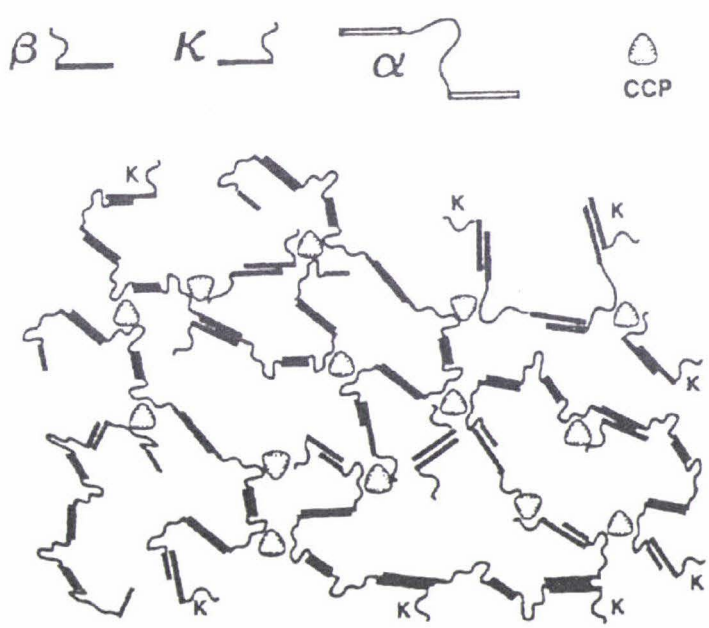

Figure 2.2 Dual bonding model of structure of casein micelle, with α-, β- and κ- casein depicted as indicated. Bonding occurs between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to colloidal calcium phosphate clusters (CCP). Molecules of κ-casein are labelled with letter 'K' (Horne, 1998).

It could also be used to explain the influence of temperature on rennet gel strength as well as the effects of fore-warming on rennet coagulation properties of milk. However, it failed to explain the appearance of substructure. Despite disagreement over the exact structure of the micelle, the concept of the casein micelle as a roughly spherical, fairly swollen particle electrostatically and sterically stabilised by a 'hairy layer' coat of κ-casein appears to be universally accepted (Walstra, 1990; Swaisgood, 1992; Horne, 1998; Walstra, 1999).

Therefore, by considering casein micelles as a collection of sterically stabilized particles with a brush of κ-casein, their behaviour in response to technological treatments could be related simply by considering the response of the κ-casein brush. Destabilizing the κ-casein by renneting, acidification, adding calcium or ethanol or combinations thereof leads to the flocculation of casein micelles (de Kruif, 1999).

2.2.2 Renneting: Enzymatic Coagulation of Milk

In general, renneting refers to the process of enzymatic coagulation of milk. Three separate but overlapping stages occur during enzymic coagulation of milk: enzymic proteolysis, aggregation, and gelation. In short, Walstra and Jenness (1984b) had distinguished two stages in milk clotting or renneting:

1. casein $\xrightarrow{\text{enzyme}}$ paracasein + glycomacropeptide
2. para-casein micelles $\xrightarrow{\text{calcium}}$ gel

Many proteolytic enzymes are able to clot milk; this means that the milk forms a gel some time after a preparation containing the enzyme has been added. The most used preparation is calf rennet, the active principle of which is chymosin.

2.2.2.1 Chymosin

Commercial rennet contained two enzymes: chymosin (EC 3.4.23.4), which contributed for about 87% to the specific proteolytic activity under normal conditions, and pepsin (EC 3.4.23.1) (van Hooydonk and Walstra, 1987). Both enzymes belong to the group of

acid proteinases. Two aspartic acid residues participate in the catalytic mechanism (Foltmann, 1981).

The chymosin molecule (sometimes called rennin) has a rod-like shape with dimensions 2.5 and 4.5 nm. It consists of two domains separated by a deep cleft running parallel to the smallest diameter. The cleft is the active site of the molecule where the two aspartic acid residues are located. The enzyme is inactivated if either of these two residues are esterified (Foltmann, 1981). Hence, chymosin is inactivated easily.

The unit of activity for milk clotting enzymes is the rennet unit (RU). Ruettimann and Ladisch (1987) defined the RU as the activity able to clot 10 mL of substrate (12 g skim milk dissolved in 0.01 M $CaCl_2$) in 100 seconds at 30°C.

The pH optimum for general proteolysis of peptides is around 4, whereas the optimum for the specific cleavage of the highly susceptible phenylalanine-methionine (Phe-Met) bond (residues 105-106) is found to be near pH 5.4 for isolated κ-casein and fragments, and around pH 6.0 in milk (van Hooydonk *et al.*, 1986). In milk, the inactivation depends much on temperature. Above 40°C, the enzyme may be inactivated before the milk clots (Walstra and Jenness, 1984b). Walstra and Jenness (1984b) also noted that the adsorption of chymosin onto paracasein is very weak at pH 6.7 and 33°C, but it increases with decreasing temperature and pH. Furthermore, calcium ions enhanced adsorption, as they have a specific influence on the stability of polyelectrolyte brushes (van Hooydonk *et al.*, 1986).

2.2.3 Gel Formation: Rennet-induced milk gel

Green (1980) and Lucey (2002) each provided a thorough review on the formation, structure and physical properties of milk protein gels including renneted milk, while Dalgleish (1981 and 1992) and Swaisgood (1992) reviewed the essential features on the clotting of milk by rennet addition.

In brief, after milk has been treated with rennet, there follows a stage of the reaction during which apparently little happens, followed after some time by a rapid coagulation

of the milk. This phenomenon, which is the first step of cheesemaking, results from two processes. The first is the attack on κ-casein by the proteolytic enzymes (chymosin, pepsin or microbial proteinases) present in the rennet; and the second is the subsequent clotting of the micelles, which have been destabilized by this enzymatic attack. These processes have been described as the primary and secondary stages of the renneting reaction (Dalgleish, 1981).

Among the casein fractions, κ-casein is rapidly hydrolyzed at the Phe_{105}-Met_{106} bond by the enzyme chymosin (EC 3.4.23.4), and by other proteases, yielding an N-terminal fragment called para-κ-casein, which contains the two cysteine residues, and a C-terminal fragment of 64 residues called the macropeptide, containing all of the carbohydrate and phosphate groups as well as the genetic substitutions (Walstra and Jenness, 1984b). When this occurs, the macropeptide diffuses into the serum, its stabilizing influence is lost, and the micelles begin to coagulate once sufficient κ-casein has been hydrolysed (Dalgleish, 1992). These reactions are shown schematically in Figure 2.3.

Van Hooydonk and Walstra (1987) have conducted an in-depth interpretation of the kinetics of the renneting reaction in milk. Since the breakdown of the κ-casein substrate is essentially a single-step enzymic reaction, it seems reasonable to suppose that the kinetics of the proteolysis should obey the standard Michaelis-Menten formulation for the kinetics of such reactions (Swaisgood, 1992). The instantaneous rate of the reaction, v (i.e. the rate at which substrate [S] is converted into product), is given as:

$$v = -\frac{d[S]}{dt} = V_{max} \cdot \frac{[S]}{(K_m + [S])} \qquad (2.1)$$

In this equation, V_{max} is the maximum rate at infinite concentration of substrate (i.e. it depends on the concentration of the enzyme) and K_m is the dissociation constant for the enzyme-substrate complex. The renneting reaction has been analysed in this way in a number of studies by Castle and Wheelock (1972), Dalgleish (1979), Chaplin and Green (1980), van Hooydonk *et al.* (1984) and Carles and Martin (1985).

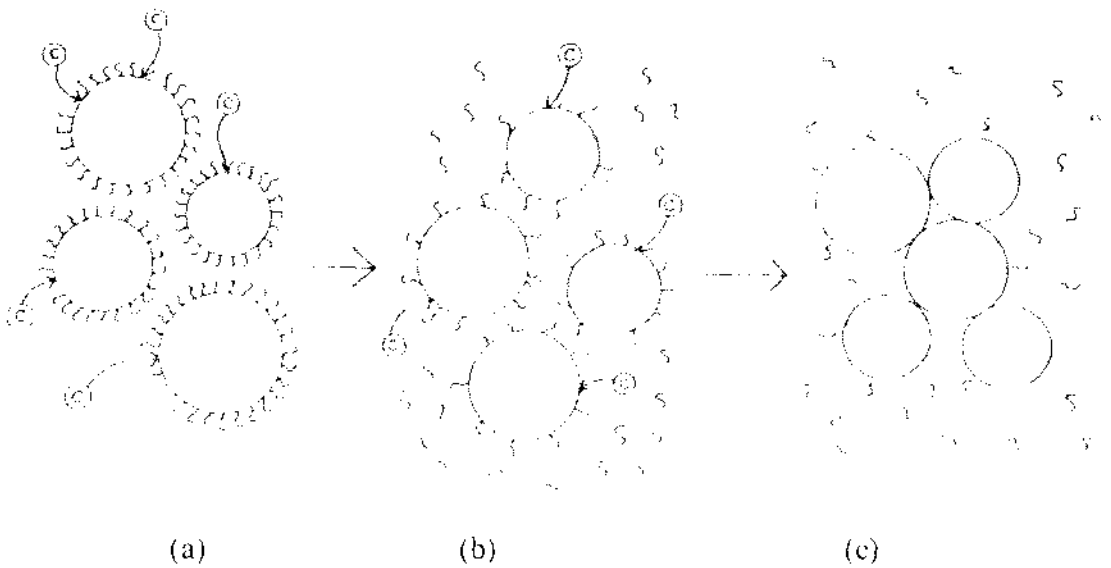

Figure 2.3 Schematic diagram of the attack by chymosin (shown as C) on casein micelles. Three different points in the reaction are illustrated: (a) the κ-casein coat of the micelles is intact, and chymosin has just been added; (b) some time later, much of the κ-casein has been hydrolyzed but sufficient remain to prevent aggregation; (c) at a later time still, nearly all of the κ-casein has been hydrolyzed and the micelles has started to aggregate (Dalgleish, 1992).

2.2.4 Gel Structure and Properties

2.2.4.1 Rheological Characteristics

James (1992a and b) provided a concise guide to the rheology of milk and cheese. The rheological properties of various types of cheese were widely different. Moreover, the rheological properties might vary markedly within one cheese and change dramatically during maturation.

Renneted skim milk, like cheese, exhibits viscoelastic behaviour. Whether an applied stress (force per area) causes predominantly elastic or viscous deformation depends not only on the properties of the product but also on the time scale over which the stress is applied. A briefly applied stress results in a more elastic response. The elastic component of renneted skim milk and cheese are expressed in a storage modulus, G' and the viscous component expressed in a loss modulus, G''. The dynamic moduli (G' and G'') can be measured simultaneously over different time scales of stress application.

For rennet skim milk gels, both G' and G'' increase with casein concentration to the power of 2.7 (Van Vliet and Walstra, 1983). This implies that the gels are inhomogeneous, consisting of aggregates connected by protein strands. Roefs *et al.* (1990), Lucey *et al.* (1997a) and Lucey and Singh (1998) have stated that most rheological parameters, namely the dynamic moduli G' and G'', characterizing casein gels depend on the number and strength of bonds between the casein particles, on the structure of the latter and the spatial distribution of the strands making up these particles.

2.2.4.2 Microstructural Characteristics

An appreciation of the microstructure of food and its components is now being recognised as a necessary prerequisite for understanding its properties. Researchers who have an interest in describing, predicting and controlling the behaviour of food materials realize the importance of a thorough knowledge on the way in which the components are organised.

Microstructural properties of rennet-induced skim milk gels can be studied using both syneresis or permeability measurements, and microscopy such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) or confocal laser scanning microscopy (CLSM). Numerous authors have attempted to study the microstructure of rennet-induced skim milk gels (Stoll, 1966; Van Dijk and Walstra, 1986; Lucey, 2001), and Aguilera and Stanley (1990b) have provided a general coverage of the microstructural aspects of milk and its products.

2.2.4.2.1 Syneresis (whey separation)

Syneresis is a phenomenon commonly occurring in gel systems, which is demonstrated by the spontaneous liberation of a liquid from a gel. It is defined as shrinkage of gel and this occurs concurrently with expulsion of liquid or whey separation. Van Vliet *et al.* (1997) had identified that in rennet-induced milk gels, extensive rearrangements of the network structure occurred after gel formation, which were related to the strong tendency of this type of gel to exhibit syneresis.

Syneresis is an essential part of cheesemaking as it is involved in dewatering curd particles, which is necessary to achieve appropriate moisture content for a cheese variety. Syneresis in cheesemaking is initiated by cutting the curd, and enhanced by stirring and increasing the temperature and acidity of the curds and whey. When modifications are made to the milk or processing conditions, the rate of syneresis may be affected.

Patel *et al.* (1971) investigated the factors, such as temperature, pH, calcium chloride ($CaCl_2$) addition, rate of heating and agitation, affecting the syneresis of cheese curd by direct acidification and rennet coagulation. It was found that the rate of heating did not have a significant effect on the syneresis of curd, whereas increasing agitation and addition of $CaCl_2$ had only slight effects. Marshall (1982) contributed to these findings by noting that increasing the amount of rennet increased the rate of syneresis of the curd slightly, while the addition of $CaCl_2$, raising the temperature and decreasing the pH, all increased the rate of syneresis. It was also discussed that in all instances, the early stages of syneresis followed the first-order kinetics where the rate depended on the

amount of whey remaining within the curd. The later stages of syneresis appeared to depend on hydrophobic interactions.

2.2.4.2.2 Microscopy

The coarseness of the network or gel structure seemed to be closely related to opacity and ease of syneresis. This ease of syneresis was associated with large pockets of solvent, which were readily expelled from the gel. Rapid gelation and high concentrations of structural materials resulted in the formation of fine gel structures, which tended to synerese slower (Stoll, 1966).

Images provided by microscopy are able to visually relate the microstructural properties of rennet-induced skim milk gels to syneresis. Aguilera and Stanley (1990a) extensively detailed the different ways of examining food microstructure, using microscopy. Since transmission electron microscopy and scanning electron microscopy involve complex or sometimes destructive sample preparation techniques, an alternative method is confocal laser scanning microscopy (CLSM).

Brooker (1995) provided a good understanding on the principles of CLSM as well as its practical applications to food systems, including dairy products. As CSLM was able to focus only on one plane of the sample, it meant that the fluorescence of the rest of the sample would not interfere with the fluorescence of the focal plane. In addition, CLSM gave not only high-resolution information about one plane but also indicated the three dimensional structure of the sample by superimposing different focal planes (Bourriot *et al.*, 1999).

2.2.5 Factors affecting gel formation, structure and properties of renneted skim milk

Green and Marshall (1977), Walstra and van Vliet (1986), Green and Grandison (1993), and Walstra (1993) discussed the mechanism as well as factors influencing the formation and structure of rennet-induced milk gels. Some of the factors which affect the properties of rennet-induced milk gels are illustrated graphically in Figure 2.4.

2.2.5.1 Storage temperature of milk

Ali *et al.* (1980) found that milk stored at 4°C or 7°C, having increased soluble casein, showed slower clotting, higher losses of fat and curd fines into the whey, weaker curds and lower curd yield than that stored at 10-20°C. Van Hooydonk *et al.* (1986) discussed that retardation of the renneting process after cold storage of reconstituted milk (which had also been observed for fresh milk) attributed to the dissociation of casein, to the solubilisation of the colloidal calcium phosphate at low temperature and to irreversible increase of the pH due to cooling (Zoon *et al.*, 1988a). Although it appeared that heating cold-stored milk to the renneting temperature virtually re-established the original partition, Zoon *et al.* (1988a) proved that the renneting process would still be greatly affected. Stirring the reconstituted milk for one hour at 30°C has been shown to be probably not enough to establish equilibrium.

However, the effects of cold storage could still at least be partially reversed by holding the milk at 60 – 65°C, which reduced the clotting time and improved curd properties (Green and Grandison, 1993). The formation of the curd, during cheesemaking, was itself influenced by the composition and treatment of the milk (Green and Grandison, 1993). Green and Grandison (1993) and Dziuba and Muzinska (1998) discussed the effect of cold storage on milk that might affect cheesemaking by both the physical effect of casein solubilization from the micelles and hydrolysis of casein and fat by enzymes in the milk. For instance, as a consequence of cold storage, the time of enzymatic coagulation was lengthened and the amount of chymosin-released peptides increased.

2.2.5.2 Calcium content

The major influence of Ca^{2+} on stabilization, rennetability, heat stability, surface and rheological properties of milk proteins has been well documented (Walstra and Jenness, 1984a and b). It is an established fact that the addition of Ca^{2+} to milk accelerated the overall clotting process, principally because of the effect on the aggregation stage of the reaction (Dalgleish, 1983). However, Bringe and Kinsella (1986) claimed that concentrations of Ca^{2+} above 8 mM decreased enzymatic activity.

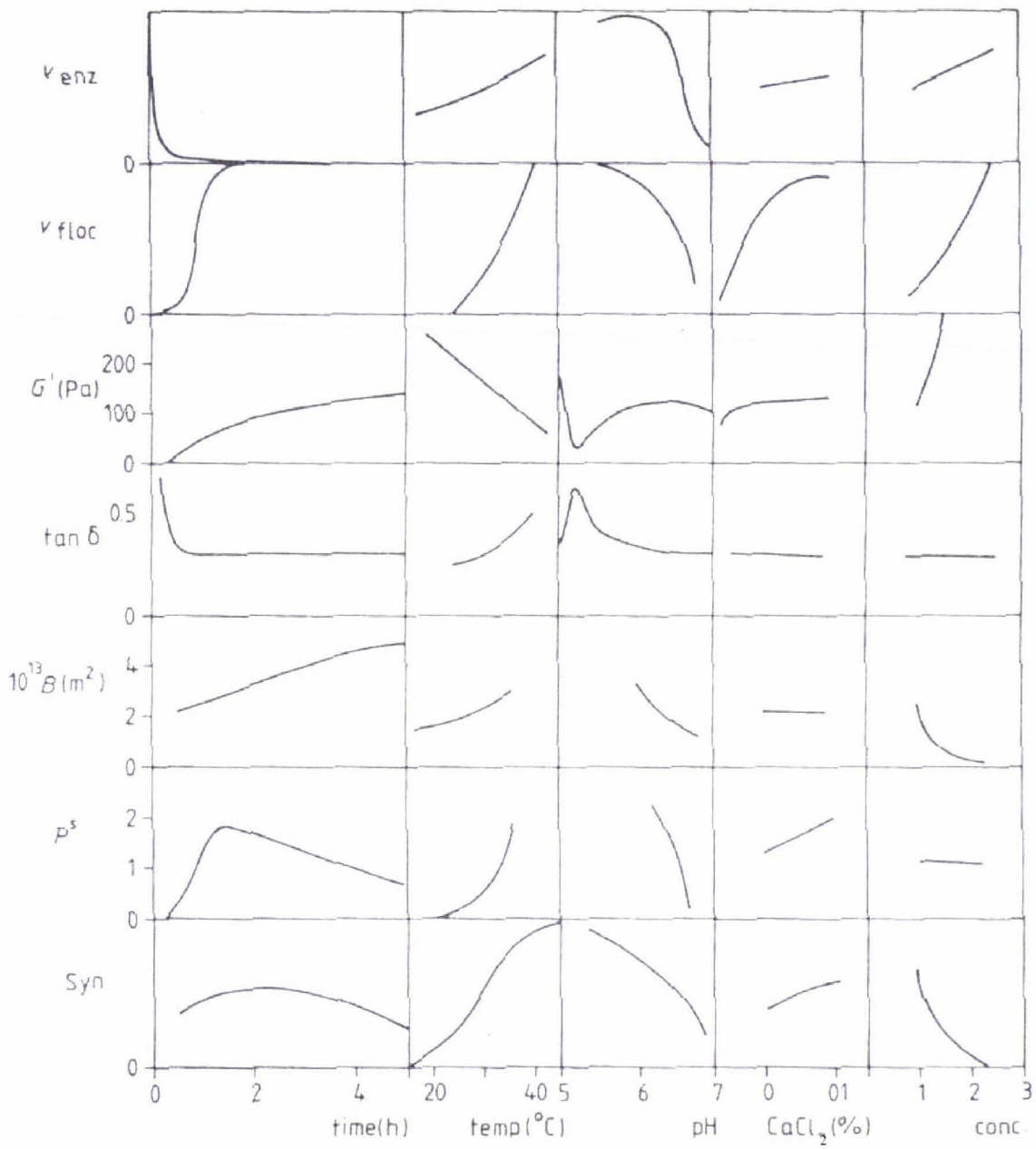

Figure 2.4 The effect of time after adding rennet, temperature, pH, added $CaCl_2$ and extent of concentrating milk by ultrafiltration on the rate of rennet enzyme reaction (V_{enz}), the rate of flocculation of micelles (V_{floc}), the dynamic storage modulus of the gel (G', Pa), the loss tangent characterizing the gel (tan δ), the permeability coefficient (B, m^2), the endogenous syneresis pressure (P^s, Pa) and the initial syneresis rate (Syn). (Walstra and van Vliet, 1986).

2.2.5.3 Rennet concentration

The inverse dependence of the clotting time of milk on the concentration of rennet is well known, and this variation is mainly attributed to the effect of enzyme concentration on the rate of proteolysis. The rate of the enzymatic reaction increases linearly with the concentration of enzyme, which accords with either a Michaelis-Menten or a first-order mechanism (van Hooydonk *et al.*, 1984). Van Hooydonk and van den Berg (1988) and Zoon *et al.* (1988a) reported an increase in gel strength (higher G' as a function of time) and shorter gelation times with increasing rennet concentrations (≤ 0.5%). In spite of that, the normal level used in commercial cheesemaking in New Zealand was reported to be 0.16 mL/L of milk (Waungana, 1995).

2.2.5.4 Renneting temperature

Temperature affects the clotting time, and although much of this variation can be attributed to the change in the rate of aggregation of renneted micelles, at least some can be attributed to the enzymatic reaction. Decreasing temperature by 10°C reduced the enzymic phase by a factor of 2 and the aggregation phase by a factor of 11 to 12 (Cheryan *et al.*, 1975). The effect of temperature on aggregation suggested that hydrophobic interactions played an important role in micelle aggregation and formation of a gel network (Kowalchyk and Olson, 1977). Van Hooydonk and van den Berg (1988) and Zoon *et al.* (1988a) found that increasing temperature (≤ 35°C) during gel formation results in an increase in firming rate as well as in G'.

2.2.5.5 pH

When pH was decreased from 6.7 to 5.6, coagulation increased 30-fold (Cheryan *et al.*, 1975). Lower pH increased enzyme activity and neutralized charge repulsion between micelles. Therefore, both primary and secondary stages of coagulation proceeded more quickly at lower pH. The effect of pH on the enzymic phase of milk coagulation was minor compared to its effect on aggregation (Cheryan *et al.*, 1975). In agreement, Zoon *et al.* (1989a) reported that decreasing pH in the range from 6.7 to 5.7 resulted in a maximum of G' near pH 6.15.

2.3 Food Polysaccharides

Polysaccharides are used to a large extent in food systems as texture agents. The widespread use of polysaccharides in food products is largely related to the fact that they impart texture, especially to dairy products, through their thickening and gelling properties. There are numerous ways to classify food polysaccharides: as according to origin, isolation method, function, texture, thermoreversibility, gelling time or charge.

The following sections are by no means an extensive coverage of the polysaccharides used in this thesis, but an attempted summary relating relevant information to current work. To begin with, Morris (1998) presented an excellent review on the gelation mechanism of the polysaccharides that had been selected for current investigation.

2.3.1 Carrageenan

Carrageenans are linear, sulfated anionic polysaccharides extracted from various species of the *Rhodophyta* (marine red algae). They existed in three main forms, namely kappa (κ), iota (ι) and lambda (λ), with κ- and ι- carrageenan having to ability to form a gel under certain conditions. All fractions are composed of galactose residues, sulphated to different degrees and alternately linked 1 → 3 and 1 → 4 (Moirano, 1977). These three carrageenan types typically have number-average molecular weights (M_n) in the range 100 200 kD and weight-average molecular weights in the range 300 600 kD (Picilell, 1995).

Extensive reviews on the structures and gelation of carrageenan have been conducted by numerous authors: Stoloff (1959); Piculell *et al.* (1994); Picilell (1995). In aqueous solutions, carrageenan polymers exist as random coils. On cooling, a three-dimensional polymer network builds up in which double helices form the junction points of the polymer chains. Further cooling leads to aggregation of these junction points and a build-up of the gel structure. It has been concluded from rheological and polarimetric measurements that the gelation of κ-carrageenan occurs at temperatures well above the coil-helix transition (> 30°C) (Picilell, 1995) and it was dependent on the ionic environment. The gelation of κ-carrageenan was promoted by cations, with K^+ ion

being more efficient than Na^{+} or Ca^{2+} ions for stabilizing the helix state and promoting gelation. Synergistic effects between these ions have been found in κ-carrageenan gels (Hermansson *et al.*, 1991). Gel strength increased initially with increased concentration of calcium salts to about 0.05 molar salt concentrations beyond which gel strength remained constant (Stoloff, 1959). Table 2.1 tabulates the properties of κ-carrageenan in relation to current investigative work.

2.3.2 Xanthan gum

Xanthan gum is a polysaccharide produced in pure culture fermentation by the microorganism *Xanthomonas campestris*, an organism originally isolated from the rutabaga plant. It has a high molecular weight of ~ 2.5 x 10^6 with low polydispersity (Nussinovitch, 1997c). Jansson *et al.* (1975) and Melton *et al.* (1976) showed the primary structure of xanthan gum to consist of a β- (1 → 4) -D-glucan backbone (cellulose) substituted, at C-3 on alternate glucose residues, with a trisaccharide side chain (Morris, 1995). Xanthan gum, which does not gel by itself and produces high viscosity solutions at low concentrations, is employed by the food industry as a thickening and stabilizing agent.

Kovacs and Kang (1977), Challen (1994), Morris (1995) and Nussinovitch (1997c) provided a valuable review of the structural and functional properties of xanthan gum. Recently, Lapasin and Priel (1995), Capron *et al.* (1997), and Rodd *et al.* (2000) conducted intensive studies on the rheological and the physical/chemical properties of xanthan. Xanthan undergoes a conformational transition from an ordered helical structure at low temperature to a disordered one at higher temperature. The transition temperature is highly dependent on the salt content, with the stability of the ordered conformation, being shifted towards higher temperature as the ionic strength increased (Morris, 1998). Its properties in relation to the current work are tabulated in Table 2.2.

Table 2.1 Properties of κ-Carrageenan

Property	
Solubility in water	Soluble above 70°C Sodium (Na^+) and Ammonium (NH_4^+) Soluble in cold water
Other factors influencing solubility	Solubility increases with decreasing Na^+, potassium (K^+) and calcium (Ca^{2+})
Solution viscosity	Low
Optimum pH range	4 to 10
Optimum soluble solids range	0 to 40%
Gelation conditions	Presence of K^+, Na^+ or Ca^{2+} Temperature below setting temperature
Gel characteristics:	
- Texture	- Strong, brittle Brittleness increases with increasing K^+ and Ca^{2+} and decreasing locust bean gum (LBG) Thermoreversible
- Setting temperature	- Increases with increasing K^+, Na^+, Ca^{2+} and sugar
- Gel strength	- Increases with increasing concentration. K^+, Ca^{2+}, and LBG

Table 2.2 Properties of xanthan gum

Property	
Solubility in water	Soluble over a wide range of temperatures
Solution viscosity	High below 100°C
Optimum pH range	1 to 13
Optimum soluble solids range	0 to 80%
Gelation conditions	Presence of locust bean gum, tara gum, cassia gum Temperature below setting temperature
Gel characteristics:	
- Texture	- Cohesive, gummy Thermoreversible Guar makes texture of xanthan/LBG gel more brittle
- Setting temperature	- Constant
- Gel strength	- Increases with increasing concentration

2.3.3 Guar gum

Galactomannans are energy-reserve polysaccharides deposited in the seed endosperms of plants of the *Leguminoseae*. Guar gum and locust bean gum are the two major galactomannans of commercial importance for the food industry (Morris, 1995). The food industry has made wide use of the ability of guar to bind large amounts of water as well as for its thickening ability.

Guar gum is a galactomannan with linear chains of D-mannopyranosyl units with side branching units of D-galactopyranose attached by (1 → 6) linkages (Meer, 1977). The unsubstituted D-mannopyranosyl units represents the so-called 'smooth' side, while the substituted D-galactopyranosyl units constitutes the 'hairy' side. The average galactose to mannose ratio in guar was 1:2 and its molecular weight ranged from 220,000 300,000 (Meer, 1977). It is non-ionic and is compatible with salts over a wide range of electrolyte concentration. Meer (1977) and Nussinovitch (1997b) have given a constructive account of the structural properties and gelation mechanism of guar gum.

Guar's inability to gel in its native form results from its 'block' conformation, which allows 'smooth' regions to aggregate in order to form junction zones. The 'hairy' regions are responsible for the network's dispersibility via hydrogen bonding with water molecules. Because of its alternating chemical structure that sterically impeded the formation of interchain junction zones, guar gum does not produce gels under typical food-system conditions (Dea *et al.*, 1977). Some properties of guar gum relative to the current work are reported in Table 2.3.

Table 2.3 Properties of guar gum

Property	
Solubility in water	Soluble over a wide range of temperatures
Solution viscosity	Hot – Low, Cold – High
Optimum pH range	4 to 10
Optimum soluble solids range	0 to 80%
Gelation conditions	Non-gelling
Gel characteristics: - Texture - Setting temperature - Gel strength	 - Non-gelling - Non-gelling - Non-gelling

2.3.4 Pectin

Pectins are a group of heterogeneous polysaccharides, consisting mainly of galacturonic acid and galacturonic acid methyl ester residues, from the primary cell walls and intercellular regions of higher plants (Voragen and Pilnik, 1995; Nussinovitch, 1997a). The average molecular weight of commercial pectin based on viscometry measurements normally fell between 50 x 10^3 and 150 x 10^3 (Nussinovitch, 1997a). The dominant feature of pectin was a linear chain of alpha- (α-) (1 $\rightarrow$ 4) -linked D-galacturonic acid units in which varying proportions of the acid groups were presented as methoxyl (methyl) esters (Voragen and Pilnik, 1995). The degree of methylation (DM) is defined as the percentage of carboxyl groups esterified with methanol. If more than 50% of the carboxyl groups are methylated, the pectins are called HM pectins; if fewer than 50% are methylated, they are called LM pectins. The esterification of galacturonic acid residues with methanol and/or acetic acid remains a very important structural characteristic of pectins.

Bender (1959), Voragen and Pilnik (1995) and Nussinovitch (1997a) had broadly investigated the structure, gelation mechanism and applications of pectins. HM pectins formed gels at low pH (below about 3.6) when a cosolute was present (typically sucrose at a concentration of greater than 55% by weight) (Oakenfull, 1991). Hence, low pH, a high soluble solids concentration and appropriate temperatures were needed for HM pectin gelation (Nussinovitch, 1997a). On the other hand, LM pectins, with more free carboxylic acid groups, formed gels only in the presence of calcium-ions (Axelos and

Thibault, 1991). Table 2.4 reports few characteristics of HM pectin, which is used in this study.

2.4 Gelatin

Gelatin is an animal protein derived by hydrolytic degradation of collagen, the principal protein component of white fibrous connective tissue (skin, tendon, bone, etc). The macromolecular unit of collagen is the tropocollagen rod, a triple helical structure composed of three separate polypeptide chains (total $M_w \approx 330\ 000$) (Ross-Murphy, 1994). The amino acid sequence varies from one source to another, but it always consists of large amounts of proline, hydroxyproline and glycine. The former is important, as it tends to promote formation of the polyproline II helix, which ultimately determines the form of the tropocollagen trimer (Ross-Murphy, 1994).

Veis (1964) and Ledward (1986) performed an extensive study on the structure and gelation mechanism of gelatin, and Ross-Murphy (1994) reported the rheological characterisation of gelatin gels. The chain segments primarily involved in the collagen-fold were the non-polar regions rich in proline and hydroxyproline. During collagen-fold formation, gelatin did not appear to have any specificity to the intersegment interaction. Hence, any random contact between non-polar chain segments might lead to the formation of a collagen-folded unit. The lack of specificity in the intersegment interaction in collagen-fold formation showed that this process was relatively independent of pH and ionic strength (Veis, 1964). These collagen-folded segments had been identified as the network junction points in gels. The rigid, structured collagen-folded aggregates were joined by flexible, unstructured individual peptide chains. However, network gel formation via random segment interactions was found to be concentration dependent and was favoured at high concentration (Veis, 1964). Table 2.5 tabulates the properties of gelatin, which may be useful in justifying its relationship with renneted skim milk in further investigative work.

Table 2.4 Properties of high methoxyl (HM) pectin

Property	
Solubility in water	Soluble over a wide range of temperatures
Other factors influencing solubility	Solubility increases with decreasing molecular weight, increasing randomness of COOH, decreasing sugar and calcium (Ca^{2+})
Solution viscosity	Low
Optimum pH range	2.5 to 4 pK_a = 3.3
Optimum soluble solids range	55 to 80%
Gelation conditions	pH below 4 and soluble solids 55-80%
Gel characteristics:	
- Texture	- Cohesive, no syneresis Thermo-irreversible
- Setting temperature	- Increases with increasing degree of esterification (DE), decreasing pH and increasing sugar
- Gel strength	- Increases with increasing concentration and molecular weight

Table 2.5 Properties of gelatin

Property	
Solubility in water	Soluble above 40°C
Other factors influencing solubility	Solubility increases with decreasing molecular weight
Solution viscosity	Low
Optimum pH range	4.5 to 10 iso-pH = 4.8 to 5.2 (limed), 6.0 to 9.5 (acid)
Optimum soluble solids range	0 to 80%
Gelation conditions	Temperature below setting temperature
Gel characteristics:	
- Texture	- Soft to strong, cohesive, gummy. Thermoreversible
- Setting temperature	- Increases with increasing molecular weight and maturing temperature
- Gel strength	- Increases with increasing concentration and decreasing salt

2.5 Milk Protein – Polysaccharides Interactions

An important feature of food systems is their multi-component nature. Dickinson and Stainsby (1982), Dickinson (1992) and Dickinson and McClements (1996) have identified proteins and polysaccharides as two of the most important functional ingredients in food colloids. These two types of biopolymers are largely responsible for the structure, mechanical and other physicochemical properties of food. As the structural functions of proteins and polysaccharides are greatly affected by their interactions with each other and with other components within the food system, it is paramount to understand the interaction mechanism of structure formation process. Hence, knowledge on protein/polysaccharides interaction as contributed by reviews of Tolstoguzov (1991), Ledward (1994), Dickinson and McClements (1996), Syrbe *et al.* (1998) and de Kruif and Tuinier (2001) are discussed.

Together with milk proteins, namely caseins and whey proteins, polysaccharides dissolved in the aqueous phase form a pseudoternary 'milk protein-polysaccharide-water' polyelectrolyte solution. Ternary polyelectrolyte solutions, for instance mixtures of polymers composed of chemically different, charge-carrying monomer units (here amino acids versus carboxylated, sulphated or unsubstituted monosaccharides) in a common solvent (here water), are known to behave all but ideally (Syrbe *et al.*, 1998).

2.5.1 The Mixing Behaviours of Biopolymer Solutions

In recent reviews, both Syrbe *et al.* (1998) and de Kruif and Tuinier (2001) cited that interaction between proteins and polysaccharides, as observed in food related systems, could be systematically discussed by separating biopolymer interactions into enthalpy and entropy dominated types. The mixing behaviour of ternary biopolymer solution was then discussed to be primarily controlled by enthalpic effects, given by the relative strength of the interactions of the polymers among each other and with the solvent, plus segment-specific excess entropy effects, such as release of bound water or counterions (Syrbe *et al.*, 1998).

It was found, in common agreement among the authors, that on mixing two biopolymers in solution, like a milk protein and a polysaccharide solution, three consequences, specifically co-solubility, incompatibility, or complexing, resulted. De Kruif and Tuinier (2001) had depicted these possibilities in Figure 2.5. Co-solubility is the least typical situation in view of the polymeric nature of proteins and polysaccharides and presence of various functional groups in their macromolecules and hence will not be discussed further. A more likely situation is that the interaction of the two biopolymers is either segregative (the biopolymers repel each other and are denoted as incompatible) or complexation/associative (the biopolymers attract one another).

Figure 2.5 Main trends in the behaviour of protein/polysaccharide mixtures (de Kruif *et al.*, 2001)

As the interaction of polysaccharides with proteins is primarily ionic in nature, it therefore depends on the charge associated with the polysaccharides (Pedersen, 1979). Protein molecules are ampholytes, containing both cationic and anionic groups, with the proportions of these depending upon the pH. In the pH range from below to slightly above the isoelectric point, these cationic groups on the protein might interact with added polysaccharides (Elfak *et al.*, 1979). At pHs below the isoelectric point of the protein, precipitation of the protein and polysaccharides often occurs. Therefore, the reaction between the protein and the negatively charged polysaccharide depends strongly on the pH of the system.

Thermodynamic incompatibility appears to be a fundamental property of proteins and polysaccharides. Grinberg and Tolstoguzov (1997) noted that proteins and

polysaccharides were only incompatible under certain conditions, which inhibited the formation of inter-biopolymer complexes. This mainly occurred at high ionic strengths and/or at pHs higher than the protein pI.

The chemical structure of polysaccharides is the factor most strongly affecting phase separation in protein/polysaccharide systems. At pHs above the protein pI, which are the most interesting for food systems, the minimal salt concentration required for phase separation to occur increases in the order: carboxyl-containing polysaccharides < neutral polysaccharides < sulphated polysaccharides. Under the same conditions (ionic strength, pH), incompatibility is enhanced (i.e. the composition area corresponding to the single-phase state is reduced) in the reverse order, i.e sulphated polysaccharides < neutral polysaccharides < carboxyl-containing polysaccharides.

Another factor influencing the phenomenon is branching of polysaccharide macromolecules, where incompatibility is reduced in branched polysaccharides compared to linear polysaccharides.

In general, it could be concluded that biopolymer concentration, the interplay of pH and ionic strength dominate the mixing behaviour of ternary protein-polysaccharide solutions.

2.5.2 Casein micelles and Polysaccharides Interaction

Casein micelles in milk are organised in large supramolecular entities that can be considered as spherical particles. A dispersion containing micellar casein is deemed closer to a particulate suspension than to a macromolecular solution. Phase separation in mixtures of polysaccharides and casein micelles/proteins are often due to a segregative interaction between these two entities. A segregative interaction between polysaccharides and casein micelles would then result in an effective attraction between the proteins through a depletion mechanism (Tuinier *et al.*, 2000), which is further discussed in Section 2.5.2.1.

The effects of adsorption or non-adsorption of polysaccharides onto milk proteins must be considered for understanding the behaviour of dairy systems containing polysaccharides. Both adsorbing and non-adsorbing polymers are able to increase or decrease the stability of colloidal dispersions, depending on their concentrations and the size ratios of polymers to colloidal particles.

The following figures (Figure 2.6, Figure 2.7 and Figure 2.8) illustrate the effect of absorbing, non-absorbing, non-gelling and gelling polymer (such as polysaccharides) in colloidal dispersions (Syrbe *et al.*, 1998). In reference to the figures illustrated, the following points were summarised (Syrbe *et al.*, 1998):

(i) Small polymer additions to a colloidal system would not induce flocculation of either the bridging or the depletion type.

(ii) Depending on its concentration, an adsorbing polymer could lead a colloidal system through the whole series of no influence → bridging → polymeric stabilization → depletion destabilization (Takigami *et al.*, 1992).

(iii) In systems with high volume ratios of colloid particles, strong destabilization could turn into pseudostability (Figure 2.6b). Destabilization made the particles sticky, so that they would aggregate into a particle network. If the attractive potential between particles was strong enough, the time scale of rearrangement into a close packing could reach the order of months (Ploehn and Russel, 1990; Parker *et al.*, 1995).

(iv) The classic path to stability opened up when the soluble polymer formed a gel network in its own, in which the colloidal particles were trapped.

(v) If the gel-forming polymer adsorbed onto the colloidal particles, a composite polymer particle gel resulted, but again flocculation became possible as the polymer concentration dropped below the gelation threshold ('indirect bridging' by self-association of several adsorbed polymer molecules).

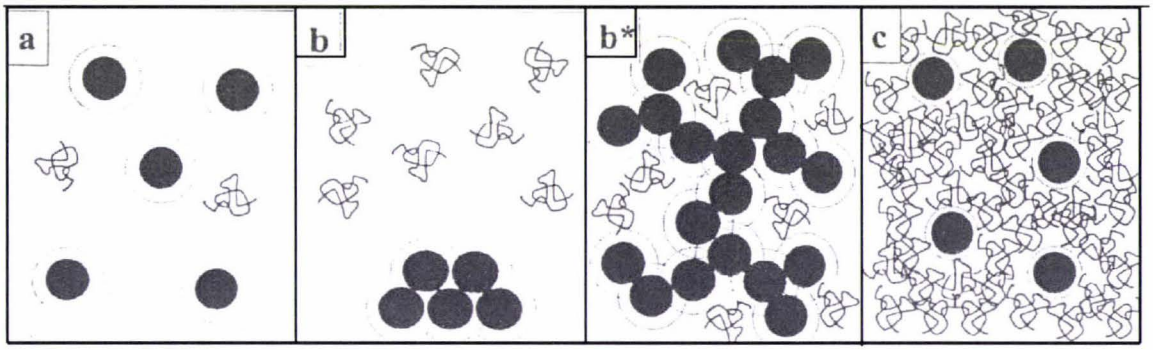

Figure 2.6 Non-absorbing, non-gelling polymer in colloidal dispersions. With increasing free polymer concentrations, the system undergoes the transition: (a) stable → (b) depletion flocculation → (c) stable. At high-dispersed phase volume fractions, a particle network could form that would need a long delay time for reorganisation into a close packing ('pseudostability', b*). The region of stability at high non-absorbing polymer concentration was linked to high viscosities and might be difficult to reach (Syrbe *et al.*, 1998).

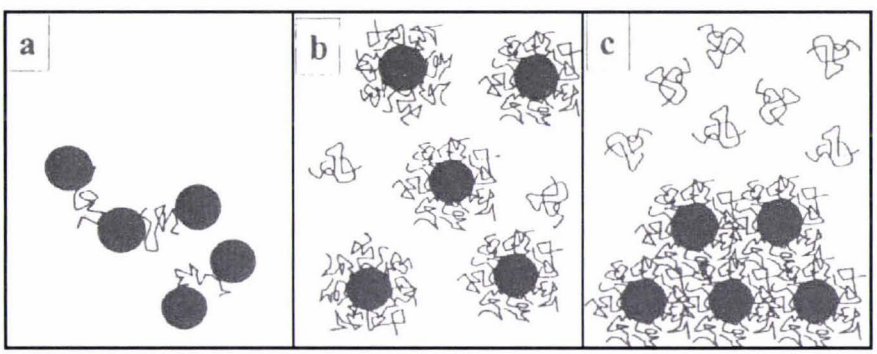

Figure 2.7 Adsorbing, non-gelling polymer in colloidal dispersion. With increasing polymer concentration, the system undergoes the transition (a) bridging flocculation → (b) polymeric stabilization → (c) depletion flocculation. Bridging sets in at very low polymer concentration, but flocculation becomes more and more effective up top about half saturation surface coverage. Excess of adsorbing polymer turned into free, non-absorbed polymer and could also lead to depletion flocculation (comparable to Figure 2.5b) (Syrbe *et al.*, 1998).

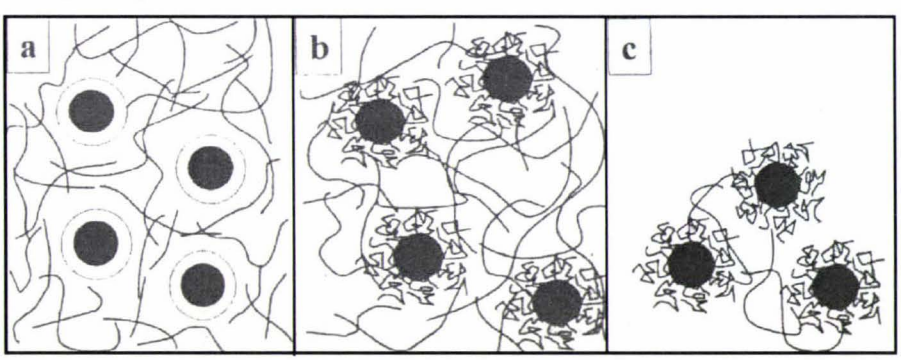

Figure 2.8 Gelling polymer in colloidal dispersions. (a) Non-absorbing polymer generate a network 'around; the colloidal particles (b) while adsorbing polymer integrate the particles into the gel structure. In both cases, the particles are stabilized against flocculation. At sub-gelling polymer concentration, adsorbing polymer could cause indirect bridging when anchored polymers self-associate with their dangling ends (c) (Syrbe *et al.*, 1998).

2.5.2.1 Depletion flocculation

In the case of depletion flocculation, the stability of a particulate suspension is reduced by the addition of polymer (Bourriot *et al.*, 1999). The fundamental explanation for this phenomenon was first given by Asakura and Oosawa (1954, 1958), later generalized by Vrij (1976). Figure 2.9 pictures a simple representation of this idea.

The polymers were excluded from the surface of the sphere molecules, as the colloidal particles and polymer had no specific interaction besides excluded volume. This resulted in an effective excluded (depletion) layer where the osmotic pressure *II*, generated by the polymer segments, was smaller than in the bulk. If two particles met as a result of Brownian motion, they shared this depleted volume. As a result, the available volume for the polymers increased by $V_{overlap}$, the overlap volume of the two depletion layers. An increase of the volume by $V_{overlap}$ was associated with a lowering of the free energy of the system by $\Delta G = -II.V_{overlap}$. Thus 'collecting' most of the particles in a separate phase (phase separation) were entropically advantageous (de Kruif and Tuinier, 2001).

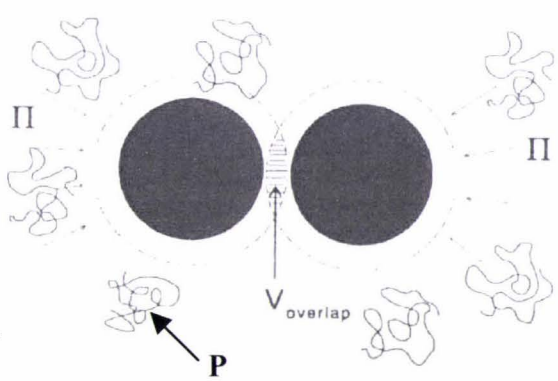

Figure 2.9 Depletion interaction between two colloidal particles induced by a dissolved polymer (P). The polymer molecules were excluded from the (overlap) volume in between the two particles (Maroziene and de Kruif, 2000).

2.5.3 Milk Proteins and Polysaccharides Interaction

In recent years, several studies have investigated the interactions of milk protein/polysaccharides mixtures.

Drohan *et al.* (1997), Langendorf *et al.* (1997 and 1999), Augustin *et al.* (1999), Tziboula and Horne (1999), Schorsh *et al.* (2000), Puvanenthiran *et al.* (2001) and Hemar *et al.* (2002) have conducted studies on skim milk/κ-carrageenan mixtures. The properties were generally determined by rheological means, while additional techniques like CLSM and quasi-elastic light scattering were also conducted. In the presence of casein micelles, gelation took place in a single step, suggesting an interaction between κ-carrageenan and the casein micelles that had to be satisfied first, and reduced the availability of carrageenan for the gelation role (Drohan *et al.*, 1997; Tziboula and Horne, 1999). Hence, the relative importance of casein micelles to gelation varied with carrageenan concentration (Drohan *et al.*, 1997) and was dictated primarily by the ionic content of the mixtures (Puvanenthiran *et al.*, 2001). The conformation of κ-carrageenan and specific interactions involving Ca^{2+} affected its interaction with the casein micelle and the rate of formation of aggregates (Augustin *et al.*, 1999). Additionally, the κ-carrageenan molecules induced flocculation of casein particles through depletion mechanism when casein micelles presented in skim milk were mixed with κ-carrageenan at temperatures above the coil-to-helix transition (Langendorf *et al.*, 1997 and 1999; Schorsh *et al.*, 2000; Hemar *et al.*, 2002).

Hemar *et al.* (2001) studied the viscosity, microstructure and phase behaviour of xanthan gum in commercial milk protein products including casein micelles. Phase separation occurring in skim milk/xanthan mixtures was reported to arise from depletion flocculation of casein micelles by the xanthan macromolecules.

Bourriot *et al.* (1999) and Tuinier *et al.* (2000) studied the interaction of guar gum with casein micelles, especially in skim milk. Phase separation in these systems was determined by rheology, CLSM and light scattering techniques. It was found that mixing guar gum with casein micelles led to a phase separation (Bourriot *et al.*, 1999; Tuinier *et al.*, 2000). Although a phase separation through thermodynamic incompatibility could not totally be ruled out, depletion flocculation had been suggested

to be more likely involved in the mechanism. When guar gum chains were added to the casein suspension, they would be excluded from the space between the micelles, provided the casein concentration is high enough. The energetic barrier would then be crossed over, leading to the aggregation of the micelles. The flocs of the casein micelles would tend to self-aggregate and form a network, which would constitute the continuous phase of the system (Bourriot *et al.*, 1999). In addition, Tuinier *et al.* (2000) reported results that were well described by the theory of Vrij (1976). The polymer concentration at the phase boundary increased with decreasing guar chain length. This was due to an increasing depletion layer thickness with increasing radius of gyration (Tuinier *et al.*, 2000).

Pereyra *et al.* (1997), Oakenfull and Scott (1998) and Maroziene and de Kruif (2000) studied the influence of pectin on the stability of milk. In particular, Maroziene and de Kruif (2000) investigated the interaction of pectin and casein micelles using the dynamic light scattering and viscometric methods. It was reported that at pH 5.3, the pectin molecule adsorbed onto the casein micelle while at 6.7, depletion flocculation of the casein micelles was observed. On increasing the pH from 5.3 to 6.7, pectin desorbed. At low pectin concentrations, a bridging flocculation was noted. On increasing the concentration further, the casein micelles became fully coated and the attraction between the particles was lowered. A phase separation through depletion flocculation would occur if the attraction between the colloidal particles were strong enough.

Salvador and Fiszman (1998), Fiszman *et al.* (1999) and Fiszman and Salvador (1999) studied the rheological and microstructural effects of gelatin in acidic milk gels formation. Hermansson *et al.* (1998) carried out a more relevant study to current work, where phase separation of milk protein/gelatin systems using CLSM was investigated. These studies showed that these systems were found to be gelatin continuous and that the gel formation increased at higher gelatin concentrations. The results showed that the kinetics of gelation determined the nature of the mixed structure. Thus, it was concluded that the speed of phase separation was determined by the kinetics of gel formation and the gelatin concentrations, where it was influenced by the milk protein concentrations and in the way the milk protein phase was dispersed in the gelatin phase.

2.5.4 Renneted Casein Micelles/Polysaccharides Systems

At the time of writing, only three papers reporting on renneted skim milk/polysaccharides were found in the literature. Hansen *et al.* (1980) and Shalabi and Fox (1982) studied the effect of κ-carrageenan on rennet coagulation of skim milk and found that rennet coagulation of skim milk containing κ-carrageenan was not significantly affected by up to 0.5% κ-carrageenan concentration. The influence of κ-carrageenan on rennet coagulation time was dependent on both the concentration of κ-carrageenan and on the concentration of milk solids but the relationship was not a simple stoichiometric one. Hansen *et al.* (1980) attributed the influence of κ-carrageenan on rennet coagulation to its effect on the calcium environment but κ-carrageenan at concentrations up to 0.5% had no effect on Ca^{2+} as measured by a Ca^{2+} sensitive electrode.

Olsen (1989) investigated the effects of xanthan, cross-linked starch, gelatin, low methoxyl pectin, ι-carrageenan, κ-carrageenan and a lactic acid bacteria produced from *Lactococcus lactis* on rennet coagulation of milk. All these polysaccharides, except LM pectin, xanthan and the *L. lactis* polysaccharide had either no effect or inhibited milk coagulation and gel formation. In Olsen's study, a rolling bottle method and a Formagraph were used to determine the clotting time, rate of curd firmness as well as the final curd firmness of the samples. The addition of xanthan was found to reduce curd formation rate and decreased gel firmness.

3 Materials and Methods

3.1 Materials

3.1.1 Low Heat Skim Milk Powder

Commercial grade, low-heat skim milk powder (SMP) was purchased from Fonterra Co-operative Group Limited, Whangarei, New Zealand. Table 3.1 shows some relevant information as provided by the supplier.

Table 3.1 Product information on low-heat skim milk powder (SMP)

	Protein (%)	Fat (%)	Moisture (%)	WPNI* (mg/g)
Skim milk powder	32.958	0.523	3.428	6.7

* WPNI: Whey protein nitrogen index

3.1.2 Polysaccharides

The polysaccharides studied were kappa (κ-) carrageenan (Bengel KK-100, Woods and Woods Pty Ltd, Australia), xanthan gum (Keltrol T, Standard 80-mesh, Germantown International Limited, New Zealand), guar gum (Grinsted guar gum 178, Danisco Cultor, United States of America) and high methoxyl (HM) pectin (GENU pectin type YM-100, CP Kelco, Denmark).

3.1.3 Gelatin

Gelatin ("A Grade") was obtained from Leiner Davis Gelatin N.Z. Limited, New Zealand.

3.1.4 Rennet

Australian double strength (ADS) rennet (Rennet Company, Etham, New Zealand) was obtained from Fonterra Research Center (FRC), Palmerston North, New Zealand.

3.1.5 Chemical Reagents

Calcium chloride 2-hydrate ($CaCl_2.2H_20$, 99.5% minimum assay, molecular weight 147.02, BDH Limited Poole, England) and antibacterial agent, sodium azide (NaN_3, BDH laboratory, Poole, England) were of technical reagent grade. MilliQ water containing 0.02 wt% sodium azide (NaN_3) was used during sample preparation (MilliQ is a registered trademark of Millipore Corporation).

3.2 Sample Preparation

3.2.1 Reconstitution of Skim Milk Powder

To obtain 20 wt% reconstituted skim milk, low-heat skim milk powder was reconstituted in milliQ water (containing 0.02 wt% NaN_3) in the ratio of 1:4 (w/w). During reconstitution, the milk sample was covered with aluminium foil to prevent evaporation and left to stir gently for at least 2 hours at room temperature (~20°C). The reconstituted milk solutions were left to equilibrate overnight at 4°C before usage. All unused solutions were discarded within 24 hours of reconstitution.

3.2.2 Dilution of ADS Rennet

ADS rennet was diluted in the ratio of 1:10 (w/w) with milliQ water containing 0.02 wt% NaN_3. It was stored at 4°C prior to use. All unused diluted rennet solution was discarded after 48 hours.

3.2.3 Preparation of 1 wt% Polysaccharide and Gelatin Stock Solution

The 1 wt% polysaccharide and 1 wt% gelatin stock solutions were prepared on the day of experiments. The mode of preparation was similar for all the five polysaccharides.

The polysaccharide powder (1 wt%) was added into milliQ water containing 0.02 wt% NaN_3. The reconstituted solution was left to hydrate by stirring for at least 30 minutes at room temperature. This solution was then heated to 75°C for 30 minutes before plunging it into an ice bath to bring the temperature down to 20°C. Any water loss

during heating was replaced and the stock solution was stored overnight at 4°C prior to usage.

3.2.4 Sample Preparation

For each experiment, 50 g of sample was prepared by mixing appropriate amounts of 20 wt% reconstituted skim milk, milliQ water containing 0.02 wt% NaN_3 and 1 wt% polysaccharide or gelatin stock solution, achieving a final concentration of 10 wt% reconstituted skim milk and polysaccharide or gelatin at concentrations of 0 wt%, 0.025 wt%, 0.05 wt%, 0.075 wt% and 0.1 wt%.

All mixed samples, except for κ-carrageenan and gelatin samples, were stirred in a temperature-controlled waterbath at 30°C for at least an hour. For skim milk/κ-carrageenan and skim milk/gelatin samples, they were first heated and stirred at 50°C for 30 minutes before reducing the temperature to 30°C and holding it for 30 minutes. This was necessary for gelling biopolymers like κ-carrageenan and gelatin to ensure that full mixing were achieved.

Prior to rennet addition, all polysaccharide-added samples were stirred continuously at 30°C and 0.2 wt% of 1:10 diluted rennet was added. This corresponded to an addition of 200 μL rennet to 100 g of skim milk sample. Renneted samples were incubated overnight (16 hours) at 30°C for syneresis experiments and CLSM analysis.

3.3 Experimental Methods

3.3.1 Rheology

The rheological properties of the renneted skim milk containing polysaccharide were determined by sinusoidal oscillation using a stress controlled UDS 200 Physica Rheometer (Physica Messtechnik GmbH, D-70567 Stuttgart, Germany). A cup and bob geometry (Z3 DIN) consisting of two coaxial cylinders (diameters 25 and 27.1 mm) was used.

The rheological properties of the sample were determined by low amplitude dynamic oscillation with the measurement of storage G' and loss G'' modulus (Bohlin *et al.*, 1984). During measurement, the cup was oscillated so that the sample was subjected to a harmonic, low amplitude shear strain, γ.

$$\gamma = \gamma_0 \cos \omega t \tag{3.1}$$

Where γ is shear strain, γ_0 is the strain amplitude, ω is the angular frequency $\omega = 2\pi f$. f is the oscillation frequency and t is the time.

The applied shear strain results in a shear stress, σ, of the same angular frequency, which is out of phase by the angle δ:

$$\sigma = \sigma_0 \cos(\omega t + \delta) \tag{3.2}$$

The elastic or storage modulus G', which is a measure of the energy stored per oscillation cycle, is determined from the component of stress that is in phase with the strain. On the other hand, the viscous or loss modulus G'', which is a measure of the energy dissipated as heat per cycle, is part of stress out-of-phase with the strain. G' and G'' are defined as follow:

$$G' = (\tau_0 / \gamma_0) \cos \delta \tag{3.3}$$

$$G'' = (\tau_0 / \gamma_0) \sin \delta \tag{3.4}$$

The complex modulus (G^*) is a measure of the energy dissipated per cycle of deformation per unit volume and is given by:

$$|G^*| = [(G')^2 + (G'')^2]^{1/2} \tag{3.5}$$

In this work, two tests, a dynamic time sweep followed by frequency sweep, were performed at 30°C. For the dynamic time sweep, an applied strain of 0.5% and a constant oscillation frequency of 0.1 Hz were used. Measurements were taken every 60 seconds for 3 hours. At the end of the test, a frequency sweep test was performed by

varying the frequency from 0.01 to 10 Hz at a constant applied strain of 0.5%. Duplicates were conducted.

3.3.2 Syneresis

Syneresis is defined as the shrinkage of gel and this occurs concurrently with the expulsion of liquid or whey separation. Spontaneous surface whey separation, which is measured here, is the contraction of a gel without the application of any external forces (e.g. centrifugation) and is related to instability of the gel network, such as large scale rearrangements, resulting in the loss of the ability to entrap all the serum phase (Walstra, 1993).

Methods for the measurement of syneresis on renneted milk had been constantly reviewed (Stoll, 1966; Marshall, 1982; Lucey *et al.*, 1998a and b). An empirical technique for surface whey quantification of rennet-induced skim milk gels as described by Lucey *et al.* (1998a) was used. Lucey *et al.* (1998b) found that the use of volumetric flasks with sloping walls induced the formation of surface whey for rennet-induced milk gels. Hence, only specially selected glass volumetric flasks (100 mL, Fortuna, West Germany) were used for current measurements. Experimental factors that could influence the syneresis in rennet-induced skim milk gels, as discussed in Chapter 2, had been taken into account. Duplicates (with ten replicates for each test) as well as consistent experimental conditions were followed and performed for reproducibility.

85 g of renneted sample was weighed into 100 mL glass volumetric flasks. The flasks were examined after 16 hours of incubation at 30°C. Any free whey was carefully decanted and weighed. The extent of syneresis was expressed as a percentage of the total initial weight of the renneted sample (~85 g). Syneresis index (%) was calculated using:

$$\text{Syneresis (\%)} = (\text{Weight of whey} / \text{Weight of sample}) \times 100\% \qquad (3.6)$$

3.3.3 Confocal Laser Scanning Microscopy (CLSM)

The fluorescent protein dye, Fast Green FCF (Merck, 64271 Darmstadt, Germany), was dissolved in milliQ water containing 0.02 wt% NaN_3 at a concentration of 1 wt% and stirred for a minimum of an hour. The solution was then filtered under 0.22 μm filter paper and kept covered in foiled at 4°C.

200 μL of the prepared dye was added to 50 g of milk sample. The stained milk was left stirring at room temperature for at least an hour. Prior to rennet addition, the temperature of the solution was brought up and maintained at 30°C. A few drops of the renneted milk mixture were transferred to glass slides with a cavity, which were then covered with a coverslip. The prepared slides were then placed in a petri-dish layered with damped paper towels. This was done to prevent drying out of the sample during incubation at 30°C. The slides were held in a temperature-controlled room for approximately 16 hours.

The gels were examined on a Leica TCS 4D confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany) with a 100x oil immersion objective (numerical aperture = 1.4). The confocal microscope had an air-cooled Argon/Krypton (Ar/Kr) laser that was used with an excitation wavelength of 488 nm.

3.3.4 Diffusing Wave Spectroscopy (DWS)

DWS measurements were performed on samples (prepared as in Section 3.2) placed in a cuvette and thermostated at 30°C using a waterbath. The DWS set-up (see Figure 3.1) consists of a Spectra Physics 125A laser, operating at λ = 632 nm and delivering 50 mW. The laser beam was expanded to approximately 1 cm diameter at the sample cell. The diffused light was collected by a single-mode fiber (P1-3224-PC-5, Thorlabs Inc. Germany) fitted with a GRIN lense (F230FC-B FC, Thorlabs Inc., Germany). The fibre was placed at the front face of the cell for the back-scattering geometry. The optic fibre was connected to a Malvern photomultiplier tube (PMT) (Malvern Instruments Ltd, Malvern, Worcester, UK), and a Malvern 7132 correlator was used to obtain the intensity autocorrelation function. The samples were contained in a 1 cm square cuvette,

and the autocorrelation functions were collected every 2 minutes over a three-hour period.

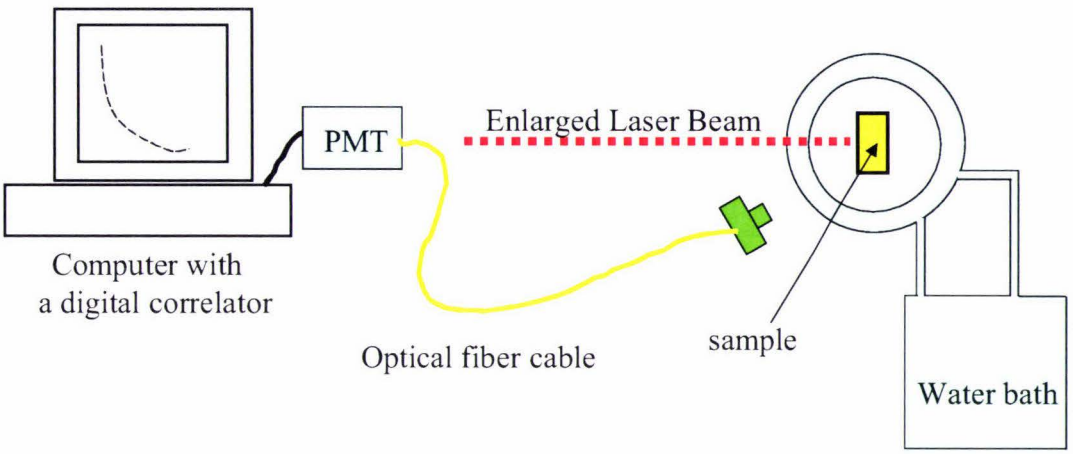

Figure 3.1 Experimental set-up of the diffusing wave spectroscopy (DWS)

In an expanded beam mode, the field autocorrelation function $g_{(1)}(t)$, is obtained from the intensity autocorrelation function using the Siegert relationship:

$$g_{(2)}(t) = 1 + \left|g_{(1)}(t)\right|^2 \tag{3.7}$$

In the back-scattering geometry, $g_{(1)}(t)$ is given by (Weitz and Pine, 1993):

$$g_{(1)}(t) = \frac{\sinh\left[\sqrt{\frac{6t}{\tau}}\left(\frac{L}{l^*} - \frac{z_0}{l^*}\right)\right] + \frac{2}{3}\sqrt{\frac{6t}{\tau}}\cosh\left[\sqrt{\frac{6t}{\tau}}\left(\frac{L}{l^*} - \frac{z_0}{l^*}\right)\right]}{\left(1 + \frac{8t}{3\tau}\right)\sinh\left[\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right] + \frac{4}{3}\sqrt{\frac{6t}{\tau}}\cosh\left[\left(\frac{L}{l^*}\right)\sqrt{\frac{6t}{\tau}}\right]} \tag{3.8}$$

where l^* is the scattering mean free path, z_0 is the penetration depth (in the present case we assume $z_0 \approx l^*$), and L is the sample thickness (= 1 cm) here corresponding to the real value of the wall-to-wall thickness of the cuvette. In the case of particles of radius a undergoing Brownian motion, the relaxation time, τ, is given by:

$$\tau = \frac{1}{Dk_0^2} \quad \text{and} \quad D = \frac{k_B T}{6\pi\eta a} \tag{3.9}$$

where $k_0 = 2\pi / \lambda$ is the incident wavevector, D the Stokes-Einstein diffusion coefficient, k_B is the Boltzmann constant, and η the viscosity of the continuous phase. Thus, knowing τ the product (ηa) can be obtained:

$$\eta a = \frac{2\, k_B\, T}{3\, \pi\, \lambda^2} \tau \tag{3.10}$$

When DWS is carried out under conditions such I/I^* is large, equation (3.7) for back scattering reduces to:

$$g_{(1)}(t) = \exp\left[-\gamma \sqrt{\frac{6t}{\tau}} \right] \tag{3.11}$$

and γ is a constant equal to 2.

In this thesis, τ was obtained by fitting $g_{(1)}(t)$ using Microcal Origin. Knowing τ, (ηa) was calculated using equation (3.10). By obtaining (ηa), the change in viscosity and particle size occurring in the sample could be monitored.

4 Effect of biopolymers addition on the rheological properties of rennet-induced skim milk gels

It has long been known that the conditions (prior heat treatment, protein concentration, protein type, pH, temperature, calcium chloride addition, rennet concentration) have a significant influence on the formation time, the rate of firming, structure and rheological properties of rennet-induced milk gels. Zoon *et al.* (1988a, b, c and 1989a and b) published a series of related publications detailing the effects of these factors on rennet-induced skim milk gels. This investigation was aimed at understanding the influence of several polysaccharides, including κ-carrageenan, xanthan gum, guar gum and HM pectin on rennet gels under defined experimental (model systems) conditions. The effect of gelatin was also obtained for comparison.

4.1 Data analysis

Experimental details for rheological determination of renneted samples are described in Chapter 3. Figure 4.1 shows typical rheology results. The aggregation (Point 1) and gelation time (Point 2) as well as the final gel strength (Point 3) were obtained from the curve of complex modulus, G^*, against time. Aggregation time, marked as Point 1, was determined as the last point before the start of the exponentially increasing curve, while gelation time was established as the time when G^* is equal or higher than 1 Pa. As G^* subsequently continued to increase with time, the gel strength of the sample was defined as the last G^* value obtained at the end of the 3-hour measurement.

Values of aggregation and gelation times as well as gel strength of the model systems studied will be presented in Chapter 7.

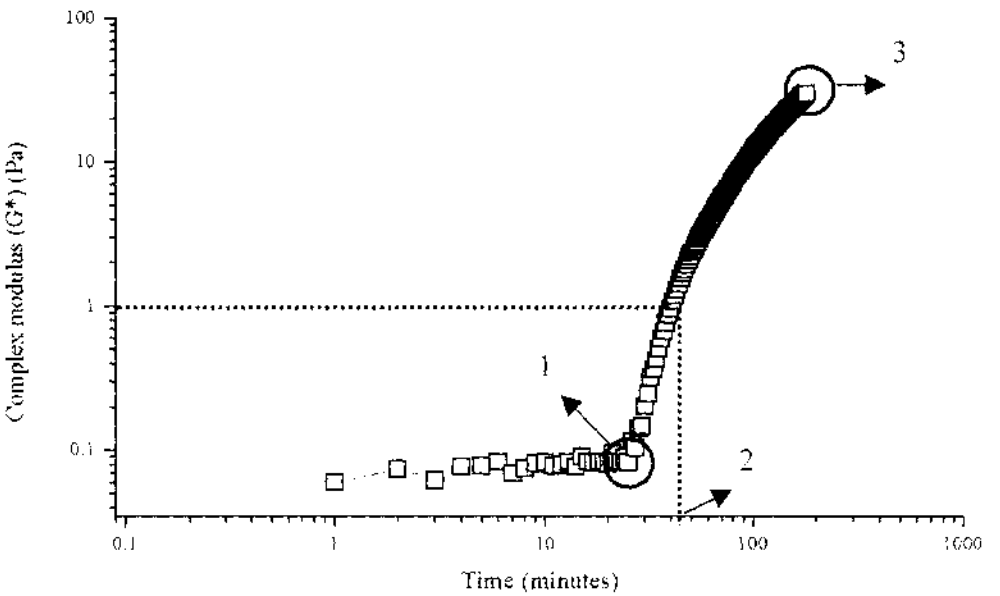

Figure 4.1 Typical graph of a rheological dynamic time sweep measurements of the control sample (10 wt% reconstituted SMP, 0 wt% polysaccharide). (1) Aggregation time; (2) Gelation time; and (3) Gel strength

4.2 Results

4.2.1 Kappa-carrageenan

Complex moduli as a function of time for renneted skim milk containing κ-carrageenan are shown in Figure 4.2. A small addition of κ-carrageenan (0.025 wt%) resulted in an increase in G^* while a decrease in G^* was observed with increasing κ-carrageenan concentrations (0.025 wt% to 0.1 wt%). This was particularly noticeable for renneted skim milk at relatively high κ-carrageenan concentrations, 0.075 wt% and 0.1 wt%, where G^* were lower than the control sample (0 wt% κ-carrageenan) after 80 minutes. These curves indicated that there was little increase in G^* with time.

The aggregation and gelation times derived from Figure 4.2, as defined in Section 4.1, decreased when κ-carrageenan was added, especially at higher concentrations.

The frequency sweep test conducted straight after the 3-hour measurement period is reported in Figure 4.3. This test was performed to investigate the viscoelastic properties of the rennet gel with and without κ-carrageenan addition as a function of frequency. Figure 4.3 shows that renneted skim milk at 0.025 wt% κ-carrageenan addition had higher G' than the control sample. Conversely, renneted skim milk with 0.05 wt%, 0.075 wt% and 0.1 wt% κ-carrageenan had lower G' than the control sample.

Furthermore, G' was higher than G'', indicating gel formation. The difference between G' and G'' at each concentration was less than a decade. This was also an indication that these samples behaved as weak gels. To summarise, renneted skim milk containing 0.025 wt% κ-carrageenan had a higher G^* than renneted skim milk without κ-carrageenan addition, but samples with 0.05 wt%, 0.075 wt% and 0.1 wt% κ-carrageenan had lower G^* values.

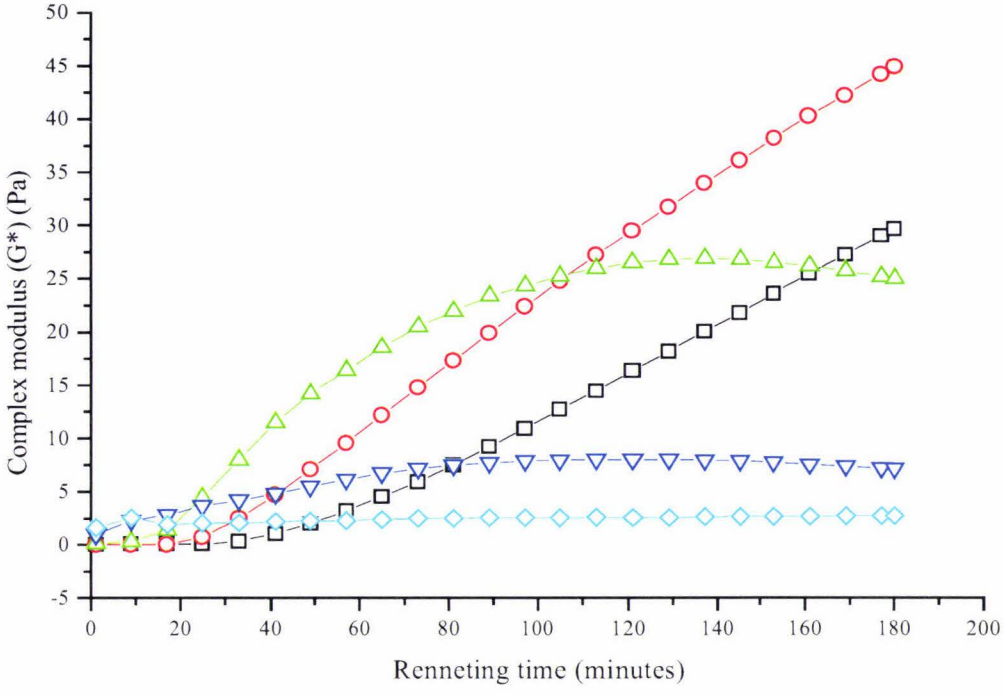

Figure 4.2 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.025 wt% (○), 0.05 wt% (△), 0.075 wt% (▽) and 0.1 wt% (◇) κ-carrageenan as a function of renneting time at 30°C.

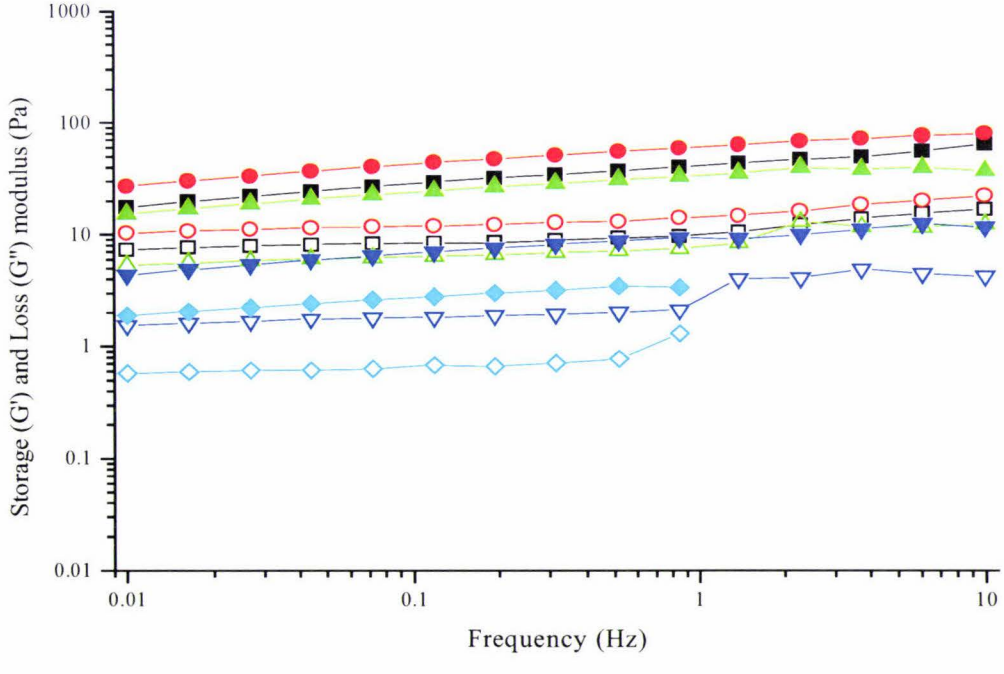

Figure 4.3 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.025 wt% (G' - ●, G'' - ○), 0.05 wt% (G' - ▲, G'' - △), 0.075 wt% (G' - ▼, G'' - ▽) and 0.1 wt% (G' - ♦, G'' - ◇) κ-carrageenan as a function of frequency.

4.2.2 Xanthan gum

Figure 4.4 depicts G^* as a function of time for renneted skim milk containing xanthan gum. G^* decreased with increasing xanthan concentrations. In particular, G^* of renneted skim milk containing xanthan showed that there was little increase in G^* with time.

The aggregation and gelation times also increased with increasing xanthan concentrations. Samples containing xanthan at 0.075 wt% and 0.1 wt% concentrations did not show any signs of gel formation as evidenced from the extremely low G^* (< 1 Pa).

The G' and G'' of renneted skim milk containing xanthan as a function of frequency are shown in Figure 4.5. All the samples generally showed higher G' than G'' with increasing frequency. In short, experimental data showed that renneted skim milk containing xanthan formed very weak gels, and did not gel at concentrations higher than 0.025wt%, as at higher xanthan concentrations as G' and G'' were lower than 1 Pa.

4.2.3 Guar gum

Figure 4.6 displays G^* of renneted skim milk containing 0 wt% to 0.1 wt% guar as a function of renneting time. For the first 50 minutes of measurement, all the guar added samples had higher G^* than the control sample (0 wt% guar). In general, the G^* of renneted skim milk containing guar increased with decreasing guar concentration, with the sample at 0.025 wt% guar addition having the highest G^* (~ 60 Pa). After 50 minutes, the G^* of renneted skim milk samples at (lower) 0.025 wt% and 0.05 wt% guar concentrations were still higher than the control sample, while at higher concentrations (0.075 wt% and 0.1 wt%), G^* were lower than the control sample. In addition, Figure 4.6 also showed that G^* of renneted skim milk with guar addition had increased with time.

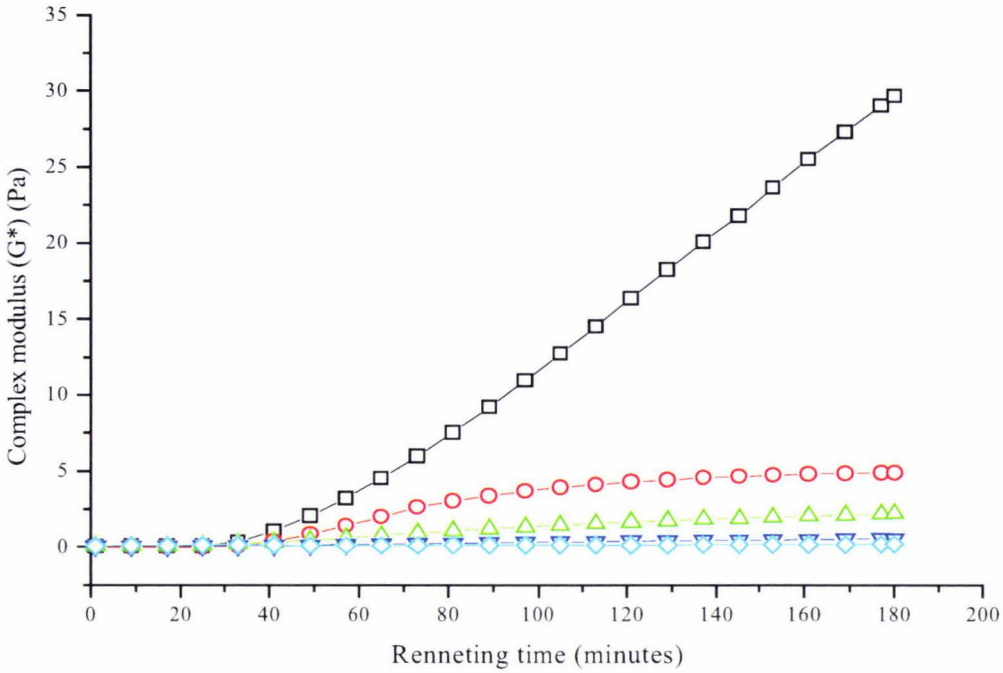

Figure 4.4 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.025 wt% (○), 0.05 wt% (△), 0.075 wt% (▽) and 0.1 wt% (◇) xanthan as a function of renneting time at 30°C.

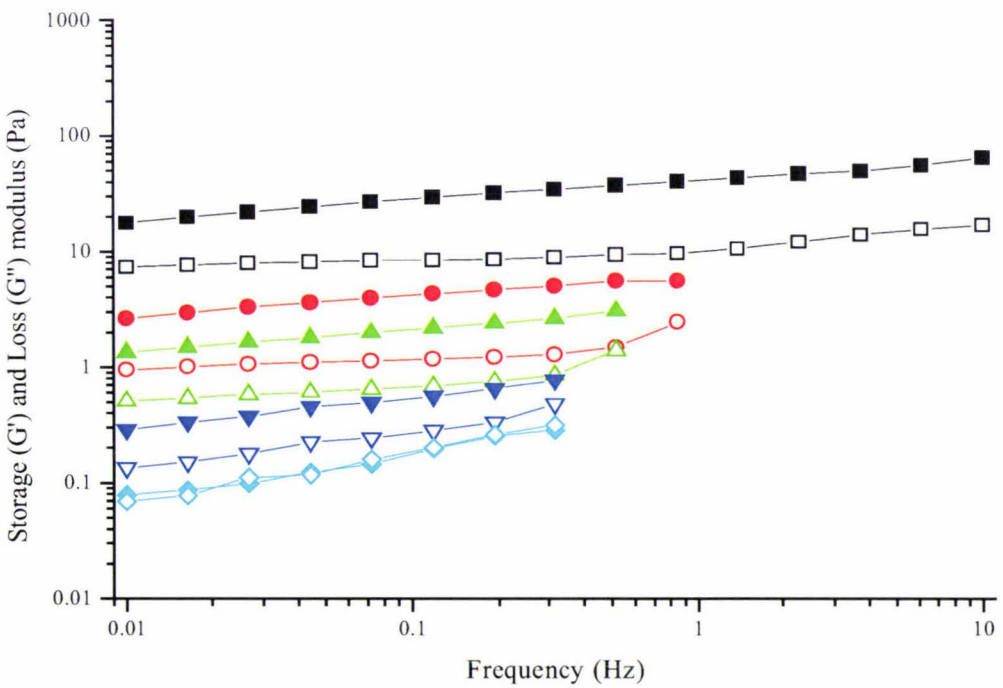

Figure 4.5 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.025 wt% (G' - ●, G'' - ○), 0.05 wt% (G' - ▲, G'' - △), 0.075 wt% (G' - ▼, G'' - ▽) and 0.1 wt% (G' - ♦, G'' - ◇) xanthan as a function of frequency.

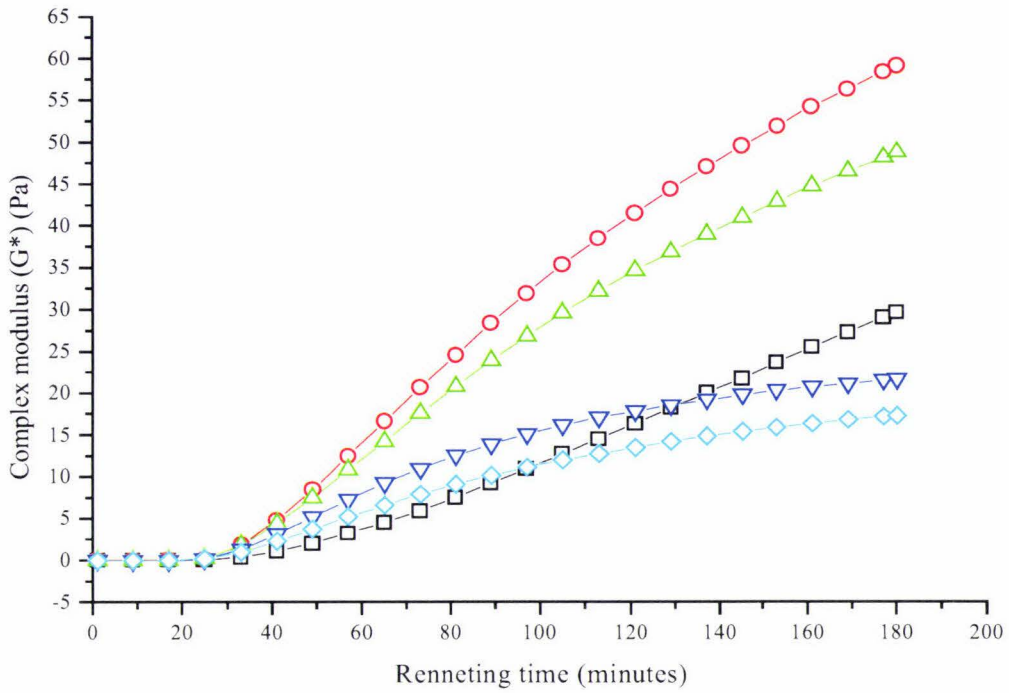

Figure 4.6 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.025 wt% (○), 0.05 wt% (△), 0.075 wt% (▽) and 0.1 wt% (◇) guar as a function of renneting time at 30°C.

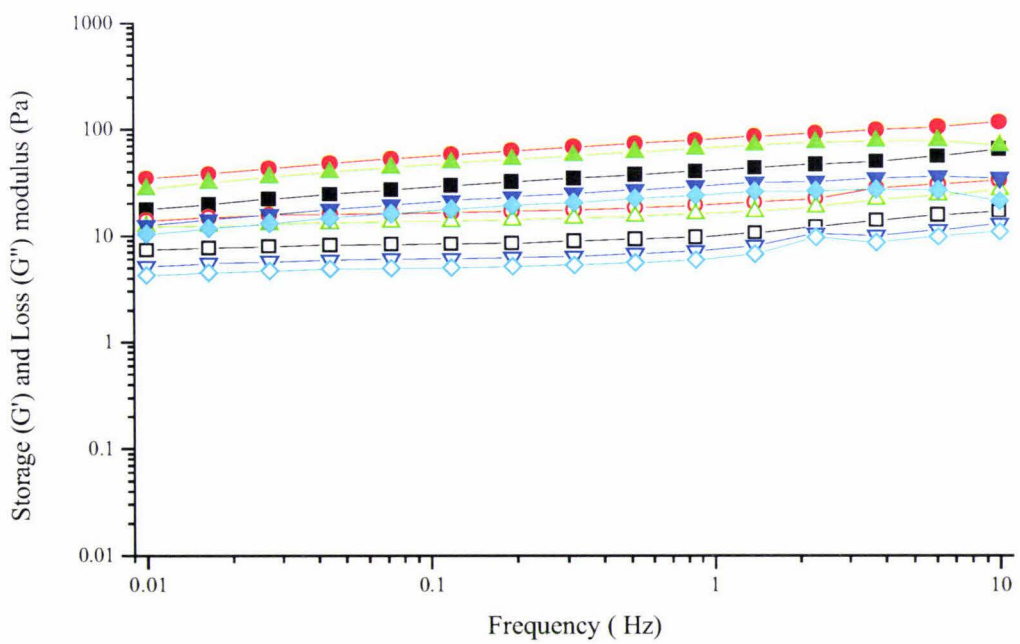

Figure 4.7 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.025 wt% (G' - ●, G'' - ○), 0.05 wt% (G' - ▲, G'' - △), 0.075 wt% (G' - ▼, G'' - ▽) and 0.1 wt% (G' - ♦, G'' - ◇) guar as a function of frequency.

The aggregation and gelation times obtained for renneted skim milk containing guar (Figure 4.6) showed different trends, with decreasing aggregation times and increasing gelation times for renneted skim milk containing increasing concentrations of guar. This indicated that at higher guar concentrations, aggregation times were shortened, while the times taken for gelation were longer. Nonetheless, experimental results showed that when compared to the control sample, both the aggregation and gelation time of renneted skim milk were shortened upon the addition of guar gum.

Figure 4.7 illustrates the G' and G'' of renneted skim milk containing guar as a function of frequency. As expected, G'' was lower than G' indicating the elastic nature of these samples. Renneted skim milk samples containing 0.025 wt% and 0.05 wt% guar had higher G' than the control sample. However, at 0.075 wt% guar concentration, G' was slightly lower than the control sample. Note that here again, a difference of less than a decade between G' and G'' was observed as an indication that these samples had behaved as weak gels.

4.2.4 High-methoxyl (HM) Pectin

Figure 4.8 exhibits G^* of renneted skim milk containing HM pectin as a function of time. Under standard experimental conditions, increasing G^* with increasing HM pectin concentrations was observed, with renneted skim milk at 0.1 wt% HM pectin concentration having the highest G^*. However, the differences in the G^* increment for renneted skim milk at HM pectin concentrations, 0.05 wt%, 0.075 wt% and 0.1 wt% were small. Nevertheless, it was obvious that renneted skim milk with added HM pectin had higher G^* than the control sample. In addition, G^* was shown to increase with time.

A decrease in aggregation and gelation times, upon HM pectin addition at increasing concentrations, was also observed.

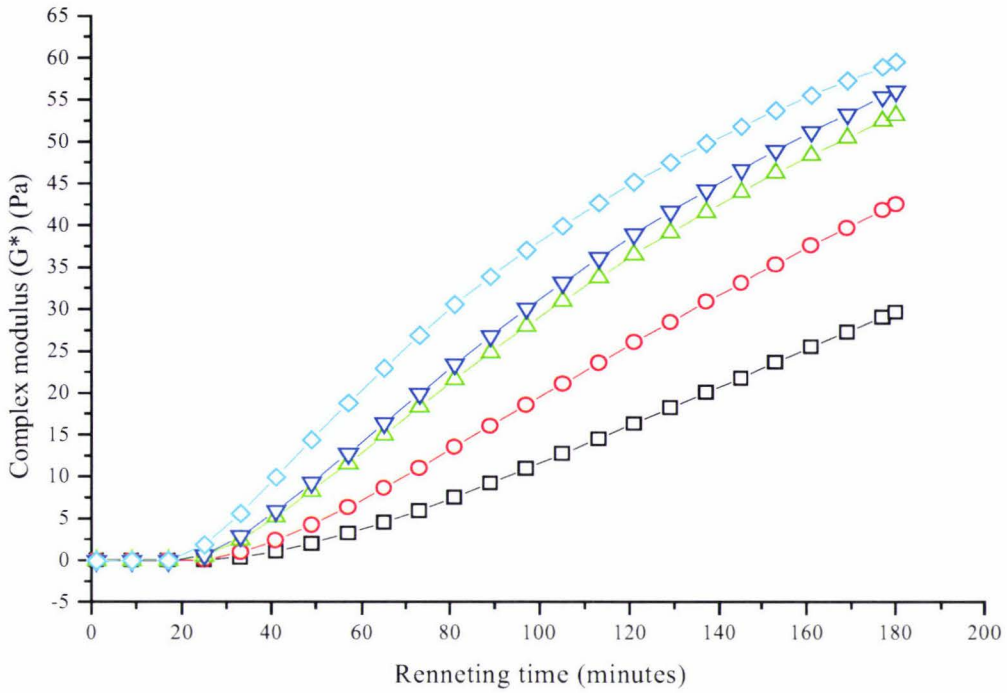

Figure 4.8 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.025 wt% (○), 0.05 wt% (△), 0.075 wt% (▽) and 0.1 wt% (◇) HM pectin as a function of renneting time at 30°C.

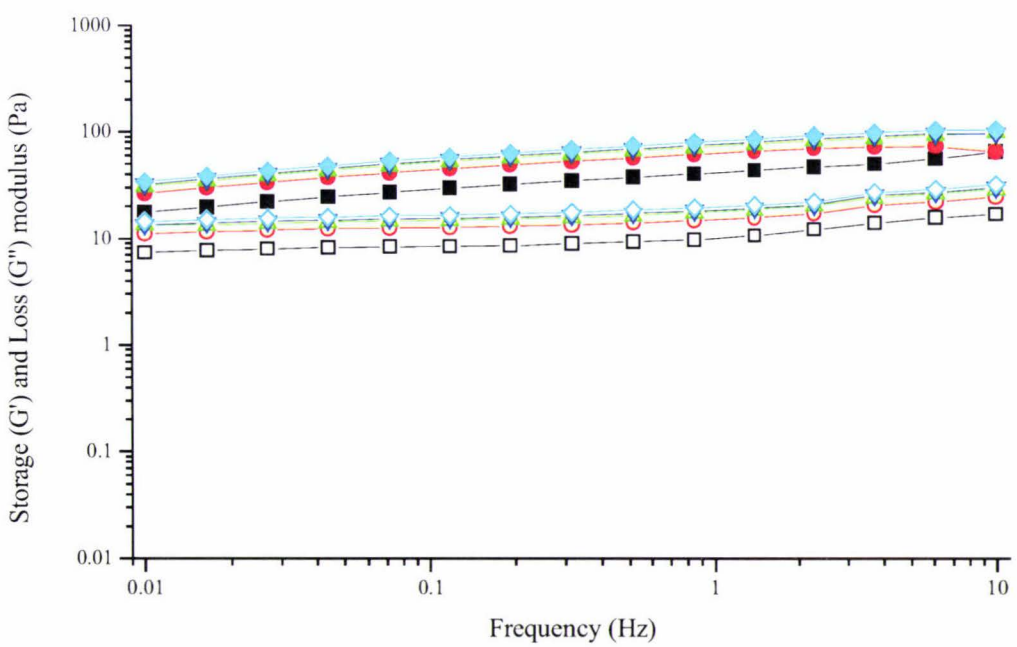

Figure 4.9 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.025 wt% (G' - ●, G'' - ○), 0.05 wt% (G' - ▲, G'' - △), 0.075 wt% (G' - ▼, G'' - ▽) and 0.1 wt% (G' - ♦, G'' - ◇) HM pectin as a function of frequency.

The frequency sweep test is reported in Figure 4.9. G' increased with increasing HM pectin concentrations. Similar results were found for G'', except that the values were lower. The measurements showed that all the gels behaved as elastic materials with G' higher than G'', and both G' and G'' showing little dependence on frequency. In addition, Figure 4.9 shows that renneted skim milk samples containing HM pectin also behaved as "weak gels".

4.2.5 Gelatin

Figure 4.10 displays an array of narrowly close G^* versus time curves for renneted skim milk containing gelatin. Despite that, it could still be distinguished that G^* did increase with an increase in gelatin concentration. Similar to renneted skim milk containing HM pectin, G^* of renneted skim milk with gelatin addition increased with time. Aggregation and gelation times were shortened when gelatin concentration was increased.

Although frequency sweep measurements showed that there was an increase in G' and G'' (Figure 4.10) with increasing gelatin concentrations, the samples behaved as weak gels. All the gels had behaved as elastic materials with G' higher than G'', and both G' and G'' showing little dependence on frequency. Experimental data had also confirmed that there was a slight increase in G' and G'' with increasing gelatin concentrations.

4.3 Comparison between the renneted samples

The earlier sections had discussed the individual rheological aspects on the addition of κ-carrageenan, xanthan, guar, HM pectin and gelatin on rennet-induced skim milk. Figure 4.12 plots G^* as a function of time for renneted skim milk containing the four different polysaccharides and gelatin at the same concentration.

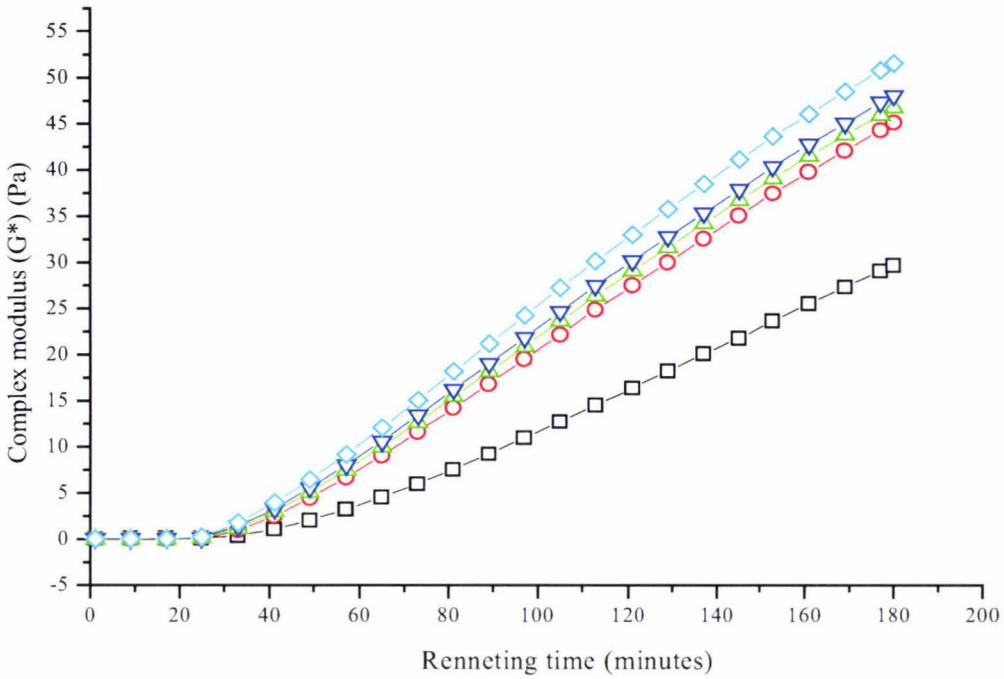

Figure 4.10 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.025 wt% (○), 0.05 wt% (△), 0.075 wt% (▽) and 0.1 wt% (◇) gelatin as a function of renneting time at 30°C.

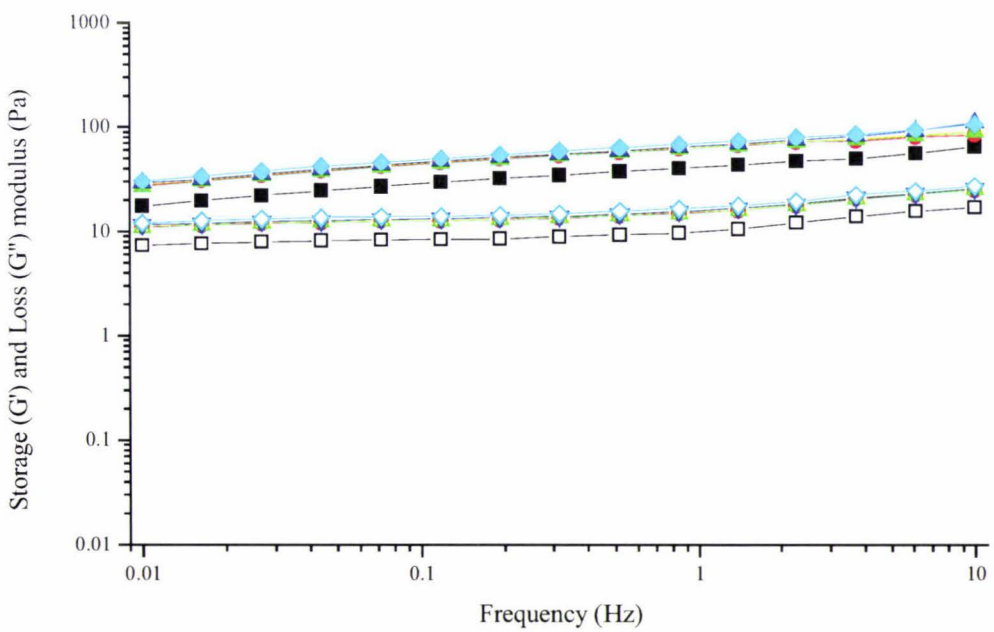

Figure 4.11 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.025 wt% (G' - ●, G'' - ○), 0.05 wt% (G' - ▲, G'' - △), 0.075 wt% (G' - ▼, G'' - ▽) and 0.1 wt% (G' - ◆, G'' - ◇) gelatin as a function of frequency.

Figure 4.12 (A) shows that for samples at 0.025 wt% polysaccharide concentration, guar had the highest G^* while xanthan had the lowest. All the polysaccharides, except xanthan, had G^* higher than the control sample (0 wt% polysaccharide). As was discussed earlier, this showed that the addition of κ-carrageenan, guar, HM pectin and gelatin, at 0.025 wt% concentration, improved the viscoelastic properties of renneted skim milk, but the addition of xanthan had the opposite effect. Additionally, Figure 4.12 (A) shows that G^* of renneted skim milk containing κ-carrageenan, HM pectin and gelatin, respectively, displayed very similar G^* values.

Figure 4.12 (B) depicts G^* of rennet milk gels with polysaccharides added at 0.05 wt% concentration, in descending order as follows: HM pectin, guar, gelatin, κ- carrageenan and xanthan gum. Similar to Figure 4.12 (A), all the polysaccharides, except xanthan, had higher G^* than the control sample.

Figure 4.12(C) and (D) display G^* of renneted skim milk with polysaccharide concentrations, at 0.075 wt% and 0.1 wt%, respectively. It could be observed from both graphs (C and D) that renneted skim milk containing respective additions of HM pectin and gelatin had higher G^* than the control sample, but κ-carrageenan, xanthan and guar containing samples displayed lower G^* than the control sample.

Figure 4.13 shows the aggregation and gelation times of renneted skim milk/polysaccharides. Aggregation time was associated with the initial proteolysis stage (see Figure 4.13 A) and the control sample started aggregation after 24 minutes and formed a gel at 41 minutes (see Figure 4.13 B). In general, all the renneted skim milk samples with added polysaccharide, except for samples with added xanthan, showed shorter aggregation and gelation times than the control sample.

It should be noted that aggregation and gelation times of renneted skim milk containing higher concentrations (0.075 wt% and 0.1 wt%) of κ-carrageenan and xanthan were not obtained, as there was no gel formation in these samples

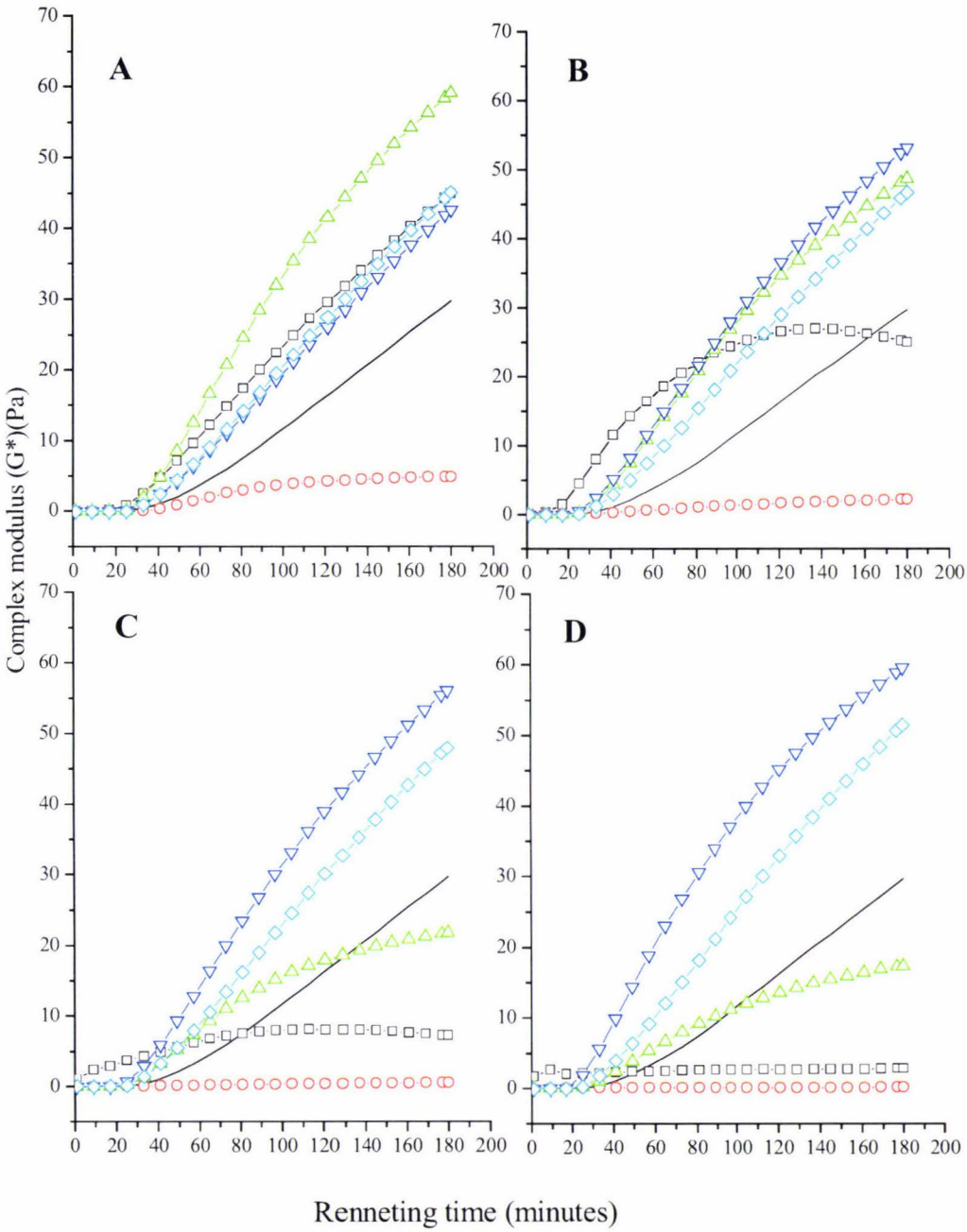

Figure 4.12 *G as a function of renneting time of renneted skim milk containing κ-carrageenan (□), xanthan (○), guar (△), HM pectin (▽) and gelatin (◇). Results from four different concentrations, 0.025 wt% (A), 0.05 wt% (B), 0.075 wt% (C) and 0.1 wt% (D) were displayed. Continuous black line across the bar chart represented the control sample (0 wt% biopolymer).**

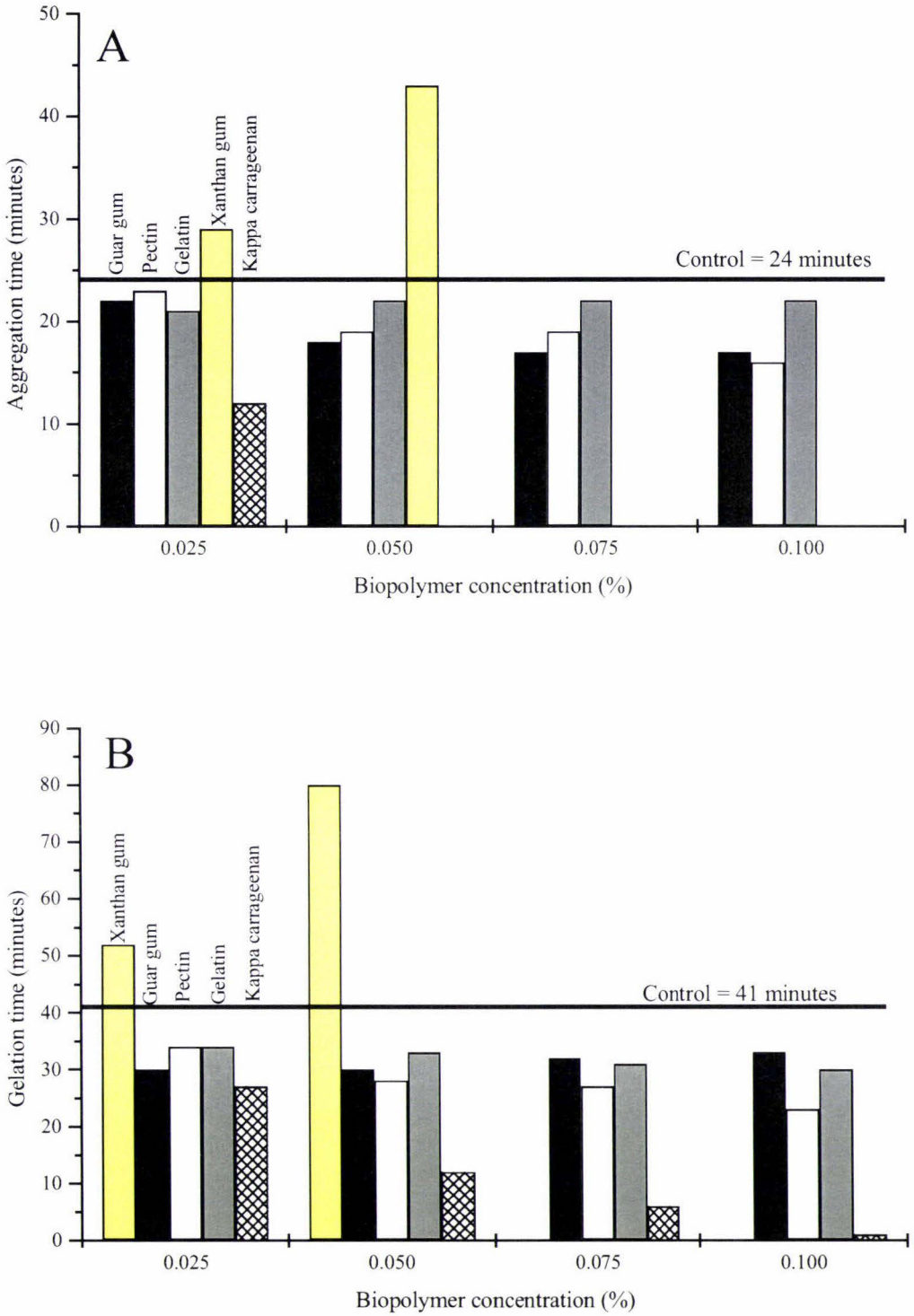

Figure 4.13 Aggregation times (A) and gelation times (B), obtained from dynamic time sweep test, of renneted skim milk containing κ-carrageenan (⊠), xanthan gum (▒), guar gum (■), HM pectin (□) and gelatin (▒). Results from four different concentrations, 0.025 wt%, 0.05 wt%, 0.075 wt% and 0.1 wt% were displayed. Continuous black line across the bar chart represented the control sample (0 wt% biopolymer).

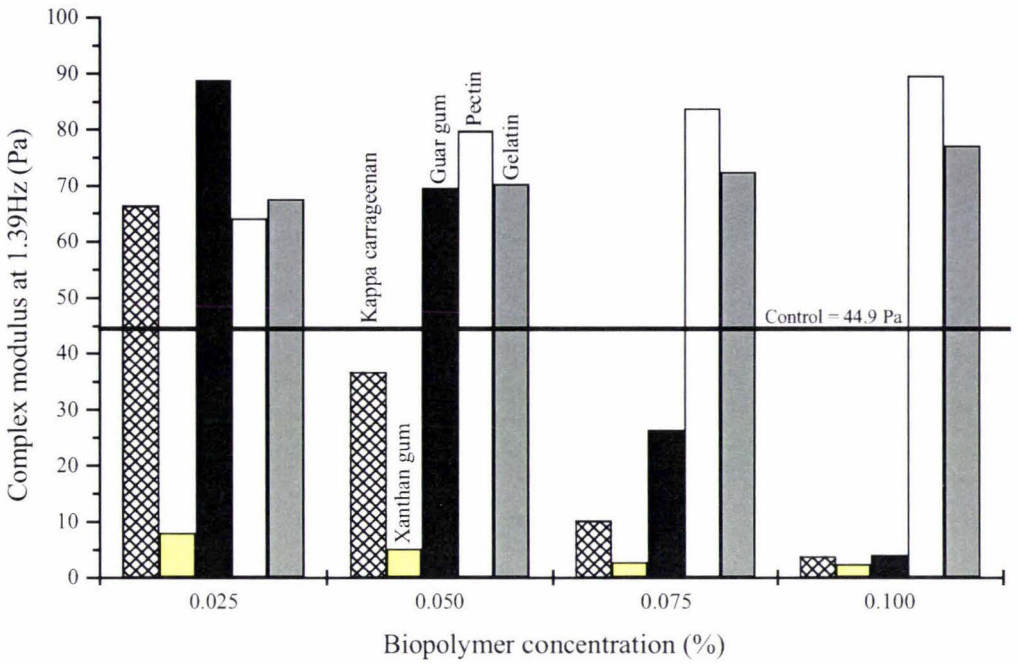

Figure 4.14 G^* at 1.39 Hz, obtained from frequency sweep test, of renneted skim milk containing κ-carrageenan (⊠), xanthan gum ( ), guar gum (■), HM pectin (□) and gelatin ( ). Results from four different concentrations, 0.025 wt%, 0.05 wt%, 0.075 wt% and 0.1 wt% were displayed. Continuous black line across the bar chart represented the control sample (0 wt% biopolymer).

A comparison on the viscoelastic properties of renneted skim milk containing polysaccharide as a function of time at different concentrations has already been discussed. A frequency sweep test at increasing frequency (0.01 to 10 Hz) was conducted straight after the dynamic time sweep measurement.

Figure 4.14 reports G^* at 1.39 Hz, obtained from the frequency sweep test, for the different polysaccharide containing samples as a function of concentration.

In general, results from Figure 4.14 duplicated results as discussed earlier in Figure 4.12. This showed that HM pectin and gelatin improved the gel properties. The explanations for the trends observed on the effects of different polysaccharides will be discussed in more detail in Chapter 8.

4.4 Summary

Dynamic rheological measurements were performed on renneted skim milk containing κ-carrageenan, xanthan, guar, HM pectin and gelatin at concentrations ranging from 0 wt% to 0.1 wt%. It was found that the respective additions of these five materials into renneted skim milk resulted in different rheological behaviour. Renneted skim milk containing xanthan showed poor gelling properties, while samples with incorporated κ-carrageenan and guar gum displayed better viscoelastic properties at lower concentrations (≤ 0.05 wt%). The addition of HM pectin and gelatin markedly improved the rheological properties of renneted skim milk. In particular, renneted skim milk containing HM pectin displayed the best viscoelastic properties in comparison to the other renneted samples studied here.

5 Effect of biopolymers addition on the syneresis and confocal laser scanning microscopy (CLSM) of rennet-induced skim milk gels

The use of rheology, in conjunction with other techniques, such as permeability and syneresis measurements (see Annex 1) as well as studies on the microstructure of the gel, were used to better understand the structural properties of rennet-induced skim milk. In this chapter, microstructure and syneresis behaviour of rennet skim milk gels containing polysaccharides are presented. Studies on samples containing gelatin are also obtained for comparison.

5.1 Whey separation (syneresis)

Van Vliet *et al.* (1997) and Lucey (2001) acknowledged the dynamic nature of casein gels, and that rearrangements of the clusters and particles forming the network might occur before or during gel formation. Lucey (2001) also noted that the gel-forming process could lead to the formation of dense aggregates and a gel network, which has large pores and is prone to syneresis.

Syneresis of curd formed by the action of rennet on milk comprised shrinkage with expulsion of whey. As defined previously, whey separation referred to the appearance of liquid (whey) on the surface of a milk gel. It could occur if the gel network was damaged or if the gel had undergone substantial structural rearrangement.

In order to investigate the effect of polysaccharides and gelatin on the syneresis behaviour of rennet-induced skim milk gels, measurements of spontaneous surface whey separation were conducted using volumetric flasks, as described in Chapter 3.

Figure 5.1 presents the results of spontaneous surface whey separation from rennet gels containing κ-carrageenan, xanthan, guar, HM pectin and gelatin at concentrations from 0 wt% to 0.1 wt%.

The mean syneresis index of the control sample (0 wt% polysaccharide) was ~ 11.65%. In general, whey separation for gels containing κ-carrageenan and xanthan increased with increasing polysaccharide concentrations (0 wt% to 0.1 wt%), whereas for gels containing HM pectin and gelatin, syneresis values decreased with increasing concentrations. Guar containing samples also depicted increasing syneresis values with increasing concentrations (0.025 wt% to 0.1 wt%), however, the syneresis index at 0.025 wt% concentration was lower than the control (0 wt% guar).

It should be noted that the decrease in the amount of whey measured for rennet skim milk gels containing increasing concentrations of gelatin was not substantial. On the other hand, significant differences for renneted skim milk with incorporation of polysaccharides like κ-carrageenan, xanthan and guar, at concentrations ranging from 0 wt% to 0.1 wt% were observed. For instance, in κ-carrageenan added samples, there was an almost two-fold increase in syneresis between 0.025 wt% and 0.05 wt% concentrations.

Syneresis measurements for renneted skim milk samples containing xanthan, especially at higher concentrations (0.075 wt% and 0.1 wt%), were unable to be conducted. After 16 hours of incubation at 30°C, unlike the other renneted skim milk/polysaccharide samples, there was no formation of any (solid) 'gel structure'. Visually, a gluey and mashy gel-like structure mixed in a pool of whey was obtained. This extremely weak gel-like structure was often decanted out together with the whey, thus making measurements inaccurate and impossible. Despite this, whey separation measurements for lower xanthan concentrations (0.025 wt% and 0.05 wt%) could still be performed and high syneresis values were obtained under these conditions.

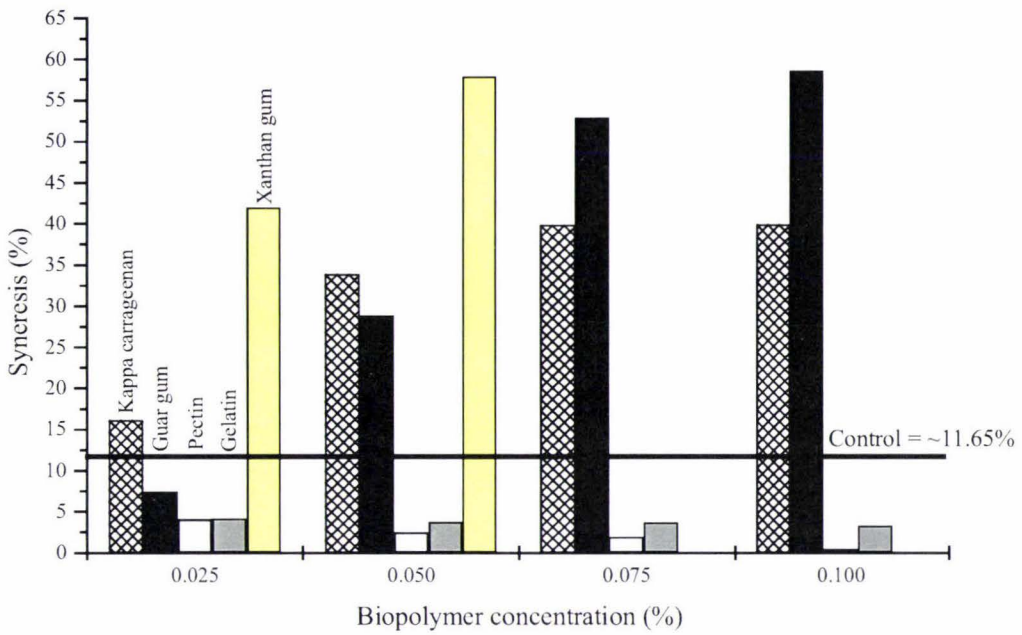

Figure 5.1 Effect of adding κ-carrageenan (⊠), xanthan gum ( ), guar gum (■), HM pectin (□) and gelatin ( ) on the whey separation behaviour of rennet-induced skim milk gels.

5.2 Microstructure

Microscopic observations were made using confocal laser scanning microscopy (CLSM) to describe the microstructure of renneted skim milk gels with and without polysaccharide addition. Fast Green FCF dye stains milk proteins. Therefore, milk proteins appeared white in the micrographs, while dark/black areas corresponded to zones devoid of milk proteins. Experimental methods for CLSM measurements are described in Chapter 3. Depth monitoring and duplicates were conducted. In each slide, micrographs from three different areas were scanned and duplicate images did not seem to differ.

5.2.1 κ-Carrageenan

Figure 5.2 (A) shows the microstructure of rennet skim milk gels without κ-carrageenan addition. A continuous and branched casein network structure was observed, indicating a compact and 'firm' gel. For the sample with 0.025 wt% added κ-carrageenan, the CLSM micrograph (Figure 5.2 B) had not shown any visual difference when compared to the control (Figure 5.2 A), with the exception that Figure 5.2 (B) projects a slightly more dense casein network arrangement. This was in contrast to the samples prepared at higher κ-carrageenan concentrations, where noticeable difference in microstructures (between Figure 5.2 A and Figure 5.2 C, D and E) could be seen. At 0.05 wt% added κ-carrageenan, large protein aggregates and bigger dark regions were observed (Figure 5.2 C).

Similar structures (Figure 5.2 D and E) were observed for samples at 0.075 wt% and 0.1 wt% κ-carrageenan. The difference between Figure 5.2 B and C was also obvious. A less compact protein network structure in Figure 5.2 (C), with sizes of the pores (dark regions) ranging from 5 to 20 μm, larger than samples with lower concentration of κ-carrageenan (Figure 5.2 B).

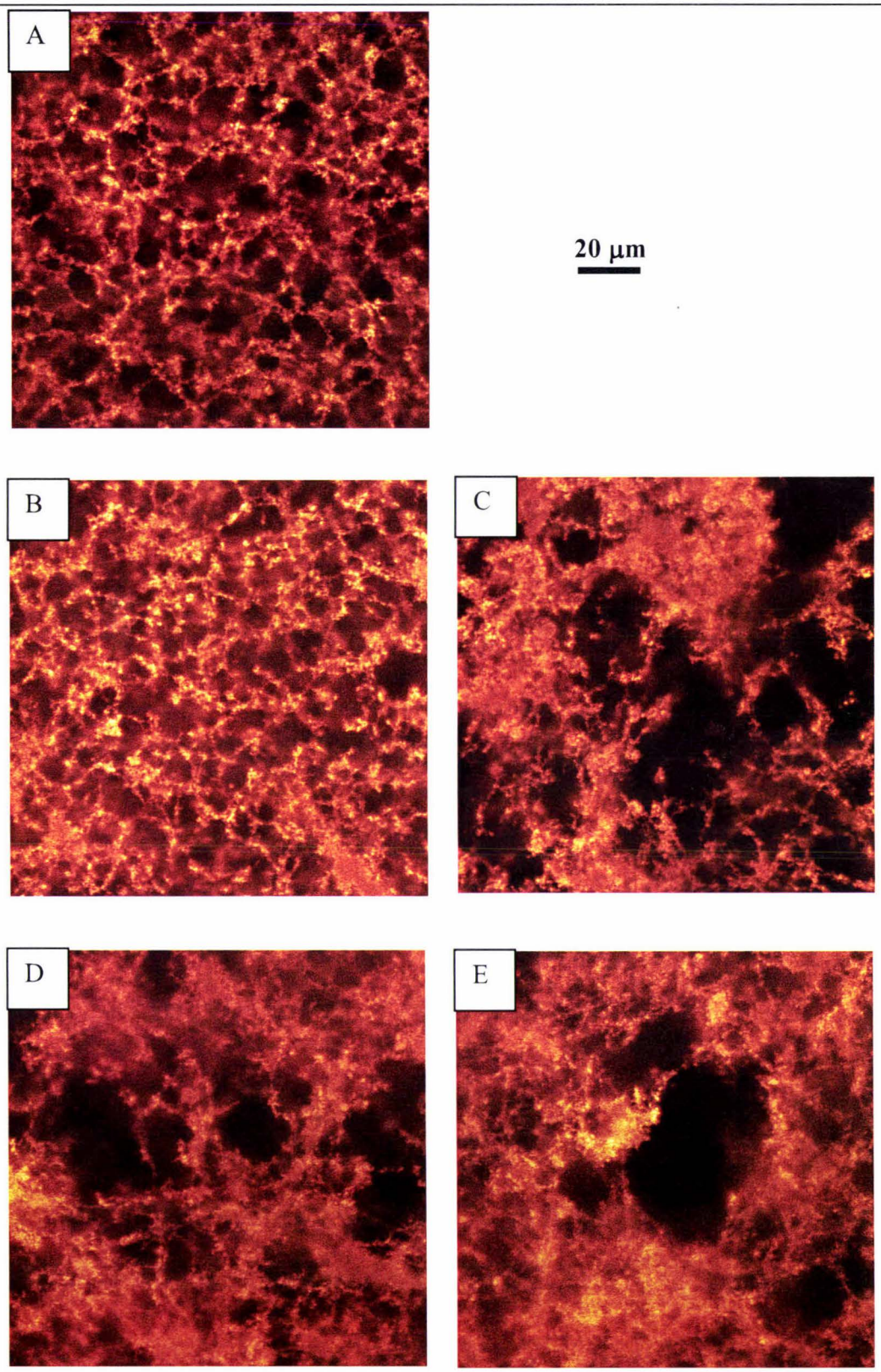

Figure 5.2 Confocal micrographs of rennet skim milk gels containing κ-carrageenan at (A) 0 wt%, (B) 0.025 wt%, (C) 0.050 wt%, (D) 0.075 wt% and (E) 0.100 wt% concentrations. The skim milk concentration was 10 wt%. Bar scale corresponded to 20 μm.

Overall, the addition of κ-carrageenan into renneted skim milk had significantly changed the microstructural state of the original renneted gel, with a noticeable increase in pore size at high κ-carrageenan concentrations.

5.2.2 Xanthan gum

The control sample (Figure 5.3 A – 0 wt% xanthan) appeared to be visually different from all the xanthan added samples (Figure 5.3 B, C, D and E). The addition of xanthan gum changed the original microstructure of the rennet-induced skim milk. This was apparent from the absence of a "branched" protein network, as was obtained in Figure 5.3 (A).

At 0.025 wt% and 0.05 wt% xanthan concentrations (Figure 5.3 B and C), clustering of milk proteins forming large aggregates were evident. These micrographs had neither displayed a branched network structure nor individual spherical aggregates of protein, instead, a slightly 'dense' protein structure with large, irregular clumps of protein was observed.

Figure 5.3 (D) and (E) had illustrated different images from Figure 5.3 (B) and (C). An 'emulsion-like' structure of spherical protein aggregates about 5-10 μm in diameter was observed. Note that Figure 5.3 (D) and (E) could explain why syneresis measurements were unable to be conducted for renneted skim milk containing 0.075 wt% and 0.1 wt% xanthan concentrations. In the place of a branched protein network, a dispersion of spherical protein particles or an 'emulsion-like' structure was obtained, suggesting the absence of gelation.

5.2.3 Guar gum

Figure 5.4 displays CLSM micrographs of rennet skim milk gels containing guar at 0 wt% to 0.1 wt% concentrations. Figure 5.4 (B) appeared to adopt a similar microstructure image as Figure 5.4 (A), with the exception that the latter exhibiting a slightly more 'open' structure.

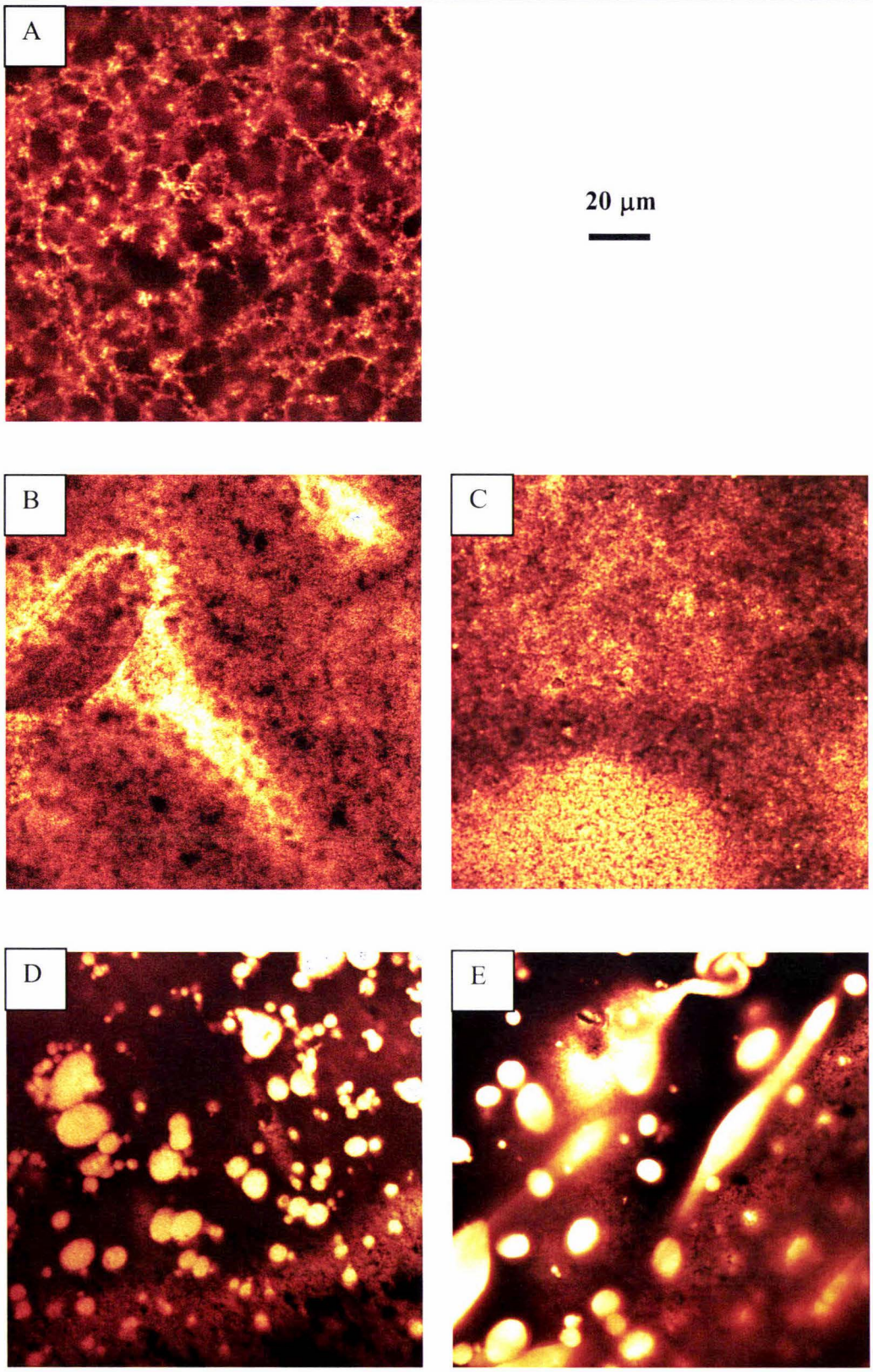

Figure 5.3 Confocal micrographs of rennet skim milk gels containing xanthan gum at (A) 0 wt%, (B) 0.025 wt%, (C) 0.050 wt%, (D) 0.075 wt% and (E) 0.100 wt% concentrations. The skim milk concentration was 10 wt%. Bar scale corresponded to 20μm.

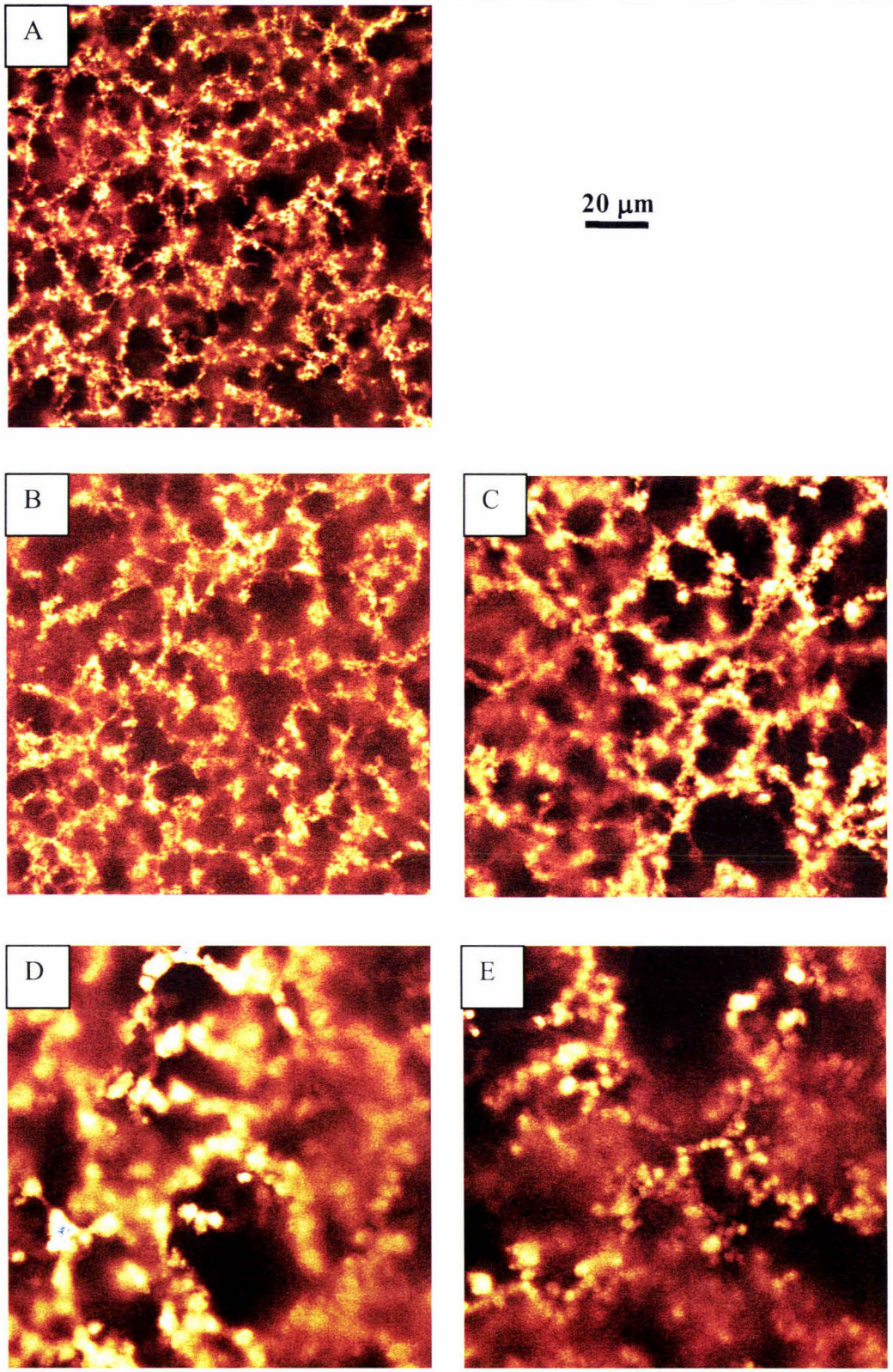

Figure 5.4 Confocal micrographs of rennet skim milk gels containing guar gum at (A) 0 wt%, (B) 0.025 wt%, (C) 0.050 wt%, (D) 0.075 wt% and (E) 0.100 wt% concentrations. The skim milk concentration was 10 wt%. Bar scale corresponded to 20μm.

Apart from the presence of larger pores indicative of a more 'open' gel structure, Figure 5.4 (C) also displayed a branched network structure similar to Figure 5.4 (A and B). The size of the dark regions ("voids") seemed to increase with increasing guar concentrations. This was especially evident in Figure 5.4 (D and E) where the partition of the fluorescence was not as regular as in Figure 5.4 (A and B). Thus, it was clear that the addition of guar alter the original microstructure of rennet-induced skim milk gels and an increase in the pore sizes was observed with an increase in guar concentration.

5.2.4 HM Pectin

Unlike the earlier three polysaccharides, CLSM micrographs of all samples containing HM pectin (Figure 5.5 A, B, C, D and E) illustrated compact, branched protein network structures. The similarity between these images had made it particularly hard to account for any microstructural differences with increasing HM pectin concentrations (0 wt% to 0.1 wt%).

In general, Figure 5.5 (A) seemed to have assumed a structure that is slightly less dense than the other four micrographs. No justifiable differences could be commented for Figure 5.5 (B), (C), (D) and (E), although they had all visually shown to have a more compact gel structure than for the sample without HM pectin addition.

5.2.5 Gelatin

There was no obvious difference in the micrographs of renneted skim milk containing gelatin at 0 wt% to 0.1 wt% concentrations (Figure 5.6). In general, Figure 5.6 (A, B, C, D and E) featured protein networks that were very densely structured. Similar to the rennet gels containing HM pectin, micrographs taken from CSLM were not able to project microstructural changes in renneted skim milk containing gelatin at the concentrations used.

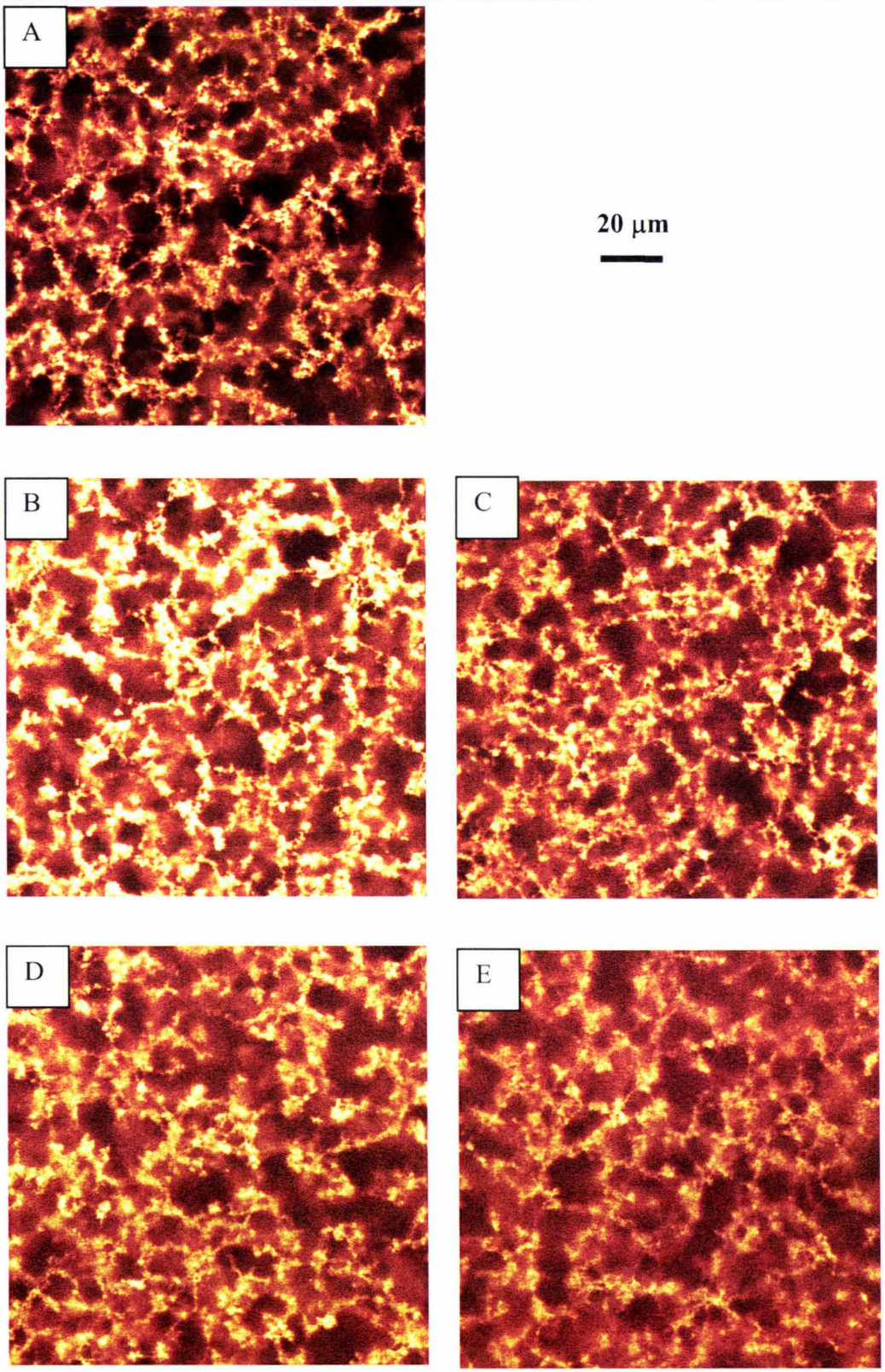

Figure 5.5 Confocal micrographs of renneted skim milk gels containing HM pectin at (A) 0 wt%, (B) 0.025 wt%, (C) 0.050 wt%, (D) 0.075 wt% and (E) 0.100 wt% concentrations. The skim milk concentration was 10 wt%. Bar scale corresponded to 20μm.

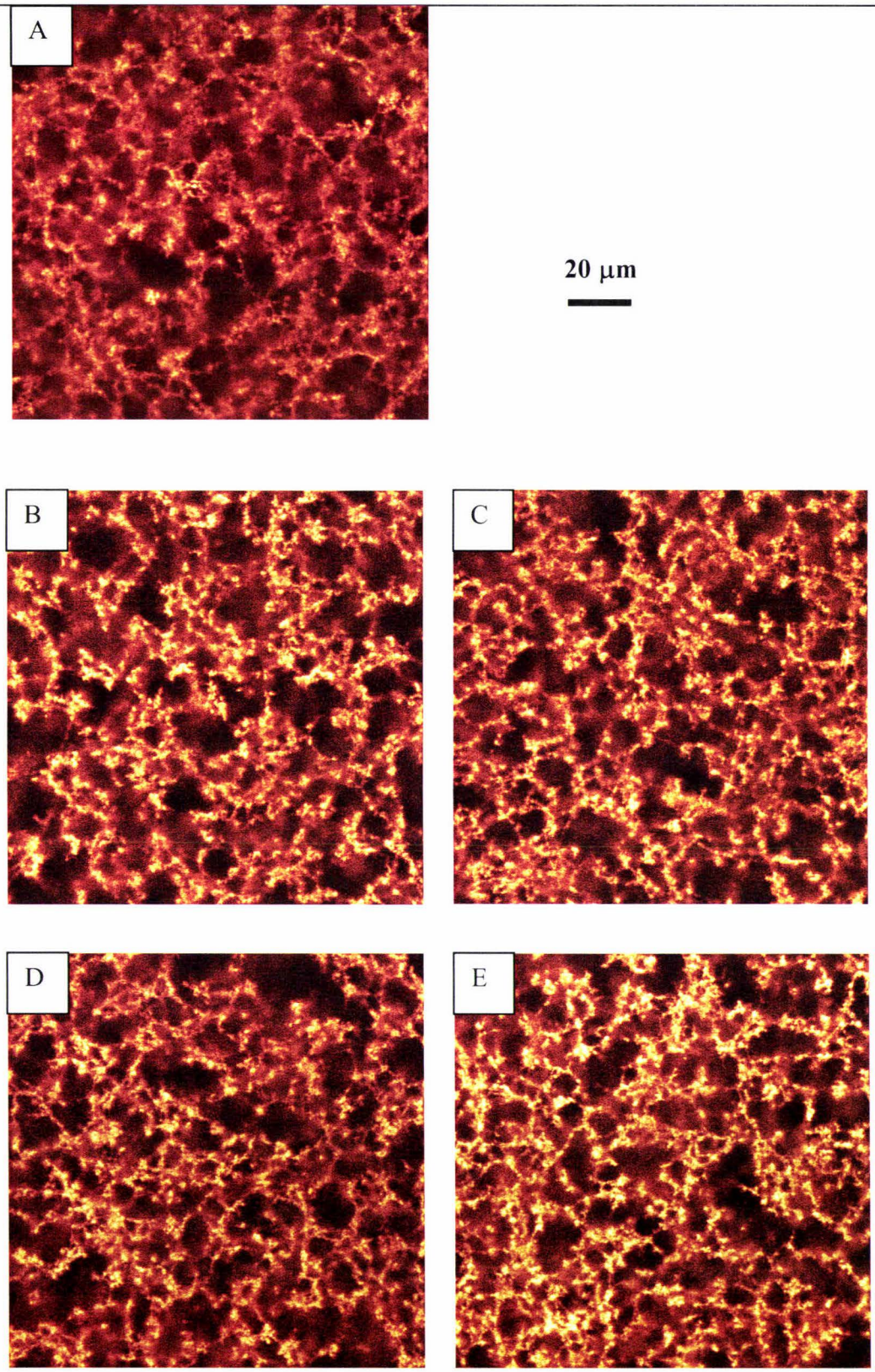

Figure 5.6 Confocal micrographs of rennet skim milk gels containing gelatin at (A) 0 wt%, (B) 0.025 wt%, (C) 0.050 wt%, (D) 0.075 wt% and (E) 0.100 wt% concentrations. The skim milk concentration was 10 wt%. Bar scale corresponded to 20μm.

5.3 Relationship between syneresis measurements and CLSM observations

The additions of κ-carrageenan, xanthan and guar in renneted skim milk induced various effects on the original microstructure properties of rennet-induced skim milk gels.

The results suggest that the respective incorporation of κ-carrageenan, xanthan and guar, considerably modified the three-dimensional organization of the casein particles gel network. This was apparent from higher syneresis values and larger pore sizes imaged with increasing polysaccharide concentrations. Through the use of a simple technique to quantify syneresis, it was found that rennet gels with respective additions of κ-carrageenan, xanthan and guar gave higher levels of whey separation as the concentrations increased.

Rennet gels without added polysaccharide had constantly exhibited a continuous and branched protein network structure. However, for gels containing κ-carrageenan, xanthan and guar, the presence of large pores and less 'dense' branched network structure was observed. The contraction of the casein network, resulting in formation of larger pores, had occurred in renneted skim milk containing 0.05 wt% to 0.1 wt% κ-carrageenan and guar gum. In addition, discontinuous protein network resulting from the formation of an emulsion-like structure explain the absence of gelation in renneted skim milk containing 0.075wt% and 0.1wt% xanthan.

The microstructure of gels containing HM pectin and gelatin were very similar. Although a decrease in syneresis was observed, CLSM observations indicated that the microstructure of rennet-induced skim milk was not affected by the addition of these two polysaccharides.

The microscopic observations and the syneresis measurements were in very good agreement as samples that had large pores exhibited higher whey separation.

5.4 Summary

Two techniques, surface whey separation measurements and confocal laser scanning microscopy (CLSM), were used to investigate the structural properties of rennet skim milk gels containing κ-carrageenan, xanthan, guar, HM pectin and gelatin.

It was found that the respective additions of κ-carrageenan and guar gum at 0.025 wt% concentrations and the respective incorporations of HM pectin and gelatin at all concentrations, had decreased whey separation in rennet-induced skim milk. In addition, the microstructural properties of rennet-induced skim milk gels had changed drastically in the presence of κ-carrageenan, xanthan and guar gum. Images from CLSM had clearly indicated that the difference in syneresis behaviour, with increasing polysaccharide concentrations, could be due to the presence of larger pores ("voids"). Furthermore, the addition of HM pectin and gelatin improved the syneresis properties, and did not show changes in microstructure.

6 Influence of biopolymers addition on rennet-induced gelation of skim milk as monitored by diffusing wave spectroscopy (DWS)

Milk coagulation by chymosin has been covered in Chapter Two. Briefly, enzymic coagulation of milk could generally be divided into two phases: primary (enzymic proteolysis and aggregation), and secondary (gelation). During the enzymic phase or 'primary reaction', κ-casein is hydrolysed according to the kinetics of Michaelis-Menten (Swaisgood, 1992; Scher and Hardy, 1993). The loss of the sterically stabilizing hydrophilic peptide, which then diffuses away from the micelle, leads to a progressive destabilization of the micelle as the proteolysis of its κ-casein coating continues. The subsequent aggregation of the destabilized micelles through to gel formation constituted the secondary stage of the process which can be followed using diffusing wave spectroscopy (DWS) (Horne, 1990).

6.1 Data analysis

Experimental methods for DWS measurements are described in Chapter 3. Figure 6.1 shows typical DWS results.

In a single experiment, the light-scattering behaviour of coagulating 10 wt% skim milk, over a three-hour measurement period, provided three characteristic times at which significant changes occurred. Aggregation time, marked as Point 1, was defined as the last point before divergence away from the initial group of points. The gelation time, marked at Point 2, was determined as the last point of the smooth increasing curve.

As the signal level became noisy once the gelation time was reached, the "strength" of the gel became quite difficult to evaluate. Hence, it would be defined as the average value of the line through the data identified as Point 3. It should be noted that the "gel strength" obtained by DWS does not necessarily equate to the values obtained from rheological results.

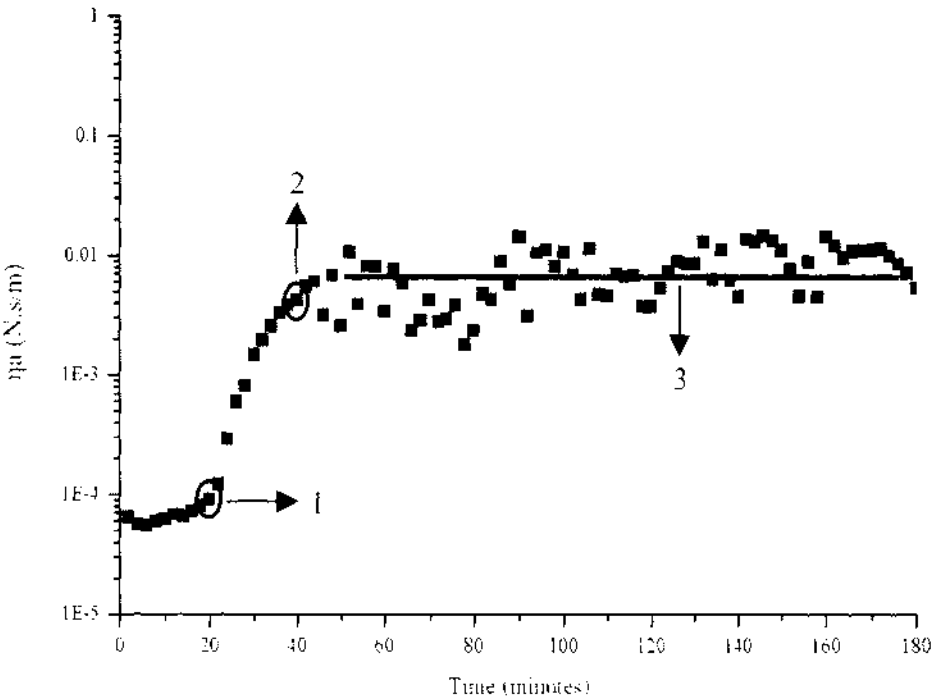

Figure 6.1 Typical graph of diffusing wave spectroscopy of renneted control sample (10 wt% reconstituted SMP, 0 wt% polysaccharide). (1) Aggregation time; (2) Gelation time; and (3) Gel strength

6.2 Results

6.2.1 Aggregation time

Figure 6.2 (A) illustrates the difference in aggregation time for renneted skim milk containing κ-carrageenan, xanthan, guar, HM pectin and gelatin at concentrations ranging from 0 wt% to 0.1 wt%. Aggregation time for the control sample (0 wt% polysaccharide) was 20 minutes. The aggregation process appeared to start earlier in polysaccharide containing samples compared with the control sample. The aggregation times were shorter for renneted skim milk containing κ-carrageenan and guar at higher polysaccharide concentrations.

Similarly, the differences in aggregation time obtained for renneted skim milk containing xanthan, HM pectin and gelatin at increasing concentrations were not large (< 2 minutes). In contrast to samples containing κ-carrageenan and guar, aggregation times for renneted skim milk with added xanthan, HM pectin and gelatin increased slightly with increasing polysaccharide concentrations.

DWS results showed that the aggregation time of the samples containing biopolymers was shorter than the aggregation time of the control sample (without biopolymers). For the samples containing biopolymers, the aggregation time decreased with increasing concentrations of κ-carrageenan and guar, but slightly increased with respective incorporations of xanthan, HM pectin and gelatin.

6.2.2 Gelation times

Figure 6.2 (B) shows the gelation times for renneted skim milk with and without polysaccharide addition. The gelation time for the control sample was 32 minutes.

Note that the aggregation and gelation times of κ-carrageenan at 0.1 wt% concentration was not reported in Figure 6.2. This indicated the absence of gelation in renneted skim milk containing 0.1 wt% κ-carrageenan.

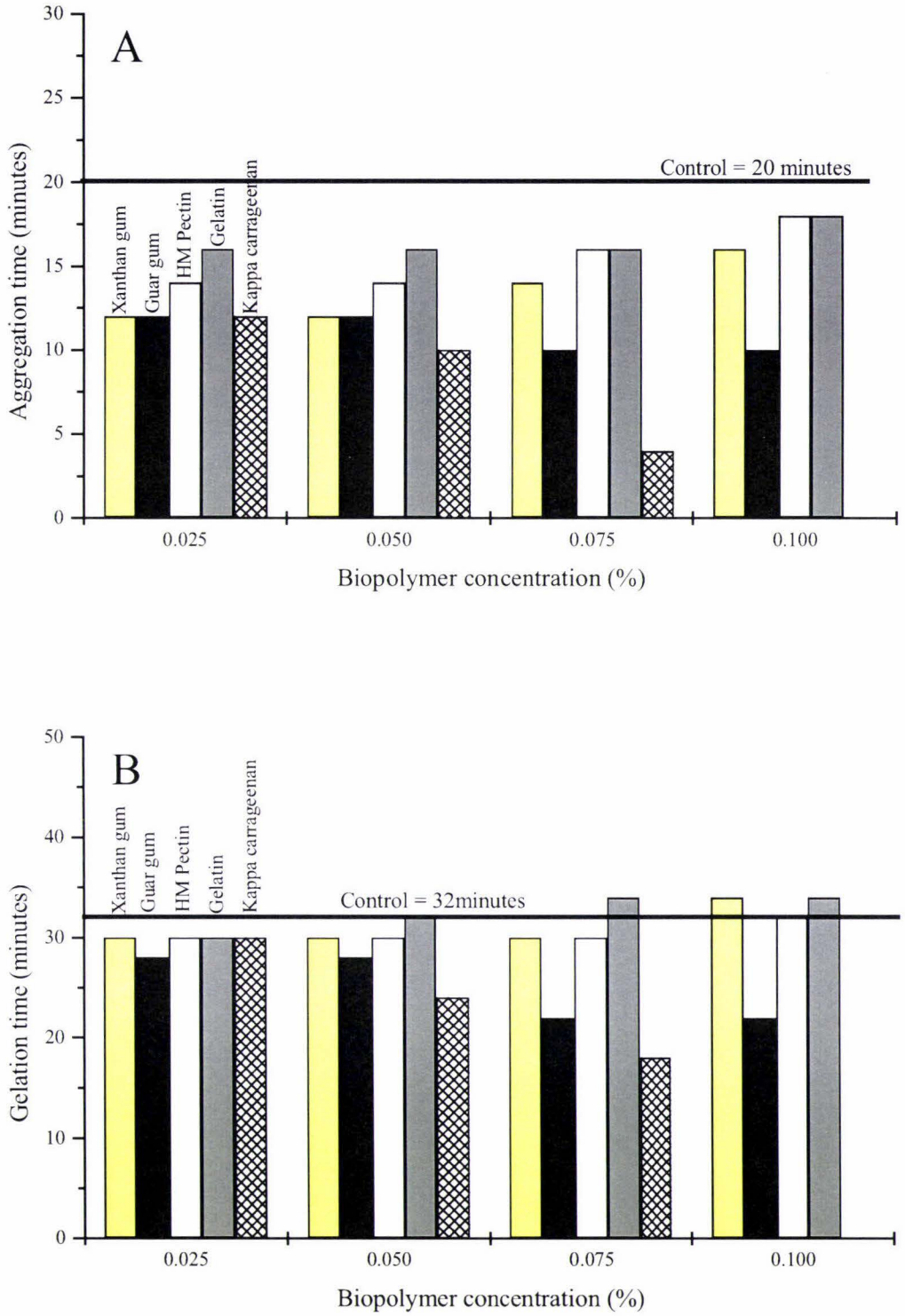

Figure 6.2 Aggregation (Graph A) and gelation (Graph B) times of renneted skim milk containing κ-carrageenan (⊠), xanthan gum (▒), guar gum (■), HM pectin (□) and gelatin (▒) at 0 wt%, 0.025 wt%, 0.050 wt%, 0.075 wt% and 0.1 wt%, as obtained by DWS. Continuous line represented the control sample (without biopolymer addition).

At the lowest polysaccharide concentration (0.025 wt%), almost all the polysaccharide containing samples gelled slightly faster than the control sample.

Figure 6.2 (B) also revealed that the renneted samples with respective incorporations of κ-carrageenan and guar gelled much faster at higher polysaccharide concentrations. The gelation times showed similar results to the aggregation times where both were faster than the control sample and decreased with increasing concentrations of κ-carrageenan and guar.

There was no change in gelation times for renneted skim milk containing HM pectin and xanthan at concentrations up to 0.075 wt%. It was only at the highest polysaccharide concentration of 0.1 wt% that the gelation of these samples took longer than the control sample (≥ 32 minutes).

Samples containing gelatin showed an increase in gelation time with increasing gelatin concentrations up to 0.05 wt%. Renneted skim milk containing 0.075 wt% and 0.1 wt% gelatin displayed similar gelation time (34 minutes) and were higher than the control sample (see Figure 6.2B).

In general, gelation time of renneted skim milk had decreased upon polysaccharide addition. However in renneted skim milk containing 0.1 wt% xanthan and gelatin, gelation time was slightly longer than the control sample.

6.2.3 Gel strength

Figure 6.3 displays the values of the "gel strength" as measured by DWS for rennet-induced skim milk gels with and without added polysaccharides addition. It must be stressed again that the "gel strength" measured by DWS is not the same as gel strength measured by a stress-strain method.

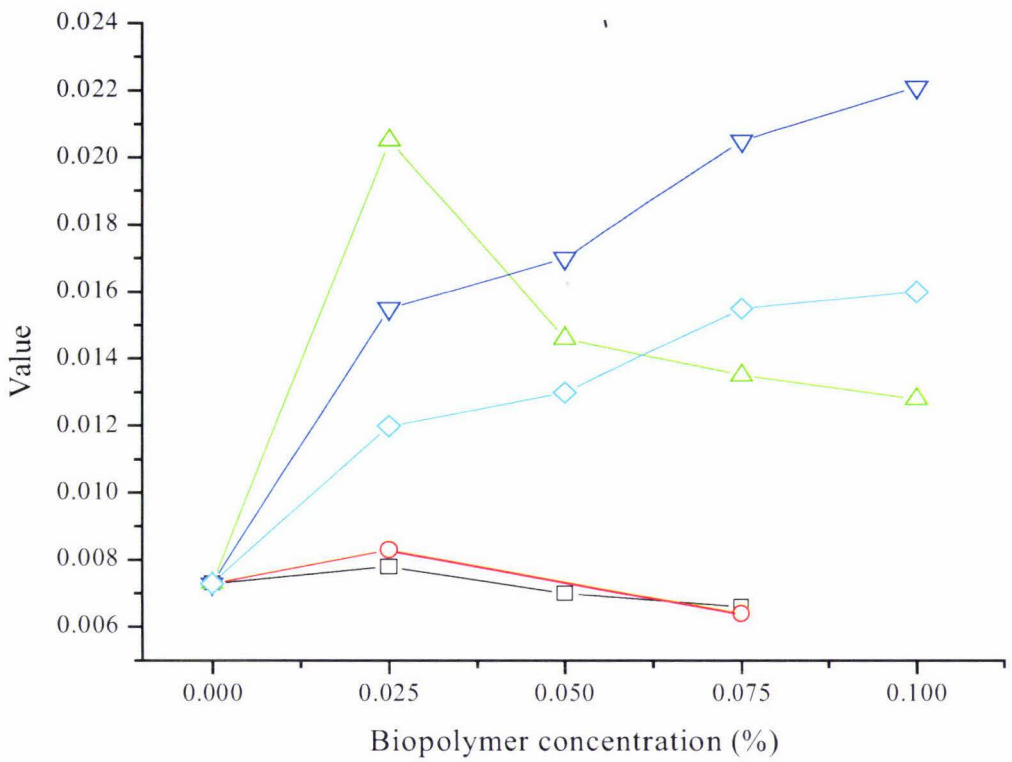

Figure 6.3 "Gel strength", obtained from DWS measurements, of rennet skim milk gels containing 0 wt%, 0.025 wt%, 0.050 wt%, 0.075 wt% and 0.1 wt% κ-carrageenan (□), xanthan gum (○), guar gum (△), HM pectin (▽) and gelatin (◇). Control sample consisted only of 10 wt% reconstituted low heat skim milk powder.

It should be noted that gel strength values of renneted skim milk containing 0.1 wt% κ-carrageenan and xanthan were not obtained, as there was no gel formation in these samples.

Most of the samples containing polysaccharides, except those with κ- carrageenan and xanthan, had gel strength values higher than the control sample.

The samples showed declining gel strength values with increasing κ-carrageenan, xanthan, and guar concentrations, while the samples containing HM pectin and gelatin displayed increased gel strengths values as the concentration increased.

6.3 Comparison between DWS and rheology

As aggregation and gelation points were both measured by DWS and rheology (Chapter 4), comparison between these methods will be attempted here. Table 6.1 compares the aggregation and gelation times of renneted samples obtained from DWS as well as from rheological measurements.

Aggregation times obtained by rheology showed similar trends as the ones obtained by DWS. Along with DWS measurements, rheological results had further ascertained the fact that the respective additions of κ-carrageenan and guar, at increasing concentrations, into rennet-induced skim milk had improved the aggregation process, which was in contrast to samples with xanthan and gelatin additions.

Gelation times of renneted skim milk/polysaccharide samples, from rheology and DWS measurements, decreased with increasing κ-carrageenan concentrations and increased with increasing xanthan concentrations. However, renneted skim milk containing guar and gelatin had displayed contrasting gelation-time results from rheology and DWS measurements. DWS had displayed shorter gelation time with increasing guar concentrations, while rheological evaluation showed the inverse and the gelation time of renneted skim milk/gelatin depicted the opposite.

In addition, aggregation and gelation times obtained respectively from DWS and rheological measurements on renneted skim milk containing HM pectin also showed contradictory trends. DWS had indicated longer aggregation time with increasing HM pectin concentrations, while rheological evaluation showed the inverse. Further investigations should be performed to elucidate this discrepancy.

In general, aggregation and gelation times obtained from DWS measurements were shorter than the ones obtained from rheology measurements.

As was earlier defined in Chapter 4, dynamic moduli were measured by a rheometer operating in oscillatory mode, which had allowed the estimation of the viscous and elastic modulus components. Given that the rheometer applied a mechanical impulse, it would require the presence of a gel of a finite strength dictated by rheometer sensitivity to produce a measurable shear modulus. Thus, the growth of shear modulus in an enzyme-treated milk would be affected, compared to the relaxation time detected by DWS (Horne, 1995). This could explain why higher aggregation and gelation times were consistently obtained by rheological measurements. In addition, another source of discrepancy could be due to the fact that DWS monitored both the changes in viscoelastic properties as well as in particle size.

The major advantage of DWS over traditional mechanical techniques of rheology is that it is a non-perturbing technique. This was particularly important for very weak gels, where the very act of applying a mechanical strain to the system might destroy or distort the growing structure. DWS simply measures the effect of spontaneous thermal fluctuations present in the system (Dalgleish and Horne, 1991). The interpretation of this behaviour in terms of system structure and interactions will remain an ongoing and active area of research. Hence, in the current investigative work, results from DWS served to complement results from rheological measurements.

Table 6.1 Comparison of aggregation and gelation times obtained by DWS and rheological measurements.

Polysaccharide	Polysaccharide concentration (%)	Aggregation time (minutes)		Gelation time (minutes)	
		DWS	Rheology	DWS	Rheology
κ-Carrageenan	0	20	24	32	41
	0.025	12	12	30	27
	0.050	10	-	24	12
	0.075	4	-	18	6
	0.1	-	-	-	1
	Trend	↓	↓	↓	↓
Xanthan gum	0	20	24	32	41
	0.025	12	29	30	52
	0.050	12	43	30	80
	0.075	14	-	30	-
	0.1	16	-	34	-
	Trend	↑	↑	↑	↑
Guar gum	0	20	24	32	41
	0.025	12	22	28	30
	0.050	12	18	28	30
	0.075	10	17	22	32
	0.1	10	17	22	33
	Trend	↓	↓	↓	↑
HM Pectin	0	20	24	32	41
	0.025	14	23	30	34
	0.050	14	19	30	28
	0.075	16	19	30	27
	0.1	18	16	32	23
	Trend	↑	↓	↑	↓
Gelatin	0	20	24	32	41
	0.025	16	21	30	34
	0.050	16	22	32	33
	0.075	16	22	34	31
	0.1	18	22	34	30
	Trend	↑	↑	↑	↓

6.4 Summary

Diffusing wave spectroscopy of renneted skim milk containing biopolymers allowed the measurements of aggregation time, gelation time and an effective "gel strength". From these measurements, it was found that the additions of κ-carrageenan and guar into rennet-induced skim milk shortened the aggregation and gelation times with increasing polysaccharide concentrations, while HM pectin and gelatin showed no significant change with the increase in concentration.

On the contrary, increasing concentrations of xanthan into rennet-induced skim milk had slowed down the aggregation as well as the gelation process. The gel strength of renneted skim milk containing 0.1 wt% κ-carrageenan and, 0.075 wt% and 0.1 wt% xanthan were not obtained due to the absence of gelation in rennet-induced skim milk containing more than 0.075 wt% κ-carrageenan and 0.05 wt% of xanthan.

7 Effect of biopolymers addition on the rheological properties of rennet-induced skim milk gels made under commercial cheesemaking conditions

In the previous chapters, the effect of adding biopolymers in rennet-induced skim milk, under defined experimental conditions (model systems), was studied by techniques such as dynamics low amplitude oscillatory rheology, confocal laser scanning microscopy (CLSM), syneresis measurements and diffusing wave microscopy (DWS). This chapter investigates the rheological behaviour of the renneted skim milk containing biopolymers under cheesemaking conditions.

7.1 Methods

The experimental conditions were carried to stimulate standard cheesemaking conditions. 0.01 wt% calcium chloride ($CaCl_2$) was added to the milk sample at pH 6.25. The final pH of milk sample was then adjusted to 6.2 using 0.1 M hydrochloric acid (HCl) and renneted at 32°C. The concentration of rennet was 80 μL of 1/10 diluted rennet in 100 g of skim milk sample. No starter bacteria was used.

Lower biopolymer concentrations (≤ 0.05 wt%) were used for this work. Four concentrations, 0 wt%, 0.010 wt%, 0.025 wt% and 0.05 wt%, were used to study the rheological effect of the four polysaccharides and gelatin on rennet-induced skim milk under cheesemaking conditions. An additional 0.005 wt% concentration was used to investigate the rheological effect of a lower xanthan concentration on rennet-induced skim milk. The preparation of samples was similar to the samples prepared in the model system described in Chapter 3. For comparison, Table 7.1 tabulates the conditions, primarily the normal experimental (model system) and the cheesemaking, used for studying the rheological effect of biopolymer addition on rennet-induced skim milk gels.

Table 7.1 Normal experimental and cheesemaking conditions used in the formation of rennet-induced skim milk gels

Factors	Experimental conditions	
	Normal (model)	Cheesemaking
pH	6.6-6.7	6.2
Temperature (°C)	30	32
Calcium chloride ($CaCl_2$) (mM)	0	0.68
Rennet concentration (μL / 100 g milk)	200	80

7.2 Results

The rheological measurements were performed as described in Chapter 4.

7.2.1 κ-Carrageenan

Complex modulus, G^* as a function of time for rennet-induced skim milk gels containing 0 wt%, 0.01 wt%, 0.025 wt% and 0.05 wt% κ-carrageenan are shown in Figure 7.1. The G^* values of κ-carrageenan containing samples were not only higher than the control sample (0 wt% κ-carrageenan), but the moduli increased with increasing concentrations. In addition, G^* of these samples also increased with time. The gelation time was shorter at higher κ-carrageenan concentrations, particularly at 0.05 wt% concentration where instant gelation was observed.

At the end of the 3-hour dynamic time sweep test, frequency sweep measurements of the rennet gel were made (Figure 7.2). It could be observed that similar viscoelastic behaviour was obtained for the control sample as well as for samples with added κ-carrageenan. The results indicated that increasing concentrations of κ-carrageenan in rennet-induced skim milk gels did not induce any drastic changes in the viscoelastic properties of the rennet gel. Nonetheless, all the samples displayed G' greater than G'', indicating gel formation.

7.2.2 Xanthan gum

Figure 7.3 shows G^* as a function of time for renneted skim milk containing 0 wt%, 0.005 wt%, 0.01 wt%, 0.025 wt% and 0.05 wt% xanthan. The moduli decreased with increasing xanthan concentrations. This meant that the control sample (0 wt% xanthan) had better viscoelastic properties than samples with added xanthan.

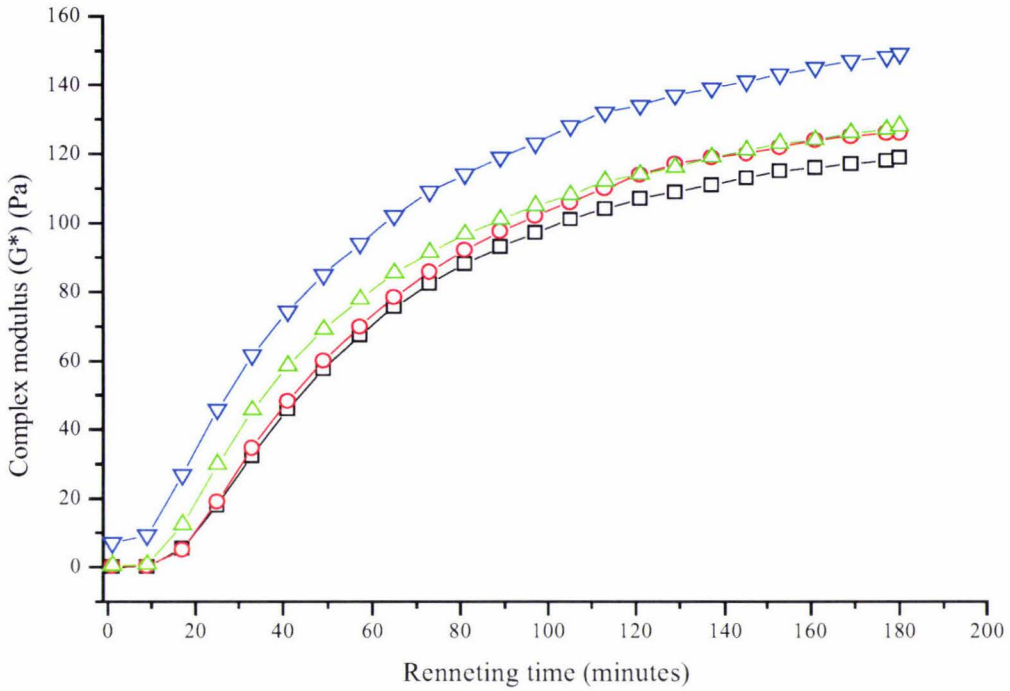

Figure 7.1 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.01 wt% (○), 0.025 wt% (△) and 0.05 wt% (▽) κ-carrageenan as a function of renneting time at 32°C.

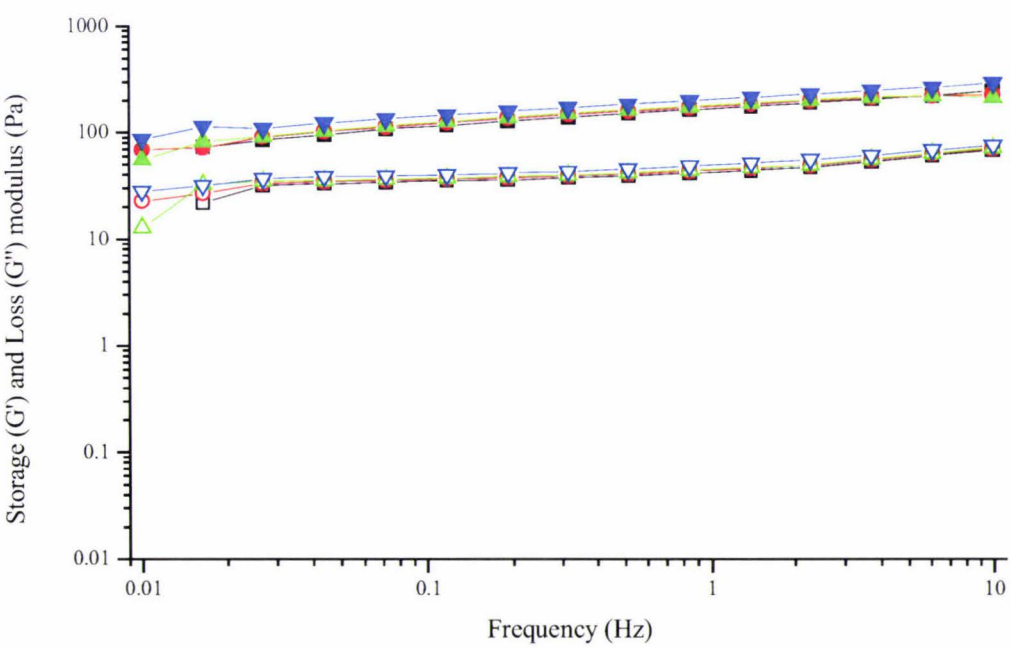

Figure 7.2 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.010 wt% (G' - ●, G'' - ○), 0.025 wt% (G' - ▲, G'' - △) and 0.05 wt% (G' - ▼, G'' - ▽) κ-carrageenan as a function of frequency.

In particular, G^* of gels containing xanthan (> 0.025 wt%) showed that there was virtually no increase in G^* with time. The gelation time also increased with xanthan concentration, especially at concentrations > 0.025 wt%.

Figure 7.4 displays G' and G'' of various rennet gels as a function of frequency. The control sample had the highest G' and G'' and the moduli declined with increasing xanthan concentrations. It could also be observed that at low xanthan concentrations of 0.005 wt% and 0.010 wt%, these samples exhibited similar viscoelastic properties as the control sample. At these concentrations, G' was higher than G'', indicating gel formation. However, the behaviour of G' and G'' with frequency indicated that these samples behaved as weak gels. This was especially evident for gels containing > 0.025 wt% xanthan. With increasing frequency, G'' became higher than G' and this revealed the weak, non-gelling nature of the xanthan, at concentrations > 0.025 wt%.

7.2.3 Guar gum

Figure 7.5 depicts G^* as a function of time for renneted skim milk containing guar under cheesemaking conditions. Renneted gels containing guar showed higher G^* when a lower guar concentration was used. Similarly to xanthan samples, the control sample (0 wt% guar) had the highest G^*. In addition, Figure 7.5 also shows that G^* of these samples had increased with time. The gelation time of rennet-induced skim milk increased upon guar additions, however the gelation time decreased with an increase in guar concentration.

Figure 7.6 presents G' and G'' of the gels as a function of frequency. It showed that gels without guar addition had the highest G' and G'', and the moduli appeared to decrease with increasing guar concentrations. Although G' was higher than G'', it was clear that the samples behaved as weak gels (Figure 7.6).

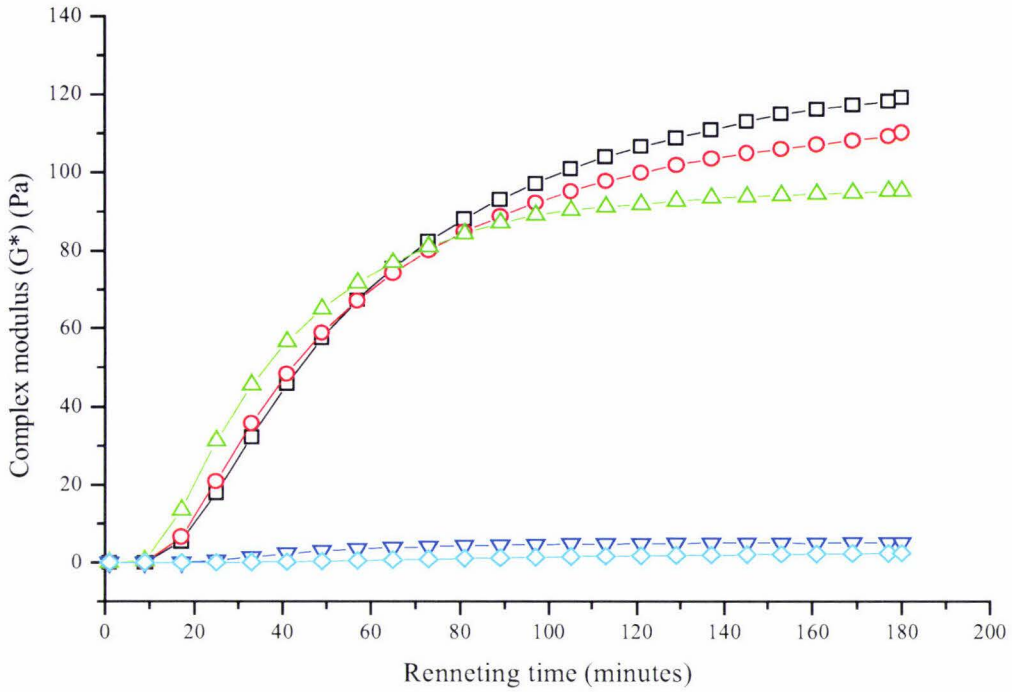

Figure 7.3 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.005 wt% (○), 0.010 wt% (△), 0.025 wt% (▽) and 0.05 wt% (◇) xanthan as a function of renneting time at 32°C.

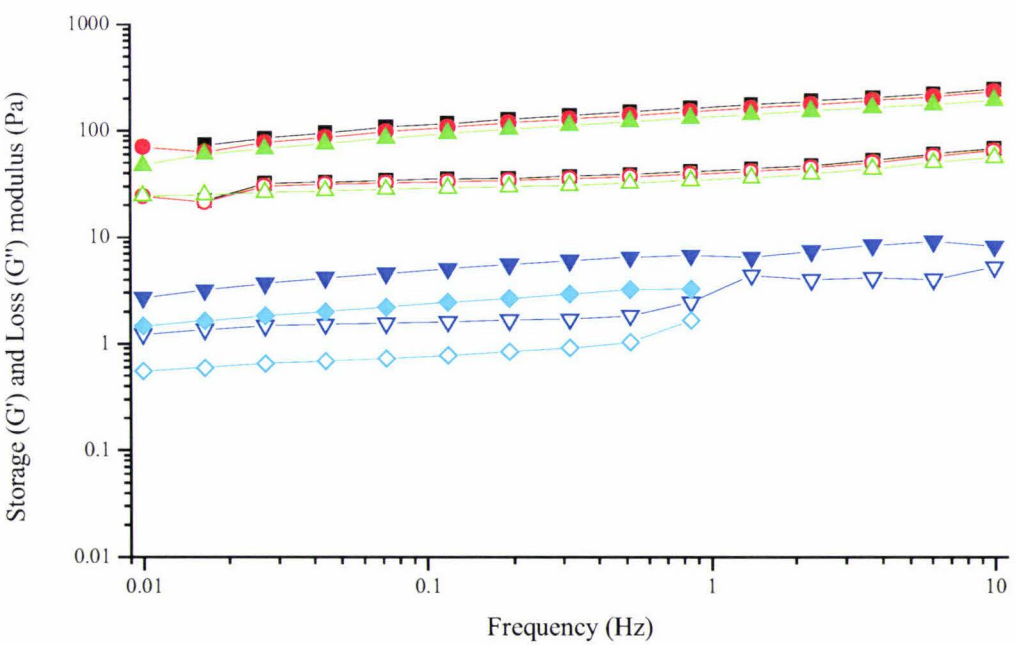

Figure 7.4 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.005 wt% (G' - ●, G'' - ○), 0.0010 wt% (G' - ▲, G'' - △), 0.025 wt% (G' - ▼, G'' - ▽) and 0.05 wt% (G' - ♦, G'' - ◇) xanthan as a function of frequency.

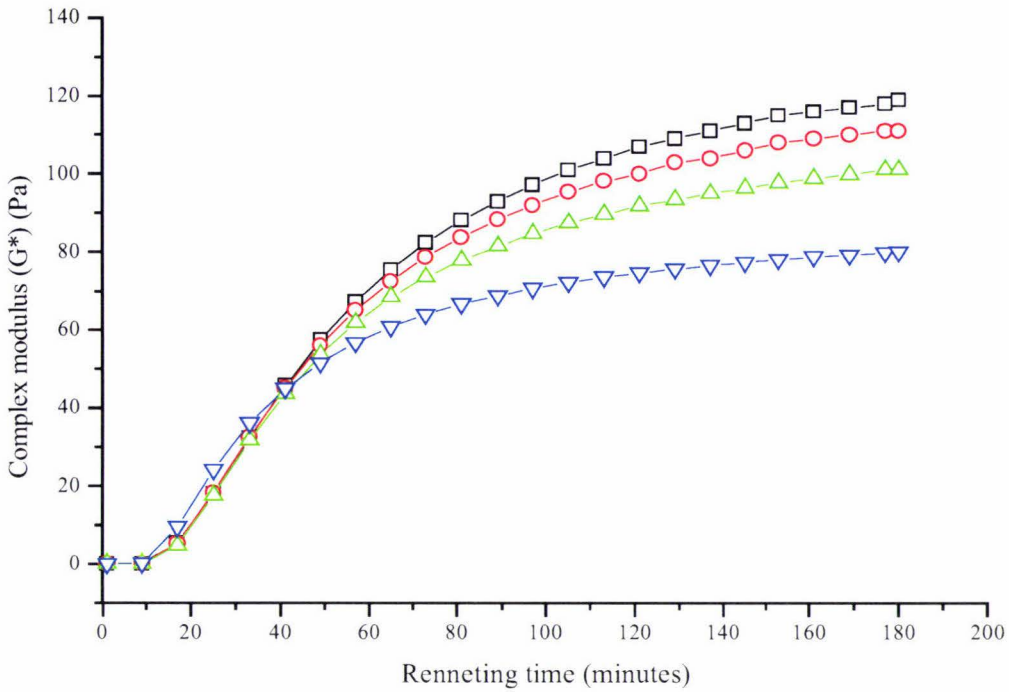

Figure 7.5 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.01 wt% (○), 0.025 wt% (△) and 0.05 wt% (▽) guar as a function of renneting time at 32°C.

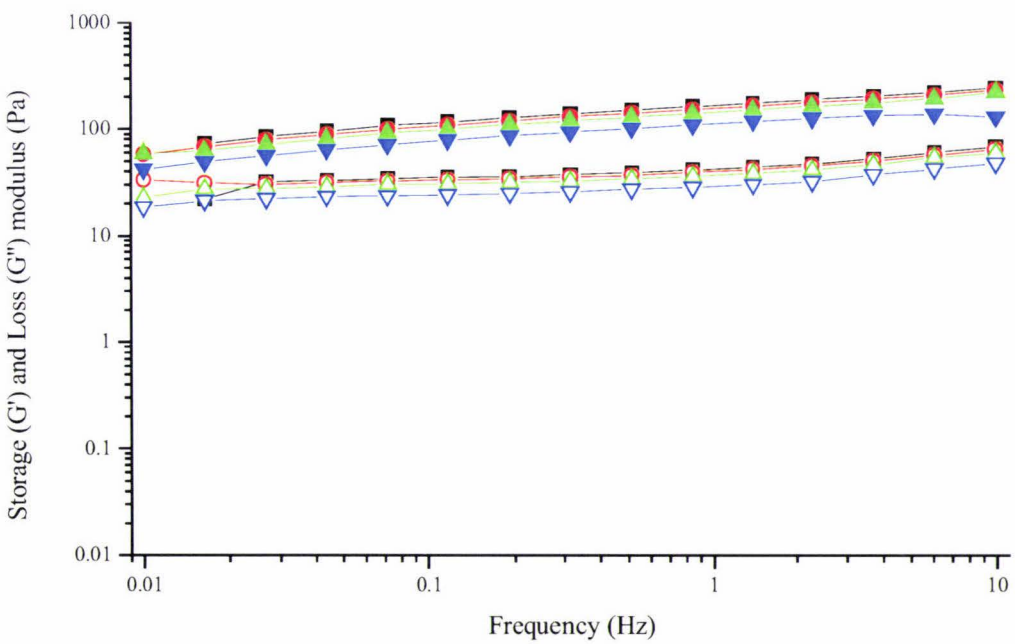

Figure 7.6 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.010 wt% (G' - ●, G'' - ○), 0.025 wt% (G' - ▲, G'' - △) and 0.05 wt% (G' - ▼, G'' - ▽) guar as a function of frequency.

7.2.4 HM Pectin

The G^* values for rennet-induced skim milk gels containing 0 wt%, 0.01 wt%, 0.025 wt% and 0.05 wt% HM pectin are shown in Figure 7.7. In general, all the HM pectin added samples had lower G^* than the control sample (0 wt% HM pectin). As the concentrations of HM pectin increased, G^* of the samples decreased. Figure 7.7 displays that G^* of the rennet gels containing HM pectin increased with renneting time. Gelation time of rennet gels decreased by adding 0.010 wt% HM pectin, however, the gelation time remained unchanged as the concentration increased.

Figure 7.8 reports G' and G'' of rennet skim milk/HM pectin gels as a function of frequency. Results showed that G' was greater than G''. It was apparent that rennet gels at HM pectin concentrations ranging from 0 wt% to 0.05 wt% had similar viscoelastic properties.

7.2.5 Gelatin

The effect of gelatin addition on G^* of rennet-induced skim milk gels is displayed in Figure 7.9. G^* of all the rennet gels containing gelatin had increased with renneting time. The G^* values increased with an increase in gelatin concentration up to 0.01 wt%, and decreased at higher levels of gelatin addition (< 0.05 wt%). Gelation time of rennet-induced skim milk decreased with gelatin addition up to 0.01 wt% but gelation time increased slightly with further increase in gelatin concentration.

The G' and G'' of renneted skim milk/gelatin as a function of frequency are shown in Figure 7.10. The results were similar to renneted skim milk containing HM pectin. Figure 7.10 shows that G' was higher than G'', and that the samples exhibited weak gel properties and no differences were observed with increasing gelatin concentrations.

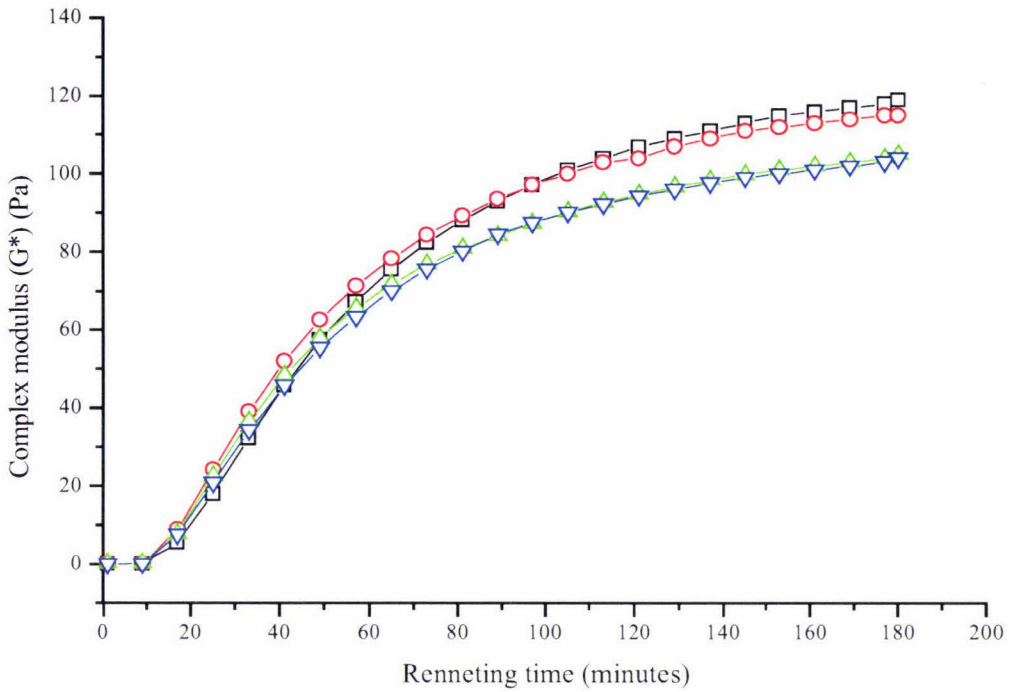

Figure 7.7 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.01 wt% (○), 0.025 wt% (△) and 0.05 wt% (▽) HM pectin as a function of renneting time at 32°C.

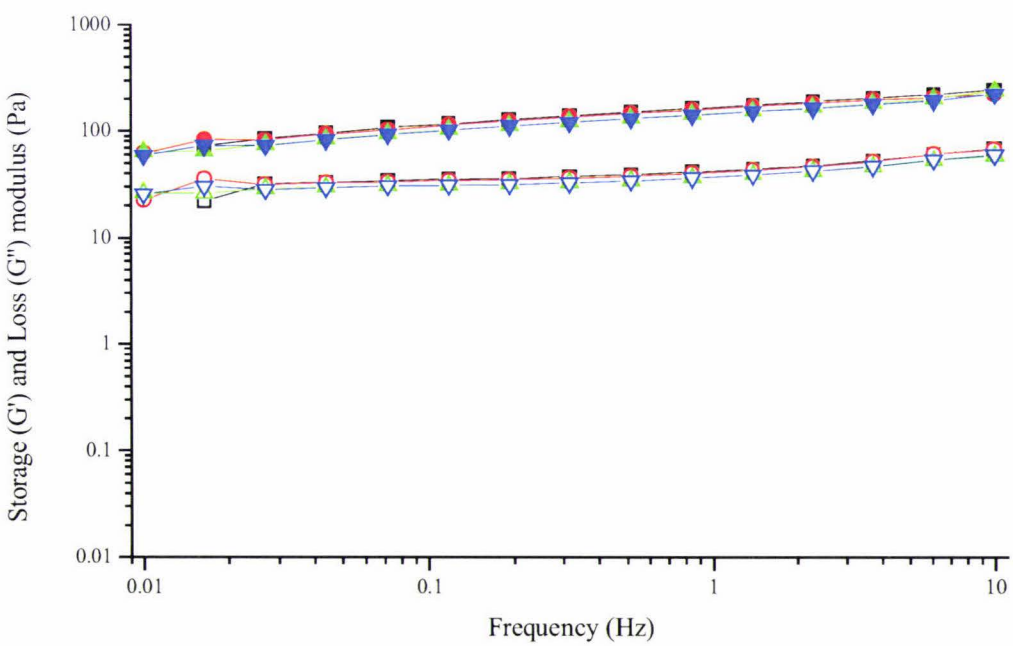

Figure 7.8 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.010 wt% (G' - ●, G'' - ○), 0.025 wt% (G' - ▲, G'' - △) and 0.05 wt% (G' - ▼, G'' - ▽) HM pectin as a function of frequency.

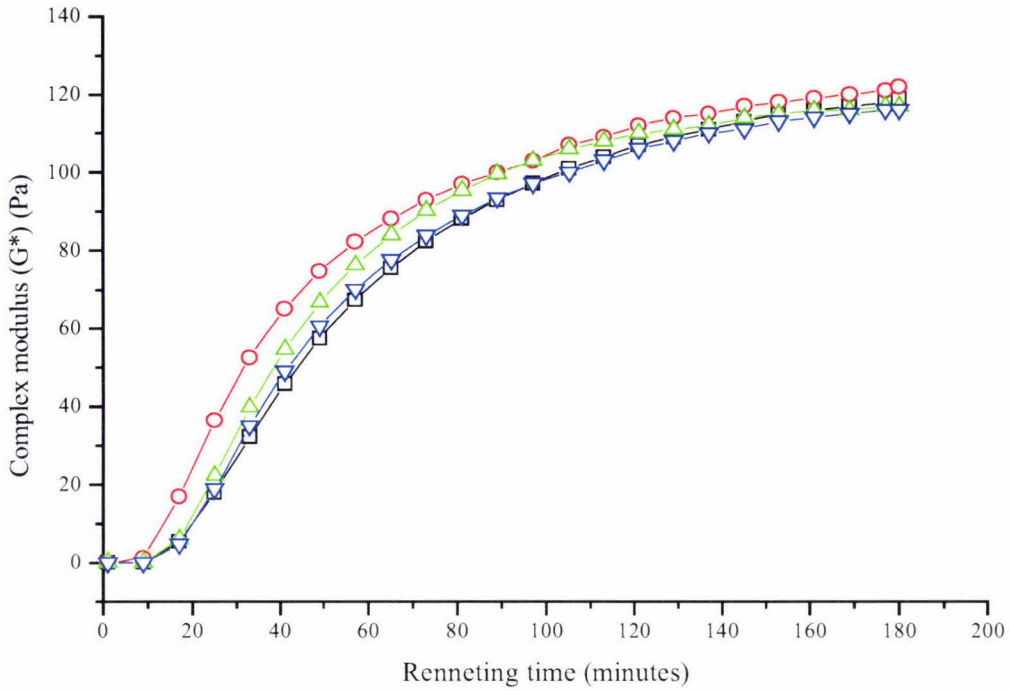

Figure 7.9 Complex modulus (G^*) of renneted skim milk with 0 wt% (□), 0.01 wt% (○), 0.025 wt% (△) and 0.05 wt% (▽) gelatin as a function of renneting time at 32°C.

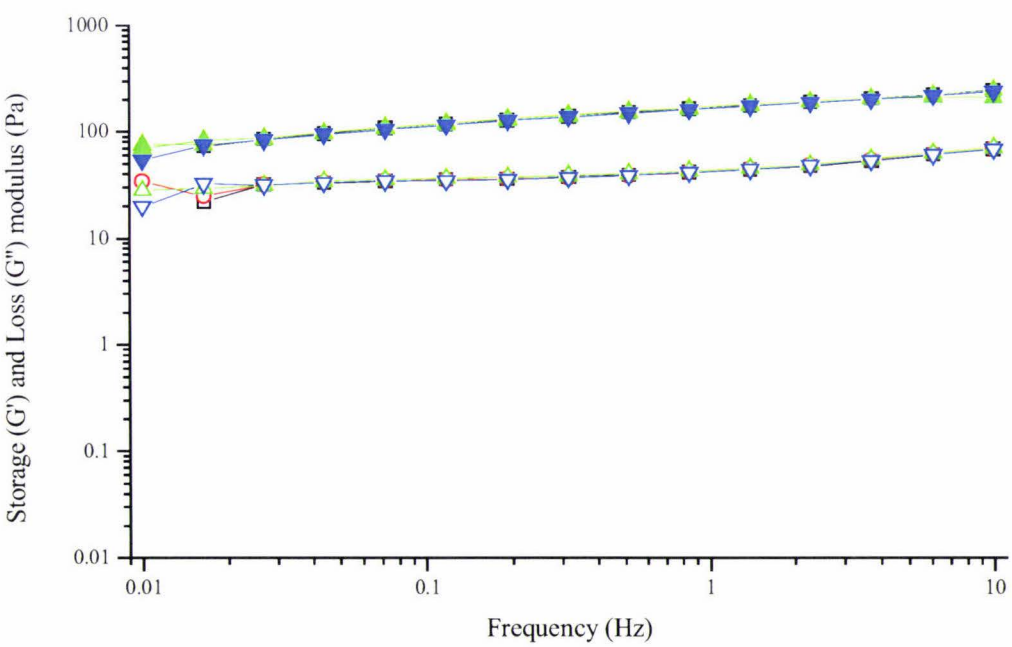

Figure 7.10 Storage (G') and Loss (G'') modulus of renneted skim milk with 0 wt% (G' - ■, G'' - □), 0.010 wt% (G' - ●, G'' - ○), 0.025 wt% (G' - ▲, G'' - △) and 0.05 wt% (G' - ▼, G'' - ▽) gelatin as a function of frequency.

7.3 Comparison between different biopolymers

7.3.1 Gelation times

The effect of biopolymer addition and the concentrations used, on the gelation times of rennet-induced skim milk from the model system and under cheesemaking conditions will be compared and discussed here. Figure 7.11 provides an illustration of this comparison. In general, under normal experimental conditions (model system), all the biopolymer added samples (0.025 wt% to 0.1 wt%) showed shorter gelation times than the control sample (0 wt% polysaccharide). Under cheesemaking conditions, the gelation times of renneted skim milk containing 0 wt% to 0.05 wt% biopolymers had displayed similar times (~ 9-14 minutes). Most of the renneted samples had either gelled at the same time or gelled faster (≤ 13 minutes) than the control sample. The only exception was renneted skim milk containing 0.05 wt% gelatin, which was the same as the control sample (14 minutes).

Figure 7.11(A) shows that renneted skim milk containing 0.01 wt% κ-carrageenan, made under cheesemaking conditions, gelled at the same time (13 minutes) as the control sample (0 wt% κ-carrageenan). As κ-carrageenan concentration increased, the time taken for gelation shortened. Similar gelling trends were also observed for renneted skim milk containing κ-carrageenan samples from the model system, where shorter gelation time was achieved with increase in κ-carrageenan concentration.

Renneted skim milk samples containing HM pectin (Graph D) showed similar trends as that for samples containing κ-carrageenan. Therefore, from Figure 7.11 (A and D), it could be concluded that renneted skim milk containing κ-carrageenan and HM pectin, made under normal experimental or cheesemaking conditions, exhibited decreased gelation time with increasing polysaccharide concentration.

Under normal experimental and cheesemaking conditions, Figure 7.11 (B) shows that the gelation times of renneted skim milk samples containing 0 wt% to 0.05 wt% xanthan increased with increasing concentrations. The time of gelation was longer, especially at higher xanthan concentrations (≥ 0.025 wt%): ~ 75 minutes and 80 minutes for normal experimental and cheesemaking conditions, respectively. Under normal

experimental conditions, no gelation time was reported for renneted skim milk containing 0.075 wt% and 0.1 wt% xanthan.

In normal experimental conditions, Figure 7.11 (C) shows that renneted skim milk containing guar took longer to gel at higher concentrations, while under cheesemaking conditions the samples gelled faster when higher guar concentrations were used. On the contrary, renneted skim milk with gelatin addition (Figure 7.11 E) showed an opposite trend: under normal experimental conditions, gelation times decreased with increasing gelatin concentrations and inverse trend was seen under cheesemaking conditions. The effect on the changes in experimental conditions, like lower pH and addition of $CaCl_2$, certainly effected the interactions between the milk proteins, polysaccharides and the activity of chymosin, which had in turn altered the gelation process. Chapter 8 will further report these interactions in order to discuss the effect of biopolymers addition on the renneted skim milk.

7.3.2 Gel strength

Figure 7.12 presents the gel strength of renneted samples, under normal experimental (Graph A) and cheesemaking (Graph B) conditions, respectively.

Under normal experimental conditions, Figure 7.12 (A) shows that the gel strength of renneted skim milk containing κ carrageenan, xanthan and guar decreased with increasing concentrations from 0 wt% to 0.1 wt%. This was in contrast for renneted skim milk with respective additions of HM pectin and gelatin, where gel strength values increased with increasing concentrations. In addition, consistently high gel strength values suggested that renneted skim milk with HM pectin additions had better viscoelastic properties than the other biopolymers added samples.

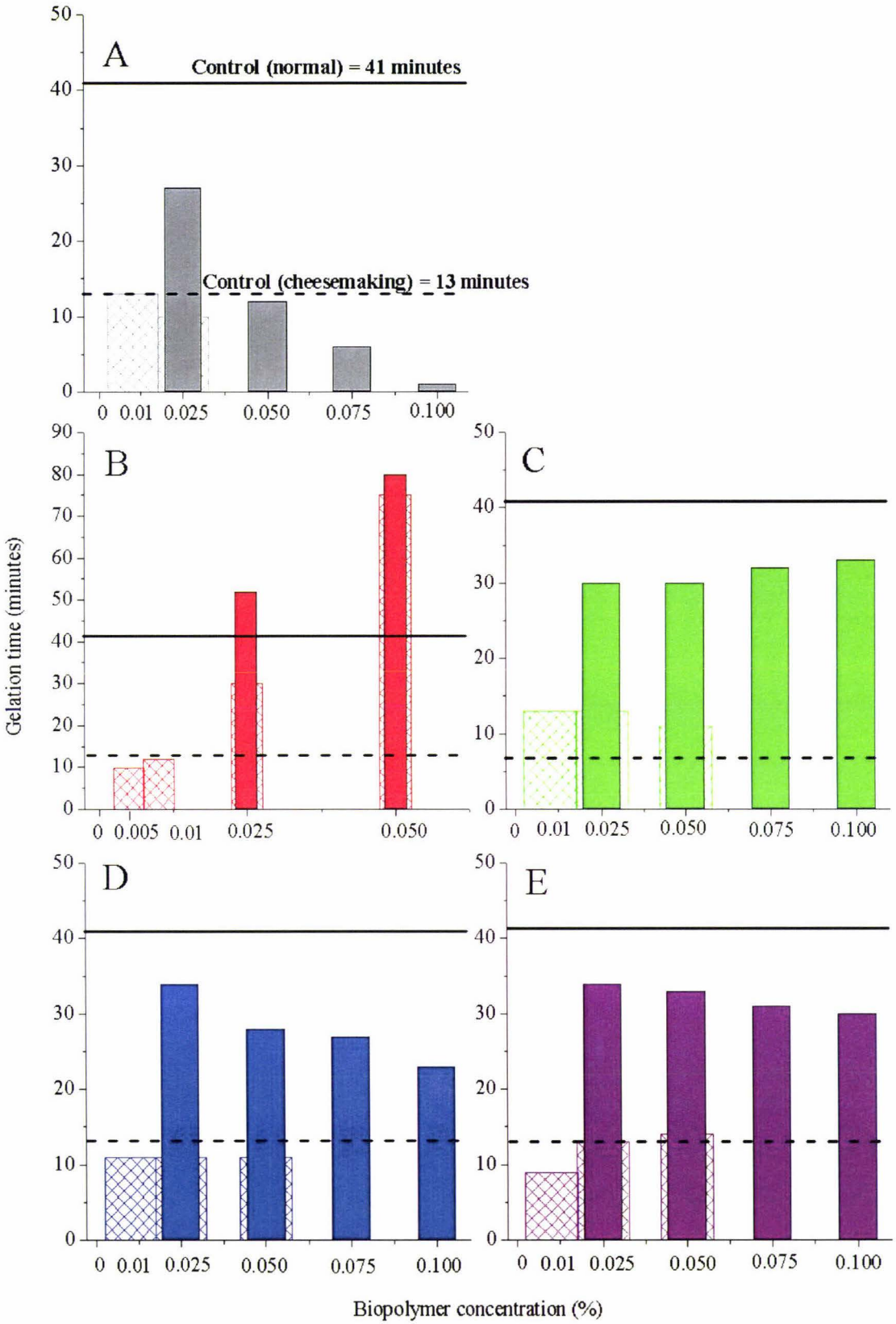

Figure 7.11 Gelation times of renneted skim milk containing κ-carrageenan (A), xanthan gum (B), guar gum (C), HM pectin (D) and gelatin (E), under normal experimental (■) conditions at 0 wt%, 0.025 wt%, 0.05 wt%, 0.075 wt% and 0.1 wt% concentrations, and under cheesemaking (⊠) conditions at 0 wt%, 0.01 wt%, 0.025 wt% and 0.05 wt% concentrations, respectively.

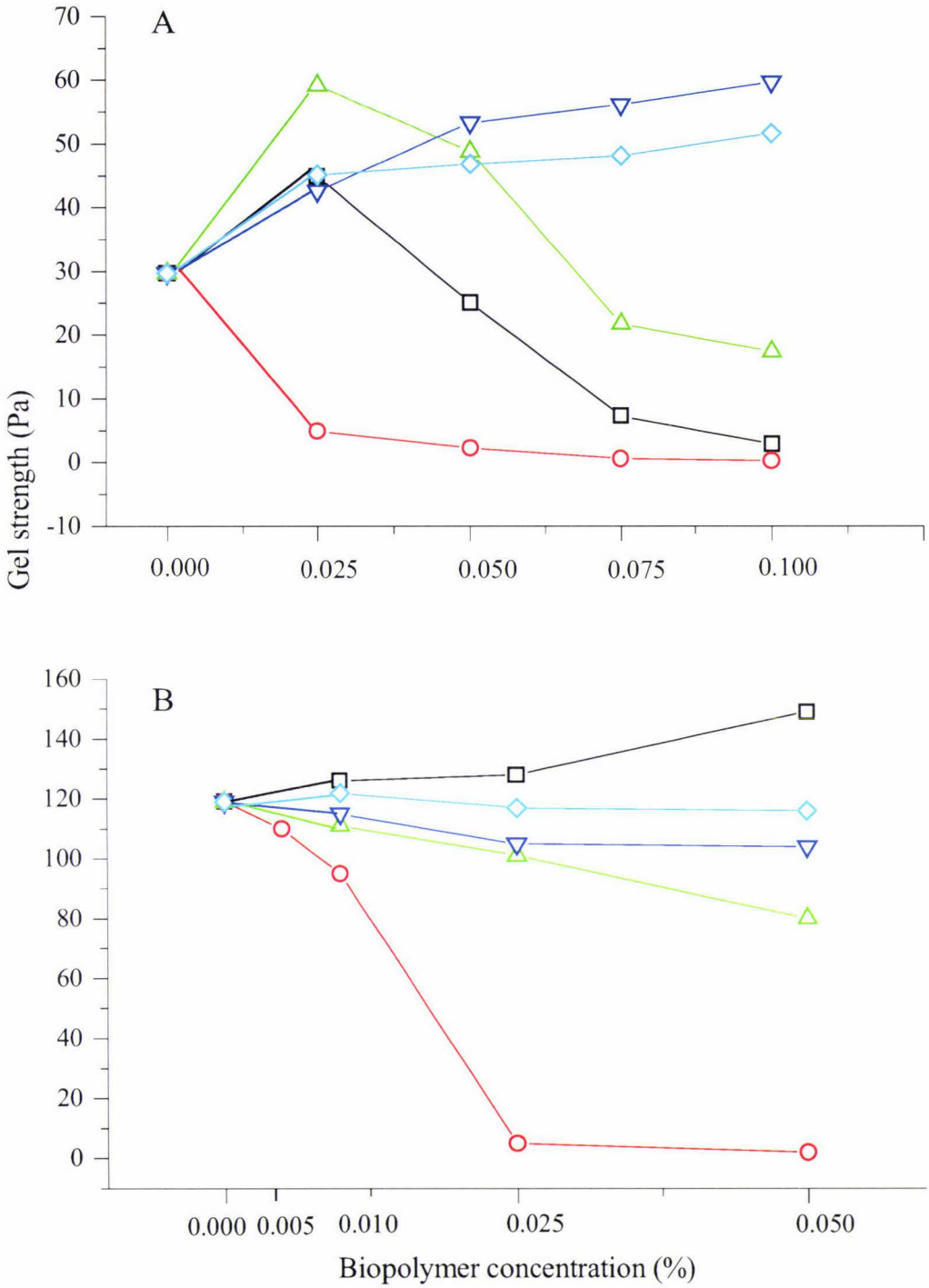

Figure 7.12 "Gel strength" as a function of biopolymer concentration of renneted skim milk containing κ-carrageenan (□), xanthan gum (○), guar gum (△), HM pectin (▽) and gelatin (◇). Graph (A) represented model system samples and Graph (B) represented samples under cheesemaking conditions.

However, under cheesemaking conditions (Figure 7.12 B), different results were obtained. Only renneted skim milk containing κ-carrageenan showed an increase in gel strength value with an increase in concentration.

An extremely sharp decline in gel strength was noted for samples with increasing xanthan concentrations. For samples containing guar, HM pectin and gelatin, the samples either showed a slight decrease (guar) or remained relatively constant (HM pectin and gelatin) in gel strength. As for gelation time, these discrepancies will be addressed in Chapter 8.

7.4 Summary

In this chapter, the influence of biopolymers addition, namely κ-carrageenan, xanthan, guar, HM pectin and gelatin, to skim milk during renneting, under cheesemaking conditions, were studied. Among all the samples, only renneted skim milk samples containing κ-carrageenan at concentrations ranging from 0.01 wt% to 0.05 wt% concentrations and gelatin at 0.05 wt% concentration, respectively, exhibited better rheological properties (higher G^*, shorter gelation times and higher gel strength values) than the control sample (0 wt% polysaccharide). G^* as well as gel strength values of the remaining samples containing polysaccharides like xanthan, guar and HM pectin, respectively, decreased with increasing polysaccharide concentrations.

8 General Discussion

Studies on the effect of biopolymers addition in rennet-induced skim milk were conducted under two different conditions, the normal experimental conditions (model skim milk dispersion) and cheesemaking conditions. These two systems will be discussed separately.

8.1 Model systems (renneted skim milk/biopolymers)

8.1.1 κ-carrageenan

Experimental results showed increasing G^* for renneted skim milk samples with increasing κ-carrageenan concentrations up to 0.025 wt%, which was in agreement with microstructural results that showed a more compact network formation. As the concentration of κ-carrageenan was further increased, G^* decreased, which also resulted in increased whey separation. At a microscopic level, CLSM observations showed clearly that there was an increase in pore size of rennet skim milk gels at higher κ-carrageenan concentrations.

κ-carrageenan was reported to react specifically with κ-casein (Grindrod and Nickerson, 1968, Payens, 1972; Drohan *et al.*, 1997) via electrostatic interaction between the positively charged segment (residues 20-115) of κ-casein and the negatively charged sulphated groups of κ-carrageenan. The association of κ-carrageenan with κ-casein on the micelle surface, resulting in bridging of the casein micelles could not be excluded (Hemar *et al.*, 2002). However, when casein micelles were mixed with κ-carrageenan at temperatures above the coil-to-helix transition (> 30 °C), the κ-carrageenan molecules induced flocculation of casein particles, through a depletion flocculation mechanism (Langendorf *et al.*, 1997, 1999; Schorsh *et al.*, 2000; Hemar *et al.*, 2002). Schorsh *et al.* (2000) pointed out that even in the case of the adsorption of κ-carrageenan to casein micelles, an excess of κ-carrageenan coils could cause depletion flocculation of the casein micelles.

Although our studies were performed on skim milk/κ-carrageenan mixtures, it was likely that the phase separation observed in this current study was also caused by depletion flocculation. In fact, phase separation was expected be to enhanced in rennet induced skim milk/κ-carrageenan system, as the size of casein micelles increased during the renneting process. It appears that a critical concentration of κ-carrageenan (> 0.025 wt%) is required to induce depletion flocculation.

It has to be noted that Shalabi and Fox (1982) reported that κ-carrageenan caused destabilisation in renneted skim milk at concentration higher than 0.05 %.

8.1.2 Xanthan gum

Rheological results of renneted skim milk containing xanthan showed longer aggregation and gelation times with very low values of complex modulus, and increased whey separation at increasing xanthan concentrations. At high xanthan concentrations, the absence of gelation as well as phase separation was clearly illustrated in the CLSM micrographs.

As the casein micelles and xanthan molecules have an overall negative charge at neutral pH, net repulsive interactions between them may lead to thermodynamic incompatibility. Hemar *et al.* (2001) reported that depletion flocculation was the most likely cause of phase separation for skim milk/xanthan mixtures at neutral pH. As in the case of κ-carrageenan addition, phase separation in renneted skim milk containing xanthan was also likely to be due to the flocculation of casein micelles aggregates by depletion mechanisms.

8.1.3 Guar gum

Rheological measurements showed that G^* increased with increasing guar concentrations up to 0.025 wt%, which was in agreement with CLSM micrographs where a more compact protein network was observed. As the concentrations increased

further, G^* decreased and whey separation increased. These experimental findings were in agreement with CLSM observations that showed larger pore size at high guar concentrations.

Bourriot *et al.* (1999) and Tuinier *et al.* (2000) reported that phase separation in casein micelle/guar mixtures originated from a depletion interaction leading to an effective attraction between the casein micelles by non-adsorbing guar. Mixture of guar gum and casein micelles phase separate when a certain concentration was exceeded (Tuinier *et al.*, 2000).

Once again as for the previous two polysaccharides, it was possible that phase separation occurred as a result of depletion interaction of casein micelles by the non-adsorbing guar. This explained the larger pore size as illustrated in CLSM micrographs for renneted skim milk at high guar concentration.

8.1.4 HM Pectin

In current findings, the microstructural results of rennet gels containing HM pectin were in accordance with rheological evaluation. Higher G^* values corresponded to a gel with firmer and more compact structure, leading to a lower syneresis.

Maroziene and de Kruif (2000) found pectin to be a non-adsorbing polymer when it was in solution with skim milk at pH 6.7. At pH 6.7, 0.2 % of HM pectin in skim milk caused depletion flocculation. In accordance with Maroziene and de Kruif (2000), as the concentrations of HM pectin used in the present work were lower than 0.1 %, phase separation due to depletion flocculation of renneted skim milk containing HM pectin was not observed.

8.1.5 Gelatin

Current investigations showed higher G^* and lower whey separation in rennet-induced skim milk gels containing increasing amounts of gelatin. Similar to renneted skim milk/HM pectin, CLSM micrographs illustrated dense protein network structures.
At the point of investigation, no literature on the rheology of renneted skim milk containing gelatin at neutral pH was found. However, Fiszman *et al.* (1999) showed that the mechanical behaviour of the acidified milk gels (pH 5.3) containing gelatin corresponded to a structure basically constituted by gelatin and without any phase separation. It is likely that gelatin does not induce depletion-flocculation in this system.

To summarise, it appeared clearly that polysaccharides such as κ-carrageenan, xanthan and guar, which were known to induce phase separation when added to skim milk, do induce phase separation when they were added to renneted skim milk. Furthermore, it is even expected that phase separation will be even more effective since the size of the casein aggregates in renneted systems is larger than casein micelles present in skim milk.

8.2 Cheesemaking conditions

The incorporation of polysaccharides may be used to increase moisture levels in cheese. As was previously reported, under normal experimental conditions, the addition of xanthan and guar gum, respectively, in renneted skim milk had impaired the coagulation and gel firming process, but others, such as κ-carrageenan, HM pectin and gelatin, accelerated both coagulation and gel firming.

In general, the addition of polysaccharides to renneted skim milk under cheesemaking conditions did not affect the gelation times as much as in the samples made under the normal conditions. This could be due to accelerated gelation and the higher moduli of rennet skim milk gels caused by the addition of $CaCl_2$ and lowering the pH to 6.2 (van Hooydonk *et al.*, 1986; Zoon *et al.*, 1988c and 1989a). However, the change in renneting conditions had resulted in higher G^* in comparison to the samples obtained

under normal experimental conditions (see Annex 2 and 3). Despite higher G^* and faster gelation times, the viscoelastic properties of rennet gels did not improve upon adding xanthan, guar, HM pectin and gelatin, but did improve when κ-carrageenan was added.

Experimental results showed that gels containing κ-carrageenan displayed higher G^* and shorter gelation times with increasing κ-carrageenan concentration. Although pH had little effect on the gelation of κ-carrageenan (Stoloff, 1959; Black, 1966), it is well known that addition of Ca^{2+} promoted the gelation of κ-Carrageenan (Michel *et al.*, 1997). It is possible that under cheesemaking conditions, κ-carrageenan formed a gel instantly, and thus did not induce flocculation by depletion, which in turn improved the rheological properties of rennet gel containing κ-carrageenan.

The addition of xanthan into renneted skim milk under cheesemaking conditions resulted in similar rheological behaviour as renneted skim milk containing xanthan in the model system, where the gelation times increased and the G^* decreased with increasing xanthan concentrations. Because xanthan is not sensitive to changes in pH and salt addition (Kovacs and Kang, 1977; Nussinovitch, 1997c), similar effects were noted in both the systems.

Renneted skim milk with added guar displayed similar behaviour to that observed under the normal conditions, where G^* decreased with increasing guar concentrations. Similarly to xanthan, guar is stable to pH change, between pH 1 and pH 10.5, and as a non-ionic it is compatible with salts over a wide range of electrolyte concentration (Meer, 1977).

The addition of HM pectin and gelatin on the rheological properties renneted skim milk under cheesemaking was also similar to the effect of their addition under normal condition. Conditions had not shown much rheological changes as the polysaccharide concentrations increased. These two biopolymers were not significantly affected by change in pH to 6.3 and the salt concentration used in this study (Nussinovitch, 1997a; Ledward, 1986).

Overall, compared to the normal conditions, only the addition of κ-carrageenan significantly affected the rheological behaviour of the rennet skim milk gels under cheesemaking condition, and this was likely due to the effect of salt on the gelling properties of κ-carrageenan. While the addition of xanthan, guar, HM pectin and gelatin were qualitatively similar to their addition to renneted skim milk under normal conditions, these four biopolymers were known to be not affected by the slight change in pH and the concentration of $CaCl_2$ used in this study.

8.2.1 Summary

In this chapter, the phase separation in renneted milk containing polysaccharide was explained in term of a depletion-flocculation mechanism. This was based on several previous studies performed by different research groups, which reported phase separation in skim milk/polysaccharide mixtures and explained their observation by depletion-flocculation. As the systems here investigated were under the action of rennet, it was expected that phase separation would be enhanced.

Under cheesemaking conditions (lower pH with addition of $CaCl_2$) the addition of biopolymers into rennet skim milk gels had a similar effect as in samples under normal condition (neutral pH without salt addition). This was due to the fact that the cheesemaking conditions were known not to affect these biopolymers. This was true for xanthan, HM pectin, gelatin and guar, but not for κ-carrageenan which was very sensitive to ions, such as calcium, and pH.

9 Conclusions and Recommendations

From the present study, the following conclusions could be drawn.

1. Rheological results for model systems (renneted skim milk/biopolymers) had showed that the addition of κ-carrageenan, xanthan, guar gum, HM pectin and gelatin affected the viscoelastic properties of rennet-induced skim milk gels.
   - The G^* of renneted skim milk containing biopolymers decreased with increasing κ-carrageenan, xanthan and guar, and increased with increasing HM pectin and gelatin concentrations.
   - The aggregation and gelation times of these samples decreased with increasing κ-carrageenan, HM pectin and gelatin, and increased with increasing xanthan and guar concentrations.

2. Microstructure observations had showed the change in the microstructural properties of the renneted milks upon biopolymer addition.
   - CLSM micrographs were effective in drawing conclusive microstructural properties of renneted skim milk containing κ-carrageenan, xanthan and guar. For renneted skim milk with the incorporation of HM pectin and gelatin, the micrographs did not exhibit observable differences.
   - There was an increase in pore sizes for rennet skim milk gels containing increasing concentrations of κ-carrageenan and guar.
   - At xanthan concentrations higher than 0.05 wt%, CLSM micrographs exhibited emulsion-like microstructure.

3. Syneresis behaviour of rennet-induced skim milk was altered upon biopolymer additions. Syneresis of rennet-induced skim milk decreased by adding guar, HM pectin and gelatin, and increased with increasing κ-carrageenan, xanthan and guar, and decreased with increasing HM pectin and gelatin concentrations.

4. Diffusing-wave spectroscopy (DWS) was found to be a useful tool to monitor changes in these systems and allowed determination of the aggregation time, gelation time and gel strength of renneted skim milk/biopolymer mixture.
   - Aggregation and gelation time of renneted skim milk decreased upon biopolymer addition compared to control.
   - Renneted samples containing biopolymer had shorter aggregation time with increasing concentrations of κ-carrageenan and guar, while aggregation time increased with increasing concentrations of xanthan, HM pectin and gelatin.
   - Gelation time of these samples increased with increasing xanthan, HM pectin and gelatin, and decreased with increasing concentrations of κ-carrageenan and guar.
   - Gel strength of rennet-induced skim milk increased with 0.025wt% biopolymer addition.
   - Gel strength of renneted skim milk containing biopolymer increased with increasing HM pectin and gelatin, and decreased with increasing concentrations of κ-carrageenan, xanthan and guar.

5. Rheological results for renneted skim milk with biopolymer additions under cheesemaking conditions showed that the addition of κ-carrageenan had improved the viscoelastic properties of rennet-induced skim milk, while the incorporation of xanthan, guar, HM pectin and gelatin had not.
   - The G^* of renneted samples containing biopolymer had increased with increasing κ-carrageenan, and decreased with increasing concentrations of xanthan, guar, HM pectin and gelatin.
   - The gelation time of these samples had decreased with increasing κ-carrageenan and guar, and increased with increasing xanthan and gelatin concentrations. Gelation time for renneted skim milk containing HM pectin remained unchanged with increasing concentrations.
   - As expected, when compared to model systems, G^* of renneted skim milk containing biopolymer were found to be higher for samples under cheesemaking conditions.

- Because of the difference in rennet concentration, salt and pH, G^* of these samples were found to be higher for samples under cheesemaking conditions. In addition, the gelation time was shorter for samples made under cheesemaking than those made under normal conditions.

The effect of polysaccharide addition was believed to be associated with the phase separation in renneted milk containing polysaccharide, and was explained in term of a depletion-flocculation mechanism.

9.1 Recommendations

Based on findings in this dissertation, the following areas are recommended for further study.

1. The characteristics of rennet gel, such as water-holding capacity and gel strength, were important parameters and affected characteristics such as yield, moisture content, and textural attributes. In the current study, the water-holding capacity of the polysaccharides studied was determined by syneresis measurements. Attempts to investigate the adsorption of polysaccharide to milk proteins were conducted using a chemical (phenol sulphuric) assay and high performance liquid chromatography (HPLC). Unfortunately, these two methods were not sensitive enough (results not shown). The use of other techniques, such as TEM and SEM, to investigate the adsorption of polysaccharides into milk protein under renneted system is recommended.

2. Investigate a wider range of pHs and polysaccharide concentrations, especially at the lower concentration (< 0.025 wt%), under cheesemaking conditions.

3. Determine the kinetics of aggregation and gelation by varying the rennet concentration and temperature, and study its effect on phase separation. For instance, would phase separation still occur if the renneting process were very fast?

4. More remains to be investigated to have a clear understanding on the effect of biopolymer additions that influence the properties of rennet-induced skim milk and their impact on cheese production. The following are suggestions recommended for further investigations.

- Polysaccharide concentration

  Properties of rennet-induced skim milk containing a wider range of polysaccharide concentrations could be investigated to determine the optimum concentration to be recommended for use in a cheesemaking process.

- $CaCl_2$ concentration

  Rheological properties of model rennet-induced skim milk system had improved tremendously at cheesemaking conditions. Since the addition of Ca^{2+} was known to promote the gelation, especially in the case κ-carrageenan, further investigations by varying the concentration of $CaCl_2$ is recommended.

- Rennet concentration

  A range of rennet concentration should be investigated in order to study the effect of the kinetic of aggregation and gelation.

- Scale-up trial

  A pilot-plant trial on renneted skim milk containing the selected polysaccharide at the determined-optimum conditions will be useful to justify the results obtained as well as for commercial benefits.

References

Aguilera, J.M. and Stanley, D.W. (1990a). Examining food microstructure. In *Microstructural Principles of Food processing and Engineering*, Elsevier Science Publishers Ltd, New York, USA, 1-53

Aguilera, J.M. and Stanley, D.W. (1990b). Microstructural aspects of a fluid food: Milk. In *Microstructural Principles of Food processing and Engineering*, Elsevier Science Publishers Ltd, New York, USA, 193-235

Ali, A.E., Andrews, A.T. and Cheeseman, G.C. (1980). Influence of storage of milk on casein distribution between the micellar and soluble phases and its relationship to cheese-making parameter. *Journal of Dairy Research*, Volume **47**, 371-383

Asakura, S. and Oosawa, F. (1954). *Journal of Chemistry and Physics*, Volume **22**, 1255

Asakura, S. and Oosawa, F. (1958). *Journal of Polymer Science*, Volume **33**, 183

Augustin, M.A., Puvanenthiran, A. and McKinnon, I.R (1999). The effect of kappa carrageenan conformation on its interaction with casein micelles. *International Dairy Journal*, Volume **9**, 413-414

Axelos, M.A.V. and Thubault, J.F. (1991). In *The Chemistry and Technology of Pectin*. Walter, R.H. (ed.), Academic Press, San Diego, Chapter **6**, 109

Bender, W.A. (1959). Pectin. In *Industrial Gums*. Whistler, R.L. and BeMiller, J.N. (ed.), Academic Press, New York, 377-432

Black, W.A.P. (1966). Preparation and some of the properties and uses of the κ- and λ-carrageenans. In *The Chemistry and Rheology of Water-Soluble Gums and Colloids*. The Society of Chemical Industry, 33-44

Bohlin, L., Hegg, P.O. and Ljusberg-Wahren, H. (1984). Viscoelastic properties of coagulating milk. *Journal of Dairy Science*, Volume **67**, 729-734

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

Brooker, B.E. (1995). Imaging Food Systems by Confocal Laser Scanning Microscopy. In *New Physico-Chemical Techniques for the Characterization of Complex Food Systems.* Dickinson, E. (ed.), Chapman and Hall, Great Britain, 53-68

Bourriot, S., Garnier, C. and Doublier, J.L (1999). Phase separation, rheology and microstructure of micellar casein-guar gum mixture. *Food Hydrocolloids*, Volume **13**, 43-49

Capron, I., Brigand, G., and Muller, G. (1997). About the naïve and renatured conformation of xanthan exopolysaccharide, *Polymer*, Volume **38**, 5289-5295

Carles, C. and Martin, P. (1985). Kinetic study of the action of bovine chymosin and pepsin A on bovine κ-casein. *Archives of Biochemistry and Biophysics.* Volume **242**, 411-416

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*, Volume **39**, 15-22

Challen, I.A. (1994). Xanthan gum: A multifunctional stabilizer for food products. In: *Food Hydrocolloids: Structures, Properties and Functions.* Nishinari, K. and Doi, E. (ed.), Plenum Press, New York, 138-140

Chaplin, B. and Green, M.L. (1980). Determination of the proportion of κ-casein hydrolyzed by rennet on coagulation of skim milk. *Journal of Dairy Research*, Volume **47**, 351-358

Cheryan, M., van Wyk, P.J., Olson, N.F. and Richardson, T. (1975). Secondary phase and mechanism of enzymic milk coagulation. *Journal of Dairy Science*, Volume **58**, 477-481

Dalgleish, D.G. (1979). Proteolysis and aggregation of casein micelles treated with immobilized or soluble chymosin. *Journal of Dairy Research,* Volume **46**, 643-661

Dalgleish, D.G. (1981) Effect of milk coagulation on the nature of curd formed during renneting – a theoretical discussion, *Journal of Dairy Research,* Volume **48**, 65-69

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

Dalgleish, D.G. and Horne, D.S. (1991) Studies of gelation of acidified and renneted milk using diffusing wave spectroscopy. *Milchwissenschaft*, Volume **46 (7),** 417-422

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

Dea, I.C.M., Morris, E.R., Rees, D.A. (1977). Associations of like and unlike polysaccharides: mechanism and specificity in galactomannans, interacting bacterial polysaccharides, and related systems. *Carbohydrate Research*, Volume **57 (1)**, 249-272

De Kruif, C.G., Jeurnink, Th.J.M, and Zoon, P. (1992). The viscosity of milk during the initial stages of renneting. *Netherlands Milk and Dairy Journal*, Volume **46**, 123-137

De Kruif, C.G. (1999). Casein micelle interactions. *International Dairy Journal*, Volume **9**, 183-188

De Kruif, C.G. and Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids*, Volume **15**, 555-563

De Kruif, C.G. and Holt, C. (2001). Casein micelle structure substructure and interactions. In: *Advanced Dairy Chemistry Volume 1: Proteins*. Fox, P.F. and McSweeney, P. (ed.), 3rd edition, Gaithersburg, MD: Aspen Publications.

Dickinson, E., and Stainsby, G. (1982). Macromolecular Adsorption and Colloid Stability. In *Colloids in Food*, Applied Science Publishers, London, Chapter **3**, 67-105

Dickinson, E. and McClements, D.J. (1996) Protein - Polysaccharides Interactions. In *Advances in Food Colloids*, Blackie Academic and Professional, UK, 81-101

Drohan, D.D., Tziboula, A., McNulty, D. and Horne, D.S. (1997). Milk protein-carrageenan interactions. *Food Hydrocolloids*, Volume **11**, 101-107

Dziuba, J. and Muzinska, B. (1998). Effect of cold storage of milk on functional properties of casein. *Polish Journal of Food and Nutrition Sciences*, Volume **7/48 (3)**, 485-492

Elfak, A.M., Pass, G. and Phillips, G.O. (1979). The effect of casein on the viscosity of solutions of hydrocolloids, *Journal of Science, Food and Agricultural*, Volume **30**, 994-998

Fiszman, S.M. and Salvador, A. (1999). Effect of gelatin on the texture of yoghurt and of acid-heat-induced milk gels. *Zeitschrift für Lebensmittel-Untersuchung und – Forschung A*, Volume **208**, 100-105

Fiszman, S.M., Lluch, M.A. and Salvador, A. (1999). Effect of addition of gelatin on microstructure of acidic milk gels and yogurt and on their rheological properties. *International Dairy Journal*, Volume **9**, 895-901

Foltmann B. (1981). Mammalian milk-clotting protease: Structure, function, evolution and development. *Netherlands Milk and Dairy Journal*, Volume **35**, 223-231

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,* Volume **44**, 521-531

Green, M.L. (1980). The formation and structure of milk protein gels. *Food Chemistry*, Volume **6**, 41-49

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 One: General Aspects*. Fox, P.F. (ed.), 2nd Edition, Chapman and Hall, Great Britain, 101-140

Grinberg, V,Ya., and Tolstoguzov, V.B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, Volume **11**, 145-158

Grindrod, J. and Nickerson, T.A. (1968). Effect of various gums on skim milk and purified milk proteins. *Journal of Dairy Science*, Volume **51**, 834-841

Hansen, P.M.T. (1968). Stabilization of αs-casein by carrageenan. *Journal of Dairy Science*, Volume **51**, 192-195

Hansen, P.M.T., Huang, T.V., Renoll, M. and Mangins, M.E. (1980). Stabilization of rennet treated skim milk by carrageenan. *Journal of Dairy Science*, Volume **75**, 50 (abstract)

Hemar, Y., Tamehana, M. Munro, P.A. and Singh, H. (2001). Viscosity, microstructure and phase behavior of aqueous mixtures of commercial milk protein products and xanthan gum. *Food Hydrocolloids*, Volume **15**, 565-574

Hemar, Y., Hall, C.E., Munro, P.A., and Singh, H. (2002). Small and large deformation rheology and microstructure of κ-carrageenan gels containing commercial milk protein products. *International Dairy Journal*, Volume **12**, 371-381

Hermansson, A.M., Eriksson, E., and Jordansson, E. (1991). Effects of potassium, sodium and calcium on the microstructure and rheological behavior of kappa-carrageenan gels. *Carbohydrates Polymers*, Volume **16**, 297-320

Hermansson, A.M., Altskar, A, and Jordansson, E. (1998). Phase separated mixed gelatin-milk protein systems. In *Gums and Stabilisers for the Food Industry 9.* Williams, P.A. and Philips, G.O. (ed.), The Royal Society of Chemistry, Bookcraft (Bath) Ltd, UK, 107-116

Holt, C. (1992). Structure and stability of bovine casein micelles, *Advance Proteins Chemistry*, Volume **43**, 63-151

Holt, C. and Horne, D.S. (1996). The hairy casein micelle: Evolution of the concept and its implications for dairy processing. *Netherlands Milk and Dairy Journal*, Volume **50**, 1-27

Holt, C., and Roginski, H. (2001). Milk Proteins: Biological and Food Aspects of Structure and Function. In *Chemical and Functional Properties of Food Proteins.* Zdzislaw, E.S. (ed.), Technomic Publishing Company, Inc, Pennsylvania, USA, 271-334

Horne, D.S. and Davidson, C.M. (1990). The use of dynamic light-scattering in monitoring rennet curd formation. *Milchwissenschaft*, Volume **45 (11)**, 712-715

Horne, D.S. (1995). Light scattering studies of colloid stability and gelation. In *New physico-chemical techniques for the characterization of complex food systems.* Dickinson, E. (ed.), Chapman and Hall, Great Britain, 240-267

Horne, D.S. (1998). Casein interactions: Casting light on the black boxes, the structure in dairy products. *International Dairy Journal*, Volume **8**, 171-177

James, H. P. (1992a). Milk. In *Dairy Rheology – A concise guide.* YCH Publishers, Inc, USA, 49-56

James, H. P. (1992b). Cheese. In *Dairy Rheology – A concise guide*. YCH Publishers, Inc, USA, 85-112

Jansson, P.E., Kenne, L. and Lindberg, B. (1975). Structure of the extracellular polysaccharide from *Xanthomonas compestris, Carbohydrate Research,* Volume **45**, 275

Kovacs, P. and Kang, K.S. (1977). Xanthan Gum. In: *Food Colloids*. Graham H.D. (ed.), The AVI Publishing Company, Inc., Westport, Connecticut, 500-521

Kinsella, J.E. (1984). Milk Proteins: Physicochemical and functional properties. *CRC Critical Review in Food Science and Nutrition*. CRC Press, Inc., Boca Raton, Florida, Volume **21**, Issue **3**, 197-262

Kowalchyk, A.W., and Olson, N.F. (1977). Effects of pH and temperature on the secondary phase of milk clotting by rennet. *Journal of Dairy Science,* Volume **60**, 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*, Volume **59 (5)**, 956-959

Langendorff, V., Cuvelier, G., Launay, B., and Parker, A. (1997). Gelation and flocculation of casein micelle/carrageenan mixtures, *Food Hydrocolloids*, Volume **11**, 35-40

Langendorff, V., Cuvelier, G., Michon, C., Launay, B., Parker, A. and De Kruif, C.C. (1999). Casein micelle/ iota carrageenan interactions in milk: Influence of temperature. *Food Hydrocolloids*, Volume **12**, 211-218

Lapasin, R., and Pricl, S. (1995). Rheology of polysaccharides systems. In *Rheology of industrial polysaccharides: theory and applications*, Blackie Academic and Professional, Chapman and Hall, Glasgow, UK, 32-43 and 250-477

Ledward, D.A. (1986). Gelation of gelatin. In: *Functional Properties of Food Macromolecules*. Mitchell, J.R. and Ledward, D.A. (ed.), Elsevier Applied Science Publishers, London and New York, 171-202

Ledward, D.A. (1994). Protein-Polysaccharide Interactions. In *Protein Functionality in Food Systems*. Hettiarachchy, N.S. and Ziegler, G.R. (ed.), Marcel Dekker, Inc, United States of America, 225-259

Lin, C.F., and Hansen, P.M.T. (1968). Stabilization of calcium caseinates by carrageenan. *Journal of Dairy Science*, Volume **51**, 945 (abstract)

Lin, C.F. (1977). Interaction of sulfated polysaccharides with proteins. In: *Food Colloids*. Graham, H.D (ed.). The AVI Publishing Company, INC. Westport, Connecticut. 360

Lucey, J.A., Van Vliet, T., Grolle, K., Geurts, T. and Walstra, P. (1997a). Properties of acid casein gels made by acidification with glucono-δ-lactone. 1. Rheological properties. *International Dairy Journal*, Volume 7, 381-388

Lucey, J.A., Van Vliet, T., Grolle, K., Geurts, T. and Walstra, P. (1997b). Properties of acid casein gels made by acidification with glucono-δ-lactone. 2. Syneresis, Permeability and Microstructural Properties, *International Dairy Journal*, Volume 7, 389-397

Lucey, J.A. and Singh, H. (1998). Formation and physical properties of acid milk gels: a review. *Food Research International*, Volume **30 (7)**, 529-542

Lucey, J.A., Munro, P.A. and Singh, H. (1998a). Whey separation in acid milk gels made with glucono-δ-lactone: Effect of heat treatment and gelation temperature. *Journal of texture studies*, Volume **29**, 413-426

Lucey, J.A., Tamehana, M, Singh, H. and Munro, P.A. (1998b). A comparison of the formation, rheological properties and microstructure of acid skim milk gels made with a bacterial culture or glucono-δ-lactone. *Food Research International*, Volume **31(2)**, 147-155

Lucey, J.A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids*, Volume **15**, 603-608

Lucey, J.A. (2002). Formation and Physical Properties of Milk Proteins Gels. *Journal of Dairy Science*, Volume **85**, 281-294

Maroziene, A. and de Kruif, C.G. (2000). Interaction of pectin and casein micelles. *Food Hydrocolloids*, Volume **14**, 391-394

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

Meer, W.A. (1977). Plant hydrocolloids. In: *Food Colloids*, Graham H.D. (ed.), AVI Publishing Co., Westport, Connecticut, 522-539

Michel, A.S., Mestdagh, M.M. and Axelos, M.A.V. (1997). Physico-chemical properties of carrageenan gels in presence of various cations. *International Journal of Biological Macromolecules*, Volume **21**, 195-200

Melton, L.D., Mindt, L., Rees, D.A. and Sanderson, G.R. (1976). Covalent structure of the extracellular polysaccharide from *Xanthomonas campestris*: evidence from partial hydrolysis studies, *Carbohydrate Research*, Volume **46**, 245

Moirano, A.L. (1977). Sulfated seaweed polysaccharides. In *Food Colloids*. Graham H.D. (ed.), AVI Publishing Co., Westport, Connecticut, 347-381

Morr, C.V. (1967). Effect of oxalate and urea upon ultracentrifugation properties of raw and heated skim-milk casein micelles. *Journal of Dairy Science*, Volume **50**, 1744

Morr, C.V. (1979). Conformation and Functionality of milk proteins. In *Functionality and Protein Structure*. Pour-El, A. (ed.). ACS Symposium Series 92, American Chemical Society, Washington D.C. 65-80

Morris, V.J. (1995). Bacterial Polysaccharides. In: *Food Polysaccharides and it Applications*, Stephen, A.M. (ed.), Marcel Dekker, Inc, New York, 342-365

Morris, V.J. (1998). Gelation of polysaccharides. In *Functional properties of food macromolecules*. Hill, S.E., Ledward, D.A. and Mitchell, J.R. (ed.), 2nd Edition, Aspen Publishers, Inc, Great Britain, 143-226

Nussinovitch, A. (1997a). Pectins. In *Hydrocolloid Applications – Gum technology in the food and other industries*. Blackie Academic and Professional, UK, 83-104

Nussinovitch, A. (1997b). Seed Gums. In *Hydrocolloid Applications – Gum technology in the food and other industries*. Blackie Academic and Professional, UK, 140-153

Nussinovitch, A. (1997c). Xanthan gum. In *Hydrocolloid Applications – Gum technology in the food and other industries*. Blackie Academic and Professional, UK, 154-168

Oakenfull, D. (1991). *The Chemistry and Technology of Pectin*. Walter, R.H. (ed.), Academic Press, San Diego, Chapter **5**, 87

Oakenfull, D. and Scott, A. (1998). Milk gels with low methoxyl pectins. In: *Gums and Stabilizers for the Food Industry* – 9, Williams, P.A. and Phillips, G.O. (ed.), The Royal Society of Chemistry, Cambridge, 212-221

Olsen, R.L. (1989). Effect of polysaccharides on rennet coagulation of skim milk proteins. *Journal of Dairy Science*, Volume **72**, 1695-1700

Parker, A., Gunning, P.A, Ng, K., and Robins, M.M. (1995). How does xanthan stabilize salad dressing?. *Food Hydrocolloids*, Volume **9(4)**, 333-342

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

Payens, T.A.J. (1971). Light scattering of protein reactivity of polysaccharides, especially of carrageenans. *Journal of Dairy Science*, Volume **55**, 141-150

Pedersen, J.K. (1979). The selection of hydrocolloids to meet functional requirements. In *Polysaccharides in Food*, Blanshard J.M. and Mitchell, J.R (ed.). Butterworths & Co Publishers Ltd, London, 219-227

Pereyra, R., Schmidt, K.A. and Wicker, L. (1997). Interaction and Stabilization of acidified casein dispersions with low and high methoxyl pectins, *Journal of Agricultural Food Chemistry*, Volume **45**, 3448-3451

Piculell, L., Nilsson, S., Viebke, C., and Zhang, W. (1994). Gelation of (some) seaweed polysaccharides. In *Food Hydrocolloid: Structure, Properties and Functions*. Nishinari, K., and Doi, E. (ed.), Plenum Press, New York, 35-44

Piculell, L. (1995). Gelling Carrageenans. In *Food Polysaccharides and their Applications*. Alistair M.S. (ed.), Marcel Dekker Inc., New York, 205-244

Ploehn, H.J. and Russel, W.B (1990). Interactions between colloidal particles and soluble polymers. *Advances in Chemical Engineering*, Volume **15**, 137-227

Puvanenthiran, A., Goddard, S.J. and Augustion, M.A. (2001). Gelation of mixed gels containing κ-carrageenan and skim milk components. *Journal of Food Science*, Volume **67 (2)**, 573-577

Rodd, A.B., Davis, C.R., Dunstan, D.E., Forrest, B.A. and Boger, D.V. (2000). Rheological characterization of 'weak gel' carrageenan stabilized milks. *Food Hydrocolloids*, Volume **14**, 445-454

Roefs S.P.F.M, van Vliet, T., van der Bijaart, H.J.C.M, de Groot Mostert, A.E.A., and Walstra, P. (1990). Structure of gels made by combined acidification and rennet action. *Netherlands Milk and Dairy Journal*, Volume **44**,150-188

Rollema, H.S. (1992). Casein association and micelle formation. In: *Advanced Dairy Chemistry Volume 1: Proteins*, P.F. Fox (ed.), London and New York, Elsevier Applied Science, 111-140

Ross-Murphy, S. (1994). Recent advances in the rheological characterization of gelatin gels. In *Gums and Stabilizers for the food industry 7*. Philips, P.A. and Wedlock, D.J (ed.). Oxford University Press Inc. US, 243-255

Ruettimann, K.W. and Ladisch, M.R. (1987). Casein micelles: structure, properties and enzymatic coagulation. *Enzyme Microbiology Technology*, Volume **9**, 578-589

Salvador, A. and Fiszman, S.M. (1998). Textural characteristics and dynamic oscillatory rheology of maturation of milk gelatin gels with low acidity. *Journal of Dairy Science*, Volume **81**, 1525-1531

Scher, J. and Hardy, J. (1993). Study of the evolution of casein micelle size distribution after renneting by means of quasielastic light scattering. *The Australian Journal of Dairy Technology*, Volume **48**, 62-65

Schmidt, D.G., and Payens, T.A.J. (1976). Colloidal aspects of casein, Surface *and Colloid Science*, Volume **9**,165

Schmidt, D.G. (1980). Colloidal Aspects of casein, *Netherlands Milk and Dairy Journal*, Volume **34**, 42-64

Schmidt, D.G. (1982). Association of caseins and casein micelle structure. In: *Developments in Dairy Chemistry Volume 1: Proteins*. Fox, P.F. (ed.), Applied Science Publishers, London, UK, 61-86

Schorsh, C., Jones, M.G., and Norton, I. (2000). Phase behaviour of pure micellar casein/κ-carrageenan systems in milk salt ultrafiltrate. *Food Hydrocolloids*, Volume **14**, 347-358

Seaman, J.K. (1980). *Handbook of water-soluble gums and resins*. ed. Davidson R.L., The Kingsport Press, United States of Amercia, Chapter **6** 11 and Chapter **14** 8

Shalabi, S.I. and Fox, P.F. (1982). Effect of κ-carrageenan on the heat stability and rennet coagulation of milk. *Irish Journal of Food Science and technology*, Volume **6**, 183-187

Shimmin, P.D., and Hill R.D. (1964). An electron microscope study of the internal structure of casein micelles. *Journal of Dairy Research*, Volume **31**, 121-123

Slattery, C.W. (1976). Review: casein micelle structure: an examination of models. *Journal of Dairy Science*, Volume **59**, 1547-1555

Stoll, W.F. (1966). Syneresis of rennet-formed milk gels a thesis, University of Minnesota, Michigan, 1-181

Stoloff, L. (1959). Carrageenan. In: *Industrial Gums*. Whistler, R.L. and BeMiller, J.N. (ed.), Academic Press, New York, 106-115

Swaisgood, H.E. (1982). Chemistry of milk protein. In *Developments in Dairy Chemistry Volume 1*. Fox, P.F. (ed.), Applied Science Publishers, London, Chapter **1**

Swaisgood, H.E. (1985). Characteristics of edible fluids of animal origin: Milk, In *Food Chemistry*, Fennema, O.R. (ed.). 2nd Edition, Marcel Dekker, New York, 791-827

Swaisgood, H.E. (1992). Chemistry of the caseins. In: *Advanced Dairy Chemistry Volume 1: Proteins*, Fox, P.F. (ed.), Elsevier Applied Science, London and New York, 64-110

Syrbe, A., Bauer, W.J. and Klostermeyer, H. (1998). Polymer Science Concepts in Dairy Systems – An overview of milk protein and food hydrocolloid interaction. *International Dairy Journal*, Volume **8**, 179-193

Takigami, S., Williams, P.A. and Philips, G.O. (1992). Interfacial properties of xanthan gum. In *Gums and Stabilizers for the Food Industry 6*. Philips, G.O., Williams, P.A. and Wedlock D.J (ed.), IRL Press, Oxford, 371-377

Tolstoguzov, V.B. (1991). Review: Functional properties of food proteins and role of protein-polysaccharide interaction. *Food Hydrocolloids*. Volume **4(6)**, 429-468

Tuinier, R., Grotenhuis, E.T and De Kruif, C.G. (2000). The effect of depolymerised guar gum on the stability of skim milk. *Food Hydrocolloids*, Volume **14**, 1-7

Tziboula, A. and Horne, D.S. (1999). Influence of milk proteins on kappa carrageenan gelation. *International Dairy Journal*, Volume **9**, 359-364

Van Dijik, H.J.M. and Walstra, P. (1986) One-dimensional syneresis of rennet curd in constant conditions. *Netherlands Milk and Dairy Journal*, Volume **40**, 3-30

Van Hooydonk, A.C.M., Olieman, C. and Hagedoorn, H.G. (1984). Kinetics of the chymosin-catalyzed proteolysis of κ-casein in milk. *Netherlands Milk and Dairy Journal*, Volume **37**, 207-222

Van Hooydonk, A.C.M., Hagedoorn H.G. and Boerrigter I.J. (1986). The effect of various cations on the renneting of milk. *Netherlands and Milk Dairy Journal*, Volume **40**, 369-390

Van Hooydonk, A.C.M. and Walstra, P. (1987). Interpretation of the kinetics of the renneting reaction in milk. *Netherlands Milk and Dairy Journal*, Volume **41**, 19-47

Van Hooydonk, A.C.M. and van den Berg, G. (1988). Control and determination of the curd-setting during cheesemaking, *International Dairy Federation (IDF)*, **225**

Van Vliet, T. and Walstra, P. (1983). Rheology of curd and cheese: Abstract. *Netherlands Milk and Dairy Journal*, Volume **37**, 93-94

Van Vliet, T., Lucey, J.A., Grolle, K. and Walstra, P. (1997). Rearrangements in acid-induced casein gels during and after gel formation. In *Food Colloids – Proteins, Lipids and Polysaccharides*, Dickinson, E. and Bergenstahl B. (ed.), The Royal Society of Chemistry, UK, 335-345

Veis, A. (1964). The macromolecular chemistry of gelatin. Horecker, B., Kaplan, N.O. and Scheraga, H.A (ed.). Academic Press, New York and London, 267-411

Voragen, A.G.J. and Pilnik, W. (1995). Pectins. In: *Food polysaccharides and it applications*, Stephen, A.M. (ed.), Marcel Dekker, Inc, New York, 287-339

Vrij, A. (1976) *Journal of Pure Applied Chemistry*, Volume **48**, 471

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

Walstra, P., and Jenness R. (1984a). Proteins. In *Dairy Chemistry and Physics*. John Wiley, New York, NY, Chapter 6, 98-122

Walstra, P., and Jenness R. (1984b). Casein micelles, In *Dairy Chemistry and Physics,* John Wiley, New York, NY, Chapter 13, 229-253

Walstra, P. and van Vliet, T. (1986). The physical chemistry of curd-making. *Netherlands Milk and Dairy Journal*, Volume **40**, 241-259

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

Walstra, P. (1993). The syneresis of curd. In *Cheese: Chemistry, Physics and Microbiology – Volume 1, General Aspects*. Fox, P.F. (ed.), 2nd Edition, Chapman and Hill, Great Britain, 141-191

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

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

Weitz, D. A., and Pine, D. J. (1993). In *Dynamique light scattering, the method and some applications*. Brown, W. (ed.), Clarendon Press, Oxford, 652

Whitney, R.McL. (1977). Milk Proteins. In *Food Colloids*. Graham, H.D (ed.). AVI Publisher Co., Westport, 66-141

Whitney, R. McL. (1988). Proteins of milk. In *Fundamentals of Dairy Chemistry*. Wong, N.P. (ed.), 3rd Edition, AVI book, Van Nostrand Reinhold, New York, 81-169

Xu, S., Stanley, D.W., Goff, H.D., Davison, V.J. and Le Mahuer, M. (1992). Hydrocolloid/milk gel formation and properties. *Journal of Food Science*, Volume **57**, 96-102

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

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

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

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

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

Websites:

World Trade atlas (2002) – New Zealand Dairy industry
http://www.marketnewzealand.com/home/index/0,1455,SectionID%253D4590%2526ContentID%253D3615,00.html

Annex 1 – Permeability

Liquid permeation is the flow of solvent through a fixed matrix. For a laminar flow through a homogenous fixed matrix, the liquid flux, v, obeys Darcy's law. In one direction, this flow is given as follow.

$$v = \frac{-B}{\eta} \nabla P \qquad \text{(Annex 1 –1)}$$

Where v is the liquid flux [volume flow rate/ cross-sectional area (m/s)], B is the permeability coefficient (m^2), η is the dynamic viscosity of the flowing liquid (Pa.s) and ∇P is the pressure gradient over the fixed matrix (Pa/m).

1-1 Methods

Gel permeability coefficient (B) could be determined by the technique of van Dijik and Walstra (1986). Renneted samples were made in glass tubes with an inner diameter of 3 mm and a length of 25 cm, which were open at both ends (Figure 1-1). After allowing gelation to proceed within the tubes (for ~ 16 hours), they were withdrawn and submerged in a whey bath, so that the resulting hydrostatic pressure ($\triangle P$) would cause the whey to permeate upward through the gel in the tube: $\Delta P = \rho g h_0$ at a level h_0 below the whey surface (Figure 1-2). The rate of permeation of whey was used as a measure of gel permeability (Roefs *et al.*, 1990). Measurements were taken from a microscope-recorder (Figure 1-3). Figure 1-4 describes the permeability measurement schematically and B is given by:

$$B = \frac{-In(\frac{h_0 - h_{t2}}{h_0 - h_{t1}}).\eta.h_1}{\rho.g.(t_2 - t_1)} \qquad \text{(A1-2)}$$

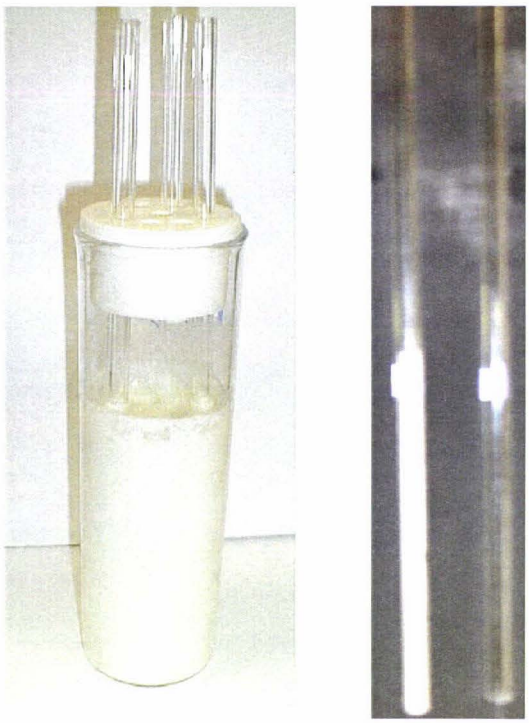

Figure 1-1 Right: glass cylinder for gel formation. Left: glass tubes open at both ends with a length of 25 cm and an inner-diameter of 3 mm.

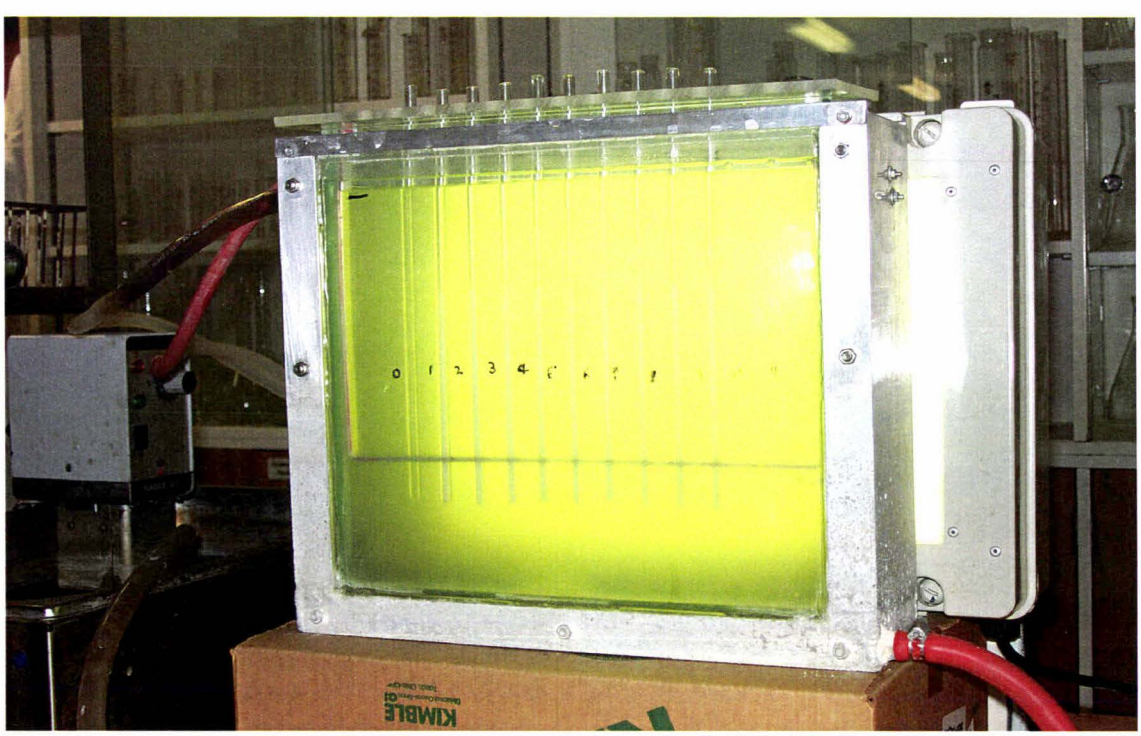

Figure 1-2 Illustration of the thermostated (30°C) transparent glass tank with whey and tubes of rennet-induced skim milk gels, with a luminated light board at the back

Figure 1-3 Illustration of the recording machine

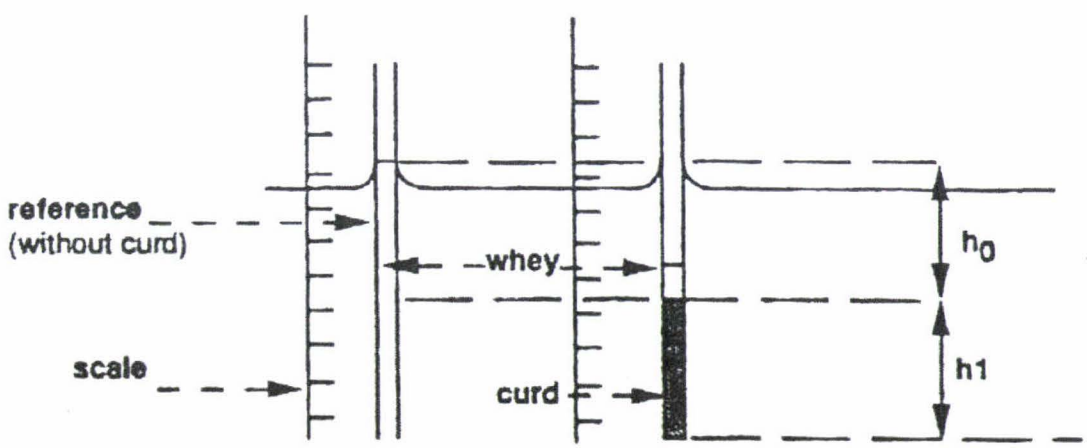

Figure 1-4 Schematic diagram of permeability measurement (Lagoueyte *et al.*, 1994)

Where B is the permeability coefficient [m^2], h_0 is the height of the whey in the reference tube h_t is the height of serum level in the gel tube [m] at time t, h_1 is the length of the gel [m], g is acceleration due to gravity [ms^{-1}], ρ is the density of the whey [kg/m^3], and t_1 and t_2 represents the start and end time of the measurement respectively.

1-2 Discussion

Gel permeability coefficient (B) served as a good representation for the measure of the number and the size of the largest 'capillaries' (pores) present in a gel. A typical discussion for a high B generally implied that large holes had been formed in the network. High B usually arisen from a 'loose' gel structure (van Dijik and Walstra, 1986; Roefs *et al.*, 1990).

To measure the permeability of the gel, it was necessary to ensure that the gel did not shrink during the experiment. Hence, before permeability measurements, it was important to select glass tubes with gel visually showing no spaces void of gel in between. Any 'openings' in between the gel and the glass tube would lead to a false analysis.

The mean value of B obtained for rennet-induced skim milk without polysaccharides addition was 1.3 x 10^{-12} m^2. Further investigative work on renneted skim milk after the addition of polysaccharides could not be carried out. This was because upon the addition of polysaccharides like κ-carrageenan, xanthan and guar gum, the formation of a shrunk "spaghetti-like" gel was observed (Figure 1-5). This phenomenon had made permeability measurements impossible. Several attempts, like longer incubation time (from 3 hours to 16 hours), were tried but to no avail.

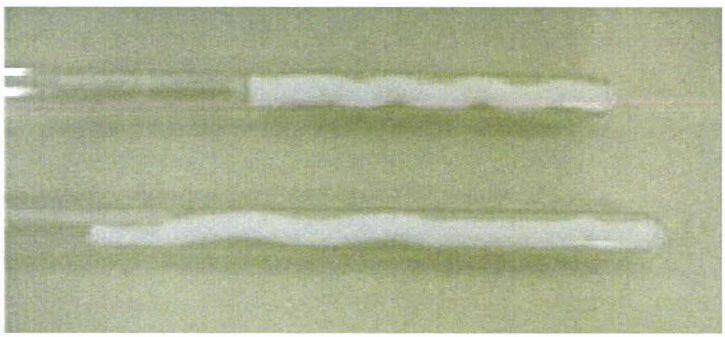

Figure 1-5 Permeability tubes containing renneted skim milk with 0.1 wt% κ-carrageenan after 16 hours incubation at 30°C

It could therefore be concluded that gel permeability measurements, as described by Lagoueyte *et al.* (1994), were not suitable for renneted skim milk containing polysaccharide, like κ-carrageenan, xanthan and guar. This was mainly due to the weak nature of the gel formed.

Annex 2- Properties of rennet-induced skim milk (control)

Studies on the rheological effect of adding polysaccharides in rennet skim milk gel required the properties of rennet-induced skim milk. This section compares the rheological properties of the properties of rennet-induced skim milk under the normal condition (model system) to rheological properties of rennet-induced skim milk under cheesemaking conditions.

Rheological measurements of rennet-induced skim milk under model system were described in Chapter 3 and the experimental conditions of rennet-induced skim milk under cheesemaking conditions were presented in Chapter 7.

2-1 Effect of reconstituted skim milk preparation method

The total milk-solids content of the rennet-induced skim milk was 10 wt%, obtained by a 1:1 dilution (from 20 wt%) with milliQ water, as described in Chapter 3. This exclusive manner of milk prepared for renneting does not appear to have been cited in any related area of research. However, this preparation method was deemed compulsory for current investigation to allow for standardised comparison as well as incorporation of the polysaccharides (1 wt% stock solution).

Two different skim milk reconstitution methods and their effect on the rheological properties of rennet-induced skim milk were investigated. Figure 2-1 presents G^* as a function of time of renneted skim milk prepared from undiluted (20 wt%) and diluted (10 wt%) reconstituted skim milk stored overnight at 4°C, 20°C and 30°C. Rheological results showed that for all storage temperatures, renneted skim milk from undiluted reconstituted skim milk had higher G^* than renneted skim milk from diluted reconstituted skim milk. In addition, higher G^* were obtained for renneted skim milk samples at a higher overnight-storage temperature (30°C versus 4°C).

Table 2-1 tabulates the gelation times of rennet-induced skim milk from undiluted and diluted reconstituted skim milk. The gelation time for the control model system used in current investigation was 41 minutes. Similar to G^* results, gelation time was faster for renneted skim milk prepared from undiluted reconstituted skim milk. It was also evident that gelation time had decreased with increasing storage temperature. This could be explained by the changes in the physico-chemical properties of milk due to cold storage, which was known to prolong coagulation time, lower curd firmness and cheese yield (Ali *et al.*, 1980; Van Hooydonk *et al*, 1986).

In addition, despite standardized experimental methods, the G^* of renneted skim milk from diluted reconstituted skim milk was lower than sample from undiluted reconstituted skim milk. Zoon and co-workers (1988a) had discussed that although reconstituted milk always had the same composition, its gelling behaviour could be different if not prepared in a standardized way.

2-2 Effect of pH, salt addition and rennet concentrations

Two sets of (1:10 diluted) rennet concentrations, 80 μL/ 100 g (similar to cheesemaking rennet concentration) and 200 μL/ 100 g (normal experimental concentration) for renneted skim milk prepared under normal experimental conditions, and two renneting temperatures, 30°C and 32°C for renneted skim milk prepared were investigated.

Figure 2-2 displays the complex modulus (G^*) as a function of time for rennet-induced skim milk at four different experimental conditions. It was obvious that G^* of renneted skim milk under cheesemaking conditions were higher than renneted skim milk from normal experimental conditions. This was caused by calcium chloride ($CaCl_2$) addition, pH lowering and renneting temperature which were known to influence the rheological properties (Zoon *et al.*, 1988a, b, c and 1989a). Cheese milk was usually enriched with $CaCl_2$ to accelerate the renneting process. This acceleration was due to the combined effect of the increased calcium concentration and a drop of pH (van Hooydonk *et al.*, 1986).

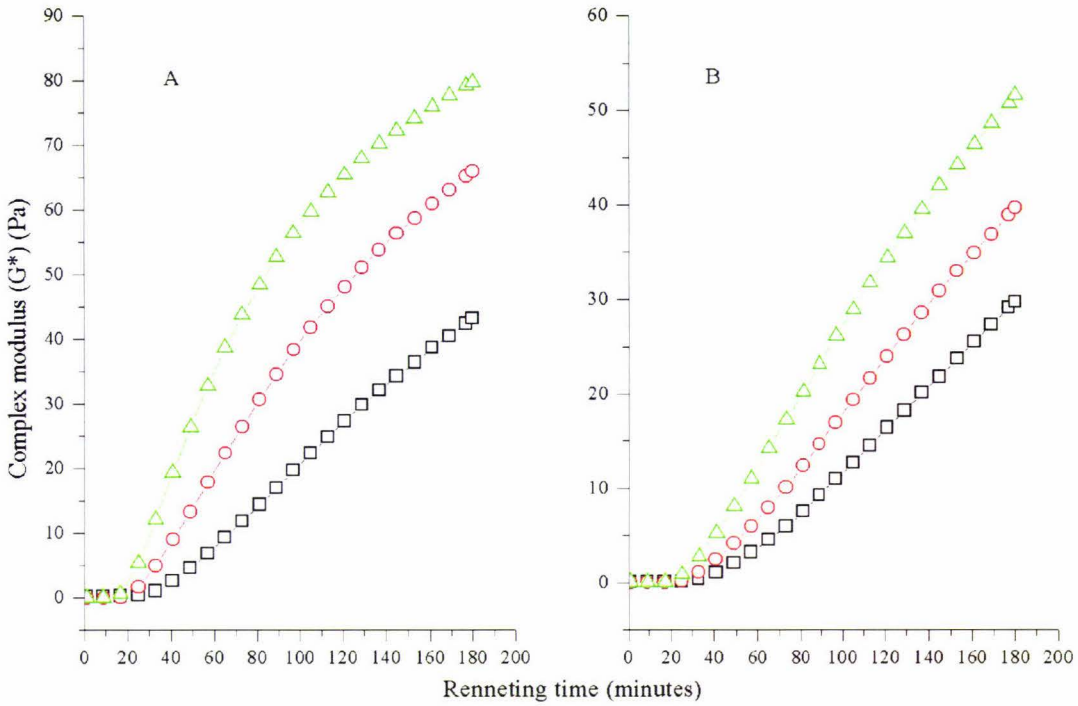

Figure 2-1 Complex modulus, *G of renneted skim milk as a function of renneting time at 30°C. Graph A showed samples from "*undiluted*" 10 wt% reconstituted skim milk and Graph B showed samples from '*diluted*' (from 20 wt%) 10 wt% reconstituted skim milk. All reconstituted solutions were stored overnight at 4°C (□), 20°C (○) and 30°C (△).**

Table 2-1 Gelation times of rennet-induced skim milk, using undiluted and diluted 10 wt% reconstituted skim milk

Storage temperature (°C)	Gelation times (mins) of rennet-induced skim milk	
	Undiluted	Diluted 1:1 dilution (from 20wt%)
4	33	41
20	23	32
30	18	26

The addition or removal of calcium would not influence the enzymic reaction if the pH were kept constant. In addition, Zoon *et al.* (1988c) noted that calcium ion activity influenced the clotting time and the moduli.

A lower pH in rennet-induced skim milk accompanied a faster rearrangement of strands and fusion of micelles, resulting in a faster increase of the moduli directly after the onset of gelation and the earlier attainment of a plateau value of the modulus (Zoon *et al.*, 1989a). Van Hooydonk *et al.* (1986) and Zoon *et al.* (1988b) showed that the dynamic moduli increased with temperature ($\leq 40^{\circ}C$). Thus, the addition of calcium chloride (0.1 wt% $CaCl_2$) as well as the reduction of pH (from 6.7 to 6.2) of skim milk had helped tremendously in improving its viscoelastic properties.

In addition to the effects of salt addition and pH, the effect of rennet concentration was also considered. It was found (Figure 2-3A) that the increase of rennet concentration did increase G^* of renneted skim milks made under normal condition (no salt and neutral pH). However, unlike samples from the model system, G^* of renneted skim milk under cheesemaking conditions had not increased (Figure 2-3A) with increasing rennet concentration. However, unlike samples from the model system, G^* of renneted skim milk under cheesemaking conditions had not increased with increasing rennet concentration (Figure 2-3B).

Zoon *et al.* (1988a) and van Hooydonk and van den Berg (1988) reported that at a higher rennet concentration, rennet-induced skim milk was formed sooner after rennet addition and that the increase of G' as a function of time was faster. Van Hooydonk and van den Berg (1988) had suggested that the reason for the increase was that at a higher rennet concentration, more rapid aggregation took place, leading to a coarser network with fewer junctions but with more bonds per junction.

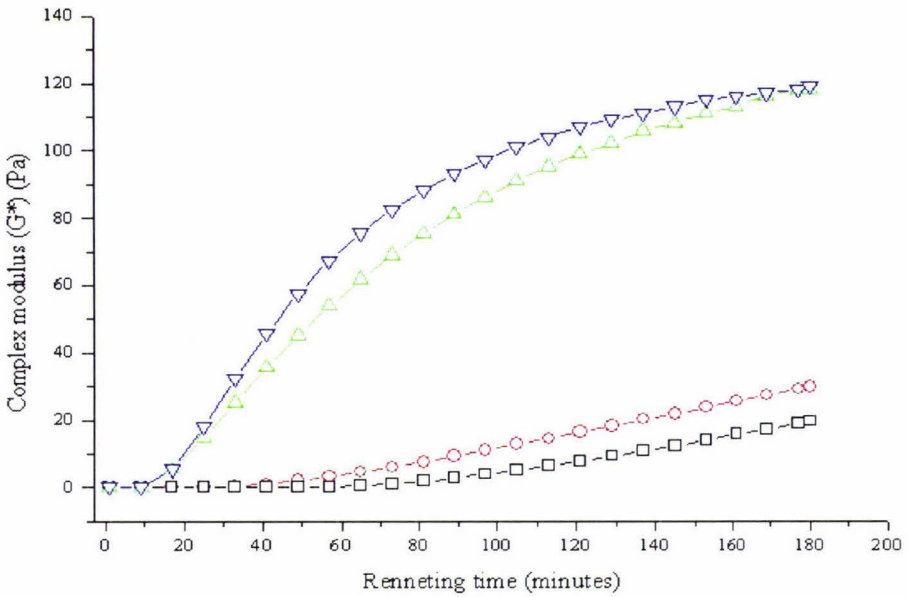

Figure 2-2: Model system: G^* of renneted skim milk at 80 µL (□) and 200 µL (○) of rennet in 100g sample. The temperature was held at 30°C throughout the 3-hour measuring period. Cheesemaking condition: G^* of renneted skim milk at renneting temperature of 30°C (△) and 32°C (▽). Rennet was used at 80µL/100g.

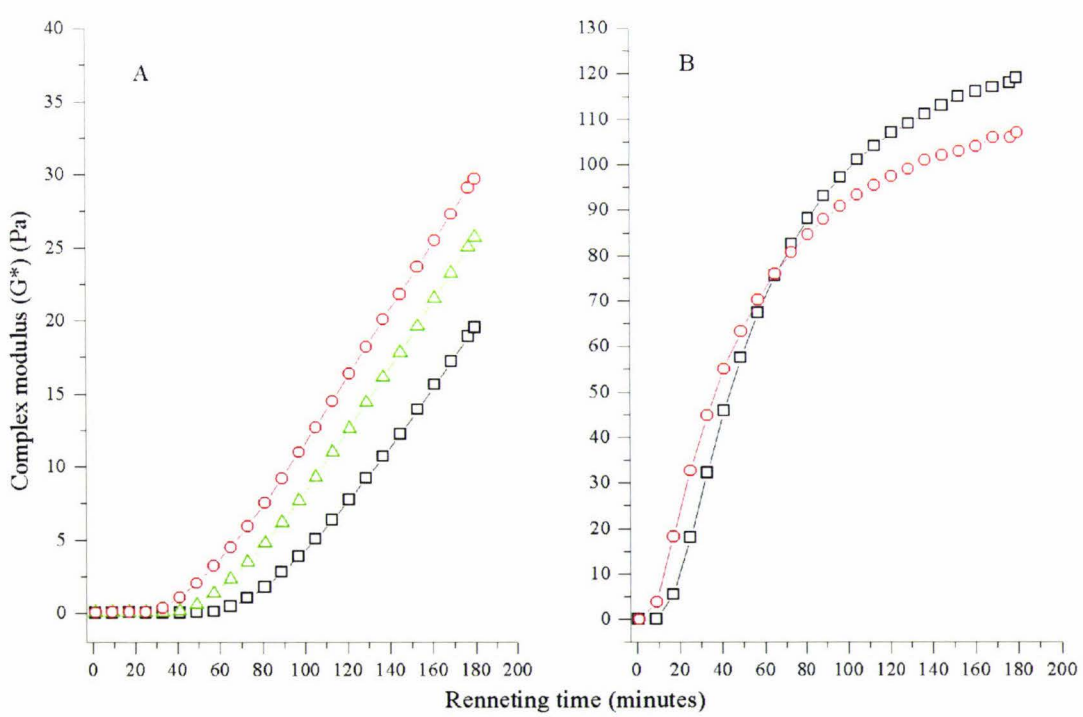

Figure 2-3: G^* of renneted skim milk under normal condition (A) and cheesemaking condition (B) as a function of renneting time at 30°C. Rennet concentration was 80µL (□), 120µL (△) and 200µL (○) in 100g sample.

Annex 3 - pH of model renneted skim milk/biopolymer

The pH remains to be an important factor in the manufacture of several types of dairy products such as yoghurt, quarg and cheese. pH is an important cheesemaking parameter, affecting for instance, the rate of renneting and syneresis (Walstra and van Vliet, 1986). The objective of this section was to monitor the change in pH of model renneted skim milk containing biopolymer.

The pH of the samples was measured at 30°C using the Radiometer model PHM 84 Research pH meter (Copenhagen, Denmark). Before measurement, the pH meter was calibrated at 21°C using Radiometer Analytical IUPAC Standard pH 4.005 and 7.005 ± 0.010, France.

In order to observe if there would be any significant pH changes over the 16-hour incubation period at 30°C, four pH measurements were conducted. The pH readings were recorded for each sample before rennet addition and after the 16-hour incubation. Three pH measurements were taken on renneted skim milk containing biopolymer, namely on the gel (curd) formed, on the whey expelled and on their mixture (mixture of the whey expelled and the curd). Triplicate measurements were performed.

Table 3-1 reports the pH measurements of renneted skim milk/biopolymer mixtures.
Table 3-1 shows that pH values of the skim milk/biopolymer mixtures before rennet addition ranged from 6.64 to 6.71. For all the four polysaccharides and gelatin investigated in this study, changes in pH observed after rennet addition and 16 hours incubation for the gel formed, the whey expelled and their mixtures were minimal.

Table 3-1 Tabulation of pH measurements for rennet-induced skim milk, before and after rennet addition. Measurements of samples taken after rennet addition were incubated at 30°C for 16 hours.

Biopolymers	Concentration (wt%)	Rennet addition			
		Before	After (16 hours incubation at 30°C)		
			Gel	Whey	Mixture (gel and whey)
Kappa carrageenan	0	6.66	6.66	6.66	6.65
	0.025	6.65	6.66	6.65	6.65
	0.050	6.66	6.66	6.66	6.66
	0.075	6.65	6.67	6.65	6.67
	0.100	6.65	6.68	6.66	6.68
Xanthan gum	0	6.68	6.68	6.69	6.68
	0.025	6.69	6.68	6.68	6.67
	0.050	6.70	6.68	6.67	6.67
	0.075	6.70	6.68	6.68	6.68
	0.100	6.69	6.68	6.68	6.68
Guar gum	0	6.71	6.67	6.68	6.67
	0.025	6.66	6.67	6.66	6.67
	0.050	6.69	6.68	6.68	6.67
	0.075	6.68	6.68	6.67	6.68
	0.100	6.68	6.69	6.68	6.68
High methoxyl (HM) Pectin	0	6.68	6.67	6.67	6.67
	0.025	6.66	6.63	6.63	6.62
	0.050	6.65	6.65	6.64	6.64
	0.075	6.66	6.66	6.65	6.66
	0.100	6.65	6.62	6.61	6.62
Gelatin	0	6.66	6.67	6.65	6.66
	0.025	6.65	6.63	6.62	6.61
	0.050	6.65	6.66	6.65	6.66
	0.075	6.65	6.64	6.64	6.67
	0.100	6.64	6.67	6.66	6.66