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Formation and physical properties of acid milk gels: a review

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Acidified milk products like yoghurt are an important food product but there are relatively few reports on the mechanisms involved in gel formation and the effects of processing variables such as heat treatment and gelation temperature on the important physical properties (such as whey separation) of acid-induced gels. Most previous reviews have described the microbiology of the starter cultures and technologies used in yoghurt manufacture. Recent developments are reviewed including the use of techniques such as dynamic low amplitude oscillatory rheology to monitor the gel formation process; confocal scanning laser microscopy to examine gel microstructure; and various models for the aggregation of particle gels are discussed in terms of possible mechanisms involved in the formation of acid-induced milk gels. © 1998 Canadian Institute of Food Science and Technology. Published by Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Fermented milk products are produced throughout the world with yoghurt (or yogurt) being the most popular. The worldwide production of fermented milk products probably exceeds 20 million tonnes (mton) and the production of related milk products such as fresh acid cheese is ~3 mton (IDF, 1995). The popularity of fermented milks is due at least in part to various health claims and therapeutic benefits that have been associated with some of these products. This review concentrates on the properties of (plain) set-style acid milk gels, i.e. gels that are formed (undisturbed) in the retail pot. Stirred-type yoghurt is made by breaking a set gel before mixing with fruit and filling into retail containers; it is likely that many of the properties of the resultant 'semi-viscous liquid' are influenced by the original properties of the undisturbed gel. The microbiology of the starter culture used as well as the technologies involved in yoghurt manufacture were reviewed extensively (e.g. Robinson and Tamime, 1993; Tamime and Marshall, 1997). In spite of the importance of acidified milk products, much less is known about the formation and physical properties of acid-induced gels. For example, Holt and Horne (1996) recently stated that "unfortunately, in spite of much effort, the mechanism of acid-induced gelation is still largely unknown". Although the formation of acid-induced gels is a complex subject, the objective of this review is to summarize recent developments which have greatly improved our understanding of the factors and mechanisms involved in the formation and properties of acid milk gels. In this regard, techniques such as dynamic low amplitude oscillatory rheology, confocal scanning laser microscopy (CSLM), the concepts of fractal aggregation theory as applied to aggregation of casein particles and the use of glucono-δ-lactone (GDL) in model acidification studies are described.

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FORMATION AND STRUCTURE OF ACID MILK GELS

Method of acidification of milk

Milk can be acidified by bacterial cultures, which ferment lactose to lactic acid, by the addition of chemical acids such as HCl, or by the use of GDL where the

hydrolysis of GDL to gluconic acid results in a reduction in pH. An extensive study of the formation and rheological properties of acid gels formed by cold acidification of milk and subsequent heating to the gelation temperature was reported (Roefs, 1986; Roefs *et al.*, 1990; Roefs and van Vliet, 1990). Most studies on the formation of acid gels have been with GDL-induced gels. The rate of acidification is different between milk acidified with GDL and bacterial cultures; GDL is rapidly hydrolysed to gluconic acid (especially at high temperatures) whereas after the addition of starter bacteria the pH does not usually change very much initially. The final pH that is attained in GDL-induced gels is a function of the amount initially added to milk, whereas starter bacteria can continue to produce acid until a very low pH (e.g. <4.0) is attained when bacteria become inhibited by the low pH; in practice bacterial gels are cooled when sufficient acidity has been attained. The rate of pH change during fermentation or addition of acid is controlled by the acid-base buffering properties of milk (see review by Singh *et al.*, 1997). Some preliminary work has indicated that the rheological and physical properties of gels made with GDL differs from fermentation derived gels particularly at high gelation temperatures (Lucey *et al.*, unpublished results).

Effect of acidification on the properties of casein micelles

Caseins constitute approximately 80% of the protein in bovine milk, with four main types (α_{s1}-, α_{s2}-, β-, and κ-caseins) in combination with appreciable quantities of micellar or colloidal calcium phosphate (CCP) in the form of casein aggregates called casein micelles. At least two models for the structure of casein micelles were proposed; one suggestion is that the micelle core is divided into discrete subunits (submicelles) with a distinctly different character from an outside hairy layer (Schmidt, 1982, Walstra, 1990); while the other model suggests that the internal substructure resembles a mineralized, entangled or cross-linked web of chains of casein molecules (Holt, 1992) or is an aggregate of individual casein molecules with α_{s1}-casein providing a skeleton or backbone (Visser, 1992). Originally, it was assumed that submicelles were kept together ('cemented') in the micelle by bridges of CCP (Schmidt, 1982) but it is now known that CCP does not have an exclusive role in maintaining the integrity of the micelle (Holt, 1992; Visser, 1992). Hydrophobic and hydrogen bonding are important for micelle integrity since the addition of urea disrupts the micelle structure (McGann and Fox, 1974); CCP is important since its removal also disrupts the micelle (McGann and Pyne, 1960); while Ca^{2+} also plays a role in the integrity of the micelle (Holt *et al.*, 1986).

During acidification of milk, many of the physicochemical properties of casein micelles undergo considerable change, especially in the pH range 5.5 to 5.0, including a voluminosity maximum (of the sedimentable caseins) and dissociation of the caseins (Roefs *et al.*, 1985; Walstra, 1990). As the pH of unheated milk is reduced, CCP is dissolved (Pyne and McGann, 1960) and the caseins are liberated into the milk serum phase (Roefs *et al.*, 1985; Dalgleish and Law, 1988). The extent of liberation of caseins is dependent on temperature; at 30°C, a decrease in pH causes virtually no liberation; at 4°C about 40% of the caseins are liberated in the serum at pH ~5.5 (Dalgleish and Law, 1988). Aggregation of casein occurs as the isoelectric point (pH 4.6) is approached. Apparently little change in the average hydrodynamic diameter of casein micelles occurs during acidification of (unheated) milk to pH ~5.0 (Roefs *et al.*, 1985). Vreeman *et al.* (1989) concluded that the lack of change in the size of micelles on reducing the pH of milk to 5.5 may be a result of concomitant swelling of the particles as CCP was solubilized.

Virtually all milks used in yoghurt manufacture are subjected to an extensive heat treatment. Heating of milk can lead to many changes including denaturation of whey proteins and their interaction with casein micelles, changes in salt equilibria, inactivation of enzymes, destruction of heat-labile vitamins, reduction in the redox potential as well as non-enzymatic browning and Maillard reactions (Singh and Creamer, 1992; Mulvihill and Grufferty, 1995). Heat treatment of milk above 70°C causes denaturation of whey proteins, some of which associate with casein micelles, involving κ-casein, via hydrophobic interactions and the formation of intermolecular disulphide bonds (Haque and Kinsella, 1988; Singh, 1995). Moderate heating does not appear to affect the sizes of casein micelles although these treatments cause the whey proteins to denature and bind to micellar κ-casein; more extensive heat treatment causes some degree of micellar aggregation and an increase in particle size (Dalgleish *et al.*, 1987). Heat treatment of milk has a profound effect on the formation and properties of acid gels and this is described in detail in a later section.

The effect of heat treatment on the solubilization of CCP and the release of caseins during acidification of milk was studied recently (Law, 1996; Singh *et al.*, 1996). In general, heat treatment had little effect on the extent of solubilization of calcium and Pi from the micelles (Law, 1996; Singh *et al.*, 1996). When acidification was carried out at 5°C more caseins were dissociated from the micelles in heated milks than in unheated milks; the reverse occurred when milks were acidified at 22°C (Singh *et al.*, 1996).

It was suggested that the yoghurt gel network is formed from micelle-like particles whose internal and surface arrangements could be largely unchanged from the original micelles in terms of the location of the individual caseins (Holt and Horne, 1996). However, during acidification of milk to pH values ≥ 5.3, most of

the CCP in the micelles was solubilized, the charge on individual caseins was altered and the ionic strength of the solution increased. As a result, the forces responsible for the integrity of these 'micelle-like' CCP-depleted casein particles are considerably different from native micelles even if their average hydrodynamic diameter appears largely unchanged. Hydrophobic interactions are also important for the stability of these casein particles as evidenced by the temperature-dependence of the dissociation of caseins. Thus, the character of the casein particles that aggregate to form an acid-induced gel would appear to be different from native casein micelles.

Rheological properties of acid milk gels

Most studies of the textural properties of set-style yoghurt gels were measured empirically as firmness or viscosity (Dannenberg and Kessler, 1988*b*). The relevance of viscosity measurements in a set gel is unclear, although it is important in stirred-style yoghurts. It is well accepted that denaturation of whey proteins increases the firmness and viscosity of yoghurt (Parnell-Clunies *et al.*, 1986; Dannenberg and Kessler, 1988*b*). Most rheological parameters characterizing casein gels (e.g. the dynamic moduli) 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 (Roefs *et al.*, 1990).

Dynamic testing, which involves an oscillatory applied strain or stress, can provide very useful information on the gel formation process. In the strain controlled version of this experiment, the sample is subjected to a sinusoidally oscillating strain. This generates a sinusoidally oscillating stress response, which is, to some extent, out of phase with the strain. The amplitude of this stress, and the difference in phase between it and the strain, are measured, and the strain is resolved into one component (elastic response), completely in phase with the strain, and another (viscous response) 90° out of phase (Ferry, 1980). Some of the main parameters that are usually determined from these responses include the elastic or storage modulus (G′), which is a measure of the energy stored per oscillation cycle, the viscous or loss modulus (G″), which is a measure of the energy dissipated as heat per cycle, and the loss tangent (tan δ), which is the ratio of the viscous to elastic properties. These parameters are defined as follows (Vélez-Ruiz and Barbosa Cánovas, 1997):

$$G' = (\tau_0/\gamma_0) \cos \delta \quad (1)$$

$$G'' = (\tau_0/\gamma_0) \sin \delta \quad (2)$$

$$\tan \delta = G''/G' \quad (3)$$

where τ_0 is the amplitude of the shear stress, γ_0 is the amplitude of the strain and δ is the phase angle.

The rheological properties of acid gels made from heated and unheated milk, at two gelation temperatures, are shown in Fig. 1. Unheated milks form weak gels and the pH at gelation is generally ~4.8 (Lucey *et al.*, 1997*a*). After gelation, G′ increases rapidly but starts to plateau during ageing of the gel; tan δ decreased to < 0.4 soon after gelation and decreased to ~0.25 during ageing of the gel. If the pH of gel decreases to very low values (e.g. ≤4.0) G′ may start to decrease (Lucey *et al.*, unpublished results). Roefs (1986) demonstrated that for acid gels made by cold acidification and quiescent heating, G′ continues to increase for periods of up to several days, due presumably to slow ongoing fusion of casein particles.

There have been few reports on the effects of heat treatment on the rheological properties of acid gels determined by dynamic low amplitude oscillation (van Vliet and Keetels, 1995; Lucey *et al.*, 1997*a*). van Vliet and Keetels (1995) reported that acid skim milk gels made from reconstituted low heat skim milk powder (SMP) had much lower dynamic moduli than gels made from high heat SMP. Lucey *et al.* (1997*a*) studied the effects of heat treatment of milk on the rheological properties at small and large deformations of acid skim milk gels made at 30°C with GDL. Heating milks at temperatures ≥80°C greatly increased the G′ compared to unheated milk (~15 Pa) and produced gels with G′ in the range 350 to 450 Pa. In gels, the resistance against

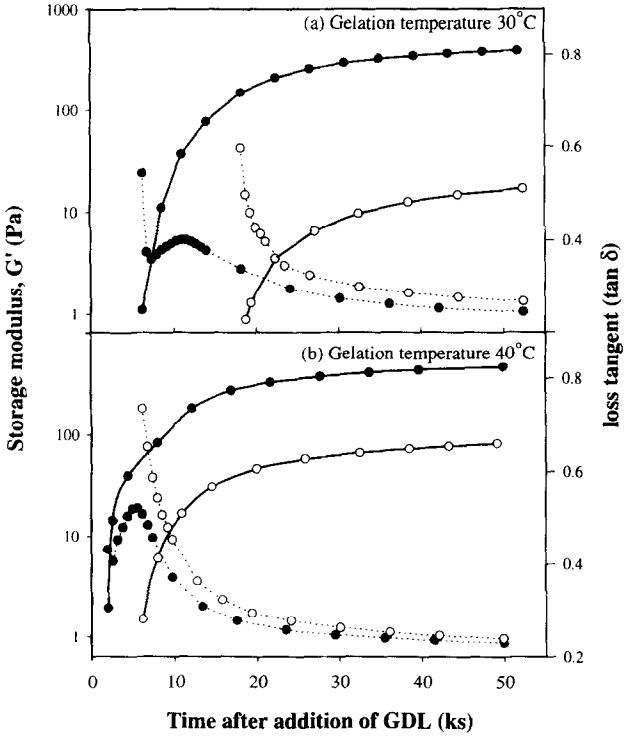


Fig. 1. Effects of heat treatment and gelation temperature on the rheological properties of acid milk gels made by acidification with 1·3% (w/w) glucono-δ-lactone (GDL). Gelation temperatures: (a) 30°C and (b) 40°C. (○) unheated milk and (●) milk heated at 80°C for 30 min prior to GDL addition. Storage modulus (G′) (–) and loss tangent (tan δ) (·····).

deformation is proportional to the number of contact points per cross-section of the network (van Vliet and Keetels, 1995). Cross-linking or bridging, possibly by denatured whey proteins, within gels made from heated milk could be responsible for the increased rigidity and G′ of the network (Lucey *et al.*, 1997*a*,*b*, 1998*b*,*c*).

An unusual rheological phenomenon is observed soon after the formation of acid gels made from heated milk; tan δ initially decreases but then increases to a maximum value before decreasing again (Fig. 1) (Biliaderis *et al.*, 1992; Rönnegård and Dejmek, 1993; van Marle and Zoon, 1995). A high tan δ may increase the susceptibility of bonds and strands in the gel to break or relax, thus facilitating more rearrangements of the gel (van Vliet *et al.*, 1991).

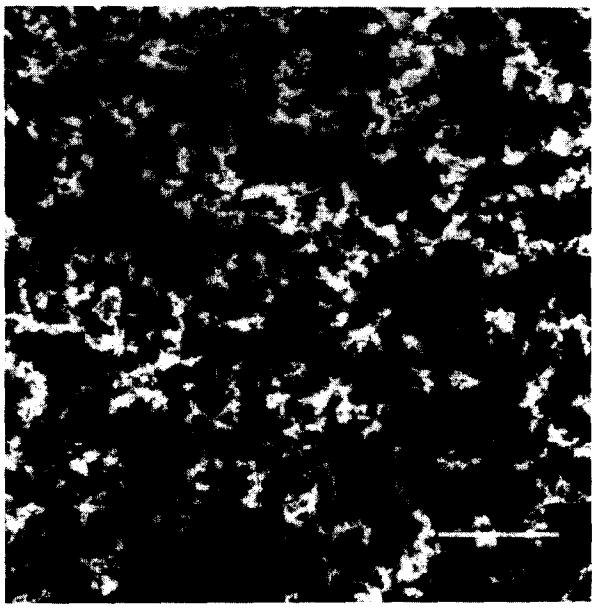
In experiments where the timescale of the applied deformation was varied (frequency sweeps) log G′ versus log angular frequency (i.e. the mechanical spectrum) gave linear curves with a slope of ~0.15, for various types of acid casein gels (Roefs and van Vliet, 1990; Lucey *et al.*, 1997*a*,*b*). Heat treatment of milk had little effect on the slope of the log G′ versus log angular frequency curves (Lucey *et al.*, 1997*a*). In acid milk gels, the value of G′ is always substantially higher than that of G″ for all frequencies. Mechanical spectra are useful for classifying a given system, e.g. into a weak or strong gel or an entangled polymer solution (Ross-Murphy, 1995).

Large deformation studies or destructive tests provide information on properties that may be related to the consistency of the gel during consumption. Large deformation studies also provide information on the resistance of the gel to processes such as stirring, pumping and other shearing operations, which are used in the production of stirred-style yoghurt. There is little information on the large scale deformation properties of yoghurts (Rönnegård and Dejmek, 1993). An apparent 'yield stress' of stirred yoghurt was estimated from experiments where the shear rate is varied (flow curves), e.g., by applying the Casson (Parnell-Clunies *et al.*, 1986) or Hershel-Buckley models (Ramaswamy and Basak, 1991), although this approach is of little relevance in set gels. Mixing and stirring of set gels prior to rheological testing means that reported (Dannenberg and Kessler, 1988*b*) yield properties are not those of the original 'set' gel. Large deformation fundamental rheological properties of acid casein gels were reported (Bremer *et al.*, 1990; van Vliet *et al.*, 1991; van Vliet and Keetels, 1995; Lucey *et al.*, 1997*a*,*b*). Gross fracture of acid casein gels, made with GDL, in creep experiments was observed at a strain of 0.5 to 0.6 (Roefs, 1986; van Vliet *et al.*, 1991). van Marle and Zoon (1995) reported in strain sweep experiments, with both GDL and fermentation derived acid gels, that with strains above ~0.45, the value of the dynamic moduli decreased sharply, implying fracture of the network. Lucey *et al.* (1997*b*), using a low constant shear rate technique for acid casein gels made *in situ* found that the shear stress at fracture increased with decreasing gelation temperature. The shear stress at fracture of acid casein gels increased with ageing while the strain at fracture decreased somewhat (Lucey *et al.*, 1997*b*). Lucey *et al.* (1997*a*) using the low constant shear technique reported that heat treatment of milk prior to acidification resulted in a large reduction in the strain at fracture, from ~1.5 in gels made from unheated milk to 0.5 to 0.8 for gels made from milks heated at temperatures ≥80°C. Lucey *et al.* (1997*a*) found that the shear stress at fracture decreased when milk was heated at 75°C; heating at higher temperatures resulted in an increase and maximum was observed when milk was heated at 85°C for 15 min after which the shear stress (at fracture) decreased to values similar to that of unheated reconstituted milk (made from ultra-low heat SMP).

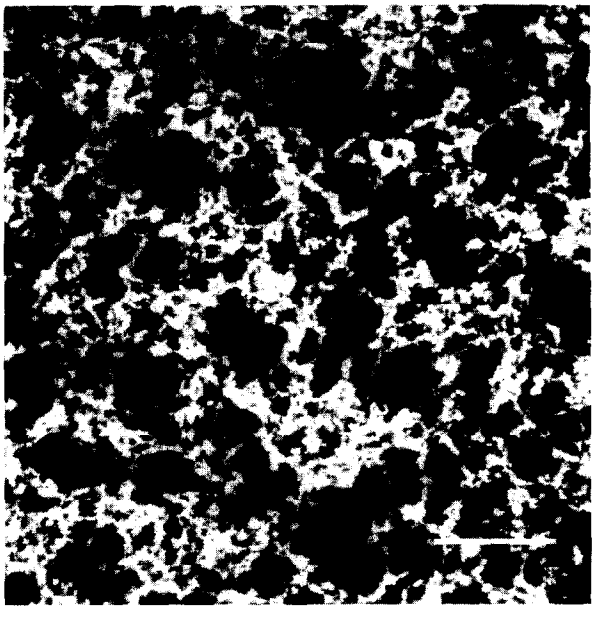
Microstructure of acid gels

Electron microscopy (EM) studies on acid gels have demonstrated that these gels consist of a coarse particulate network of casein particles linked together in clusters, chains and strands (Kalab *et al.*, 1983). The network has pores or void spaces where the aqueous phase is confined. The diameter of these pores may vary from 1–30 μm, with larger pores in gels made at high gelation temperatures and from milks with a low protein content. There were many EM studies on the microstructure of gels formed by acidification of heated milk (Davies *et al.*, 1978; Parnell-Clunies *et al.*, 1987; Mottar *et al.*, 1989). Harwalkar and Kalab (1980) proposed, from the examination of electron micrographs, that yoghurt gels made from unheated milk had larger protein clusters than gels made from heated milk, which were described as highly branched.

Many of the preparation steps required in EM, including dehydration, fixation, embedding, sectioning, and staining, can disrupt the native structure of milk products and lead to the creation of artifacts, unless great care is taken. Confocal laser scanning microscopy (CSLM) enables samples to be observed with minimal preparation procedures because of its unique optical sectioning capabilities and high spatial resolution (Brooker, 1995) and is very suitable for observing the overall microstructure of milk gels but not the finer details or internal structure of features such as casein micelles or protein clusters (Hassan *et al.*, 1995; Lucey *et al.*, 1997*c*, 1998*c*; van Marle, 1998). Confocal micrographs of acid gels made from unheated or heated milk are shown in Fig. 2. Lucey *et al.* (1998*c*) found using CSLM that heating milks at temperatures ≥80°C resulted in a microstructure of GDL-induced acid gels that appeared 'branched' and had a higher 'apparent interconnectivity' (in the thin optical section of the *x*-*y* plane) of aggregates compared to unheated or less severely heated milks, which had tortuous, bent or irre-



(a)



(b)

Fig. 2. Confocal scanning laser micrographs of acid milk gels made at 30°C by acidification with 1·3% (w/w) glucono-δ-lactone (GDL) of (a) unheated milk and (b) heated milk. Milk was heated at 85°C for 30 min prior to GDL addition. Scale bar = 20 μm. The protein matrix appears white while pores appear dark. Figures reproduced from *Food Hydrocolloids* (Lucey *et al.*, 1998*c*) with kind permission from Oxford University Press, Oxford, OX2 6DP, UK.

gular clusters and strands making up the gel network and less 'apparent interconnectivity' of strands and clusters. There did not appear to be major differences in the microstructure of acid milk gels formed from milk heated in the range 80 to 90°C. van Vliet and Keetels (1995) suggested heat treatment of milk resulted in 'straightening' of the strands in the acid gel network compared to unheated milk, which may have tortuous strands.

Many of the preparation steps used in EM of whole milk yoghurt can result in partial extraction or alterations to fat globules, unless great care is taken (Allan-Wojtas and Kalab, 1984). Barrantes *et al.* (1996) reported that in yoghurt made from recombined milk, the fat globules were not noticeable using scanning EM but could be observed using transmission EM.

Permeability of acid milk gels

Permeability measurements give information about the inhomogenities at the level of the gel network (van Dijk and Walstra, 1986; Roefs *et al.*, 1990) and the permeability of acid gels can be calculated as follows:

$$B = -[\ln \frac{(h_\infty - h_{t_2})}{(h_\infty - h_{t_1})}]\eta H/[\rho\, g(t_2 - t_1)] \qquad (4)$$

where B is the permeability coefficient, h_∞ is the height of the whey in the reference tube, h_{t1} is the height of the whey in the gel tube at t_1, h_{t2} is the height of the whey in the gel tube at t_2, η is the viscosity of the whey, H is the length of the gel, ρ is the density of the whey and g is acceleration due to gravity. In acid gels, made at 30°C with GDL, the B is usually in the range $1\text{–}2\times10^{-13}\,m^2$ (Roefs *et al.*, 1990 Lucey *et al.*, 1998*c*). Acid gels made from Na caseinate at high gelation temperatures and with a low ionic strength had a very high B (Lucey *et al.*, 1997*c*). Bacterial fermentation of milk with 'ropy' cultures produced gels with a slightly lower B than non-ropy strains (van Marle and Zoon, 1995). The permeability of acid milk gels is relatively unaffected by heating, which suggests that the number and size of the largest pores in gels made from unheated or heated milk are similar (van Vliet and Keetels, 1995; Lucey *et al.*, 1998*c*).

In rennet-induced milk gels B increases with time, which was taken as evidence of 'microsyneresis' or breakage and rearrangement of strands in the network causing the formation of larger pores and thus a higher B (Walstra, 1993). Studies on the B of acid-induced gels have concluded that B does not change with time (Roefs *et al.*, 1990; Lucey *et al.*, 1997*c*). However, in these studies the B was determined in aged gels or at least when the pH of the gel was ~4.6. In acid gels made from heated milk, there is other evidence that young gels are undergoing structural rearrangements since cracks can be seen on the surface of the gel and concomitantly whey (serum) is expelled and can be observed on the surface of the gel (Lucey *et al.*, 1998*a*). In acid casein gels, van Vliet *et al.* (1997) found that rearrangements could occur during or after gelation.

Mechanisms involved in the formation of acid-induced gels

Theoretical models

Acid milk gels like yoghurt are examples of particle gels. Fractal aggregation theory was applied to the formation

of various casein gels (Bremer *et al.*, 1989, 1990) and is described in the following section. In particle gels, a fractal scaling regime may occur only over small length scales, which are of the order of the aggregating clusters. At longer length scales, the microstructure appears homogeneous. Fractal behaviour is not usually expected in gels made from high volume fraction systems, i.e. $\phi > 0.3$ (Dickinson, 1997*a*). Fractal aggregation assumes that spherical particles of radius a can move by Brownian motion and that they can aggregate when they encounter each other. The aggregates (or clusters) formed then also aggregate with each other. These fractal clusters may be considered as the building blocks of the gel. If no further changes occur among the particles in an aggregate, once they are incorporated, this cluster–cluster aggregation process leads to aggregates obeying the scaling relation:

$$N_p = (R/a)^D \tag{5}$$

where N_p is the number of particles in an aggregate of radius R, and D is a constant called the fractal dimensionality. Since the number of sites for particles in a cluster N_S, is given by $(R/a)^3$, the volume fraction of particles in an aggregate, ϕ_A is:

$$\phi_A = \frac{N_p}{N_S} = \frac{(R/a)^D}{(R/a)^3} = (R/a)^{D-3} \tag{6}$$

Because D is invariably < 3, ϕ_A decreases as aggregation proceeds (R increases) and at a certain moment the average ϕ_A will equal the volume fraction of particles in the system ϕ, and all aggregates will touch, forming a continuous network; hence a gel is formed. The radius of the aggregates at the point of gel formation R_g is given by:

$$R_g = a\phi^{1/(D-3)} \tag{7}$$

The structure of a gel can be characterized by the parameters a, ϕ and D. Bijsterbosch *et al.* (1995) suggested that for gels that do possess some fractal character, the correlation length, which defines the length scale beyond which the structure starts to pass over gradually into the homogeneous regime as well as some other structural parameter related to the spatial distribution of voids (pores) within the network would be useful additional parameters for characterizing a gel. For casein gels (both rennet and acid), $D \approx 2.3$ was observed generally (Bremer *et al.*, 1989). However, in gels with a similar D, both a and ϕ can vary, and as a result the properties of the gel can be substantially different (Lucey *et al.*, 1997*c*). van Vliet and Keetels (1995) reported that D was similar in acid gels made from low and high heat treated milk. Bijsterbosch *et al.* (1995) used Brownian dynamic (computer) simulation to investigate the influence of different types of interparticle interactions on the fractal structure of particle gels and these models included the possibility of local restructuring of the aggregate, which may occur in practice during the formation of acid milk gels. The simple fractal approach (outlined earlier), although it has successfully described semi-quantitative features in irreversibly aggregating systems, does appear to have some deficiencies including the lack of any allowance for aggregate rearrangement (before, during and after gelation) and interpenetration, and the assumption that all aggregates have the same size at the gel point (Dickinson, 1997*a*). It was also suggested that in reality, in a fairly concentrated dispersion, when the biggest clusters are just beginning to join together to form a system-spanning network, a significant proportion of the particles could still exist as very small clusters and that these clusters may only get incorporated into the developing network at times well beyond the gel point (Dickinson, 1997*a*). It is possible that the slow increase in the G′ of acid gels after gelation reflects the incorporation of additional protein clusters in the gel network as well as the continued fusion and rearrangement of bonds and strands in the network. van Vliet *et al.* (1997) found that acid casein gels could undergo rearrangement at the particle (or cluster) level during or after gelation.

The aggregation of casein particles during the acidification of milk has also been modelled using the adhesive hard sphere theory (de Kruif *et al.*, 1995; de Kruif and Roefs, 1996; de Kruif, 1997). In this model it is proposed that the glycomacropeptide (GMP) part of κ-casein sterically stabilizes casein micelles and the GMP is considered as a polyelectrolyte brush which collapses on the surface of the micelle as the pH of the system approaches the pKa of the charged groups on the brush (lowered charge density).

Dickinson (1997*b*) has attempted to incorporate the concept of fractal aggregate formation into the sticky hard-sphere model, thereby including the simultaneous effects of strong bonding and weak reversible interactions into the same description of particle gelation.

Possible physico-chemical mechanisms involved in the formation of gels from unheated and heated milk

In milks of normal pH, the casein micelles are stabilized by hydration, negative charge and steric repulsion (Mulvihill and Grufferty, 1995). On acidification, casein particles aggregate as a result of charge neutralization, leading to the formation of chains and clusters that are linked together to form a three-dimensional network. Considerable changes occur in casein micelles during acidification such that the casein particles that form a gel at pH ~4.8 do not contain any CCP and have a different character to native casein micelles, as discussed earlier. Lucey *et al.* (1997*a*) suggested that gels made from unheated milks could undergo extensive particle rearrangements during the gel formation stage, resulting in the formation of dense clusters of aggregated casein

particles, which in turn aggregate to form a gel. From these clusters, many particles would hardly contribute to cross linking of the network, thus, unheated milk gels would have a low G′ value.

Acid gels made from heated milk have higher pH at gelation (Heertje *et al.*, 1995; Horne and Davidson, 1993) and produce considerably firmer gels than unheated milk (Lucey *et al.*, 1997*a*). Many of the effects of heat treatment on the microstructure of acid milk gels were explained by suggesting that aggregation of denatured whey proteins occurs during the acidification of heated milk (Lucey *et al.*, 1997*a*). High heat treatment of milk causes denaturation of whey proteins and subsequently a proportion of denatured whey proteins associates with the casein micelles, involving κ-casein (Singh, 1995). These whey proteins appear as appendages or filaments on the micellar surface in electron micrographs (Davies *et al.*, 1978; Kalab *et al.*, 1983; Mottar *et al.*, 1989). It is likely that when heated milk is acidified, the denatured whey proteins in the serum, as well as those associated with the casein micelles become susceptible to aggregation, as the net repulsive charge on the proteins is reduced. There are enhanced protein–protein interactions between denatured whey proteins near their isoelectric pHs caused by the heat-induced exposure of previously buried hydrophobic groups (Zhu and Damodaran, 1994). For example, the isoelectric pH of the major whey protein, β-lactoglobulin, is ~5.3 (Kinsella and Whitehead, 1989). This would explain the high pH of gelation of heated milk. Denatured whey proteins associated with casein micelles could act as bridging material by interacting with other denatured whey proteins associated with micelles. This is likely to increase the number and strength of bonds between protein particles. Moreover, the concentration of gelling protein would be increased because of active participation of denatured whey proteins in the gel structure. Both these factors could be responsible for the increased G′ of acid gels made from heated milks. This is likely to result in the formation of the 'branched' microstructure, which was observed in acid gels made in heated milks (Lucey *et al.*, 1998*c*). In addition, the presence of denatured whey proteins on the surface of casein particles would also hinder the close approach of other casein particles and lessen the likelihood that dense clusters of casein particles could be formed.

Other explanations were proposed to explain the increase in the pH of gelation in heated milks (Heertje *et al.*, 1985; Horne and Davidson, 1993). Heertje *et al.* (1985) proposed that the interaction of whey proteins with casein micelles on heating milk at its natural pH may increase the hydrophobicity of the micelle surface and decrease the hydration barrier against aggregation, or that on heating milk there may be an increase in the concentration of serum β-casein and a decrease in serum α_s-casein leading to a micellar network that is more sensitive to Ca^{2+}. Horne and Davidson (1993) speculated that the complexation of whey proteins with casein micelles (as a result of heating) diminishes the capacity of the κ-casein hairs to sterically stabilize the casein pseudo-micelle, allowing these particles to coagulate at a higher negative charge.

Heertje *et al.* (1985) proposed, from an electron microscopy study of acidified milk, that at pH ~5.5, some dissociation of casein occurred (mainly β-casein), but β-casein reassociated with micelle at pH ~5.2 which coincided with a 'stage of contraction and rearrangement' and at pH ~4.8 the 'final' gel network was formed. It should be noted that these microstructural changes were only observed in heated milk (90°C for 15 min), which is understandable since gelation in unheated milk occurs at pH ~4.8 while heated milks form gels at pH values > 5.0. Recent studies (Law, 1996; Singh *et al.*, 1996) have shown that at the temperatures (e.g. 30°C) used for the formation of acid gels from heated milk, no preferential dissociation of β-casein from the micelle occurs at pH values between 5.5 to 5.2. In unheated milks, there is also little dissociation of caseins from the micelle during acidification at temperatures ≥20°C (Dalgleish and Law, 1988). Heertje *et al.* (1985) did not suggest a significant role to denatured whey protein during the formation of acid gels from heated milk.

PHYSICAL PROPERTIES AND DEFECTS OF ACID MILK GELS

Appearance

Set-style yoghurt gels should have a smooth, custard-like, semi-solid consistency with no surface whey. The appearance of a set gel should be smooth with no cracks, holes or other 'blemishes'. Schmidt *et al.* (1980) reported that yoghurt made from milk heated at 90°C for 30 min was 'grainy', while yoghurts prepared from milk heated at 80 or 85°C for 30 min were described as 'smooth and firm bodied'. Acid gels made from severely heated milks with GDL had a 'rough' surface with visible cracks and some whey separation (Lucey *et al.*, 1998*c*). Rearrangement of the network just after gel formation may be responsible for these defects. Gels made from severely heated milk had a low strain at fracture compared to gels made from unheated milk and possibly this makes heated gels more susceptible to localized fracturing of strands in the network (Lucey *et al.*, 1997*c*). The continued aggregation (rearrangements) of casein particles after the aggregation/gelation of whey proteins was initiated may cause local stresses in the network (Lucey *et al.*, 1998*a*,*c*). If the protein–protein bonds have a relatively short lifetime, this may lead to yielding of a junction and with that, to breaking of a strand (van Vliet *et al.*, 1991).

Whey separation

Whey separation, which refers to the appearance of liquid (whey) on the surface of a milk gel, is a common defect in fermented milk products. Whey separation can occur if the gel network is damaged or if the gel undergoes substantial structural rearrangement. Syneresis is defined as shrinkage of a gel and this occurs concomitantly with expulsion of liquid or whey separation. Spontaneous syneresis is contraction of a gel without the application of any external forces (e.g. centrifugation) and is related to instability of the gel network (i.e. large scale rearrangements) resulting in the loss of the ability to entrap all the serum phase (Walstra, 1993). Yoghurt manufacturers try to prevent whey separation by increasing the total solids content of milk and in the case of stirred yoghurt by adding stabilizers (e.g. pectin, gelatin).

Lucey *et al.* (1998*a*) have recently developed a simple method to quantify spontaneous whey separation in acid gels. Previous studies had determined the quantity of whey expelled from yoghurt as result of high speed centrifugation or drainage through a screen (Harwalkar and Kalab, 1983; Harwalkar and Kalab, 1986; Dannenberg and Kessler, 1988*a*). The drainage of whey from a broken gel distributed over a screen measures whey separation when a very large surface area is available and is more relevant to products, such as cottage cheese or casein, where whey is separated from curd by screen drainage, than to set gels like yoghurt (Lucey *et al.*, 1998*a*). High speed centrifugation measures water holding capacity under relatively high forces. Therefore, these whey expulsion methods are not relevant to the spontaneous whey separation defect that may occur in some set gels. The approach of Lucey *et al.* (1998*a*) was to make gels in containers of different geometries and determine the amount of surface whey that was spontaneously expelled during gelation. Making gels in volumetric flasks proved to be a useful method, having the advantages of sloping walls which appeared to encourage whey separation, easy collectability of the whey which was poured-off through the neck of the flask, and the amount of gel (~220 g) was in the usual size range for many retail yoghurt pots. The effects of gelation temperature and milk heat treatment on whey separation were studied (Lucey *et al.*, 1998*a*) using response surface methodology. Whey separation (in volumetric flasks) was significantly increased by heat treatment ($P \leq 0.001$) and gelation temperature ($P \leq 0.01$); a regression equation adequately predicted the effects of heat treatment and gelation temperature on whey separation ($R^2 = 0.89$) (Lucey *et al.*, 1998*a*).

In practice, rapid acidification of milk and high incubation temperatures may be two of the main causes of whey separation in acid gels like yoghurt (Table 1). For example, yoghurt made at high temperatures (43–44°C) using a high concentration of starter inoculum (4–5%) from unheated (and unfortified) skim milk (Kalab *et al.*, 1976) or from skim milk that was heated at 88°C for 25 min (Modler *et al.*, 1983) exhibited extensive wheying-off. Acid-induced milk gels formed by slow acidification of milk at low temperatures and quiescent heating exhibits little wheying-off or spontaneous syneresis (Roefs, 1986). Lucey *et al.* (1997*c*) found that acid Na caseinate gels formed at a high gelation temperature (i.e. 40°C) with GDL, formed coarse gels that had a strong tendency to undergo 'spontaneous' wheying-off. Acid Na caseinate gels formed at a low gelation temperature (i.e. 20°C), had small pores and considerably less tendency to spontaneously whey-off.

Table 1. Some possible causes of wheying-off in acid gels

Very high incubation temperatures
Excessive heat treatment of the mix
Rapid rate of acidification
Low total solids content (protein and/or fat) of the mix
Containers with sloping walls or an excessive height to width ratio
Movement or agitation during or just after gel formation
Very low acid production (pH≥4.8)
Use of rennet enzyme

It was reported that heat treatment of milk reduces the quantity of whey expelled from yoghurt gels during centrifugation tests or drainage through a screen (Harwalkar and Kalab, 1983, 1986; Dannenberg and Kessler, 1988*a*). The release of whey during centrifugation or drainage through a screen is difficult to relate to the whey separation defect, which may occur spontaneously in some 'set' gels. The release of whey under high external forces (e.g. centrifugation) may be related to the rigidity of the network (i.e. its ability to resist large external forces) (Harwalkar and Kalab, 1986) whereas the method developed by Lucey *et al.* (1998*a*) is more closely related to the whey separation defect that sometimes occurs in retail containers.

Lucey *et al.* (1997*c*) used the one-dimensional syneresis technique described by van Dijk and Walstra (1986) to measure the shrinkage of acid Na caseinate gels. Acid Na caseinate gels made and measured at 30°C contracted by ~10% within 24 h of their surface being wetted by water (Lucey *et al.*, 1997*c*), compared to >60% in rennet induced gels (Walstra, 1993). Syneresis, in acid milk gels made with GDL, increased with high gelation temperatures and high pH values (van Vliet *et al.*, 1997).

It was shown (van Dijk and Walstra, 1986) that the one dimensional syneresis of rennet gels is related to the flow of liquid (whey) through the network and the rate is governed by the equation of Darcy:

$$v = \frac{B p}{\eta x} \tag{8}$$

where v is the superficial flow velocity of the syneresing liquid, B is the permeability coefficient, η is the viscosity of the liquid, p is the pressure acting on the liquid and x

the distance over which the liquid must flow. In milk gels, an endogenous syneresis pressure can occur as a result of the tendency of the casein network to rearrange after its formation (Lucey *et al.*, 1997*c*). It has recently been shown that endogenous syneresis pressure is mostly small in acid Na caseinate gels and this results in the smaller tendency for shrinkage of these gels compared to rennet gels (Lucey *et al.*, 1997*c*). It may be that acid gels made at high gelation temperatures from heated milk may have a strong endogenous pressure, at least during the initial stage just after gel formation, as this might help to explain the extensive whey separation that occurs during this period.

Differences in the ease of water removal from different casein gels can be related to the susceptibility of the network to rearrangements just after gel formation (van Vliet and Walstra, 1994). Important parameters that affect the timescale for rearrangements of bonds in a gel are the dynamic moduli, which indicate the strength and number of bonds in the network, the yield stress and shear deformation at yielding, which determine the susceptibility of the strands to breakage, and tan δ, with higher values favouring relaxation of bonds (van Vliet *et al.*, 1991; Lucey *et al.*, 1998*a*). Probably, in very young gels the number of bonds between each junction is not yet very high as indicated by the low dynamic moduli and tan δ is higher than in aged gels. The 'maximum in tan δ' which was observed in acid gels made from heated milk (Fig. 1) would favour even greater relaxation of bonds during the initial period after gel formation. Another factor that may assist rearrangements of young gels is that the pH is not yet very low (e.g. at pH values > 5.0) which may mean that electrostatic attractions between casein particles are weaker than in aged gels, which have lower pH values (van Vliet and Walstra, 1994; Lucey *et al.*, 1998*a*).

Acid milk gels that were first cooled to 5°C, before wetting their surface, actually increased in height slightly, possibly as a result of absorption of water by casein particles, which swell at low temperatures (Lucey *et al.*, 1997*c*). Surface whey that was expelled during gelation is sometimes reabsorbed into the gel on cooling and storage at low temperatures.

Textural defects

An excessively firm texture can be caused by factors such as a very high total solids content of the mix, the addition of stabilizers, or a very low gelation temperature. A weak or thin body can be caused by factors such as a very low solids content of the mix, insufficient heat treatment of the milk, low acidity and a high gelation temperature. Textural defects like 'lumpiness', 'granular' or the presence of 'nodules' are objectionable as consumers expect a smooth, fine-bodied product (Bodyfelt *et al.*, 1988). Lumpiness usually refers to the presence of large protein aggregates in yoghurt that can often range in size from 1–5 mm. Excessive production of acid at high incubation temperatures, the use of rennet and the use of excessive amounts of starter were associated with these types of defects (Humphreys and Plunkett, 1969). Excessive heat treatment of milk and the addition of high levels of whey proteins have also been associated with other textural defects. In yoghurt samples where $\geq 20\%$ of milk solids-non-fat (SNF) were replaced with whey protein concentrate (WPC), a 'grainy' texture was observed (Greig and Van Kan, 1984). Substituting WPC for skim milk powder (SMP) to elevate the total solids content of yoghurt mixes increased the 'lumpy' or 'granular' defect (Guirguis *et al.*, 1988), while replacement of casein by WPC resulted in a yoghurt with a 'less smooth and clumpy' appearance (Jelen *et al.*, 1987). In stirred-type yoghurt, stabilizers are added to control textural defects and prevent whey separation but stabilizers are not normally added to plain, set-style yoghurt.

EFFECTS OF COMPOSITIONAL AND PROCESSING PARAMETERS ON THE TEXTURAL PROPERTIES OF ACID MILK GELS

The different processing steps involved in the manufacture of acid milk gels such as yoghurt are shown in Fig. 3. The effects of each processing step on the textural properties of acid gels is considered in the following section.

Total solids content of milk

It is well known that increasing the total solids content of milk increases the firmness and viscosity of yoghurt (Robinson and Tamime, 1986). It is often considered that unless the SNF content of milk is increased to 16–18%, the yoghurt gel produced during fermentation will be weak and prone to syneresis (e.g. Robinson and Tamime, 1986). The protein or SNF content of milk can be increased by concentration of milk or by dry-matter enrichment. The sources of dry-matter are usually SMP, WPC or (occasionally) Na caseinate depending on what is legally permitted to be added to yoghurt, which varies from country to country.

The protein or SNF content of milk can be increased by concentration of milk by evaporation, ultrafiltration (UF) or reverse osmosis (RO) (Robinson and Tamime, 1986; Puhan, 1988; Becker and Puhan, 1989; Savello and Dargan, 1995). In some countries it is not permissible to add any powders or stabilizers to increase the firmness or viscosity of yoghurt, leaving concentration of milk as the usual alternative.

Yoghurts made from milk concentrated by UF are firmer than those made from milk concentrated by evaporation or by the addition of SMP (Becker and Puhan, 1989; Savello and Dargan, 1995), to similar protein

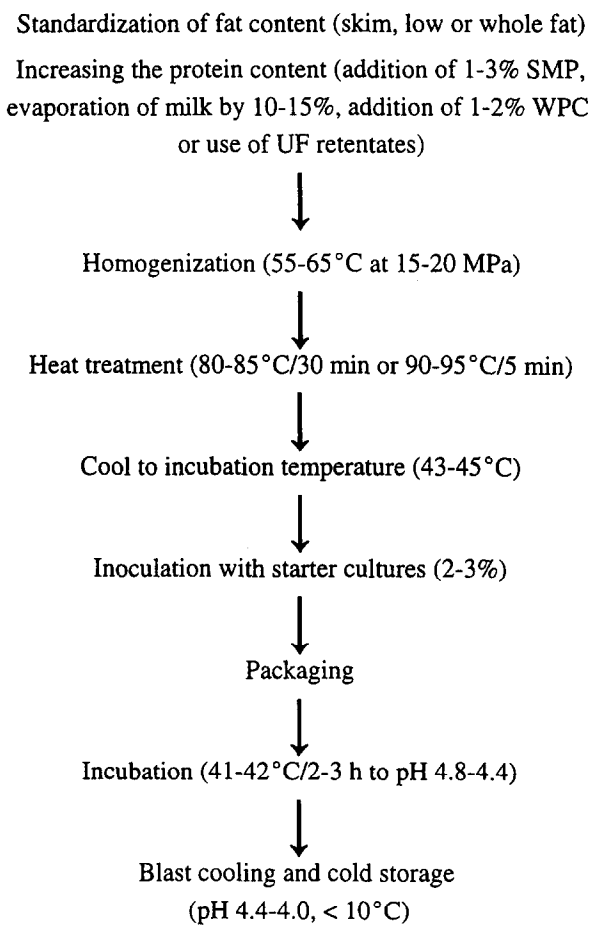


Fig. 3. Flow diagram for the manufacture of plain, set-style yoghurt.

levels. Commonly, the SNF content is increased by 1–3%, which corresponds to evaporation by 10–25% (Puhan, 1988).

Commercial yoghurts are often produced from milk that has been fortified by the addition of 1–3% SMP. At similar protein levels, yoghurts enriched with Na caseinate show higher viscosities or firmness compared with yoghurts enriched with SMP or WPC (Modler *et al.*, 1983; Guinee *et al.*, 1995). The use of whey proteins in yoghurt has been investigated many times (Modler *et al.*, 1983; Jelen *et al.*, 1987; Morris *et al.*, 1995). Morris *et al.* (1995) found that at similar protein concentrations, yoghurt fortified with both SMP and WPC were not significantly different in firmness compared to yoghurt fortified with SMP only. After stirring, the gels containing WPC alone retained more whey than those made with SMP (Morris *et al.*, 1995). The addition of 1% WPC to milk and heat treatment resulted in an increase in G′ and a reduction in the gelation time for acid milk gels (Lucey *et al.*, 1998*b*). It was suggested that during heat treatment, the added whey proteins, as well as the original whey proteins in milk, were denatured and associated with casein micelles to provide additional cross-linkages within acid-induced gels (Lucey *et al.*, 1998*b*).

Substitution of milk protein (casein) with a WPC solution (protein content 3.1%) up to 10 to 15% level had no effect on the final viscosity or sensory attributes of yoghurt, but at high levels of substitution, flocculation occurred during heat treatment of the mix (Greig and Van Kan, 1984). The firmness of yoghurt gels made from milk with various casein to whey protein ratios was similar (Jelen *et al.*, 1987).

Homogenization

Yoghurts can have fat contents ranging from 0–10%. A fat content of 0.5–3.5% is, however, more common. Fat provides a perception of creaminess and improves the mouth feel of yoghurt products. Homogenization of milk for yoghurt manufacture is considered to prevent fat separation during storage, improve consistency, increase whiteness and reduce whey separation (Tamime and Robinson, 1985; Puhan, 1988). Milk is usually homogenized at pressures in the range 10–20 MPa, at temperatures in the range 55–65°C and prior to heat treatment of the mix.

It was claimed that homogenized whole fat milk produces firmer gels than those made from skim milk, even in milks with similar SNF or protein contents (Becker and Puhan, 1989). An increase in homogenization pressure was reported to result in an increase in the viscosity of full fat yoghurt (Puhan, 1988). In contrast, Schmidt and Bledsoe (1995) reported that there were no significant differences in the firmness or apparent viscosities of yoghurts made from low fat (1.5%) or full fat (3.5%) milk even if these samples were given very different homogenization pressures. It was reported (Tamime and Robinson, 1985; Puhan, 1988) that heat treatment of milk has a much greater effect on the consistency and texture of acid milk gels than homogenization. Cobos *et al.* (1995) concluded that the conditions of homogenization did not have a major influence on the rheological properties of acid gels made from recombined milk. Lucey *et al.* (unpublished results) found that increasing the fat content of recombined milk increased the G′ of acid milk gels but to a much lesser extent than heat treatment of milk.

Fat globules act as an inert filler if the native fat globule membrane is intact since the native membrane does not interact with casein particles (van Vliet and Dentener-Kikkert, 1982; van Vliet, 1988). The G′ value of acid milk gels decreases with an increasing volume fraction of fat, which has an intact native fat globule membrane (van Vliet and Dentener-Kikkert, 1982; van Vliet, 1988). In homogenized or recombined milk, the native membrane is replaced largely with casein and some whey protein so that the (surfaces of) fat particles can interact with the protein matrix (largely casein but some denatured whey proteins when made from heated milks) of acid milk gels (van Vliet and Dentener-Kikkert, 1982; van Vliet, 1988). In acid gels made from

recombined milk, G′ increases with an increasing volume fraction of fat (van Vliet and Dentener-Kikkert, 1982; van Vliet, 1988). Acid milk protein gels containing fat globules with artificial protein membranes, had higher compressive strengths and dynamic moduli than gels where the fat globules had a surfactant (non-interacting) membrane (Xiong and Kinsella, 1991; Xiong *et al.*, 1991).

Heat treatment

Heat treatment of milk is one of the most important processing parameters affecting the texture of acid milk gels, like yoghurt (Mulvihill and Grufferty, 1995). Although there were many studies on the effects of heat treatment on the texture of yoghurt (e.g. Dannenberg and Kessler, 1988*b*) most studies have used empirical tests to determine attributes described as 'firmness' or 'curd tension'. Several reports (e.g. Dannenberg and Kessler, 1988*b*) have related the firmness and viscosity of yoghurt gels to the extent of denaturation of whey proteins during heat treatment. Heat treatment also results in a reduction in the gelation time and an increase in the pH at gelation (Heertje *et al.*, 1985; Lucey *et al.*, 1997*a*). High heat treatments of milk increases the dynamic moduli of acid milk gels (van Vliet and Keetels, 1995; Lucey *et al.*, 1997*a*) although the fracture strain decreases with increasing heat treatment making these gels more brittle (Lucey *et al.*, 1997*a*). The effect of heat treatment on the formation of acid milk gels was discussed in an earlier section.

UHT treatment of milk for yoghurt manufacture generally results in yoghurts that are less firm and viscous than yoghurt manufactured by conventional (Vat) heating methods, e.g. lower temperatures, long holding times (e.g. Savello and Dargan, 1995). It is likely that the method of heating (i.e. direct and indirect) also affects the degree of denaturation of individual whey proteins. Acid gels made from reconstituted high- or medium-heat SMP had higher G′ than gels made from reconstituted low-heat SMP (Lucey *et al.*, 1997*a*).

Inoculation and gelation temperature

The thermophilic starter bacteria, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are normally used as cultures in the fermentation of yoghurt. The incubation temperatures for yoghurt are usually in the range 40 to 45°C and fermentation often takes up to 4 h, depending on the amount of starter addition. After the pH of yoghurt has decreased to the desired values (usually $\leq$4.6), the gel is then cooled to $<$ 10°C. The final pH of most yoghurts varies from 4.6–4.0.

In general, an excessive rate of acid development at a high incubation temperature contributes to the 'whey-ing-off' defect and poor gel formation (Kosikowski, 1977). Several researchers have recommended lowering the incubation temperature from ~44°C to $\leq$ 38°C to improve gel firmness, viscosity and reduce whey separation (Kosikowski, 1977). At lower incubation temperatures (e.g. ~30°C) the fermentation time can be extended for up to 12 h (overnight incubation) but good quality yoghurt can be produced. Gels with very high pH values (e.g. 4.8) have a much greater tendency to exhibit syneresis than gels with a low pH value (van Vliet *et al.*, 1997).

In various types of acid milk gels formed with GDL, a lower gelation temperatures (e.g. 20°C) results in longer gelation times but these gels can have higher G′ values compared to gels made at a high gelation temperature (e.g. 40°C) (Cobos *et al.*, 1995; Lucey *et al.*, 1997*b*). The dynamic moduli of acid gels increase with decreasing measuring temperature (Lucey *et al.*, 1997*a*,*b*). Whey separation also decreases in gels made at lower gelation temperature (Lucey *et al.*, 1998*a*).

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