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# Effect of milk solids concentration on the gels formed by the acidification of heated pH-adjusted skim milk

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**Abstract**

Reconstituted skim milk of 10–25% total solids was adjusted to pH values between about 6.2 and 7.1 and heated at 80 °C for 30 min. Gels were formed from the heated milks by slow acidification to pH 4.2 and the gelation process and final gels were analyzed for their rheological properties. At each milk concentration, the final acid gel firmness (final $G'$) and breaking stress could be changed markedly by manipulation of the pH during heating. The final gel firmness and breaking stress could also be modified by changing the concentration of the milk solids prior to heating and acidification. The results indicated that similar gel firmness and breaking stress could be achieved over a range of milk concentrations by control of the pH of the milk during heating. When expressed as a percentage change in final $G'$ or breaking stress relative to that obtained at the natural pH, plots of the change in final $G'$ or breaking stress versus pH fell close to a single curve, indicating that the same mechanism may influence the gelation properties at all milk concentrations. The final $G'$ and breaking stress were related to the denaturation and interaction of the whey proteins with the casein micelles, and the formation of non-sedimentable casein when the milk was heated.


*Keywords:* Milk; Concentrated milk; Denatured whey proteins; Casein micelles; Rheology, acid gelation; Heating; pH; Strain; Stress

## 1. Introduction

Recent studies have demonstrated that small changes in the pH of milk prior to heat treatment can markedly influence the level of denatured whey protein interacting with the casein micelles (Anema & Li, 2003; Donato & Dalgleish, 2006; Rodriguez del Angel & Dalgleish, 2006; Vasbinder & de Kruif, 2003). At pH 6.5, about 70% of the denatured whey proteins are associated with the casein micelles, and the level of association decreases with increasing pH, so that only about 30% are associated at pH 6.7 and even lower levels are associated at higher pH.

As the pH of the milk is increased from about pH 6.5 to pH 7.1 before heating, κ-casein progressively dissociates from the casein micelles so that, at pH 6.5, the majority of the κ-casein is associated with the casein micelles whereas, at pH 7.1, about 60–70% of the κ-casein is found in the milk serum (Anema, 2007; Donato & Dalgleish, 2006). As the denatured whey proteins interact with the casein micelles via disulfide bonding with the κ-casein, this dissociation of κ-casein probably explains why the association of the whey proteins with the casein micelles is pH-dependent (Anema, 2007).

Further studies have also shown that the pH of the milk during heat treatment has a marked effect on the gels formed during subsequent acidification. As the pH of the milk was increased from pH 6.5 to pH 7.1 prior to heat treatment and acidification, the $G'$ of the acid gels progressively increased so that the firmness (based on storage modulus, $G'$) at pH 7.1 was approximately twice that observed at pH 6.5 (Anema, Lee, Lowe, & Klostermeyer, 2004; Guyomarc'h et al., 2007; Rodriguez del Angel & Dalgleish, 2006). This increase in $G'$ could be related to the distribution of the denatured whey proteins between

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the serum and colloidal phases. The milk samples with a greater level of non-sedimentable denatured whey proteins produce acid gels with a higher $G'$ than those where most of the denatured whey proteins are associated with the casein micelles (Anema et al., 2004; Rodriguez del Angel & Dalgleish, 2006).

There is considerable interest in controlling and manipulating the texture of food gels by modifying functional properties of the gels (e.g. firmness or breaking properties) while maintaining a constant composition, or by maintaining constant functional properties of the gels while altering the composition (Euston, Piska, Wium, & Qvist, 2002; Hallab, Kohen, Grandison, Lewis, & Grandison, 2007; Mezzenga, Schurtenberger, Burbidge, & Michel, 2005; Singh, Ye, & Havea, 2000). The results with milk at its natural concentration suggest that the properties of gels prepared by the acidification of heated milk could be manipulated by altering the pH before heating. Although it is known that milk concentration (or composition) affects the properties of these gels (Biliaderis, Khan, & Blank, 1992; Kristo, Biliaderis, & Tzanetakis, 2003; Pereira, Matia-Merino, Jones, & Singh, 2006), it is unknown whether similar pH behaviour is observed at all concentrations of milk, or whether similar functional properties of gels can be achieved over a range of milk concentrations by altering the pH during heating before the formation of the gels.

Therefore this study was conducted to examine the effects of milk solids concentration and pH during heating on the rheological properties of gels prepared by acidification of the heated milk. In addition, the denaturation of the whey proteins and their interaction with the casein micelles in the heated milks were also determined and related to the properties of the acid gels prepared from the heated milks. The results from this study will give an indication of the range of textural modifications that can be achieved by altering milk concentration and pH during heating, and whether these changes can be related to fundamental changes in the interactions between the milk proteins induced by heating at all milk concentrations.

## 2. Materials and methods

### 2.1. Milk supply

Low heat skim milk powder was obtained from Fonterra Co-operative Group, New Zealand. Reconstituted skim milk samples were prepared by adding low heat skim milk powder to purified (reverse osmosis, followed by filtration through a Milli-Q apparatus) water to a final concentration of 10–25% (w/w) total solids (TS). The milk samples were mixed using an overhead stirrer and paddle apparatus for a minimum of 2 h to ensure thorough dispersion and reconstitution of the milk powder. The reconstituted skim milk samples were allowed to equilibrate at ambient temperature (about 20 °C) for at least 10 h and then stirred for a further 1 h before use. A small amount of sodium azide (0.01% w/v) was added to all milk samples as a preservative.

### 2.2. Adjustment of pH and heat treatments

The pH of the milk samples was adjusted by the slow addition of 3 M HCl or 3 M NaOH to stirred solutions. The pH was allowed to equilibrate for at least 2 h and then minor re-adjustments were made. The milk samples were transferred to glass tubes and heated, with continuous rocking, for 30 min in a thermostatically controlled oil bath preset to 80 °C. The heating time included the time to reach the experimental temperature, which was about 30 s. After heat treatment, the milk samples were cooled by immersion in cold running water until the temperature was below 30 °C. The samples were stored for 6 h at ambient temperature after heat treatment and before any further analysis.

### 2.3. Formation of gels by acidification

The milk samples were carefully re-adjusted back to the natural pH with 3 M HCl or 3 M NaOH. They were acidified using glucono-δ-lactone (GDL) at 30 °C to form acid gels. Standard curves of pH versus GDL level for each milk concentration were prepared by adding different levels of GDL to the milk, and measuring the pH after 6 h at 30 °C. The level of GDL required to achieve a pH of approximately 4.20 after 6 h of reaction was calculated from these standard curves. From this, it was shown that 2.0%, 2.56%, 3.62%, and 4.36% (w/w) GDL were required for the 10%, 15%, 20%, and 25% skim milk samples, respectively.

### 2.4. Rheological measurements

The rheological properties of the acidified milks were monitored with time using low amplitude dynamic oscillation. A TA AR2000 rheometer (TA Instruments UK, Cirencester, Gloucestershire, England) and a cone (4 cm, 4°) and plate arrangement were used for all samples. The GDL was added to the milk, the milk was stirred for 30 s, and then 1.2 ml was transferred to the rheometer plate and the cone was lowered to give the required gap between the cone and the plate. A water trap and cover arrangement was placed over the sample to prevent evaporation. The strain applied was less than 1%.

Initial measurements involved monitoring gelation as the milk was acidified. The samples were oscillated at a frequency of 0.1 Hz and the temperature of the sample was maintained at 30 °C. Measurements were taken every 5 min for 6 h, and the storage modulus ($G'$) at this point was considered to be the final $G'$ at 30 °C.

Commercial acidified milk products are usually stored at refrigerator temperatures (~5 °C) and often consumed at these temperatures. As a consequence, the rheological properties of the set gels at a temperature of 5 °C are of interest. Therefore, after gelation was complete (i.e. after

gelation for 6 h at 30 °C), the temperature of the system was reduced to 5 °C. The rheological properties of the set gel were monitored as the temperature was lowered to 5 °C, and then for an additional 15 min at 5 °C. The $G'$ at this point was considered to be the final $G'$ at 5 °C. The final measurements involved monitoring the large deformation properties of the set gel at 5 °C. The strain was progressively increased at a constant rate until the gel broke. From this, the stress and the strain at breaking were obtained.

To ensure that no geometry effects existed, selected samples were also run, under identical conditions, on a Physica MCR 301 rheometer with a concentric cylinder geometry (Anton Paar GmbH, Graz, Austria), and the sample was overlaid with a light mineral oil to prevent evaporation. In all cases, similar results and trends to those obtained with the cone and plate arrangement were obtained, indicating that the cone and plate geometry was suitable for these gelation experiments.

### 2.5. Centrifugation

Non-sedimentable whey proteins were defined as those that did not sediment from the milk during centrifugation at 14,000 rev/min (25,000*g* average) for 1 h at 20 °C in an Eppendorf centrifuge Type 5417C. The concentrated milks (15–25% TS) were diluted so as to be directly comparable with the 10% TS milk, and then the pH values of the milks were adjusted to the natural pH of the 10% TS milk (pH 6.67). A sample (1 ml) of each milk was placed in a small plastic tube of 1.5 ml total volume and centrifuged. The clear supernatant was carefully poured from the pellet. The protein content and the composition of the supernatants were determined by gel electrophoresis and laser densitometry.

### 2.6. Gel electrophoresis and laser densitometry

The level of non-sedimentable casein and whey protein was determined by analyzing the centrifugal supernatants by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions, as has been described previously (Anema & Klostermeyer, 1997). The levels of native α-lactalbumin and β-lactoglobulin in the heated milk samples were determined, using native polyacrylamide gel electrophoresis (native-PAGE), as has been described previously (Anema & McKenna, 1996). The casein and the denatured whey protein were removed from the milk by adjusting the pH to 4.6 and centrifuging out the precipitate in a bench centrifuge. The resultant supernatant was analyzed by native-PAGE.

Native-PAGE and SDS–PAGE gels were scanned using a molecular dynamics model PD-SI computing densitometer (Molecular Dynamics Inc., Sunnyvale, CA) and integrated using the Imagequant software associated with the densitometer. The quantity of each protein in the ultracentrifugal supernatants was determined as a percentage of that in the original milk samples.

All experiments were repeated at least twice, and the significance of differences was determined by an analysis of variance test. Results were reported as significant for $P \leqslant 0.05$.

## 3. Results and discussion

### 3.1. Small strain rheological properties during acidification of skim milk

For all gelation experiments, the pH at heating refers to the pH the milk was adjusted to before heating and acidification, the final $G'$ refers to the $G'$ value measured after a gelation time of 6 h. All four milk concentrations showed the same general effect of the pH at heating on the acid gelation properties of the milks. Fig. 1A and B shows the changes in $G'$ with time after GDL addition for 10% and 20% TS reconstituted skim milk samples, respectively, that were pH-adjusted prior to heat treatment (80 °C/30 min). These are representative of milks close to the natural milk concentration (10% TS milk) and of those twice the natural milk concentration (20% TS milk). Table 1 provides the final $G'$ measured at 30 °C for all milk concentrations and all pH's during heating. It should be noted that the pH of all samples was re-adjusted to the natural pH for each milk concentration prior to starting the gelation experiments, and that the gelation was stopped when the pH of the milk was 4.2.

At their natural pHs, increasing the concentration of the milk prior to heating caused a marked increase in the final $G'$ of the acid gels when measured at 30 °C, from about 200 Pa for the 10% TS milk to over 1200 Pa for the 25% TS milk (Fig. 1A and B; Table 1). Increasing the milk concentration increases the protein concentration and, as protein is responsible for structure formation, an increase in gel firmness would be expected as there are increases in the number of protein components, structural interconnectivity and contact points within a given unit area. For all milk concentrations, there was a clear effect of the pH during heating on the $G'$ versus time curves (Fig. 1A and B) and in particular the final $G'$ of the acid gels (Table 1). For all milk concentrations, reducing the pH during heating (from that naturally observed) shifted the $G'$ versus time curves to lower values and reduced the final $G'$, whereas an increase in pH during heating (from the natural pH) shifted the $G'$ versus time curves to higher values and increased the final $G'$. In effect, the final $G'$ could be markedly modified over a 0.6 unit pH range centred about the natural pH for each milk concentration (Fig. 1A and B, Table 1).

The final $G'$ values for the acid gels prepared from the 10% TS milks were lower than those from the milks at higher concentrations at all pHs during heating; however, for the 15–25% TS milks, there was some overlap in final $G'$ for the acid gels prepared from the milks at these higher

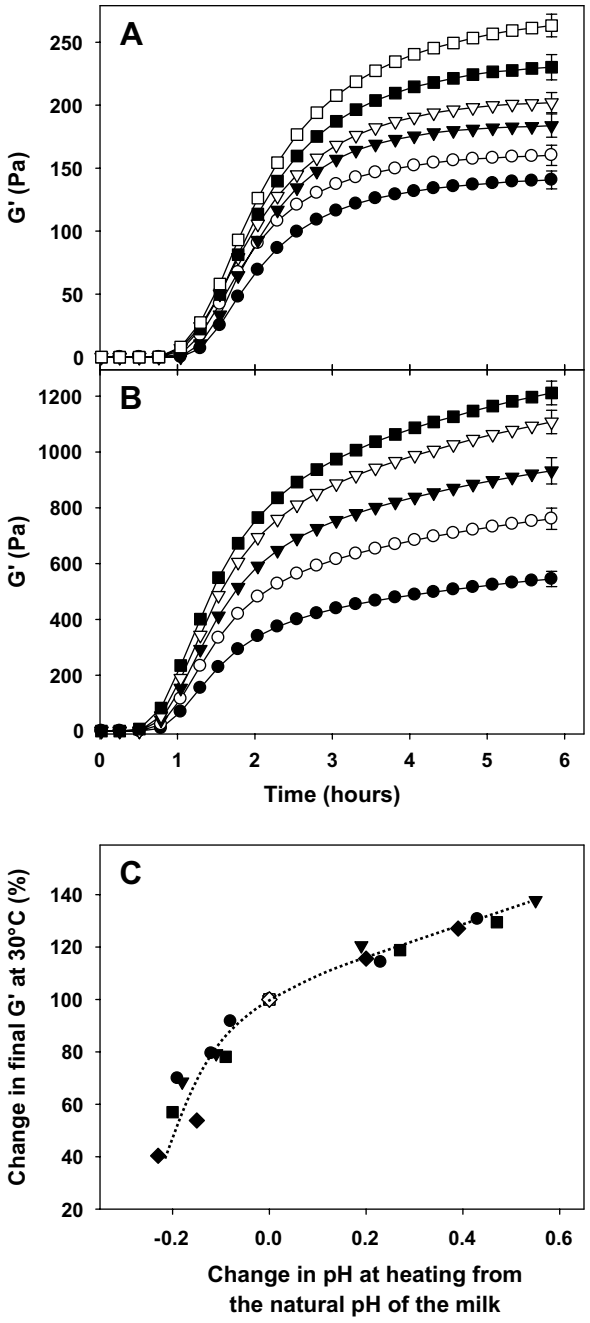


Fig. 1. A: Changes in storage modulus, $G'$, with time after GDL addition for heated (80 °C/30 min) 10% TS skim milk samples; ●: pH 6.48; ○: pH 6.55; ▼: pH 6.59; ▽: pH 6.67; ■: pH 6.90; □: pH 7.10. B: Changes in storage modulus, $G'$, with time after GDL addition for heated (80 °C/30 min) 20% TS skim milk samples; ●: pH 6.28; ○: pH 6.39; ▼: pH 6.48; ▽: pH 6.75; ■: pH 6.95. C: Percentage change in final $G'$ versus change in pH at heating from the natural pH of the milk. ●, ○: 10% TS milk samples; ▼, ▽: 15% TS milk samples; ■, □: 20% TS milk samples; ♦, ◇: 25% TS milk samples. Open symbols: samples at the natural pH; filled symbols: samples that were adjusted in pH before heating. The error bars represent the standard deviation of triplicate measurements.

milk concentrations (Table 1). This indicates that it is possible to produce gels at lower milk concentrations, with similar or higher firmness compared with gels at higher milk concentrations by manipulation of the pH before heat treatment.

Fig. 1C shows the percentage change in final $G'$ at 30 °C relative to that observed for the milks heated at the natural pH for each of the milk concentrations. When the final $G'$ was expressed in this way, the final $G'$ for all four milk concentrations fell very close to a single curve. This indicates that a change in pH of the milk during heating had a similar relative effect on the final $G'$, regardless of the milk concentration.

For the 10% TS milks, the final $G'$ values for the acid gels at 5 °C were approximately 2.2 times those at 30 °C, regardless of the pH during heating. However, as the milk concentration increased, the relative change in final $G'$ between 30 and 5 °C increased so that, at 25% TS, the final $G'$ at 5 °C was about 3.5 times that at 30 °C (Table 1). Hydrophobic interactions are strongly dependent on temperature and play an important role in the assembly of casein micelles, and in the structure of acid casein gels (Horne, 1998, 2003). It has been proposed that the decreased strength of hydrophobic interactions when the temperature is reduced may lead to a less compact conformation of casein molecules, thereby increasing the size of the casein particles within acid gel networks. This alters the balance between inter-particle and intra-particle bonds so that there are more inter-particle bonds between casein particles as the temperature is reduced, resulting in marked increases in $G'$ (van Vliet, Roefs, Zoon, & Walstra, 1989). As milk is concentrated, the number of particles within a unit area will increase, and therefore the number of inter-particle bonds may increase to a greater extent with decreasing temperature, resulting in proportionally higher increases in $G'$ at the lower temperatures. Other factors, such as the increased viscosity of the aqueous phase due to increasing lactose concentrations may also contribute to the proportionally larger increase in the final $G'$ at lower temperatures for the milks at higher concentrations.

### 3.2. Large strain rheological properties of acid skim milk gels

Once the gel was set and the temperature was reduced to 5 °C, the strain was increased at a constant rate and the stress was monitored against time. As with the gelation properties at 30 °C, all four milk concentrations showed the same general effect of the pH during heating on the large strain deformation properties of the set gels at 5 °C. Typical stress versus strain curves for the acid gels prepared from the 10% and 20% TS milk samples heated at different pH values are shown in Fig. 2A and B, respectively. The curves showed a characteristic increase in stress to a maximum, followed by a marked decrease in stress as the gel broke. The maximum in the curve was considered to be the breaking point and the stress and the strain at this point were considered to be the breaking stress and breaking strain, respectively (Table 1). For each milk concentration, the breaking strains of the acids gels were not significantly

Table 1
Selected rheological properties of gels formed from the acidification of heated pH-adjusted skim milk samples of different concentrations

| | pH during heating | Final $G'$, 30 °C (Pa) | Final $G'$, 5 °C (Pa) | Breaking stress (Pa) | Breaking strain (%) |
|---|---|---|---|---|---|
| 10% TS milk | 6.48 | 141 (7)[a] | 317 (15)[a] | 50 (3)[a] | 15 (2)[a] |
| | 6.55 | 160 (8)[b] | 370 (16)[b] | 53 (3)[a] | 15 (2)[a] |
| | 6.59 | 186 (9)[c] | 441 (18)[c] | 63 (3)[b] | 15 (3)[a] |
| | 6.67[A] | 202 (8)[c] | 486 (16)[d] | 69 (4)[b,c] | 14 (2)[a] |
| | 6.90 | 231 (10)[d] | 524 (19)[e] | 70 (3)[c] | 13 (2)[a] |
| | 7.10 | 265 (9)[e] | 602 (18)[f] | 77 (3)[d] | 14 (3)[a] |
| 15% TS milk | 6.38 | 340 (17)[a] | 919 (35)[a] | 128 (5)[a] | 18 (2)[a] |
| | 6.45 | 394 (20)[b] | 1086 (42)[b] | 148 (6)[b] | 21 (3)[a] |
| | 6.56[A] | 496 (25)[c] | 1315 (49)[c] | 175 (7)[c] | 19 (3)[a] |
| | 6.75 | 598 (30)[d] | 1604 (55)[d] | 182 (9)[c] | 19 (3)[a] |
| | 7.11 | 683 (34)[e] | 1886 (67)[e] | 208 (12)[d] | 18 (3)[a] |
| 20% TS milk | 6.28 | 536 (27)[a] | 1800 (48)[a] | 240 (14)[a] | 26 (2)[a] |
| | 6.39 | 734 (38)[b] | 2349 (69)[b] | 299 (18)[b] | 23 (2)[a] |
| | 6.48[A] | 940 (47)[c] | 3187 (78)[c] | 346 (20)[c] | 21 (2)[a] |
| | 6.75 | 1117 (42)[d] | 3580 (81)[d] | 347 (17)[c] | 23 (3)[a] |
| | 6.95 | 1217 (43)[e] | 3877 (83)[e] | 382 (14)[d] | 21 (2)[a] |
| 25% TS milk | 6.19 | 485 (24)[a] | 1811 (50)[a] | 206 (12)[a] | 26 (2)[a] |
| | 6.27 | 648 (32)[b] | 2156 (65)[b] | 281 (17)[b] | 25 (3)[a] |
| | 6.42[A] | 1203 (60)[c] | 4005 (98)[c] | 388 (23)[c] | 25 (2)[a] |
| | 6.62 | 1391 (49)[d] | 5050 (89)[d] | 465 (28)[d] | 24 (3)[a] |
| | 6.81 | 1529 (66)[e] | 5443 (105)[e] | 463 (27)[d] | 26 (3)[a] |

The numbers in parentheses represent the standard deviations from at least two repeated measurements.
For each milk solids concentration, superscripts with different letters are significantly ($P \leqslant 0.05$) different from each other.
[A] Natural pH of the milk.

affected by the various pH values during heating. The breaking strain increased slightly but significantly ($P \leqslant 0.05$) as the milk concentration was increased.

At any given pH, the breaking stress of the acid gels increased markedly with increasing milk concentration, and, at any given concentration, the breaking stress of the acid gels increased significantly with increasing pH during heating, although some adjacent points were not significantly different from each other (Table 1). As with the final $G'$, there was some overlap between the breaking stresses for the acid gels at the different milk concentrations; especially at the higher milk concentrations.

When the breaking stress was plotted as the percentage change relative to that observed for acid gels prepared from milks heated at the natural pH, the curves for all four milk concentrations fell close to a single curve (Fig. 2C), indicating that a change in pH has a similar relative effect on the breaking stress. This was similar to the effect observed for the final $G'$ (Fig. 1C) and, as a consequence, the breaking stress and the final $G'$ were correlated (not shown).

### 3.3. Whey protein denaturation and distribution of casein and whey protein between colloidal and serum phases

In further experiments, the heated and pH-adjusted milk samples were analyzed for the level of native whey protein, and the level of non-sedimentable whey protein and casein (Fig. 3). The level of total native whey protein (α-lactalbumin and β-lactoglobulin combined) increased slightly with decreasing pH; however, there was only a small effect of milk concentration on the level of denaturation of the total whey protein (Fig. 3A). Similar effects were observed for the individual whey proteins (not shown).

At each milk concentration, the level of non-sedimentable whey protein increased markedly with increasing pH. At any given pH, the level of non-sedimentable whey protein also appeared to increase with increasing milk concentration, particularly at the lower pH; however, many of the differences were not statistically significant, although the same trend was observed in all repeated experiments. This increase in non-sedimentable whey proteins at the lower pH may have been partly due to the slightly higher levels of native whey protein (Fig. 3A). Again, similar effects were observed for the individual whey proteins (not shown).

The level of non-sedimentable ($\alpha_s + \beta$)-casein was generally low, although the 10% TS sample at about pH 7.1 had slightly higher levels than those from the other milk samples (Fig. 3B). In contrast to the ($\alpha_s + \beta$)-casein, the level of non-sedimentable κ-casein increased markedly with increasing pH at all milk solids concentrations (Fig. 3B). As with the non-sedimentable whey proteins, at any given pH, the level of non-sedimentable κ-casein appeared to increase with increasing milk concentration, especially at the lower pH values; however, only those for the 25% TS milk were statistically higher than for the lower milk concentrations. When the level of non-sedimentable denatured

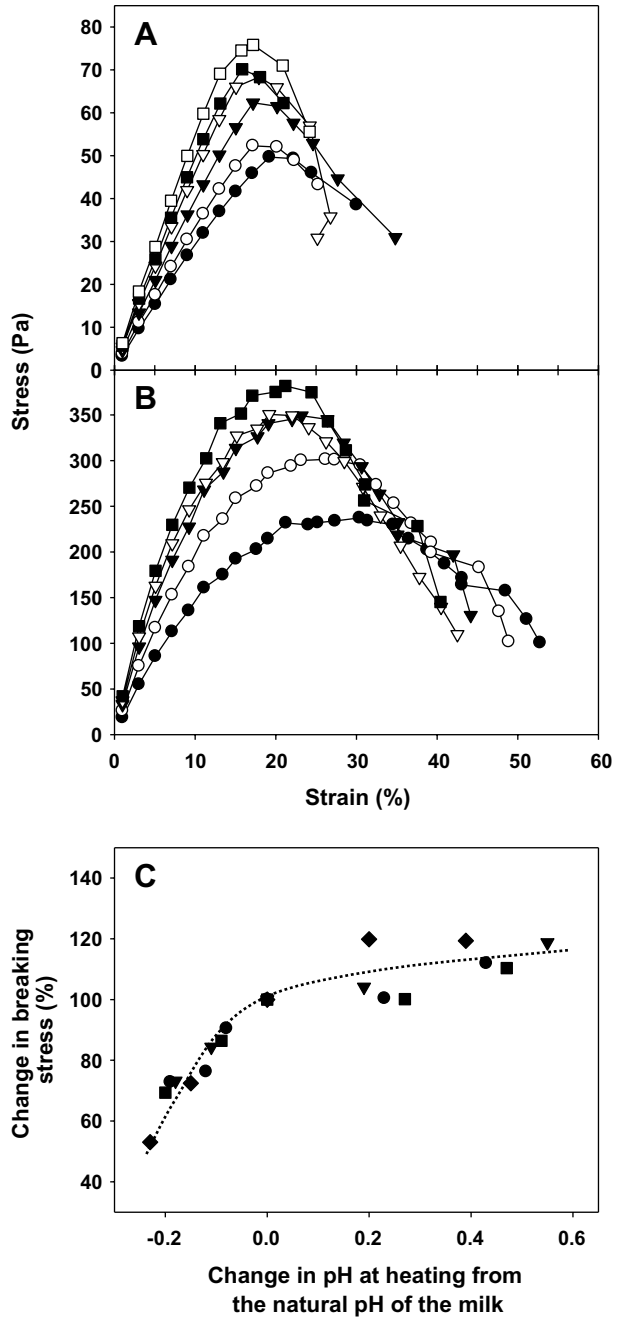


Fig. 2. A: Stress versus strain curves for acid gels prepared from heated (80 °C/30 min) 10% TS skim milk samples; ●: pH 6.48; ○: pH 6.55; ▼: pH 6.59; ▽: pH 6.67; ■: pH 6.90; □: pH 7.10. B: Stress versus strain curves for acid gels prepared from heated (80 °C/30 min) 20% TS skim milk samples; ●: pH 6.28; ○: pH 6.39; ▼: pH 6.48; ▽: pH 6.75; ■: pH 6.95. C: Percentage change in breaking stress versus change in pH at heating from the natural pH of the milk. ●: 10% TS milk samples; ▼: 15% TS milk samples; ■: 20% TS milk samples; ♦: 25% TS milk samples.

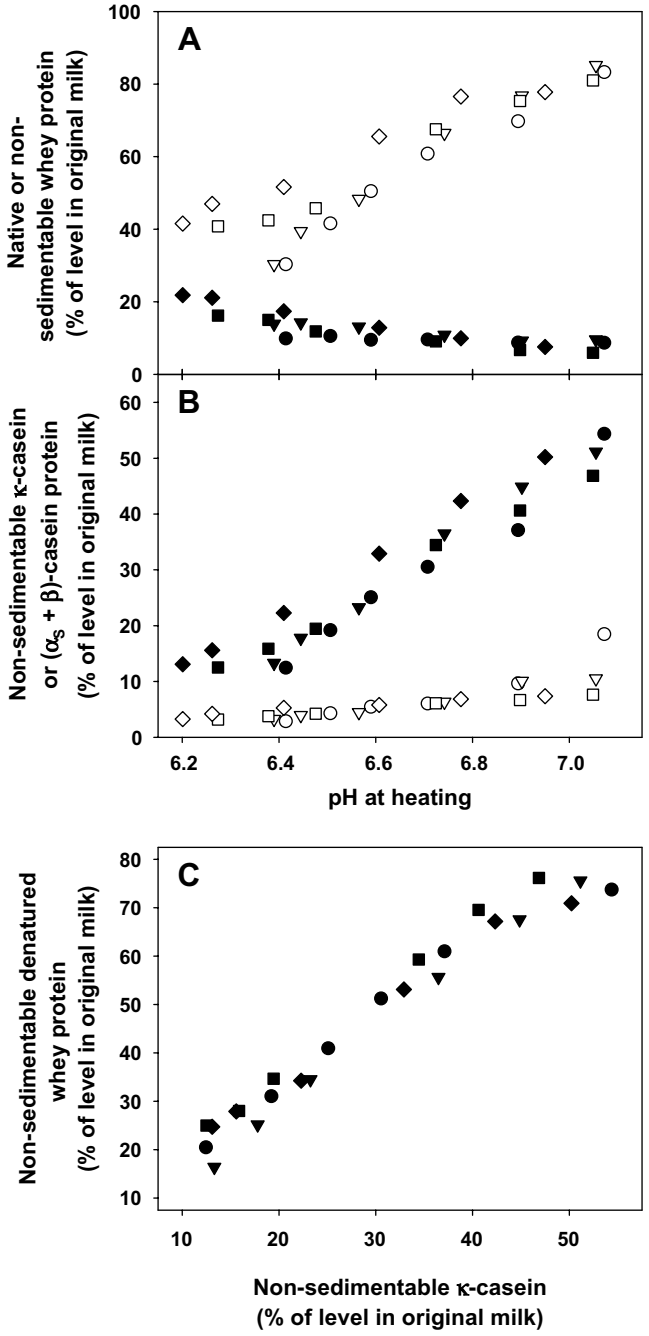


Fig. 3. A: Level of native (closed symbols) and non-sedimentable whey proteins (open symbols) in heated (80 °C/30 min), pH-adjusted skim milk. B: Level of non-sedimentable ($\alpha_s + \beta$)-casein (open symbols) and κ-casein (filled symbols) in heated (80 °C/30 min), pH-adjusted skim milk. C: Relationship between non-sedimentable κ-casein and non-sedimentable denatured whey protein in heated (80 °C/30 min), pH-adjusted skim milk. ●, ○: 10% TS milk samples; ▼, ▽: 15% TS milk samples; ■, □: 20% TS milk samples; ♦, ◇: 25% TS milk samples.

whey protein was plotted against the level of non-sedimentable κ-casein for all milk concentrations, a linear relationship was observed (Fig. 3C). This clearly indicates that the distribution of denatured whey proteins between the colloidal and serum phases, for heated milks at all milk concentrations, is influenced by the distribution of κ-casein between these two phases. This is consistent with results observed for unconcentrated (10% TS) milk (Anema, 2007; Donato & Dalgleish, 2006; Guyomarch, Law, & Dalgleish, 2003).

### 3.4. Relationships between the distribution of protein (between serum and colloidal phases) after heating and the rheological properties of acid gels

When the change in final $G'$ of the acid gels was plotted against the level of non-sedimentable denatured whey proteins in the milk, the results for all pH values and for all milk concentrations were close to a single curve (Fig. 4A). Similarly, plots of the change in final $G'$ and the level of non-sedimentable κ-casein were also close to a single curve (Fig. 4B), which is expected as the levels of non-sedimentable κ-casein and non-sedimentable denatured whey protein were correlated (Fig. 3C). Plots of the change in breaking stress against the level of non-sedimentable denatured whey protein (Fig. 4C) and non-sedimentable κ-casein (Fig. 4D) were also close to a single curve.

When milk is heated prior to acidification, the whey proteins denature and can interact with other milk proteins (Fig. 3). Unlike native whey proteins, the denatured whey proteins are insoluble at their isoelectric points and the denatured whey proteins can play a role in the gel structure formed during acidification. Therefore, heated milk samples will gel when the pH of the milk approaches the isoelectric point of the whey proteins (about pH 5.3 for β-lactoglobulin), and, as a consequence, heated milk samples gel more rapidly and at a higher pH than do unheated milk samples (Bikker, Anema, Li, & Hill, 2000; Lucey & Singh, 1998; Lucey, Teo, Munro, & Singh, 1997; van Vliet & Keetals, 1995). In this study, the levels of denaturation of the whey proteins were similar in all samples, regardless of the pH at heating or milk concentration (Fig. 3A), which indicates that the marked differences in final $G'$ (Fig. 1, Table 1) cannot be attributed to differences in whey protein denaturation.

It is observed that the relative change in final $G'$ (Fig. 1C) and the relative change in breaking stress against the pH during heating (Fig. 2C) fall close to a single curve. This suggests that similar mechanisms are responsible for the effect of the pH during heating on the final acid gel properties, regardless of the milk concentration. It is apparent that the pH during heating markedly affected the distribution of the denatured whey proteins between the serum and colloidal phases for the milk samples at all concentrations (Fig. 3). The strong relationship between the level of non-sedimentable denatured whey proteins or non-sedimentable κ-casein and the change in final $G'$ or breaking stress (Fig. 4) indicates that milk samples with high levels of non-sedimentable denatured whey proteins

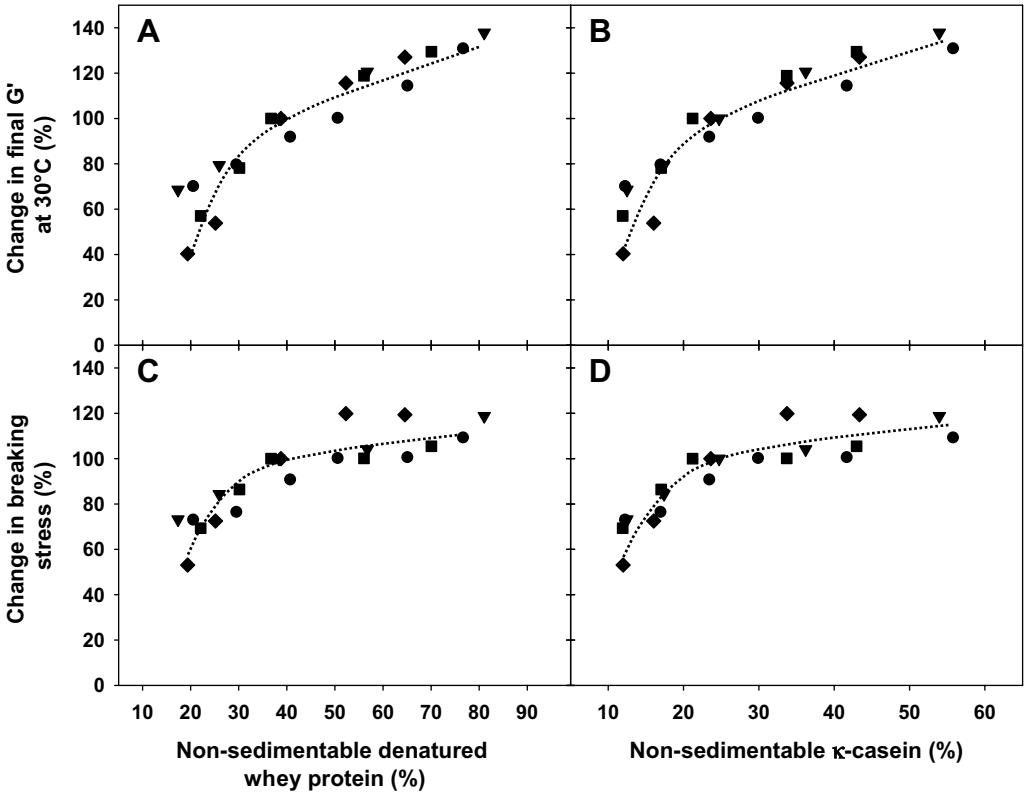


Fig. 4. A: Relationship between the change in final $G'$ for acid skim milk gels and the level of non-sedimentable denatured whey protein in heated (80 °C/30 min), pH-adjusted skim milk. B: Relationship between the change in final $G'$ for acid skim milk gels and the level of non-sedimentable κ-casein in heated (80 °C/30 min), pH-adjusted skim milk. C: Relationship between the change in breaking stress for acid skim milk gels and the level of non-sedimentable denatured whey protein in heated (80 °C/30 min), pH-adjusted skim milk. D: Relationship between the change in breaking stress for acid skim milk gels and the level of non-sedimentable κ-casein in heated (80 °C/30 min), pH-adjusted skim milk. ●: 10% TS milk samples; ▼: 15% TS milk samples; ■: 20% TS milk samples; ♦: 25% TS milk samples.

produced acid gels with a higher firmness and breaking stress than those where the denatured whey proteins were predominantly associated with the casein micelles for all milk concentrations. Recent studies on acid gels prepared from unconcentrated milks have suggested that the soluble denatured whey proteins are important in the formation of acid gel structures (Anema et al., 2004; Donato, Alexander, & Dalgleish, 2007; Rodriguez del Angel & Dalgleish, 2006).

The relative firmness (final $G'$) of particulate gels can be related to the number of contact points and the strength of these contact points (van Vliet & Keetals, 1995). At any given pH during heating, increasing the milk concentration will increase the number of particles, strands, and contact points within a given unit area, and this will increase the gel firmness. Changing the pH of the milk at heating changes the level of serum-phase κ-casein and denatured whey proteins (Fig. 3). For milk samples heated at high pH, the denatured whey proteins and much of the κ-casein are in the serum phase and therefore there are considerably more aggregating particles, and the potential for more complex acid gel formation than for the milk samples heated at low pH, where most of the denatured whey proteins and κ-casein are associated with the casein micelles. In the latter case, the acid gel process will probably involve only entire whey protein/casein micelle complexes. Therefore, there may be fewer contact points in the acid gels formed from milk with the denatured whey proteins associated with the micelles than in those formed from milk with soluble denatured whey proteins, and hence a gel with a lower firmness is observed.

Large strain deformation properties could give some indication of the types of bonding involved in the gel network. The breaking strain for the acid gels at each milk concentration did not change markedly with the pH during heating. In contrast, the breaking stress was markedly affected, increasing with increasing pH (Fig. 2, Table 1). It has been reported that, before a gel will break, all strands within the network must be straightened and breaking results in the rupture of these strands (or bonds within) as stretching is continued (Mellema, van Opheusden, & van Vliet, 2002; van Vliet & Keetals, 1995). In this scenario, the breaking strain will be dependent on the degree of curvature of the strands, giving a higher breaking strain with higher strand curvature as these strands will need to be straightened and then stretched before breaking. At each milk concentration, the observation that the breaking strain did not change with the pH during heating, despite the marked change in final $G'$, indicates that the relative curvature of the individual strands within the gel network was not affected by the pH during heat treatment. However, the increase in breaking strain as the milk was concentrated suggests that there was a greater degree of strand curvature as the milk concentration increased.

In contrast to the breaking strain, the breaking stress of the set gels was markedly dependent on the pH during heating (Fig. 2, Table 1). The type of bonding involved in the gel network will have an influence on the breaking stress (Mellema et al., 2002). The rupture of strands containing covalent bonds would require a greater force than those held together by noncovalent bonds as covalent bonds have higher bond energies. Therefore, a change in the number or distribution of covalent bonds within the gel network may explain the differences in breaking stress as the pH of the milk during heating was changed.

As the levels of whey protein denaturation were similar in samples at all milk concentrations, regardless of the pH during heating (Fig. 3) and as all samples were adjusted back to the natural pH before acidification, it seems unlikely that the difference in breaking stress can be due to a greater degree of disulfide bonding within the gelled sample (although there may be continuing thiol–disulfide exchange reactions occurring during acidification (Vasbinder, Alting, Visschers, & de Kruif, 2003), the physical number of disulfide bonds is unlikely to be markedly different). The denatured whey proteins, along with some of the κ-casein, are progressively transferred to the serum phase when the pH of the milk is increased before heating (Fig. 3). As these interactions involve disulfide bonding, this indicates that the interaction between the denatured whey proteins and κ-casein is transferred from the colloidal phase (casein micelle) to the serum phase. On subsequent acidification, both non-sedimentable and colloidal phase denatured whey proteins are incorporated in the acid gel structure.

The non-sedimentable denatured whey protein/κ-casein complexes can form strands that may be involved in interconnecting the colloidal particles. As the non-sedimentable aggregates are disulfide-bonded, those samples heated at high pH and with high levels of non-sedimentable whey protein/κ-casein aggregates will have a greater number of these strands interconnecting the residual casein micelles. In contrast, the samples heated at lower pH will have the denatured whey proteins predominantly associated with the casein micelles and therefore fewer of the whey protein/κ-casein aggregates interconnecting the colloidal particles. Therefore, the samples heated at higher pH may have a greater number of disulfide bonds interconnecting the colloidal particles and therefore a higher breaking stress, whereas, for the samples heated at lower pH, most of the disulfide bonds are on the colloidal particles and fewer disulfide bonds interconnect the colloidal particles, and this may explain the lower breaking stress.

As the milk is concentrated, similar proportions of the denatured whey proteins/κ-casein are distributed between the colloidal and serum phases at any pH during heating relative to the natural pH (Fig. 3). Therefore the relative effects on the breaking stress were similar at all milk concentrations (Fig. 2C). However, as the concentration of all components is increased, the number of interconnecting particles and strands will increase per unit area of gel structure. Therefore the breaking stress versus pH at heating curves would be expected to increase to higher stresses as the milk concentration is increased (Fig. 2C, Table 1).

This study has demonstrated that adjusting the pH of milk prior to heat treatment can influence the final acid

gel properties, and that similar effects are observed at all milk concentrations. It is possible to alter the final acid gel firmness ($G'$) and final breaking stresses over a very wide range at each milk concentration, so that some overlap in these properties could be achieved for milks at different concentrations. At all milk concentrations, the firmness of the acid gels appears to be related to the distribution of the denatured whey proteins and κ-casein between the serum and colloidal phases. Increasing the pH prior to heat treatment increased the level of non-sedimentable denatured whey protein and κ-casein on heating and this produced acid gels with markedly higher firmness than those heated at lower pH and with markedly lower levels of non-sedimentable denatured whey proteins.

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