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Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels

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Abstract

In this study, the effects of heat treatment of milk on the rheological properties and microstructure of acid milk gels formed by the hydrolysis of glucono-δ-lactone were investigated. Gels were formed from reconstituted skim milk, with or without added whey protein concentrate (WPC), which had been heated at temperatures in the range 75–90°C for 15 or 30 min. The rheological properties of these gels were determined using dynamic low-amplitude oscillation in the Bohlin rheometer. The large deformation and fracture properties were studied using a low, constant shear rate method with gels formed in the rheometer. Microstructure was determined using confocal scanning laser microscopy and permeability measurements. Heating milk above 80°C resulted in an increase in the pH of gelation, a reduction in the gelation time and a marked increase in the storage modulus compare to unheated milk. Addition of WPC to milk followed by heat treatment at 80°C caused further increase in pH of gelation, reduction in gelation time and increase in storage modulus. Heat treatment resulted in a large decrease in shear deformation at fracture. The microstructure of gels made from unheated milk appeared to be irregular with areas of dense protein clusters. In contrast, the gels made from heated milk had a microstructure that appeared more regular with clearly defined pores and thinner, straighter strands compared to unheated milk. The large differences in the rheological properties at small and large deformations also suggested that the microstructure of gels was considerably altered by heat treatment of milk. These results are discussed in terms of denaturation of whey proteins and subsequent interactions of proteins occurring during acidification and gel formation. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Acid milk gels; Heat treatment; Rheological properties

1. Introduction

Yoghurt is one of the most popular fermented milk products. The formation and physical properties of acid milk gels have been reviewed (Lucey & Singh, 1997). Protein fortification and heat treatment of milk are two of the most important processing parameters affecting the textural properties of yogurt. Whey protein concentrate (WPC) has been added to milk for yogurt manufacture to reduce whey separation and increase the firmness and viscosity of yogurt (Robinson & Tamime, 1986). However, little is known about the effects of added whey proteins on the fundamental rheological properties of acid milk gels. Usually, empirical penetration tests have been used to characterise firmness of yogurt or the viscosity of the disturbed system has been measured (e.g. Dannenberg & Kessler, 1988), although the relevance of viscosity in a 'set' gel is unclear.

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Fundamental studies on the effects of heat on the dynamic rheological properties of acid milk gels have been reported (van Vliet & Keetels, 1995; Lucey, Teo, Munro & Singh, 1997). Heat treatment of milk increases the storage moduli (G') but decreases the shear deformation at yielding of acid milk gels (van Vliet & Keetels, 1995; Lucey et al., 1997). Lucey et al. (1997) suggested that denatured whey proteins in heated milk become susceptible to aggregation during acidification, as the isoelectric points of whey proteins are approached. Denatured whey proteins associated with the micelles (or in the serum) after heat treatment could act as bridging material by interacting with other denatured whey proteins associated with casein micelles. It was proposed that cross-linking or bridging, by denatured whey proteins, within gels made from heated milk was responsible for the large increase in G' (Lucey et al., 1997).

The objective of the present study was to investigate the effects of added WPC and heat treatment of milk on the rheological properties of acid gels.

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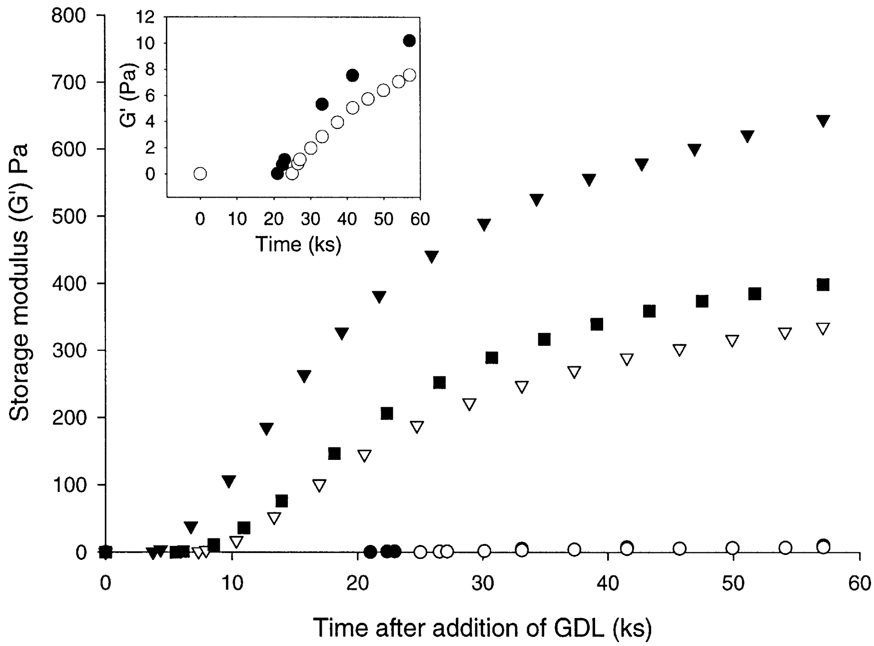

Fig. 1. Storage modulus (G') of acid milk gels made at 30°C with glucono-δ-lactone from unheated milk (●), unheated milk with 1% WPC added (○), heated milk (■), milk with 1% WPC added and then heated (▼) and milk that was heated and then 1% WPC added (▽). Heat treatment was 80°C for 30 min.

2. Materials and methods

Ultra-low heat skim milk powder was reconstituted to 10.7% total solids in demineralized water, allowed to equilibrate overnight at room temperature and NaN_3 (0.02%) was added to prevent bacterial growth.

A commercially available WPC powder (ALACEN 193) was used in this study. This WPC powder contained 79.8% protein, 4.1% moisture, 6% fat and 5.5% ash. Reconstituted skim milk was fortified with 1% of this powder with mixing for approximately 1 h at ambient temperature (~22°C). Milk with added WPC was then heated at 80°C for 30 min in water baths. In some cases milk was first heated at 80°C for 30 min before addition of WPC.

Milks were heated (unless otherwise stated) at 80°C for 30 min as described by Lucey et al. (1997). Milks were acidified by addition of 1.3% (w/w) glucono-δ-lactone (GDL) at 30°C. The pH of the gels was ~4.5 to 4.6 after 16 h. The rheological properties, at small and large deformations, were determined as described by Lucey et al. (1997). A Bohlin VOR Rheometer operating in dynamic low-amplitude oscillation mode was used to determine G' every 10 min for 16 h. Samples were oscillated at 0.1 Hz and the maximum strain applied was <0.01. The large deformation properties were determined on gels made in situ and fractured 16 h after addition of GDL at 30°C. Gels were subjected to a low ($0.00185\ s^{-1}$), constant shear rate up to yielding of the gel, defined as the point when the shear stress started to decrease.

3. Results

In agreement with Lucey et al. (1997), heat treatment of reconstituted milk at 80°C for 30 min caused a marked increase in G' (Fig. 1). Addition of WPC to milk (1% w/w) followed by heat treatment at 80°C for 30 min resulted in a large increase in G'. Addition of WPC to milk that had already been heated resulted in the acid gel having a lower G' value compared to milk that had WPC added prior to heat treatment. The effects of WPC addition on the gelation properties are shown in Table 1.

Table 1
Effects of addition of whey protein concentrate (WPC) and heat treatment on the gelation properties of acid milk gels made at 30°C with glucono-δ-lactone (GDL)

Milk sample	Gelation			
	pH		Time (ks)	
	Mean	SD	Mean	SD
Control (unheated)	4.83	0.01	22.8	1.8
Unheated with 1% WPC	4.76	0.07	26.5	0.9
Heated at 80°C for 30 min	5.17	0.02	7.3	0.4
Heated at 80°C for 30 min then 1% WPC added	5.19	0.03	7.9	0.2
Milk with 1% WPC added then heated at 80°C for 30 min	5.24	0.02	5.0	0.5

Note. Gelation was defined as the point when a storage modulus (G') of ≥ 1 Pa was attained; means from two replicates.

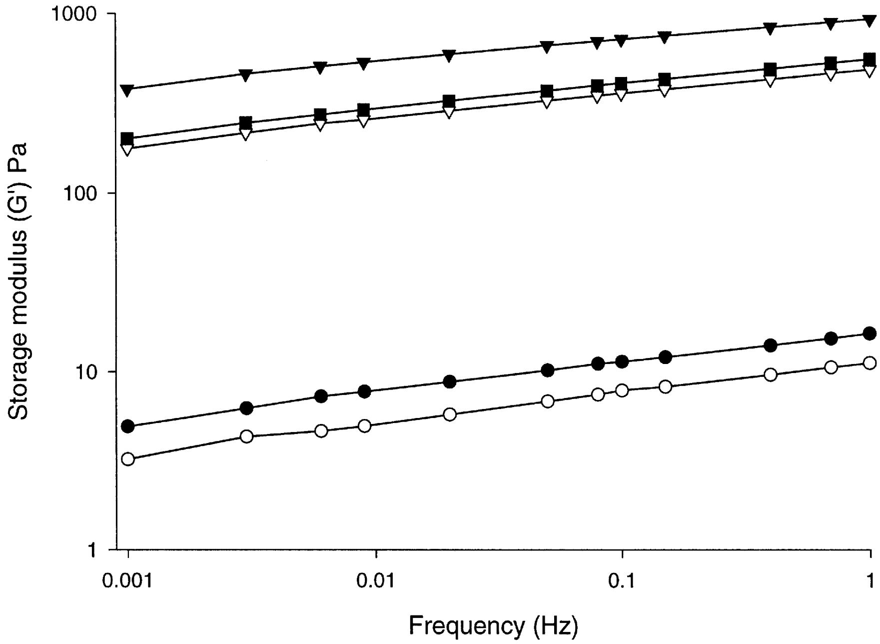

Fig. 2. Storage modulus (G') as a function of frequency for acid milk gels made at 30°C with glucono-δ-lactone from unheated milk (●), unheated milk with 1% WPC added (○), heated milk (■), milk with 1% WPC added and subsequently heated (▼) and milk that was heated and then 1% WPC added (▽). Heat treatment was 80°C for 30 min.

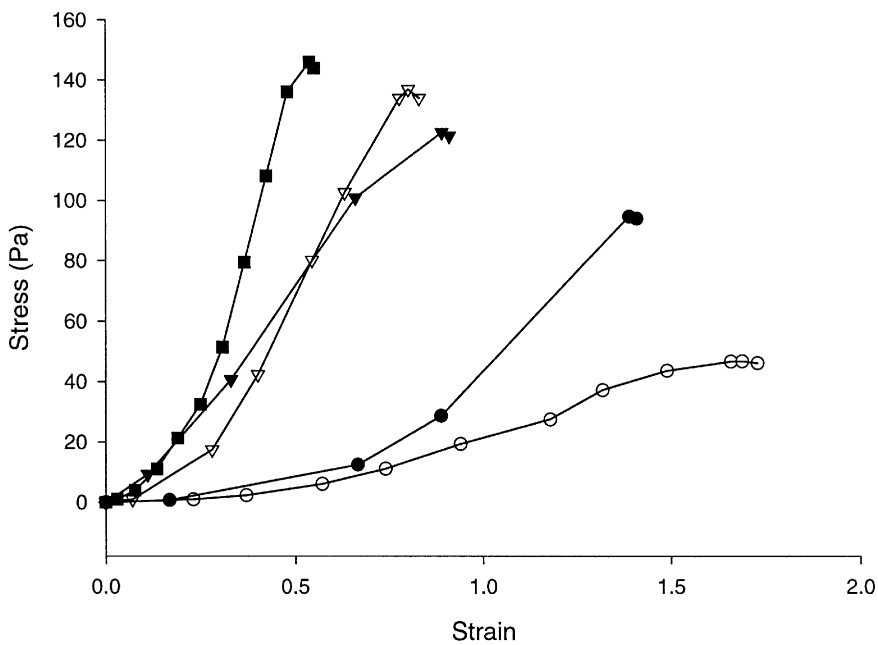

Fig. 3. Shear stress as a function of applied deformation at a low, constant shear rate (0.00185 s^{-1}) for acid milk gels formed at 30°C from unheated milk (●), unheated milk with 1% WPC added (○), heated milk (▼), milk with 1% WPC added and subsequently heated (■) and milk that was heated and then 1% WPC added (▽). Gels were formed by acidification with 1.3% glucono-δ-lactone (GDL) and analysed approximately 16 h after addition of GDL. Shearing was stopped when the stress started to decrease, which was taken as the yield point. Heat treatment was 80°C for 30 min.

Heated milk had a shorter gelation time and higher pH at gelation than unheated milk, as was expected. Addition of whey protein to milk and subsequent heat treatment resulted in a decrease in the gelation time and an increase in the gelation pH. Addition of WPC to milk that had already been heated resulted in an increase in the gelation time and a decrease in the gelation pH compared to milk that had WPC added prior to heat treatment. The addition of WPC to unheated milk resulted in a decrease in G'.

The effects of varying the time scale of the applied deformation on the G' of acid gels are shown in Fig. 2. Log G' versus log frequency gave linear curves with a slope of $\sim$0.15, which is in agreement with values reported for other types of acid casein gels (Roefs & van Vliet, 1990).

The effects of WPC addition on the large deformation properties of acid milk gels are shown in Fig. 3. Heat treatment of milk at 80°C for 30 min resulted in a large reduction in the shear deformation at fracture and an increase in the shear stress at yielding. The shear stress at yielding was reduced and the shear deformation at fracture was increased in gels made from unheated milk with added WPC. Heat treatment of milk, with and without the addition of WPC, decreased the shear deformation at yielding (i.e., gels became more brittle).

The effects of heat treatment on the microstructure of acid milk gels is shown in Fig. 4. Gels made from unheated milk appeared to have a 'tortuous' clustered network whereas gels made from heated milk had a 'branched' kind of network (Lucey et al., 1997b).

4. Discussion

The present results showed that native whey proteins do not contribute to the gel matrix, since addition of WPC to milk resulted in a reduction in the G' and shear stress at yielding. This suggests that native whey proteins may act as an inert filler in acid milk gels. Greig and Van Kan (1984) noted that the addition of WPC to yoghurt milk after the milk had been heated resulted in 'poor' gel formation. In fresh acid cheese made using ultrafiltration (UF), Mahaut and Korolczuk (1992) concluded that native whey proteins had little effect on consistency, however, denaturation of whey proteins (those present in milk and added WPC) during heat treatment of the milk resulted in an increase in viscosity.

When milk is heated to high temperatures (e.g., 80°C for 30 min) whey proteins are almost completely denatured and some of the denatured whey proteins associate with the casein micelles, involving κ-casein via thiol-disulphide interchange (Singh & Creamer, 1992). During acidification, the denatured whey proteins (both those associated with the casein micelles and those in the serum), may aggregate, as the isoelectric points of the whey proteins are approached (Lucey et al., 1997). This results in increased cross-linking or bridging within the gels, and is responsible for the increase observed in G'. Addition of WPC to milk followed by heat treatment would denature the whey proteins of WPC which subsequently can interact with casein micelles or the original whey proteins in milk. In acid gels made from milk that was heated prior to the addition of WPC the original whey proteins in milk were almost completely denatured and contributed to an increase in G' however, the added WPC remained undenatured and probably acted as a filler in the gel matrix causing a reduction in G' compared to heated milk.

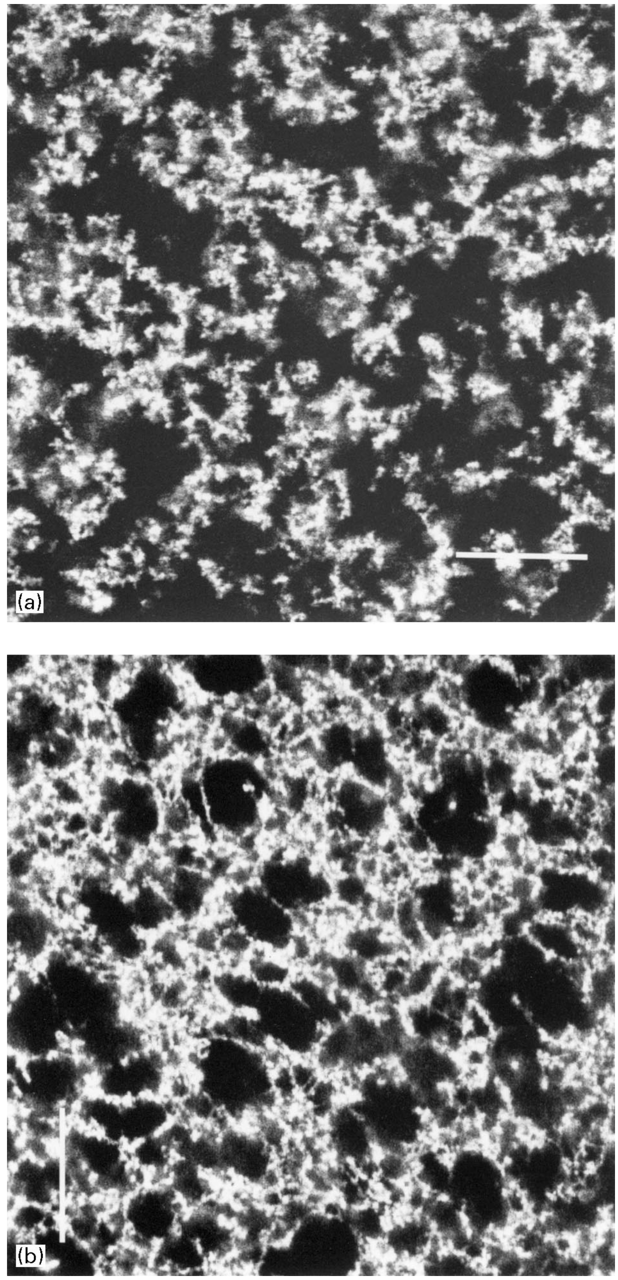

Fig. 4. Confocal scanning laser micrographs of acid milk gels made at 30°C by acidification with 1.3% glucono-δ-lactone (GDL) from unheated milk (a) and heated milk (b). Heat treatment was 80°C for 30 min. Bar = 20 μm. (taken from Lucey et al., 1998, *Food Hydrocolloids*, permission requested).

This work suggests that denatured whey proteins that are associated with casein micelles during heat treatment may act as bridging material by interacting with other denatured whey proteins. This is likely to have caused the branched microstructure observed in acid gels made from heated milk (Lucey, Teo, Munro & Singh, 1998).

The branched microstructure also contributed to the brittle consistency of heated milk gels. This work demonstrates that the 'state' of whey proteins, both the original proteins in milk and those added as WPC, greatly affects the formation and properties of acid milk gels.

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