

Original article

## A rheological study of acid-set “simulated yogurt milk” gels prepared from heat- or pressure-treated milk proteins

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(Received 2 November; accepted 26 February 2001)

**Abstract** — The application of high pressure as an alternative to heat treatment in the acid-set gelling of milk proteins was studied using a “simulated yogurt milk” (SYM) system, containing phosphocasein and whey protein isolate (WPI) in a ratio of 4:1. Gels were made by acidification of SYM with glucono- $\delta$ -lactone (GDL) at 40  C to pH 4.6 and their properties measured by dynamic rheology using a Bohlin CVO rheometer. Gelation was studied in heat – (90  C  $\times$  10 min) or pressure – (700 MPa  $\times$  20 min) treated SYM or SYM containing heat – (78  C  $\times$  30 min) or pressure – (0–700 MPa  $\times$  20 min) treated WPI. For a constant time (20 min) and temperature (25  C), the extent of whey protein denaturation was dependent on the applied pressure. Although pressures of  $\leq$ 400 MPa caused as much as 57% denaturation, they did not support acid-set gelation when pressure-treated WPI was incorporated into SYM. Pressurisation of WPI at 600 and 700 MPa, which resulted in 86.5 and 91.4% denaturation, respectively, resulted in the formation of cohesive gels when SYM was acidified with GDL. The acid-induced gelation profiles of SYM pressurised at 700 MPa  $\times$  20 min and SYM containing WPI pressurised under the same conditions were different, suggesting that the kinetics of aggregation were different, presumably due to the disruption of casein micelles in the SYM system during the pressurisation step. Gels prepared from SYM containing pressure-treated WPI were weaker, i.e., they had lower values for  $G'$  throughout acidification, than those prepared from SYM containing heat-treated WPI. The gelation properties of heated SYM containing native or pressurised WPI were similar, indicating that the combination of pressurisation of WPI followed by heating SYM does not have an additive effect in relation to acid-induced gelation. Heating was more efficient at producing casein/whey protein interaction products that were suitable for the formation of gels on acidification.

high pressure / heat treatment / whey protein isolate / phosphocasein / acid gel

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**R sum  —  tude rh ologique de gels acides simulant le yaourt pr par s   partir de prot ines laiti res trait es thermiquement ou par hautes pressions.** L'application de hautes pressions comme alternative au traitement thermique pour la g lification acide de prot ines laiti res a  t   tudi e sur un syst me   l'aide d'un lait simulant le yaourt (SYM), contenant de la phosphocas ine et un isolat de prot ines de lactos rum (WPI) dans un rapport 4:1. Les gels ont  t  obtenus par acidification du SYM par la glucono-delta-lactone (GDL)   40  C,   pH 4,6, et leurs propri t s mesur es par rh ologie dynamique   l'aide d'un rh om tre Bohlin CVO. La g lification a  t   tudi e sur le SYM trait  thermiquement (90  C – 10 min) ou par hautes pressions (700 MPa – 20 min) ou sur le SYM contenant le WPI trait  thermiquement (78  C – 30 min) ou par hautes pressions (0–700 MPa – 20 min). Pour un temps de pressurisation constant (20 min) et une temp rature constante (25  C), l' tendue de la d naturation des prot ines de lactos rum  tait d pendante de la pression appliqu e. Bien que les pressions  $\leq 400$  MPa provoquaient jusqu'  57 % de d naturation du WPI, elles ne conduisaient pas   la g lification acide quand ce WPI  tait incorpor  au SYM. La pressurisation du WPI   600 et 700 MPa, provoquant respectivement 86,5 et 91,4 % de d naturation, entra nait la formation de gels coh sifs quand le SYM  tait acidifi  avec la GDL. Les profils de g lification acide du SYM pressuris    700 MPa – 20 min et du SYM contenant le WPI pressuris  dans les m mes conditions,  taient diff rents, sugg rant que des interactions diff rentes avaient lieu, probablement dues   la dissociation des micelles de cas ine dans le SYM au cours de la pressurisation. Les gels pr par s   partir de SYM contenant le WPI trait  par hautes pressions  taient plus mous, i.e. avaient des valeurs de  $G'$  plus basses tout au long de l'acidification, que ceux pr par s   partir de SYM contenant le WPI trait  thermiquement. Les propri t s de g lification du SYM chauff  contenant le WPI natif ou pressuris   taient similaires, indiquant que la combinaison de la pressurisation du WPI suivie du chauffage du SYM n'a pas d'effet suppl mentaire sur la g lification acide. Le chauffage  tait plus efficace que les hautes pressions sur l'obtention de produits d'interaction cas ine/prot ine de lactos rum favorables   la formation de gels acides.

**haute pression / traitement thermique / isolat de prot ines de lactos rum / phosphocas ine / gel acide**

## 1. INTRODUCTION

Heat treatment of milk is one of the most important processing parameters affecting the texture and consistency of yogurt [25]. High heat treatment of milk ( $> 70$   C) causes unfolding and aggregation of whey proteins, some of which interact with casein micelles, involving  $\kappa$ -casein [35, 36]. These whey proteins appear as appendages or filaments on the micellar surface in electron micrographs [18]. Denatured whey proteins could act as bridging material by interacting with the whey proteins which are associated with the micelles, which would increase the number and strength of bonds between protein particles. The concentration of potential gelling protein would also be increased due to active participation of denatured whey protein in the

gel structure [22]. While denatured whey proteins are known to affect the formation of acid milk gels [22], the mechanism by which they affect the rheological properties is not adequately explained. The denatured whey protein load on the casein micelle and the degree of whey protein aggregation both at the casein micelle surface and in the serum phase are two major areas requiring elucidation. Simulating yogurt milk using phosphocasein, WPI and lactose-free simulated milk ultrafiltrate (SMUF) has proven to be a useful tool for studying casein/whey protein interactions during acidification [28]. It is commonly accepted [22, 23, 28] that whey protein denaturation and subsequent interaction with micellar casein is a prerequisite for optimal structure development during acidification and subsequent stability to syneresis. It has already been

shown that when pre-denatured whey proteins (10% w/w WPI, heated 78 °C × 30 min, pH 7) are introduced to phosphocasein/SMUF suspensions at room temperature, and the mixture acidified to pH 4.6, gels with a high storage modulus are formed [28]. However, the inclusion of soluble denatured whey protein aggregates (SDWP), formed by heat treatment of milk serum following high speed centrifugation, had relatively little effect on the rheological properties of acid milk gels made from ultra-low-heat SMP, possibly because they did not interact with casein particles during acidification [23].

In the search for new and alternative techniques to replace classical heat treatments in the food industry, high pressure processing (HPP) is attracting increasing attention. As a result, the use of high pressure (100–1000 MPa) to induce denaturation, aggregation and gel formation of milk proteins has been the subject of much recent research [1, 4, 5]. The behaviour of proteins under pressure is governed by the principle of Le Chatelier [2]. The principle states that any reaction accompanied by a decrease in volume is enhanced by an increase in pressure and vice versa. Hence, hydrophobic interactions and ionic effects are liable to disruption by high pressure while the formation of hydrogen bonds is favoured by high pressure [3]. Since these bonds contribute to protein conformation and structural interactions in solution, any changes associated with them will result in modifications to the overall structure of the protein matrix. Covalent bonds, on the other hand, appear not to undergo any changes as a result of high pressure treatment. Moderate pressures ( $\leq 150$  MPa) favour dissociation of oligomeric proteins while pressures higher than 150–200 MPa induce unfolding of proteins and reassociation of subunits from dissociated oligomers.

Milk processing at 150–400 MPa caused some irreversible dissociation of casein mi-

celles, together with calcium release, increased milk viscosity and decreased milk turbidity [6, 11, 34]. The decrease in the non-casein nitrogen fraction of skim milk subjected to pressures of 200–600 MPa for periods of up to 2 h, observed by Johnston et al. [16] was attributed to aggregation of the whey proteins and/or their association with the dissociated casein polymers. These authors also observed that exposure of hydrophobic groups increased with increasing severity and duration of pressure, indicating that considerable irreversible protein unfolding was occurring; this effect persisted for at least 8 d at 5 °C. While the exact changes in conformational state of the caseins and whey proteins is not known, the gel strength and water-holding capacity of acid-set gels is improved and there is an increased resistance to syneresis after milk pressurisation [17]. However, these results were compared to unheated milk where acid gel strength was low and syneresis high.

Dumay et al. [8] showed that high pressure processing at 450 MPa for 15 min at pH 7.0 induced partial unfolding and aggregation of a  $\beta$ -lactoglobulin ( $\beta$ -Lg) isolate, upon treatment of aqueous solutions containing 2.5 or 5.0% (w/w) protein. Soluble protein aggregates with molecular weight in the range 36 to 103 kDa were formed as a result of pressure processing. Aggregation was partly reversible with storage time after pressure release. Dufour et al. [7] found that  $\beta$ -Lg unfolded extensively and irreversibly at pH 7, but less extensively and reversibly at pH 3, after pressure treatment in the 150–300 MPa range. The results of several other studies [10, 12, 37] have indicated that unfolding of  $\beta$ -Lg using pressure treatments at neutral pH, increased the reactivity of the SH group, and that intermolecular disulphide bonds formed through SH/S-S interchange reactions prevented reversible unfolding. Studies performed by Hinrichs et al. [11] revealed that, at the same pressure, the rate of denaturation of  $\beta$ -Lg was faster than that of  $\alpha$ -La.

Recent studies in relation to high pressure processing have included investigations regarding its effect on the gelation of milk proteins, in particular whey proteins. Van Camp and Huyghebaert [39] reported that pressure treatment of 400 MPa  $\times$  30 min induced gel formation of WPC at neutral pH at a minimum protein concentration of 11% (w/v). Kanno et al. [19] noted that >10% (w/v) protein was required for the gelation of WPI (pH 6.8) on pressurisation to 600 MPa, however for WPC at the same pH, the viscosity changed at a concentration of >12% (w/v), and gel formation started at >18% (w/v) at 400 MPa. The strength of WPI gels at a protein concentration of 20% was three times greater than WPC gels at the same protein concentration. Pressure-induced whey protein gelation is significantly influenced by pH and salt [40, 41].

This aim of this study was to compare the effects of heat and pressure treatments on whey proteins at neutral pH and their subsequent interaction with casein during the formation of acid gels.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Whey protein isolate (WPI) was obtained from Davisco Foods International, Inc. (620 North Main, Le Sueur, MN 56058, USA). The composition of WPI was as follows: 97.4% total protein on a dry weight basis, 0.8% fat, 1.7% ash and 4.3% moisture. Phosphocasein was prepared in-house as described by Kelly et al. [20]. The composition of the phosphocasein powder was as follows: 80.3% total protein, 1.9% lactose, 4.3% fat, 7.9% ash and 5.6% moisture. Glucono- $\delta$ -lactone (GDL) was supplied by Sigma (Sigma Chemical Co., St. Louis, MO 63178, USA).

### 2.2. Preparation of whey protein isolate (WPI)

WPI solution was prepared by adding WPI powder to an aliquot of distilled water and stirring for 1 h at room temperature. The pH was adjusted to 7.0 with 1N KOH

**Table I.** Pressure and/or heat treatments applied to various samples of whey protein isolate or "simulated yogurt milk" (SYM) prior to acidification of SYM with glucono- $\delta$ -lactone.

Sample code	WPI		SYM	
	Pressure $\times$ time	heat $\times$ time	Pressure $\times$ time	heat $\times$ time
	MPa $\times$ min	$^{\circ}$ C $\times$ min	MPa $\times$ min	$^{\circ}$ C $\times$ min
A (control)	–	–	–	–
B	–	–	–	90 $\times$ 10
C	–	78 $\times$ 30	–	–
D	–	–	700 $\times$ 20	–
E	700 $\times$ 20	–	–	–
F	700 $\times$ 20	–	–	90 $\times$ 10
G	600 $\times$ 20	–	–	–
H	400 $\times$ 20	–	–	–
I	250 $\times$ 20	–	–	–

and the remainder of the distilled water was added to give a final protein concentration of 10% (w/v). Untreated WPI or WPI subjected to heat or pressure (see Tab. I) were used to prepare simulated yogurt milk.

### 2.3. Preparation of simulated yoghurt milk (SYM)

WPI (native, pressure- or heat-treated) was added to a phosphocasein dispersion to give a final concentration of 1% whey protein and 4% micellar casein in simulated milk ultrafiltrate (SMUF), prepared according to the method of Jenness and Koops [15]. The pH of the final dispersion (SYM) was adjusted to 7.0 with 1N KOH.

### 2.4. Heat treatments

Two different heat treatments were used, as outlined in Table I. WPI was preheated at 78  C imes 30 min prior to preparation of SYM. Samples of SYM were placed in a waterbath at 90  C for 10 min to simulate heat treatment during yogurt manufacture.

### 2.5. Pressure treatment

Samples of WPI or SYM, intended for pressure treatment, were placed in vacuum pack bags (GB Miller & Son Ltd., Beechwood Close, Boghall Road, Bray, Co. Wicklow, Ireland) and vacuum packed (Webomatic Vacuum Packaging Systems, Bochum, Germany). Each sample was then placed in a second vacuum pack bag and again vacuum packed to ensure no contact between the hydrostatic pressurisation fluid (15%, v/v, castor oil in ethanol) and the sample. Pressure treatment was carried out in a high pressure rig (Stansted Fluid Power Ltd., Stansted, UK) of chamber size 37 mm diameter and 300 mm length. Samples were subjected to various pressure treatments (Tab. I) at 25  C. Pressure was

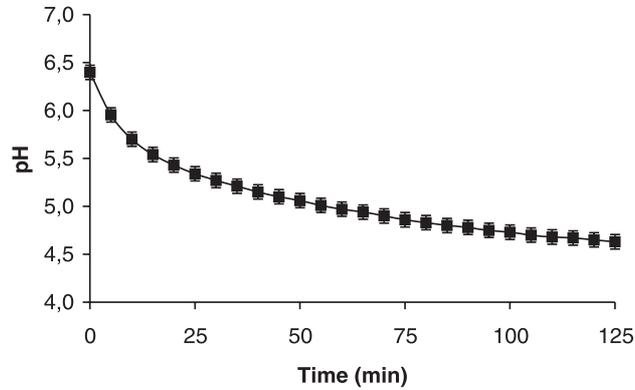
achieved in  $\leq 120$  s and depressurisation took  $\leq 60$  s. Water from a thermostatically controlled waterbath was circulated through the jacketed chamber of the pressure rig to control the temperature. The rig was equipped with a thermocouple to continuously monitor the temperature of the pressurisation fluid and samples.

### 2.6. Whey protein denaturation

The percentage protein in control or heat/pressure-treated WPI that was insoluble at pH 4.6 [14] was taken as a measure of the level of denatured protein in the sample. The analysis was carried out immediately after depressurisation in the case of the pressure-treated samples. Sample (30 mL) was transferred to a 100 mL volumetric flask, 50 mL of distilled water at 50  C and 3 mL of acetic acid (10%, v/v) were added and the mixture left stand for 10 min. Sodium acetate (3 mL, 1N) was then added and the mixture cooled to room temperature before being made up to volume with distilled water. The pH was 4.6 after addition of the sodium acetate. The solution was filtered through Whatman No. 42 filter paper and the protein content of the filtrate (A) was determined using the Macro-Kjeldahl method [13]. The total protein content of the sample (B) was also determined. The difference between A and B, expressed as a percentage of total protein, was taken as the amount of denatured protein in the sample.

### 2.7. Gel formation

Freshly prepared samples of SYM were acidified at 40  C with 2% (w/w) GDL. Pressure-treated whey protein samples were utilised within 30 min of depressurisation. The pH of the samples was measured at 1 min intervals with an Orion Ross pH8115SC combination electrode attached to an Orion model 420A pH meter (Orion



**Figure 1.** Rate of acidification of SYM at 40 °C after addition of glucono- $\delta$ -lactone (GDL) at a level of 2% (w/w). Values are means  $\pm$  S.D. for triplicate analyses.

Research, Inc., Beverly, MA 01915-6199, USA). The pH reached  $\sim$ 4.6 after 2 h (Fig. 1).

as the point at which  $G' \geq 1.0$  MPa. The final gel strength was defined as  $G'$  after 120 min.

## 2.8. Rheological properties of SYM

A controlled strain Bohlin CVO rheometer (Bohlin Instruments, Cirencester, UK) was used in the dynamic mode for small scale deformation measurements. A concentric cylinder (C25) measurement system was used. The diameter of the bob was 25 mm and the internal diameter of the cup was 27.5 mm. All measurements were taken at 40 °C. Measurements were taken at a frequency of 1 Hz and at a maximum strain of 0.0103. GDL (2%, w/w) was added to a sample of SYM and 13.2 g was weighed into the cup and inserted into the rheometer. A silicone-based oil (n-Tetradecane, Sigma Chemical Co., St. Louis, MO 63178, USA) was added to the surface of the rheometer cup to prevent evaporation.  $G'$  (elastic modulus),  $G''$  (viscous modulus) and  $\tan \delta$  (ratio between  $G''$  and  $G'$ ) were measured at 1 min intervals at 40 °C over a 120 min period. All experiments were duplicated. A duplicate sample was routinely monitored for the effect of GDL on the pH of the sample over time. The gelation time and pH were taken

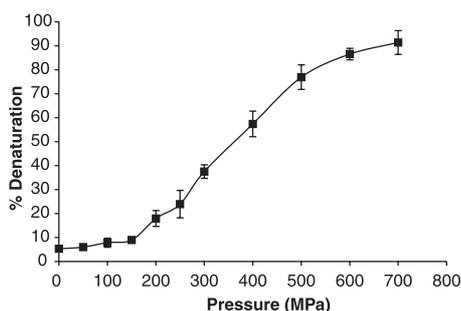
## 3. RESULTS AND DISCUSSION

While heating is the normal processing parameter used for milk in the production of yogurt, the utilisation of high pressure processing as a means of modifying protein behaviour, and therefore the textural attributes of yogurt, is of practical importance. In the manufacture of traditional quarg, unheated milk is acidified at lower temperatures in the presence of a low concentration of rennet to aid in increasing the firmness of the final gel prior to centrifugal separation. Experiments outlined in Table I were performed to determine the effect of pressure on the ability of milk proteins to create structures on acidification. Pressure and heat treatments were superimposed on a model simulated yogurt milk consisting of 4% phosphocasein, 1% WPI and lactose-free SMUF. This model attempts to duplicate the major reactions and processes that occur during heating/pressurisation and acidification as they occur in milk. The casein/whey protein ratio is 80/20 and the ionic strength is regulated by the SMUF concentration.

Since milk acid gel structure for yogurt production is dominated by the interaction between casein and denatured whey proteins, the effect of increasing pressure on the irreversible unfolding of whey proteins was initially screened. The effect of pressure on 10% WPI (w/w) solutions on the level of protein denaturation (expressed as the percentage protein insoluble at pH 4.6) is shown in Figure 2. Native WPI contained approximately 5% denatured protein. For a constant time (20 min) and temperature (25  C), pressures of  $\leq 150$  MPa resulted in a small increase in the amount of denatured protein in WPI solutions while increasing pressures above 150 MPa resulted in a steady increase in the level of denaturation. Pressures of  $\geq 400$  MPa were required to achieve  $> 50\%$  denaturation of the whey protein. Extensive denaturation (i.e., 91.4%) was evident after pressure treating WPI at 700 MPa which was comparable to, although slightly lower than, that caused by heating at 78  C  $\times$  30 min (i.e., 93.7%). These results are comparable to those of Needs et al. [26] who reported that both heat (80  C  $\times$  20 min) and pressure (600 MPa  $\times$  15 min) treatments caused  $> 90\%$  denaturation of  $\beta$ -lactoglobulin in skim milk. In an earlier study, Van Camp et al. [40] found that the amount of native  $\beta$ -lactoglobulin in WPC was reduced signifi-

cantly after pressurisation at 400 MPa for 30 min, in contrast to  $\alpha$ -lactalbumin, for which no major changes were evident. It was observed in this study that gelation of WPI occurred when pressures of 600 or 700 MPa were used but this process was reversible since the WPI samples returned to the liquid state within a few minutes of pressure release. In contrast, no gelation of WPI (10% w/w, pH 7) occurred on heating to 78  C for 30 min. Kanno et al. [19] reported that, at 600 MPa and pH 6.8, protein concentrations of above 10% (w/v) are needed for the gelation of WPI.

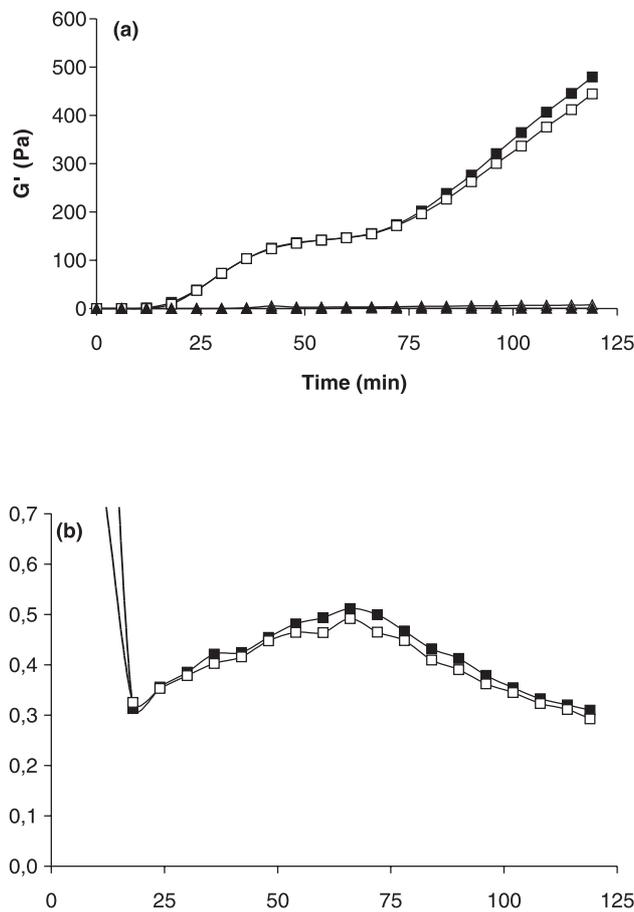
Pressure-treated whey proteins were introduced into the phosphocasein/SMUF suspension at 20  C and the development of the storage modulus during acidification at 40  C was measured, as shown in Figure 3. SYM containing native WPI (i.e., without pressure treatment) did not form a gel upon acidification. Lucey et al. [22] showed that unheated milk or heated serum protein-free milks had very low  $G'$  values ( $< 20$  Pa) after acidification at 30  C with GDL after 15 h. The final pH reached in their study was in the region of 4.5–4.6. Under our set of conditions (2 h, pH 4.6, 40  C) the ageing process may not be sufficiently advanced to register the appearance of what is undoubtedly a very weak gel. It has also been observed that phosphocasein at the 12% protein level forms an acid gel with a storage modulus of 250 Pa after acidification to pH 4.6 over a 4 h period (unpublished). This is an extremely weak gel for such a high protein level. It is probable that the phosphocasein is not exactly the same as native micellar casein, however, phosphocasein powder has been shown to exhibit micellar-like character as regards rennet coagulation [29],  $\beta$ -casein dissociation [30] and acid gelation [9]. Phosphocasein has also been introduced successfully into simulated yoghurt systems to increase the understanding of acid gelation phenomena [28]. Le Ray et al. [21], in a recent study, emphasised the determinant role of the aqueous phase, with



**Figure 2.** Protein denaturation in whey protein isolate (WPI) as a function of pressure at 25  C for 20 min. Values are means  $\pm$  S.D. for triplicate analyses.

special reference to salt type and concentration, on the physico-chemical properties of reconstituted phosphocasein dispersions in the absence of whey proteins. Roefs and van Vliet [31] also reported that increasing ionic strength of cold-acidified skim milk samples resulted in a decrease in the dynamic moduli, confirming that electrostatic interactions were important for acid casein gel formation. In fact, acid casein gel formation could be inhibited if the ionic strength was high enough. Work in progress in this laboratory (unpublished) suggests

that the pH where micellar casein gelation (5% protein) is initiated can vary from 6.2–4.7, depending on the initial ionic strength of the aqueous phase. This model has an obvious limitation in that it shows only the build up in gel consistency and not final gel structure as would be encountered in normal fermented products. However, the primary gelation events encountered during acidification will eventually affect subsequent storage and ageing behaviour. Pressure-treatment of WPI at 250 or 400 MPa for 20 min at 25  C prior to the



**Figure 3.** Elastic modulus [G'] (a) and loss tangent [tan  ] (b) as a function of time for gels made at 40  C with glucono- -lactone from simulated yogurt milk (SYM). Gels were made from SYM containing WPI that was subjected to 250 (Δ), 400 (▲), 600 (□) or 700 MPa (■) for 20 min at 25  C.

preparation of SYM did not induce acid gelation of SYM since no increase in the storage modulus occurred during the 2 h acidification period (Fig. 3a). Pressure treatment of WPI up to 400 MPa, resulting in 57% denaturation of whey protein, appeared to be insufficient for the SYM to form a gel upon acidification. Pressurisation of WPI at 600 and 700 MPa, which resulted in 86.5 and 91.4% protein denaturation, respectively (Fig. 2), supported the formation of gels when SYM was acidified with GDL (Fig. 3). These samples had similar gelation times and pH of gelation, resulting in maximum storage moduli ( $G'$ ) of 445 and 480 Pa, respectively (Tab. II, samples G, E). At the gelation point, when  $G'$  began to increase,  $\tan \delta$  also increased for both samples and reached a maximum between pH 5 and 4.9 before it decreased to a value similar to that obtained at the onset of gelation (Fig. 3b). Van Marle and Zoon [42] and more recently, Lucey et al. [23] also observed a maximum in  $\tan \delta$  during the formation of acid-set gels produced from heated skim milk. They suggested that this maximum in  $\tan \delta$  may be due to a partial loosening of the weak initial gel network due to a solubilisation of colloidal calcium phosphate, while at lower pH values there would be increased protein-protein interactions between the casein/whey protein particles as the isoelectric point is approached. It was surprising that whey proteins which had been pressure-treated to 400 MPa (57% denaturation) and subsequently incorporated into SYM did not contribute to the formation of an acid gel. It is possible that unfolding of whey proteins, in the form of WPI solutions, may show reversibility characteristics up to a certain pressure threshold. While denaturation analyses were carried out immediately following depressurisation, the formulation of SYM and initiation of the rheological evaluation took up to 30 min after depressurisation. However, no reversibility measurements of whey protein unfolding were undertaken.

Although the pH of gelation for heat-treated ( $90\text{ }^\circ\text{C} \times 10\text{ min}$ ) SYM containing native WPI and SYM containing heat-treated ( $78\text{ }^\circ\text{C} \times 30\text{ min}$ ) WPI differed (Tab. II, samples B, C), the development of the storage modulus during acidification and the final gel strength were quite similar (Fig. 4a). If anything, the initial development of gel structure, as estimated by the increase in the storage modulus ( $G'$ ), was faster where the whey proteins were heat-denatured in the absence of, rather than in the presence of micellar casein. These findings are in agreement with previous results [28]. The ability of pre-denatured whey proteins to interact with casein micelles at relatively low temperatures ( $25\text{--}40\text{ }^\circ\text{C}$ ) in the presence of SMUF results in the initiation of acid gelation at a higher pH (5.82). O'Kennedy and Kelly [28] concluded that this aggregation behaviour resembled "cold" gelation of denatured whey protein/micellar casein complexes, with the pH of gel initiation being dependent on the casein/whey protein ratio and the protein concentration of the dispersion. Further heating of denatured whey protein/casein dispersions was also shown to enhance the final strength ( $G'_{120\text{ min}}$ ) of the acid gel. This is in contrast to the work of Lucey et al. [23], who showed that the inclusion of soluble denatured whey protein aggregates (SDWP), formed by heat treatment of milk serum following high speed centrifugation, had relatively little effect on the rheological properties of acid milk gels made from ultra-low-heat SMP, possibly because they did not interact with casein particles during acidification. The model system used in this study [28] utilised pre-denatured WPI heated ( $78\text{ }^\circ\text{C} \times 30\text{ min}$ ) at pH 7.0 where maximal unfolding ( $> 90\%$  insolubility at pH 4.6) but very small increases in both turbidity and viscosity occurred. The ionic strength of 10% WPI solutions is particularly low and very limited aggregation occurred. Since acid gels with storage moduli in the range of 600 Pa were obtained when pre-denatured whey protein was mixed

with phosphocasein, (in SMUF, 80/20 ratio, 5% protein) it was concluded that the aggregation state of denatured whey proteins was an important determinant of subsequent gel strength on acidification. The results highlight the fact that denaturation of whey protein by heat has a major role to play in gelation during yogurt manufacture.

The pH of gelation (5.5) was similar for SYM pressurised at 700 MPa  $\times$  20 min and SYM containing WPI that had been pressurised under the same conditions (Tab. II, samples D, E). However, changes in the storage modulus ( $G'$ ) and  $\tan \delta$  during acidification and the final gel strength of these two systems were different. (Fig. 4a, b).  $\tan \delta$  went through a maximum in acid gels made from both formulations, however, when the micellar casein/whey protein mixture was subjected to pressure (D) this maximum occurred at a higher pH ( $\sim$ 5.2). When the whey protein was pressure-treated separately and subsequently added to non-pressure-treated micellar casein (E), the maximum in  $\tan \delta$  occurred at  $\sim$ pH 5.0. The maximum in  $\tan \delta$  has been attributed to

a partial loosening of bonds between protein particles in the gel network due to gradual solubilisation of colloidal calcium phosphate and release of  $\text{Ca}^{2+}$  from the casein micelle in the pH range 6.0–5.3 [24]. This solubilisation of calcium phosphate may alter the balance between viscous and elastic components in the gel network and lead Lucey et al. [24] to suggest that a maximum in  $\tan \delta$  would occur in any milk system that had a gel point at pH values  $\geq$  5.3, where there was subsequent acid production. At pH values acid to the pH of maximum  $\tan \delta$  there was an obvious increase in the rate of increase in  $G'$  probably due to an increase in electrostatic interactions between the protein particles. The appearance of a maximum in  $\tan \delta$  results in a change in slope of the  $G'$  versus time (pH) profiles (Fig. 3, 4) which is a reflection on the degree of bond/strand rearrangement occurring in the network. Where both casein micelles and whey proteins were present during pressure treatment (D) a relatively short period of network bond and strand rearrangement occurred (pH 5.5–5.2) com-

**Table II.** Gelation properties of various "simulated yogurt milk" samples during acidification with glucono- $\delta$ -lactone (GDL, 2%, w/v) at 40  $^{\circ}$ C for 2 h. Values are means of duplicate analyses.

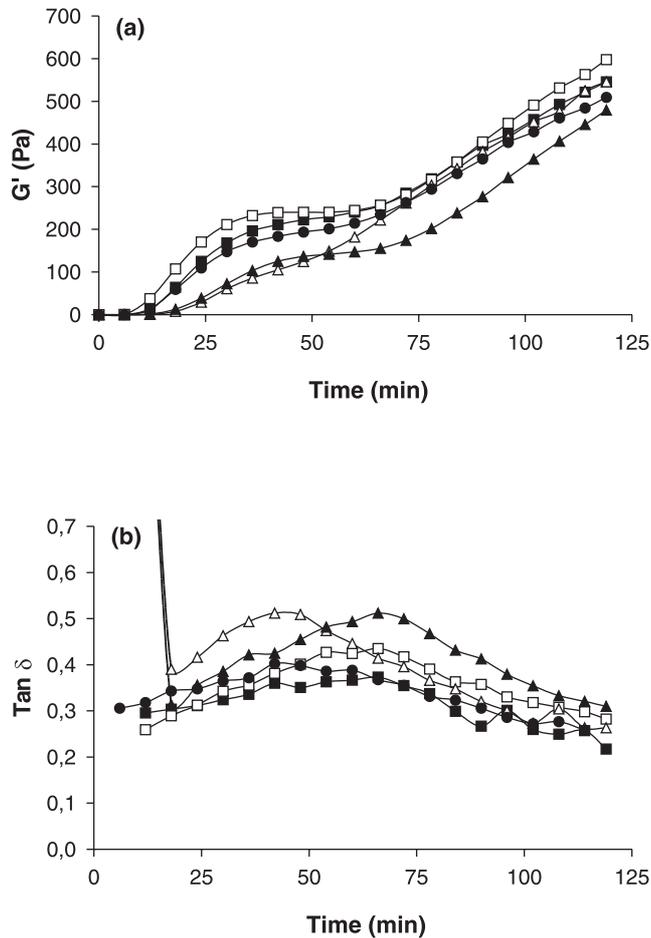
Sample Code*	Gelation time** (min)	pH of gelation	Maximum $G'$ *** (Pa)
A	no gel	no gel	no gel
B	9.5	5.72	546
C	6.5	5.83	598
D	15.0	5.55	546
E	15.0	5.56	480
F	9.5	5.72	510
G	15.5	5.55	445
H	no gel	no gel	no gel
I	no gel	no gel	no gel

Results are means of duplicates.

\* See Table I for description of each sample.

\*\* Gelation time was the time at which  $G' > 1.0$  Pa.

\*\*\* Maximum  $G'$  was that recorded 2 h after addition of GDL.



**Figure 4.** Elastic modulus [ $G'$ ] (a) and loss tangent [ $\tan \delta$ ] (b) as a function of time for gels made at 40 °C with glucono- $\delta$ -lactone from simulated yogurt milk (SYM). Gels were made from B (■), C (□), D (△), E (▲) F (●). For details of formulations and treatments, see Table I.

pared to sample E (whey protein only, pressure-treated) where  $\tan \delta$  had a maximum at  $\sim$ pH 5.0 and rearrangement occurred over the pH range 5.5–5.0. These differences were probably due to the disruption of the casein micelles in the SYM system during the pressurisation step. High pressure treatment of milk induced a partial and irreversible dissociation of casein micelles, even after pressure release [32]. The simultaneous dissociation of casein mi-

celles and whey protein unfolding and the possibility of disulphide bond formation between the denatured whey proteins and the caseins could lead to the formation of a range of interaction products, which on pressure release may reverse to a more aggregated state. Structure development during acidification of this casein/whey protein mixture would be different than the structure developed during acidification of casein/whey protein complexes formed

through the introduction of pressure-induced whey protein into an intact micellar casein suspension at room temperature.

Heat-treated (90 °C × 10 min) SYM had a shorter gelation time and a higher pH of gelation than pressure-treated (700 MPa × 20 min) SYM (Tab. II, samples B, D). Furthermore, their acid-induced gelation profiles, evident by changes in  $G'$  and  $\tan \delta$  over the 2 h acidification period (Fig. 4a, b), were considerably different although both systems yielded a maximum  $G'$  value of 546 Pa. These differences may be due to the differential stability of casein micelles to heat and pressure under the conditions used in this study. Micellar casein is known to be stable to coagulation on heating up to 140 °C at or near neutral pH although there is some dissociation of the individual caseins, especially at the higher temperatures. This dissociation, however, does not lead to a decrease in turbidity [27, 38]. On the other hand, relatively low pressures (150–400 MPa) cause partial dissociation of casein micelles and a decrease in turbidity [32, 33]. The state of aggregation of the caseins at the point where whey proteins unfold should dictate the degree and extent of the interactions between the whey protein and the casein fractions. It would seem probable that the development of structure within these heat- and pressure-treated model systems will differ because the type of casein-whey protein interactions will be different.

Acid gels prepared from SYM containing heat-treated WPI had a higher pH of gelation than those prepared from SYM containing pressure-treated WPI (Tab. II, samples C, E). While trends in the gelation patterns for these two systems were similar, values for  $G'$  were markedly lower at all stages of acidification for the pressure-treated system (Fig. 4a). Several workers have reported that gels prepared from pressure-treated WPC display a network that consists of larger aggregates and pores, when compared with those prepared from heat-treated WPC, resulting in a weaker gel

strength [39, 43]. Again, these data would suggest that the interactions between intact casein micelles and denatured whey proteins depends on whether the whey protein has been denatured by pressure or heat.

The introduction of pressure-induced denatured WPI into the model SYM mixture followed by heating the SYM to 90 °C for 10 min (Tab. II, sample F) resulted in similar pH of gelation to those of heated (90 °C × 10 min) SYM containing native whey protein (Tab. II, sample B). These samples also displayed very similar storage modulus ( $G'$ ) profiles (Fig. 4). As previously discussed, storage modulus development in SYM containing pressurised WPI was markedly lower than both of the above systems (Fig. 4). This would indicate that pressure-induced denaturation of whey proteins does not result in the same degree of interaction between the casein and whey protein in the SYM model system as that caused by heat processing. Subsequent heating of the SYM containing pressurised WPI consolidates the interaction.

#### 4. CONCLUSIONS

For a constant time (20 min) and temperature (25 °C), the extent of WPI denaturation was dependent on the applied pressure. In the absence of a subsequent heat-treatment step, low pressure treatment ( $\leq 400$  MPa × 20 min) of WPI did not result in acid-induced gelation of SYM. Pressurisation of WPI at  $\geq 600$  MPa prior to incorporation into SYM or pressurisation of SYM (containing native WPI) at 700 MPa supported the subsequent formation of acid-induced gels. Gels obtained from systems incorporating a pressurisation step were generally weaker than those involving a heat treatment step. The results confirm the fact that denaturation of whey proteins is a prerequisite for the formation of acid gels with  $G'$  values  $> 500$  Pa.

## ACKNOWLEDGEMENTS

The authors wish to thank Phil Kelly for his help with experimental design. This research has been funded by grant aid under the Food Sub-Programme of the Operational Programme for Industrial Development which is administered by the Department of Agriculture, Food and Forestry and supported by national and EU funds.

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