

Improvement of rheological properties of firm acid gels by skim milk heating is conserved after stirring

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The enhancement of the strength of set acid gels by heating milk was related to rheological parameters (water retention capacity, storage modulus) of corresponding stirred gels. To obtain accurate rheological data from stirred gel it was necessary to maintain a constant granulometry of gel particles and to recognize time after stirring as a contributing factor. Two hours after stirring, the gel exhibited a higher storage modulus when milk was heated above 80 °C. A measurement of viscosity of just-stirred yoghurt was sufficient to predict correctly the quality of a stirred gel analysed by viscoelastic measurements. Increased resistance to syneresis of just-stirred gels was related to higher viscosity. The quantity of β -lactoglobulin (β -lg) bound to casein micelles explains the improvement of these gel qualities. We have considered that the structure of the initial firm gel (mesostructure level) was conserved in fragments within the stirred gel. Consequently, the explanation given by various authors for the effect of heating milk on the properties of set gels can also be applied to stirred gels. The same mechanism, described in literature for structure formation of set gels from acidified milk is purposed to explain the role of heating milk on the recovery of gel structure after stirring. The β -lg association with casein micelles during heating favoured micelle connections during the acidification. It also favoured the association of gel fragments after stirring during the recovery in gel structure.

Keywords: Milk heating, stirred yoghurt, rheology, protein denaturation.

Heat treatment of milk improves the firmness of the yoghurt (Kalab et al. 1976; Davies et al. 1978). The texture of acid gels obtained by adding an acidogen (glucono- δ -lactone) is dependent on the preliminary heating of milk (Fox & Mulvihill, 1990).

The effects of heat treatment on the milk protein structure are governed by the conditions of heating (temperature, time, water content, pH). For mild heat treatments (below 100 °C), only structural effects have been observed. It has been known for some time that whey proteins become associated with casein micelles during the heat treatment of milk (Sawyer, 1969). The β -lactoglobulin (β -lg)/ κ -casein association occurs through a disulphide bond exchange at the natural pH of milk or by hydrophobic interactions

below pH 6.5 (Dalgleish, 1990; Rollema & Brinkhuis, 1989). Binding of β -lg onto casein micelles increases the surface hydrophobicity of the micelles (Bonomi et al. 1988; Bonomi & lametti, 1991). Consequently, the β -lg associated with the micelle favours hydrophobic interactions between the casein micelles during the gelation by acidification (Mottar et al. 1989).

The heat degradation of protein also facilitates the growth of lactic bacteria (Lorient et al. 1977). The culture conditions of microorganisms have an important effect on the rheological properties of yoghurt (Rohm & Schmidt 1993; Rohm, 1995; Beal et al. 1999) and it is obvious that microbiology also plays an important role in the gel structure. In our study, the effects of microbiological parameters were avoided by the use of glucono- δ -lactone.

Lucey et al. (1997, 1998a, b, c, 1999) studied the viscoelastic properties, the permeability and the water retention of set gels (firm gels) of acidified skim milk. They observed that the more the milk was heated (72–93 °C for 30 min),

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the higher the storage modulus (G'). Their work confirmed the role of skim milk heating on the structure of acid set gels previously observed by means of large deformation rheological measurements.

These previous studies have examined the effect of milk heating on the rheology of acid set gel but not stirred gels. A study of stirred yoghurt reported by Skriver et al. (1993) concerned the influence of ropy culture on the shear stress. They concluded that the Casson and Herschel-Buckley models gave insufficient fit with pseudo-elastic model such as stirred yoghurt. O'Donnell & Butler (2002a) demonstrated nevertheless the shear rate/shear stress data obtained with stirred yoghurt purchased directly from the manufacturer fitted to an Ostwald power law with a good correlation at both initial and equilibrium conditions. Haque et al. (2001) completed this study by observing the effect of fermentation temperature on the rheological behaviour of stirred yoghurt. Afonso & Maia (1999) studied the structure development in stirred yoghurt at different stages in the manufacturing process (from the coagulum in fermentation tank to final fruited stirred yoghurt) with steady and oscillatory rheometry. They observed a decrease of G' as a function of frequency (10^{-3} to 10^1 Hz) during the storage of yoghurt (2–14 days). The interest in stirred yoghurts is first that this product is as economically important as set yoghurts in France and some other countries. Our interest in stirred gels was also driven by the supposition that the sensory properties perceived in the mouth correspond to a partially broken gel and not on a firm gel.

The aim of the present work was to study the effect of the heating of milk on the structure and on the textural properties of stirred gels in the first moments after stirring. This study is focused only on a mild heat treatment near to those used in the French dairy industry. We had to resolve difficulties in the manipulation of stirred gels which lies in the conditions of the recovery in a gel structure and the time after stirring chosen for analysis with mechanical spectroscopy. Generally, the study of stirred gels is done by recording the flow behaviour such as the flow curves (Afonso et al. 2003), with tube viscometer (O'Donnell & Butler, 2002b) or classical viscosity measurements (de Ancos et al. 2000) and concerns gel allowed to re-equilibrate for some days after stirring. Oscillatory shear flow tests were carried out in strain sweep test and frequency sweep test on commercial stirred yoghurt (de Lorenzi et al. 1995) but indicated that the viscoelastic properties of the stirred yoghurt were non-linear. The mechanism following the stirring does not really correspond to a classical sol-gel transition. It does not correspond either to a resetting of the gel because it is the building of a new structure of gel. Because of a large variation in texture during the first minutes after stirring, it is impossible to perform an immediate comparison between stirred gels by small deformation analysis. Small differences in the timing could create large differences in the results. In this paper, we propose a method to analyse the viscoelastic properties of stirred gels during the first steps of restructuring. We tried

finally to make an hypothesis about the influence of milk heating on the mechanism of the restructuring of gel.

Materials and Methods

Skim milk was reconstituted at 117 g dry matter/l in distilled water by dispersing ultra low-heated skimmed milk powder (total nitrogen matter: 365, non protein nitrogen: 80, non casein nitrogen: 21, ashes: 79, dry matter: 950 g/kg powder), corresponding to 45 g total nitrogen matter/l and 42.4 g protein/l (coefficient for protein matter: 6.36). The powder was obtained by spray-drying (a low-heat drier method; INRA, Rennes, France; Fairise et al. 1999) and stored at 4 °C under vacuum in sealed small bags after purging oxygen with nitrogen gas. No difference between the original milk and the reconstituted milk was identified with the following tests: nitrogen measurements, evaluation of glycation level, polyacrylamide gel electrophoresis profile of casein micelles isolated by ultracentrifugation, particle size measurement, milk rennetability (Fairise et al. 1999). These characteristics of the powder were not modified by the storage during our study (4 years in all).

Heating of milks

Milks were dispersed in fine glass reaction tubes (5 ml). The tubes were plunged into a stirred oil bath regulated at 10 °C above the set temperature. The temperature of milk within the tubes was followed using a temperature probe at the centre of the tube. When the temperature reached the set point (70, 80, 90 or 100 °C), the temperature was maintained for 1, 4 or 8 min in a second regulated oil bath. The reaction tubes were then cooled in an ice bath until the temperature reached 20 °C. This procedure achieved very rapid heating and cooling, close to the industrial conditions.

Evaluation of the quantity of β -lactoglobulin associated to the micelle

After the thermal treatment, casein micelles were isolated by ultracentrifugation at 45 000 *g* for 45 min at 20 °C (Kontron Instruments, Zurich, Switzerland). Micellar casein pellets were washed three times with a synthetic ultrafiltrate (Jenness & Koops, 1962) to eliminate any free whey proteins. Resuspended pellets were applied to SDS-DTT-PAGE, the gel was stained with Coomassie Brilliant blue and scanned with a densitometer (Shimadzu, Japan). The quantification was achieved by calibration with β -lg (Sigma-Aldrich St Louis, USA). For more details, see Fairise et al. (1999).

Acidification procedure

A final pH value of 4.6 was obtained by adding 2.2 g glucono- δ -lactone (Lysactone[®] from Roquette, France) to

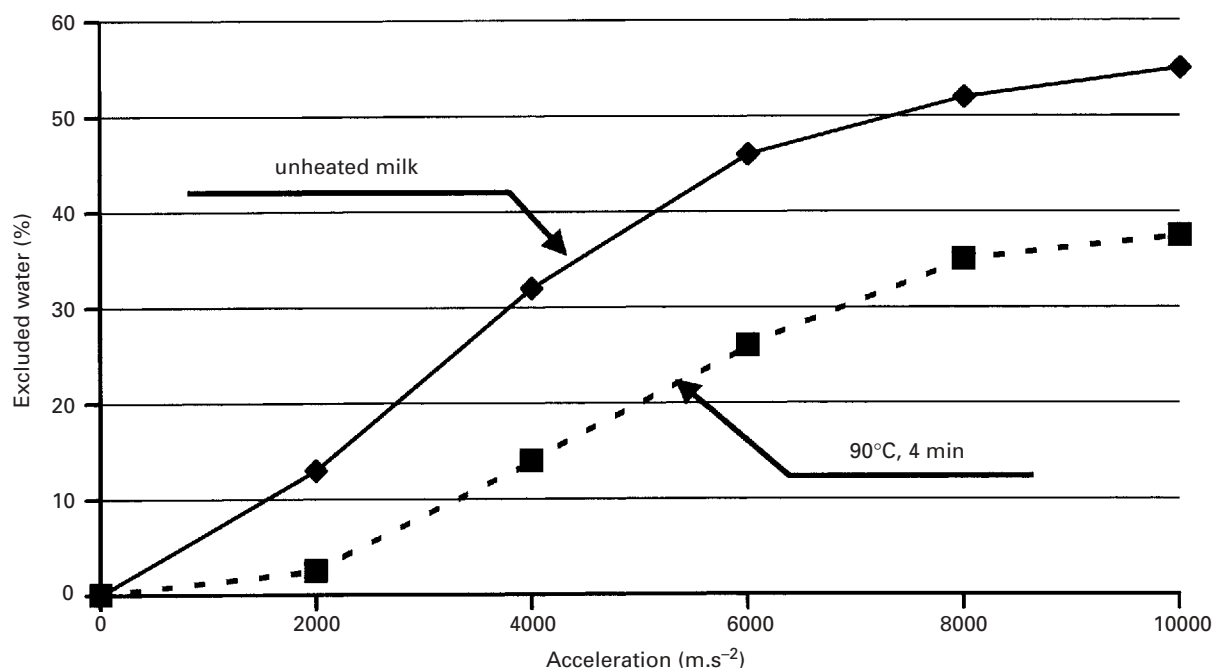


Fig. 1. Resistance of stirred gel to syneresis induced by centrifugation. The exuded aqueous phase (% m/m of gel) is given as a function of the centrifuge acceleration performed at 10 °C for 10 min.

100 ml milk. The mixture was poured into a 50 cm³ sterile plastic jar. The jars were placed in an oven at 20 °C for 6 h. The resulting gels were stored at 4 °C (10 h) and subsequently at 10 °C (10 h) before stirring and analysis.

Stirring of gel

The firm gel (set gel) was stirred with a Polytron mixer (Kinematica, stirrer PT-DA 3020/2 S, 20 mm Ø for the rotor) at 3000 rpm. Different stirring durations were performed from 30 s to 4 min to define the minimum time to obtain a good repeatability. These operating conditions were established by following the diameter of gel particles under different stirring durations using a Mastersizer MS 20 laser scattering analyser (Malvern Instrument, Orsay, France; index refraction of the medium used for dispersion i.e. water: 1.33).

Syneresis resistance of gel to a centrifugal force

Immediately after stirring 20 g stirred gel were put in a centrifugal tube. Following centrifugation (10 min at 10 °C), the liquid supernatant was taken off carefully with a syringe. The tip of the needle was put just at the gel-exudate interface. The exudate was quantified by weight. The exuded aqueous phase (mass exudates as percentage of gel) was recorded as a function of the centrifuge acceleration (0–10⁴ m.s⁻²; increment: 2 × 10³ m.s⁻²). The water holding capacities of the gels were obtained from graphs of exuded water versus acceleration parameters as shown in Fig. 1. The initial slope (sl) corresponds to the percentage

(% m/m) aqueous phase extracted at low acceleration (2 × 10³ m.s⁻²) and indicates the resistance of a gel to syneresis when the gel undergoes a small mechanical stress. The final value (Vf) is the percentage of exudate obtained at high acceleration (10⁴ m.s⁻²) when the gel undergoes a high mechanical stress. The area under the water exclusion curve (A) reflects the tendency of a gel to exclude water during a progressive centrifugation and is a synthesis of the parameters sl and Vf. The total area was calculated by the sum of the areas of the first triangle and the quadrilaterals. The lower these parameters, the greater the resistance of the gel to mechanically induced syneresis.

Rheological properties

Firm (set) gels were characterized for fracture resistance under penetration using a LFRA Texture Analyser apparatus (Stevens Ltd, St. Albans, Great Britain). The 50 cm³ jar (59 mm Ø) containing the sample was put under a 13 mm diameter cylindrical probe, which was lowered at 0.2 mm/s until fracture. The gels stored at 4 °C were equilibrated at 20 °C before performing the test.

Large deformation (flow behaviour) and dynamic oscillatory measurements (viscoelastic properties) of stirred gels were performed with a Rheometric Scientific SR-5000 apparatus (Piscataway, New Jersey, USA). A parallel plate geometry (50 mm diameter; 2 mm gap) was used.

For the measurement of the flow behaviour, the just-stirred gel was continuously and vigorously stirred with a spoon (<1 min) until a sample was transferred to the plate

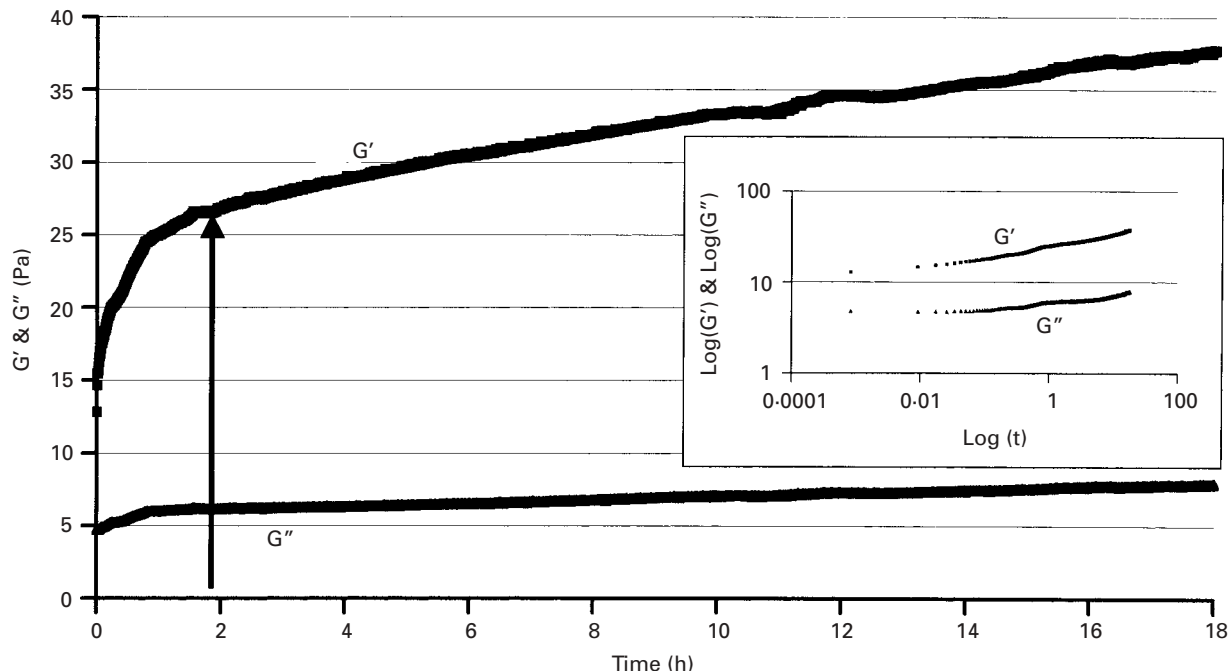


Fig. 2. Progression of the storage modulus G' and of the loss modulus G'' as a function of time, during the recovery in gel structure after stirring.

of the apparatus. The analysis was done immediately at $10\text{ }^{\circ}\text{C}$. The shear rate ($\dot{\gamma}$) increased from 0.001 to 30 s^{-1} and the corresponding shear stress (τ) was measured. The power law model (Ostwald model: $\tau = b \cdot (\dot{\gamma})^n$) was used for the determination of the consistency index, b and the flow behaviour index, n in the linear region, between 0.2 and 3 s^{-1} .

To study viscoelastic properties, oscillatory tests were made at $10\text{ }^{\circ}\text{C}$. Just after the mechanical stirring, the gel was stirred with a spoon as described previously until a sample was put on the plate of the rheometer. The stirred gel was protected from desiccation by a covering of water-logged felt. We have chosen to compare the stirred gels through the values of storage modulus (G') and loss modulus (G'') 2 h after the end of the stirring (see results). Three min with 10 points per decade and 10 min with 30 points per decade were sufficient to analyse the progression of the storage modulus G' and of the loss modulus G'' as a function of the stress. These periods were sufficiently short to establish that the gel structure was approximately at equilibrium 2 h after stirring. To identify the range of linear viscoelasticity was difficult because of the duration of analysis at very low frequencies. We chose to sweep between 0.01 Hz and 50 Hz for the definition of the linear range. The stirred gel was placed between plate and cone 2 h before the test was made at $10\text{ }^{\circ}\text{C}$ and at 0.5 Hz with an increasing shear stress from 0.1 to 20 Pa (logarithmic increment). The change of G' , G'' , and the loss angle (δ) were recorded following the stress. All the rheological analyses were repeated in triplicates, 3 fresh gels being used for each measurement.

Statistics

Principal component analysis was used to identify the relationships between all analysed parameters.

Results

Recovery in gel state after stirring

After 30 s – stirring, the mean size of particles was $D_{3,2} = 37\text{ }\mu\text{m}$ ($Dv_{0,5} = 44\text{ }\mu\text{m}$). For longer stirring (1 min, and more) at 3000 rpm , the gel fragments size remained constant ($D_{3,2} = 14\text{ }\mu\text{m}$; $Dv_{0,5} = 18\text{ }\mu\text{m}$). These size measurements demonstrate that the stirring conditions were well controlled and that stirring for 1 min was enough to obtain constant samples. For viscoelastic measurements, every gel was stirred for 1 min. The size of gel fragments were quite similar to that obtained just after stirring in commercial yoghurt production (Jean-François Boudier, Ingredia, Arras, France, personal communication) and small enough to avoid grainy sensations in mouth (the threshold value for particles is around $10\text{ }\mu\text{m}$).

After an initial period (2 h) of rapid restructuring post stirring, recovery slowed. G' continued to rise at a slow but steady rate for at least 24 h (Fig. 2). Following this observation, the time for analysis of the viscoelastic properties of the stirred gel was fixed at 2 h after the beginning of the recovery in gel structure.

Fracture force of gels

The fracture forces of the set gels obtained from the acidified milks heated at $90\text{ }^{\circ}\text{C}$ and $100\text{ }^{\circ}\text{C}$ for 1 or 4 min were

Table 1. Fracture force of set gels (firm gels) prepared by the acidification of previously heated milks. Value for unheated milk: 157 ± 8

Fracture Force (mN) values are means ± SD, for $n=3$ separate gels

Temperature	Milk heating. Duration		
	1 min	4 min	8 min
70 °C	149 ± 22	130 ± 20	n.d.
80 °C	130 ± 30	165 ± 20	n.d.
90 °C	252 ± 25	275 ± 27	n.d.
100 °C	252 ± 30	247 ± 24	240 ± 12

Table 2. Percentage (m/m) β -lactoglobulin attached to the casein micelles during heating for 1 or 4 min. The β -lg/casein micelles complexes were isolated by ultracentrifugation. β -Lactoglobulin quantification was determined by densitometry of SDS-DTT-PAGE

Temperature	β -lg (%)	
	1 min	4 min
70 °C	9	8
80 °C	33	24
90 °C	62	71
100 °C	64	68

1.6 to 1.75 times higher than that of the gel from unheated milk (Table 1). Also, the more the milk was heated the greater the quantity of β -lg associated with the casein micelles (Table 2).

Water holding capacity of stirred gels

Values of the three parameters used to describe the ability of the gels to hold water are given in Table 3. These parameters decreased globally when the heating temperature increased. The three parameters are highly connected (no statistical difference shown by variance analysis). Thus any one of these parameters should be sufficient to observe differences in water holding capacity of stirred gels.

Figure 1 clearly shows the differences in water holding capacity of gels made with unheated and heated milks. As previously demonstrated for firm gels (Fox & Mulvihill, 1990), the results (Table 3) show that the stirred gels made from milk heated above 80 °C presented the lower syneresis (i.e. a good water holding capacity).

Flow behaviour of stirred gels

The Ostwald model applied to the flow curve of the different stirred gels indicated that the flow behaviour index, n , decreased with the heating intensity (from 0.6 for unheated milk to 0.3 for 90 or 100 °C heated milk), while the consistency index, b , significantly increased for milk heating temperatures higher than 80 °C (Table 4).

Table 3. Water holding capacity of just-stirred acid gels. A curve was obtained by the sum of the exuded aqueous phase quantity (% m/m of gel) as a function of the centrifuge acceleration ($0-10^4$ m.s⁻²; increment: 2000 m.s⁻²): see Fig. 1. Three parameters of syneresis induced by centrifugation were extracted: sl , the initial slope (%); V_f , the final value (%) and A the area under the curve (%.m.s⁻²). The results are given versus heat treatment of corresponding milks

	Ability to exclude aqueous phase		
	A	sl	V_f
Milk treatment control, unheated milk	387 200 ± 39 900	11 ± 2	57 ± 5
70 °C			
1 min	385 700 ± 11 600	11 ± 2	57 ± 3
4 min	328 300 ± 6700	6 ± 1	51 ± 2
80 °C			
1 min	339 100 ± 4000	9 ± 2	53 ± 2
4 min	272 700 ± 9200	7 ± 1	51 ± 3
90 °C			
1 min	168 000 ± 1600	4 ± 1	39 ± 1
4 min	168 300 ± 39 700	4 ± 1	36 ± 5
100 °C			
1 min	168 500 ± 8400	3 ± 1	39 ± 2
4 min	170 400 ± 7000	4 ± 1	38 ± 2

Viscoelastic properties of stirred gels

A typical progression of G' and G'' as a function of the stress applied to the stirred gel is shown in Fig. 3. When the stress was increased, G' and G'' remained constant until a yield value. This value, corresponding to the beginning of the flow of the gel, was characterised by a sudden decrease of G' . This behaviour could be linked to the firmness of the restructured stirred gel and to its stress limit before breaking. The heat treatments corresponding to a sudden change in the plateau value of G' (G'_p ; Table 5) were similar to those observed for fracture force of firm gel (Table 1) and consistency index (Table 4). When milk is heated at 90 °C and more, the change of behaviour was very pronounced. The stress value corresponding to $G' = G''$ ($\sigma_{G'=G''}$) was also related to the heating temperature of milk (Table 6) but the progression of this parameter was less pronounced than G'_p .

Relation between the rheological parameters and the quantity of β -lg associated with the casein micelles

The quantity of β -lg associated with casein micelles during heating was highly related to the gel firmness (G'_p), the stress required to flow the stirred gel ($\sigma_{G'=G''}$), the flow behaviour index (n), and inversely connected to the consistency index (b) and the tendency to exclude water (V_f) as shown by principal components analysis (Fig. 4). When the quantity of β -lg associated with casein micelle increased, the strength of the gel increased. Beyond a certain intensity of heating, no change was obtained: the rheological characteristics of gels prepared with the milk heated at 90 or 100 °C were constant whatever the duration of the temperature plateau (Fig. 5).

Table 4. Consistency index (b) and flow behaviour index (n) of just-stirred gels prepared from acidification of milks previously heated for 1, 4 or 8 min. Values for control, unheated milk were $n=2.1 \pm 0.2$ and $b=1.1 \pm 0.1$

Data were obtained from the law: $\tau = b \cdot (\dot{\gamma})^n$

Flow behaviour index, n. Consistency index, b (Pa.sⁿ)

Temperature	1 min		4 min		8 min	
	n	b	n	b	n	b
70 °C	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.3 ± 0.2	n.d.	n.d.
80 °C	1.6 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.3 ± 0.2	n.d.	n.d.
90 °C	1.1 ± 0.1	4.2 ± 0.3	1.1 ± 0.1	4.4 ± 0.2	n.d.	n.d.
100 °C	1.1 ± 0.1	4.4 ± 0.4	1.1 ± 0.1	4.9 ± 0.2	1.2 ± 0.1	4.9 ± 0.3

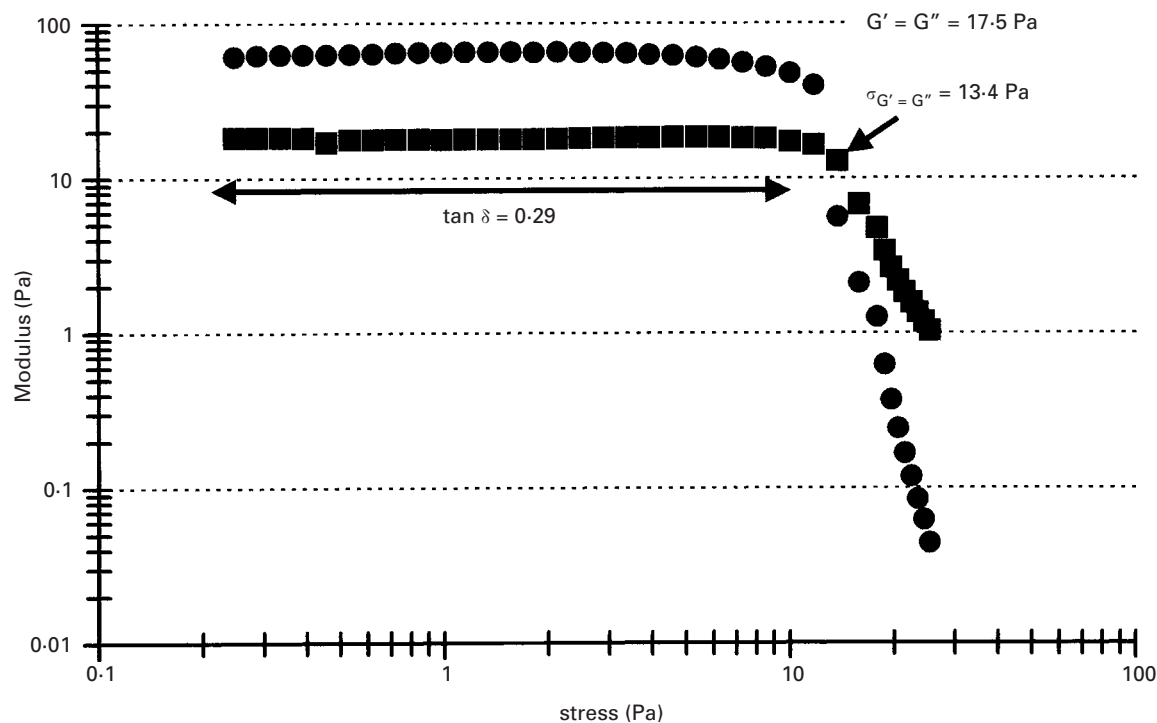


Fig. 3. Progression of the viscoelastic modulus of an acid gel (made from milk heated at 100 °C for 4 min) during the increase of the stress (0.2–25 Pa; 0.5 Hz). G' : ●; G'' : ■.

Figure 5 shows three families of heating process. The first corresponds to unheated and pasteurized milk, the second to mild heat treatments (from 70 °C/4 min to 80 °C/4 min) and the third to important heat treatments (90 and 100 °C). Heating milk at 90 °C for 1 min appears sufficient to obtain an optimum quality of stirred gel.

Discussion

To study the effect of heating milk on the quality of stirred gel, it was first necessary to resolve analytical problems associated to this type of gel. Restructuring of a gel following stirring was found to continue for up to 24 h and 2 h correspond to a minimum period to allow the initial rapid restructuring to occur. However, it is possible to

compare the effect of heating milk 2 h after stirring because changes occurring after this time were very slow. In the work of Haque et al. (2001), it seems that the resetting of the gel obtained with a ropy culture is almost complete at the end of their observation (60 min) for some of the gels. Those authors recognised that the stirred gel consisted in a 'less well-defined system than intact gels'. With ropy culture, it is possible that the resetting is quicker than in our model using glucono- δ -lactone because of the presence of exopolysaccharides.

Although the measurement of firmness (F) was done on set gels, the measurement of water holding capacity (Vf) and viscosity (n, b) on just-stirred gels and the measurement of strength ($G'p$, $st = \sigma_{G'=G''}$) on a restructured gel 2 h after stirring, all these parameters are highly connected

Table 5. Change in the plateau value of storage modulus $G'p$ (see Fig. 2) of stirred acid gels as a function of the heat treatment of milk (1, 4, or 8 min) applied before acidification. Value of control, unheated milk: 25 ± 2 Pa

Temperature	$G'p$ (Pa)		
	1 min	4 min	8 min
70 °C	26 ± 3	26 ± 2	n.d.
80 °C	27 ± 3	30 ± 2	n.d.
90 °C	62 ± 6	64 ± 4	n.d.
100 °C	61 ± 6	61 ± 4	63 ± 2

Table 6. Stress value (see Fig. 2) necessary to obtain $\tan \delta = 1$ ($G' = G''$) for stirred acid gels as a function of the heat treatment of milk (1, 4, or 8 min) before acidification. Value of control, unheated milk: 9 ± 1 Pa

Temperature	Stress ($G' = G''$) (Pa)		
	1 min	4 min	8 min
70 °C	9 ± 1	10 ± 1	n.d.
80 °C	10 ± 3	12 ± 2	n.d.
90 °C	12 ± 1	13 ± 1	n.d.
100 °C	16 ± 2	16 ± 4	18 ± 3

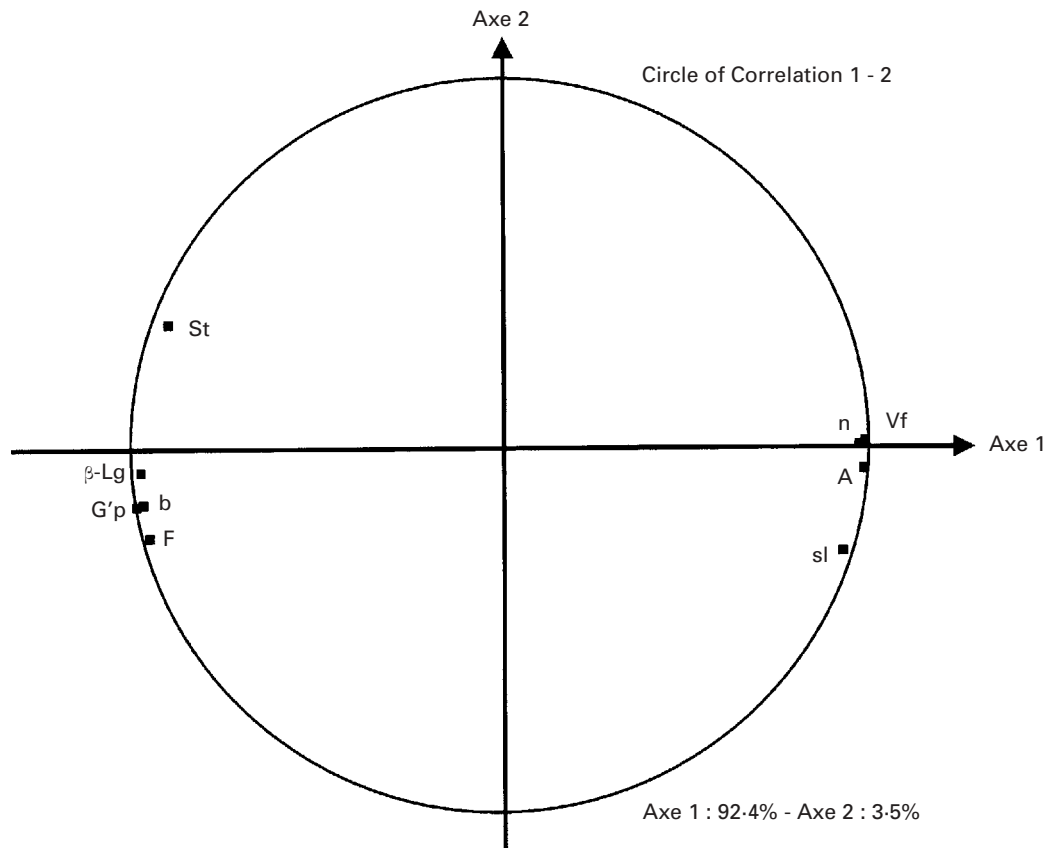


Fig. 4. Circle of correlation of principal variables. Principal component analysis of the averages Products/Attributes. β -Lg corresponds to the quantity of β -lactoglobulin associated with the casein micelles, F to the fracture force of the set gel, b to the consistency index of just stirred gel, n to the flow behaviour index, Vf (sl and A) to the ability to excluded aqueous phase during a centrifugation (see Table 3), $G'p$ to the strength of restructured gel 2 h after stirring and st to the stress necessary for the stirred gel to flow.

(Fig. 4). In this case, the simple analysis of a set gel before stirring (firmness) or a simple measurement of the viscosity of a just-stirred gel could be convenient to indicate the final quality of a stirred gel. Though these parameters seem to be disconnected, they reflect the effects of the same biochemical changes occurring in milks during heating. Just one of the parameters studied would be sufficient for comparison between stirred gels.

An explanation for the low syneresis of gels made from highly heated milk could be suggested by electronic microscopy of firm acid gels (Lucey et al. 1998b), although

our heating conditions were milder than those chosen by those authors. We have assumed that a residual structure with a partially preserved porosity remained in particles of stirred gel. This hypothesis was based on the higher viscosity of just-stirred gel, in comparison with the corresponding milks, which results from the existence of protein framework with a particle size higher than independent micelles. It appears probable that a part of the firm gel structure is preserved in the stirred gel. These gel fragments should have the same porosity as the firm gel in its totality. The porosity of firm gels was smaller with heated milk (75

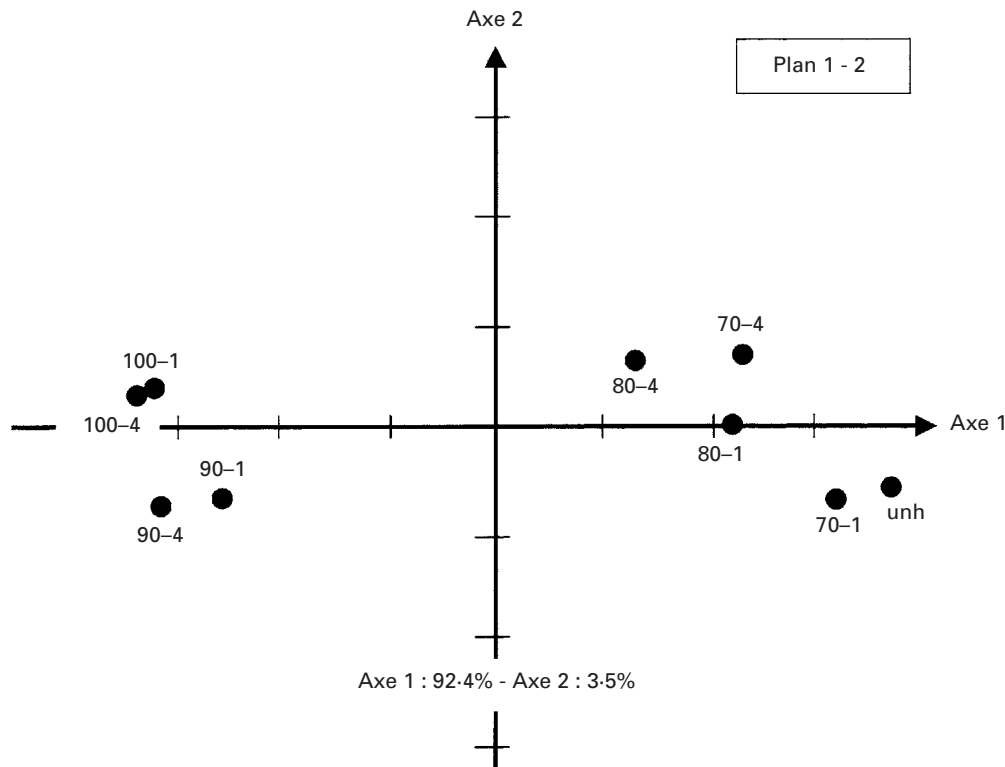


Fig. 5. Circle of correlation of principal individuals. Principal component analysis of the averages Products/Attributes. Unh corresponds to unheated milk, 70-1, 70-4, 80-1, 80-4, 90-1, 90-4, 100-1, 100-4 respectively to milk heated at 70, 80, 90, or 100 °C for 1 or 4 min.

to 90 °C, 15 or 30 min) than with non-heated milk (Kalab et al. 1976; Lucey et al. 1998b). The smaller porosity could enhance water holding in the protein network. Though the conditions of heating did not correspond to ours, the application of image analysis to electron micrographs of stirred gel by Skriver et al. (1997) showed that the heating of milk effectively modified the porosity of stirred yoghurt. They calculated a star volume of yoghurt prepared with lactic bacteria and observed an increase of this parameter value when the milk was intensively heated (85 °C – from 15 to 30 min) which corresponded to a too extensive aggregation of casein. With the milk heating at 85 °C during 15 min (corresponding to an optimum treatment according to these authors), the size of the casein aggregates in the gel was lower and the corresponding yoghurt had a fine structure with small pores.

When milk is heated there is an increase in surface hydrophobicity of the casein micelles (Iametti et al. 1993). As a result restructuring of stirred yoghurt prepared from highly heated milk may be more effective compared with unheated milk or milk heated below the denaturation temperature of β -lg. This strong re-combination of gel fragments induced a higher viscosity of just-stirred gels, which could allow a better stabilization of gel fragments during the recovery in gel state. Consequently, it should result in better water retention of stirred gels and a firmer structure ($G'p$).

From the present results and those previously published by others, it seems that the rheological characteristics of stirred yoghurt are affected by the binding of β -lg onto casein micelles during the heating of milk. In the same way that β -lg affects the surface properties and gel structure of set gels. The development with time of the storage modulus firm acid gels is related to the extent of whey protein denaturation in milk (Lucey et al. 1997, 1998c). As the association of β -lg with the micelle explains the rheology of a firm gel (Davis et al. 1978), it seems that the same explanation could apply to a stirred gel. We could admit that the hydrophobicity of the particles of stirred firm gels (set gel fragments) is increased by the high hydrophobicity of the constituted casein micelles from heated milk. It consequently enhances the ability of these particles to aggregate (or combine) after stirring. This tendency to aggregate would be retained in stirred gel and would facilitate the recovery in a gel state.

Other biochemical changes which occur during heating may be discounted, the temperatures in this study (≤ 100 °C) were too low to affect calcium repartition in milk (Pouliot et al. 1989; Wahlgren et al. 1990) or the hydrolysis of protein amide groups (Asn, Gln) (only in prolonged heat treatment at 100 °C; Chaplin & Lyster, 1986). In this study the $pH < 6.8$ was inappropriate for dissociation of casein from the micelles (Anema & Klostermeyer, 1997; Anema, 1998).

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