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Original article

Changes in the rheological properties of stirred acid milk gels as induced by the acidification procedure

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Abstract – The aim of this study was to compare the rheological properties of stirred acid milk gels, acidified using two different acidification procedures. Pre-heated milks were acidified either by means of glucono-δ-lactone (GDL gel) at 20 °C or by incubation with lactic acid bacteria (lactic gel) at 38 °C. The latter procedure gave a harder acid set gel with a more heterogeneous structure. Once formed, the gels were filtered and stirred, then immediately characterised using the low-amplitude dynamic oscillation method for 20 h at 4 °C or creep measurement at 4 °C. The stirred gels were stored at 4 °C for 28 d, during which the pH and the viscosity at 64 s^{-1} were measured. After stirring, the lactic gel made at 38 °C had a higher instantaneous elastic modulus, a higher viscosity and showed a greater increase in the elastic modulus over 20 h compared with the stirred GDL gel. The viscosity of both stirred gels progressively increased during storage at 4 °C, but that of GDL acidified gels increased 3.5 times more than that of lactic gels. The procedure used to prepare the set acid gels had notable effects on the interactions formed within 20 h and 28 d of storage of stirred gels.

stirred yoghurt / set yoghurt / acid milk gel / rheology

摘要 – 酸化过程引起搅拌型酸奶凝胶流变特性的变化。本文比较了两种不同的酸化过程引起搅拌型酸奶凝胶流变特性的变化。预热后的牛奶分别在 20 °C 用 δ-葡萄糖酸内酯酸化 (GDL凝胶) 和在 38 °C 下接种乳酸菌 (乳酸凝胶)。后者形成的凝固型酸凝胶较硬并具有较多的异性结构。将形成的凝胶立即过滤和搅拌, 然后在 4 °C、 20 h 后用低振幅动态振荡法或在 4 °C 用蠕变测量法观察凝胶特性的变化。搅拌后的凝胶在 4 °C 下贮存 28 d, 其间测定搅拌型凝胶的 pH 和黏度 (在 64 s^{-1})。与搅拌型的 GDL 凝胶相比, 38 °C 下形成的乳酸凝胶搅拌后具有较高的瞬时弹性模量和黏度, 并且在 20 h 后其弹性模量显著增加。在 4 °C 的贮藏过程中两种搅拌型凝胶的黏度均显著地增加, 但是 GDL 凝胶黏度增加的程度比乳酸凝胶多 3–5 倍。凝固型酸奶的制备过程对搅拌型凝胶在 20 h 和 28 d 贮藏期内凝胶的性质有显著地影响。

搅拌型酸奶 / 凝固型酸奶 / 酸乳凝胶 / 流变性

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Résumé – Effet de la procédure d'acidification sur les propriétés rhéologiques des gels acides brassés. Le but de ce travail est de comparer les propriétés rhéologiques des gels acides brassés acidifiés par 2 procédures différentes. Le lait traité thermiquement était acidifié soit par addition de glucono delta lactone à 20 °C (gel GDL), soit par incubation avec des bactéries lactiques à 38 °C (gel lactique). Cette dernière procédure donnait des gels plus fermes avec une structure davantage hétérogène. Une fois formés, les gels étaient filtrés et brassés, puis immédiatement caractérisés en utilisant la méthode dynamique d'oscillations en faible amplitude pendant 20 h à 4 °C et par fluage à 4 °C. Les gels brassés étaient stockés à 4 °C pendant 28 j pendant lesquels le pH et la viscosité à 64 s^{-1} étaient mesurés. Après brassage, le gel lactique préparé à 38 °C avait un plus grand module élastique instantané, une plus grande viscosité et montrait une plus grande augmentation du module élastique pendant 20 h, comparé au gel GDL brassé. La viscosité des 2 types de gel brassé augmentait progressivement pendant le stockage à 4 °C, mais elle augmentait 3,5 fois plus dans le gel GDL que dans le gel lactique. La procédure appliquée pour préparer le gel acide avait des effets notables sur les interactions formées à 20 h et à 28 j de stockage des gels brassés.

yaourt brassé / yaourt ferme / gel acide / rhéologie

1. INTRODUCTION

It is well known that the procedure and the temperature used during the acidification of pre-heated milk result in set acid gels showing different rheological properties and microstructures. Lucey et al. [15] compared gels made from heated milk and acidified using GDL at 30 °C or a bacterial culture at 42 °C. Acidification with GDL increased many parameters compared with acidification with lactic acid bacteria: the initial rate of acidification, the elastic modulus (G'), the yield stress and strain, the permeability and whey separation. The microstructure of bacterial gels is unaffected by using incubation temperatures between 30 and 42 °C, while GDL gels are more heterogeneous at 42 °C. According to many authors, increasing the incubation temperature leads to a higher gelation pH. Consequently, a higher gelation pH produces a gel composed of more mineralised particles and thus yields a microstructure with larger pore sizes and different viscoelastic behaviours. Permeability also increased, as did pore sizes and whey separation [13, 15, 16, 18]. Conversely, some authors [5, 11, 19] reported that a high acidification temperature leads to an increase in the elastic modulus and in the firmness of set yoghurt.

Changes in the microstructure of set yoghurt can be explained by many factors: a greater rearrangement of particles at 40 °C compared with 20 °C [17, 18], a temperature-dependent dissociation of casein during acidification [4], a strengthening of hydrophobic interactions with increasing temperatures [5], the rate of calcium phosphate solubilisation [13] or the balance between the rate of proton production and diffusion [9, 10]. Bacteria grow in colonies which produce lactic acid in small localised areas, therefore forming gels with large void spaces, as reported by numerous authors [2, 7, 8, 20]. The aggregation of casein micelles depends on the amino acid residues present on the micelle surface, on the physicochemical properties of these residues and therefore on the pH of the aqueous phase. When the acidification rate is high, a substantial difference between intra- and extra-micellar pH may occur and lead to a network of casein particles containing different levels of mineralisation. Acidification by GDL leads to a more homogeneous distribution of proton production and may have a different impact on the intra-micellar pH.

While the effect of the acidification procedure is well described for set acid gels, very little is known on the behaviour of stirred acid milk gels. According to many authors, the rheological properties of stirred yoghurts can be related to those of set yoghurts [3, 5, 11]. Increasing the temperature of incubation has been shown to

lead to firmer set acid gels which retained higher viscosities [5, 11] and higher elastic moduli [5] after stirring. According to Lankes et al. [11], a higher rate of acidification, which altered the kinetics of protein network formation, may explain these results. As the loss tangent (tanδ) remained unaffected by the incubation temperature and stirring, it has been suggested that similar interactions are involved in both set and stirred systems [11].

The aim of the present study was to relate the final rheological properties of stirred acid gels to the microstructures of the set acid gel and to understand the reassociation of gel particles due to stirring. Two set gels were made using two different procedures: GDL at 20 °C or incubation with lactic acid bacteria at 38 °C. These two procedures are known to induce large differences in the kinetics of acidification, gelation and in physical properties of the set gels, such as heterogeneity and porosity. The rheological properties of the two stirred acid gels were compared.

However, it must be kept in mind that the two procedures used to produce the gels cannot be directly compared, as they differ both by the mode and the temperature of acidification. The 2 methods have been used solely to produce 2 different structures and to show the effect of these different structures on the rheological properties and quality of the stirred gels.

2. MATERIALS AND METHODS

2.1. Materials

Milk was reconstituted from low-heat skim milk powder (BBA Lactalis Industrie, Bourgbarré, France) at 140 g·kg^{-1} dry matter into deionised water and equilibrated for 1 h. One litre was heat-treated through a spiral stainless steel tube immersed in a water bath, as described in Laligant et al. [9]. The temperature of the milk was measured every 5 s using a thermocouple, the SR Mini system and Specview software (TC SA, Dardilly, France). The milk was held at 90 °C for 10 min after a warm-up time of 9 min, rapidly cooled in ice-water and stored at 4 °C. Including the time taken for temperature increase, holding and decrease, the equivalent time ($\tau_{90°C}$) of the overall treatment at 90 °C was about 16 min for whey protein denaturation as calculated by:

$$\tau_{90°C} = \mathrm{d}t \sum 10^{\frac{\theta - 90°C}{z}}$$

with θ the temperature measured at each 5-s interval (dt) and z the increase in temperature that reduces the decimal reduction time by 10. According to Jeantet et al. [6], the z value is 6 for whey protein denaturation.

2.2. Acidification

Non-ropy strains of *Lactobacillus bulgaricus* (LB 340) and *Streptococcus thermophilus* (TA 060) were purchased from Danisco (Dangé St Romain, France) and added to milk at 0.002 and 0.02 unit·L^{-1}, respectively. This milk was stored between 0 and 4 °C for ≈ 7 h and then incubated at 38 °C for ≈ 12 h. Milk of another trial was directly mixed at 20 °C with GDL at 19.6 g·kg^{-1} and stored for ≈ 20 h, until a pH of 4.4 was reached.

Gelation was observed under low-amplitude dynamic oscillation using an AR1000 rheometer (TA Instruments, Guyancourt, France) with coaxial cylinders (outer and inner diameter, 5 and 4.61 cm) 0.001 strain and 1 Hz. An Inlab 423 electrode (Mettler-Toledo, Viroflay, France) was placed in the geometry to measure the pH. A plastic cover was inserted to avoid evaporation. Three bacterial gels and 2 GDL-induced gels were prepared, as the GDL acidification was more reproducible than the bacterial acidification.

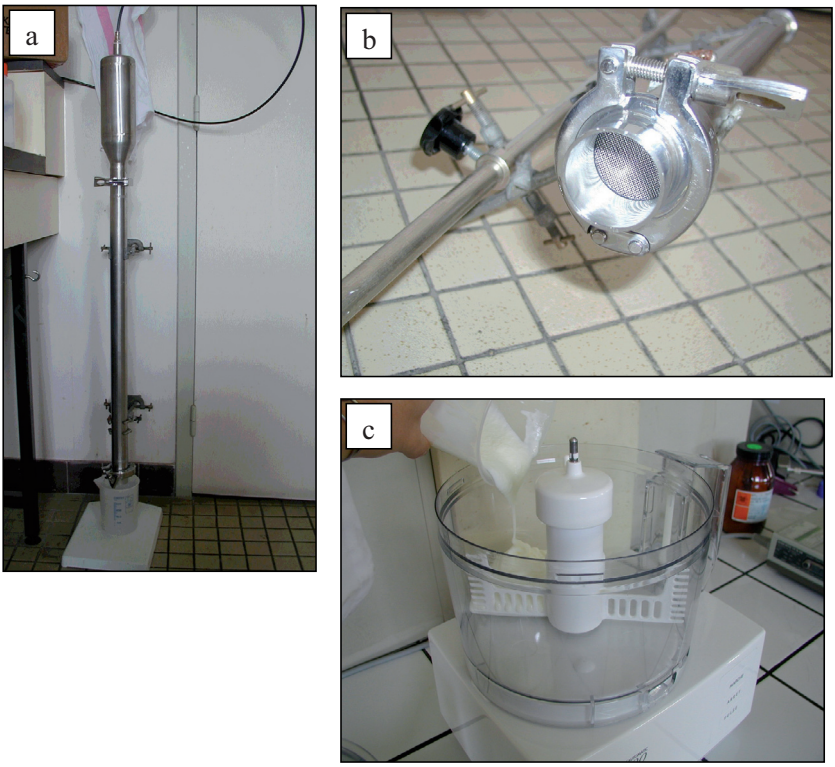

Figure 1. The stirring system used for stirring the acid gel; a: steel tube with a filter at the bottom; b: filter composed of a mesh, view from the bottom; c: the home food processor.

2.3. Confocal microscopy

Fluorescent rhodamine B isothiocyanate (RITC, Sigma, Saint-Quentin Fallavier, France) was added to milk at a final concentration of 20 mg·kg^{-1} and mixed with the milk for 15 min at 4 °C before GDL or starter addition. Forty µL of fluorescent-labelled milk were introduced into the confocal microscope on a concave slide covered by a coverslip held in place with nail varnish. Gels were observed at pH 4.4 after 20 h at 20 °C and 7 h at 38 °C, for GDL and bacterial gels, respectively. RITC binds to proteins and allowed the protein network to be observed using a Kr/Ar laser at excitation wavelengths of 568 nm and detection at 590 nm, with a 40 × oil immersion (numerical aperture 1.0) objective using a Leica TCS NT (Leica Microsystems, Heidelberg, Germany) at zoom 1 and 4. The two-dimensional images had a resolution of 1024 × 1024 pixels.

2.4. Stirring

The 3 bacterial gels and 2 GDL-induced gels (390 g) at pH 4.4 were broken by forcing the gels under compressed air through a steel cylindrical tube (70 cm high and an internal diameter of 3.5 cm) with a mesh of 350–400 µm holes at the end (Fig. 1). Stirring was then completed using a home food processor (Magimix, Vincennes, France)

for 10 s at 300 rpm. This process gave a smooth and homogeneous stirred product, with very few air bubbles. The particle size distribution in the current stirred gels measured by laser light scattering in a Mastersizer 2000 (Malvern Instruments, Malvern, UK) was very close to that of commercial nonfat yoghurt (results not shown).

2.5. Characterisation of stirred gels

Stirred gels were held at 4 °C for 10 min before four creep measurements of 5 min each were performed at increasing stresses of 0.3, 0.6, 0.8 and 1 Pa. Between every 2 creep measurements, the sample was maintained without strain for 5 min. Increasing stress allowed the linear viscoelastic model to be checked with successive curves intertwining. The compliance was best fitted with a Burger's model made up by the "in series" coupling of a Maxwell and a Voigt element, as described in Lapasin et al. [12]:

$$J(t) = 1/G_{c0} + t/\eta_{c0} + (1/G_{c1})(1 - \exp(-t \times G_{c1}/\eta_{c1})) \quad (1)$$

where J is the compliance, G_{c0} and η_{c0} are the modulus and viscosity of the Maxwell element and G_{c1} and η_{c1} are the modulus and viscosity of the Voigt element. The retardation time ($\tau_c = \eta_{c1}/G_{c1}$) was calculated for the Voigt element. Ninety-nine percent of the final value of the spring is reached at $3\tau_1$. Compliance data were fitted by this method with a confidence interval for R-square at [0.9902–0.9967]. As analysis of variance showed that the applied stress value had no significant effect on the fitted parameters, the parameter values for all stresses were therefore averaged.

The short-term recovery of viscoelastic properties of the stirred gels was measured by low-amplitude dynamic oscillation rheometry over 20 h at 4 °C using the AR1000 rheometer with an acrylic cone of 6 cm diameter and 3°59' at 0.001 strain and 0.16 Hz. The short-term recovery was defined as G′ increase over 20 h. Values of the storage modulus (G′) and loss tangent (tanδ) were adjusted with a double exponential equation:

$$G'(t) = G'_0 + G'_1 (1 - \exp(-t/\tau_{G'1})) + G'_2 (1 - \exp(-t/\tau_{G'2})) \quad (2)$$

$$\tan\delta(t) = \tan\delta_0 - \tan\delta_1 (1 - \exp(-t/\tau_{\tan 1})) - \tan\delta_2 (1 - \exp(-t/\tau_{\tan 2})) \quad (3)$$

where $\tau_{G'1}$ and $\tau_{G'2}$, or $\tau_{\tan 1}$ and $\tau_{\tan 2}$ are the time constants of the 2 successive steps for the values of G′ or tanδ, respectively. Actually, short-term recovery of viscoelastic moduli has already been described by a first post-stirring rapid procedure of 1–2 h, followed by a slower one [1, 3], and is well fitted by this 2-step equation of increase.

Dynamic viscosity measurements were also performed in triplicate on each stirred gel stored at 4 °C for 0, 7 and 28 d of storage. Measurements were carried out at 4 °C with a steel cone (2° - 5-cm diameter) in the VT550 viscometer (Haake, Thermo Electron, Cergy-Pontoise, France) for 5 min at the constant shear rate of 64 s^{-1}, and the viscosity obtained at 10 s and 5 min was analysed. Breakdown of the gels was evaluated by the decrease in viscosity between 10 s and 5 min of shear. Long-term recovery was defined as the viscosity increase during storage.

2.6. Statistical analysis

Differences between measurements were tested by a Student's t-test with $P < 0.05$.

2.7. Fitting of data

Data were fitted to equations by the non-linear least square fitter by processing successive Levenberg-Marquardt iterations (Origin 6.1, OriginLab Corporation).

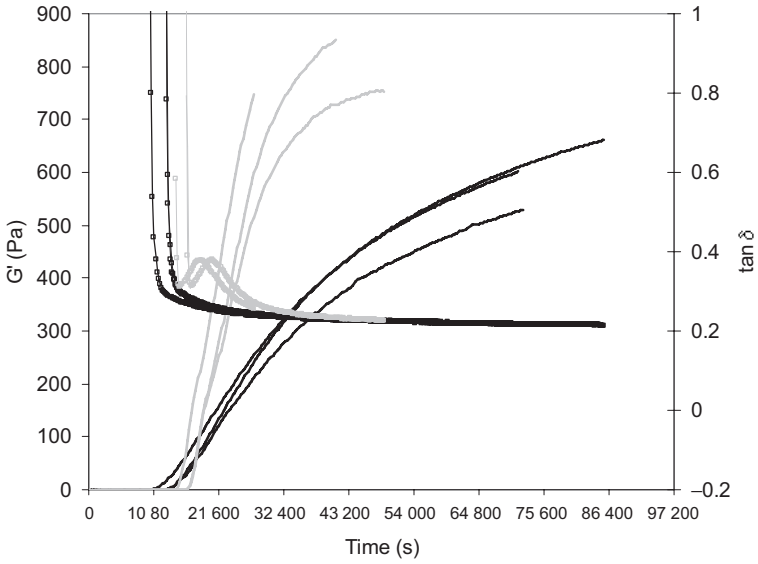

Figure 2. Viscoelastic properties of heat-treated milk during acidification with glucono-delta-lactone at 20 °C (▬: G'; —⊟—: tanδ) or a bacterial culture at 38 °C (▬: G′; —⊟—: tanδ) in coaxial cylinders versus time. Zero time for bacterial acidification was taken at the time where the temperature reached 38 °C. Three repetitions for each procedure.

3. RESULTS

As expected, the two acidification procedures used led to different set gels (Fig. 2 and Tab. I). With GDL at 20 °C, the gelation pH was lower (5.07 compared with 5.32), and the gel did not show a maximum for tanδ, while this maximum appeared at pH 4.98 ± 0.09 with the bacterial acidification. The final moduli and tanδ of GDL gels were lower than for the bacterial acidification, resulting in a softer gel with a more solid-like behaviour as compared with the bacterial gel.

In the bacterial gel, magnification (× 40) revealed large spaces or pores without fluorescence (Fig. 3a), while in the GDL gel, the structure was more homogeneous, with a regular porosity (Fig. 3b). These spaces are thought to be occupied by macro-colonies of bacteria. Figures 3c and 3d show the microstructure in an area of the gel not containing macro-colonies (× 40 – zoom 4). The two structures were not very different, except that the GDL gel appeared a little denser compared with the bacterial gel, with a slightly smaller porosity.

In the following discussion, the acid gel obtained by the GDL procedure will be referred to as the soft gel and the gel obtained by fermentation will be referred to as the hard gel. Also, the hard bacterial gel had a more heterogeneous structure compared with the soft GDL gel.

The pH of the hard and soft gels (4.18 ± 0.03 and 4.12 ± 0.01, respectively) after 28 d of storage was not significantly different ($P > 0.05$).

The compliance data of the just-stirred gels were fitted with Burger's model (Fig. 4 and Tab. II). Stirred gel behaved with an instantaneous compliance which indicated the stiffness of the material, a retarded compliance indicating a time-dependent

Table I. Parameters obtained by the dynamic rheological method during acidification of heated milk (mean ± standard deviation). Gelation was defined when G′ = 1 Pa.

Parameters	Chemical acidification at 20 °C (n = 2)*	Bacterial acidification at 38 °C (n = 3)
Gelation pH	5.07^{a} ± 0.05	5.32^{b} ± 0.11
Max tanδ	–	0.3773 ± 0.0016
pH of max tanδ	–	4.98 ± 0.09
G′ at pH 4.6 (Pa)	484^{a} ± 39	655^{b} ± 82
tanδ at pH 4.6	0.223^{a} ± 0.005	0.258^{b} ± 0.015

* n: Number of measurements.
–: No local maximum in tanδ.
^{a,b}: Means with different superscripts on the same row differ significantly ($P < 0.05$).

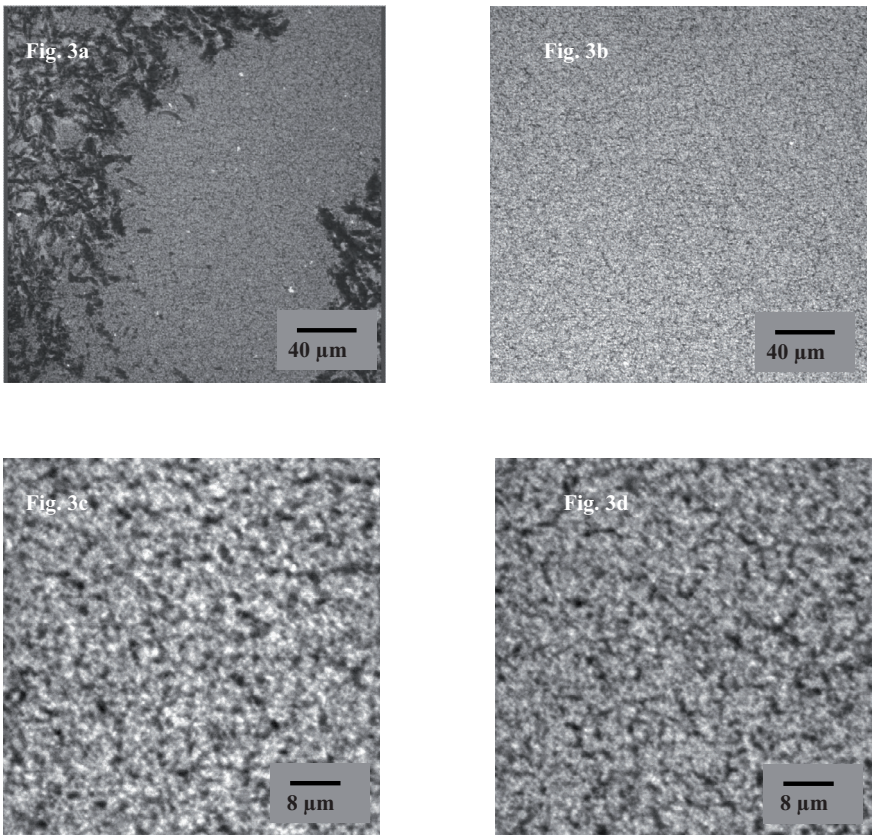

Figure 3. Microstructure of acid gels obtained with yoghurt starters at 38 °C for 12 h (a (× 40); c (× 40-zoom 4)) and with GDL addition at 20 °C for 20 h (b (× 40); d (× 40-zoom 4)).

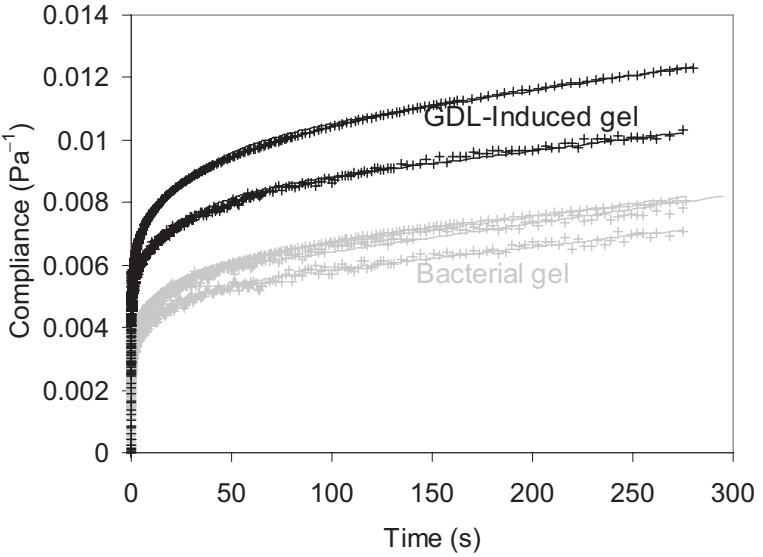

Figure 4. Creep measurements on stirred acid gels with cone-plate geometry. Stirred gel at day 0 (the day of stirring) was subjected to 4 creep measurements of 5 min each at 4 °C at increasing stresses of 0.3, 0.6, 0.8 and 1 Pa. Milk was acidified with glucono-delta-lactone at 20 °C (+) or a bacterial culture at 38 °C (+).

Table II. Parameters of the Burger's model obtained from creep measurements on GDL and bacterial stirred acid gels at day 0 (mean ± standard deviation):
$J(t) = 1/G_{c0} + t/\eta_{c0} + (1/G_{c1})(1 - \exp(-t \times G_{c1}/\eta_{c1}))$.

Parameters	GDL (n = 8)*	Bacterial culture (n = 12)
G_{c0} (Pa)	$175^a \pm 18$	$279^b \pm 21$
η_{c0} (Pa·s)	$105\,605^a \pm 11\,181$	$127\,542^b \pm 23\,243$
G_{c1} (Pa)	$344^a \pm 48$	$514^b \pm 73$
η_{c1} (Pa·s)	$4118^a \pm 590$	$4877^a \pm 1158$
τ_1 (s)†	$23.5^a \pm 2.2$	$17.6^b \pm 4.5$

* n: Number of measurements.
† τ_1: The recovery times, η_{c1}/G_{c1}.
a,b: Means with different superscripts on the same row differ significantly ($P < 0.05$).

behaviour and a final flow. Except for the viscosity of the Voigt element, parameters of the Burgers model were significantly higher for the hard gel compared with the soft gel, meaning that the hard bacterial stirred gel had a higher instantaneous modulus and a higher viscosity of the dashpot component.

During the 1–2 h after stirring, the gels showed a strong recovery in G′ and G″ and a decrease in tanδ (Fig. 5), but it was not completed by 20 h. Upon stirring, small pieces of gel were generated which were supposed to reassociate during storage, with an increased solid behaviour, probably due to the formation of new interactions. The fitted parameters of the double exponential equation are shown in Table III for G′ and tanδ. Stirred gels from the soft GDL gels showed less rebodying with time. Their elastic moduli at 20 h was half that obtained from the hard gel. Initial values for G′ and tanδ and G′ increase over the first step were different between the two gels, while there was no difference afterwards. However, the time constants of increase or decrease in

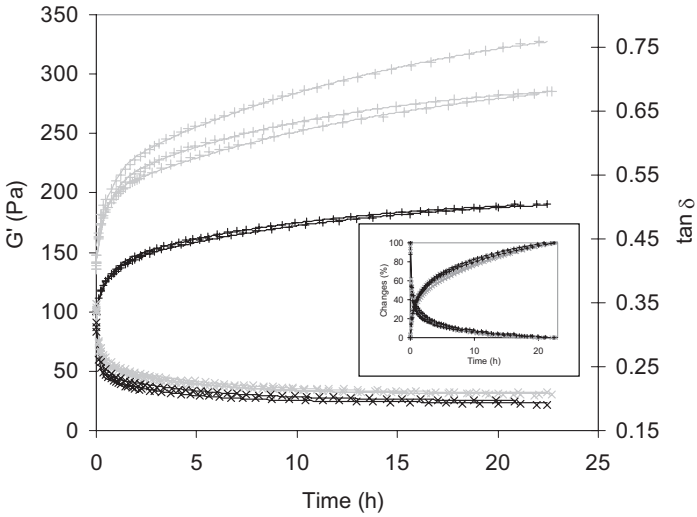

Figure 5. Storage modulus (+, +) and tanδ (×, ×) versus time after stirring of acid gels at pH 4.4, in a cone-plate geometry at 4 °C. Milk was acidified with GDL at 20 °C (+, ×) or a bacterial culture at 38 °C (+, ×). Lines are for the calculated values with the double exponential model. Relative changes in G′ and tanδ in the inserted curve.

Table III. Parameters of the double exponential equation for the increase in G′ or the decrease in tanδ for 20 h after stirring, for GDL and bacterial stirred gels (mean ± standard deviation): $G'(t) = G'_0 + G'_1(1 - \exp(-t/\tau_{G'1})) + G'_2(1 - \exp(-t/\tau_{G'2}))$
$\tan\delta(t) = \tan\delta_0 - \tan\delta_1(1 - \exp(-t/\tau_{\tan 1})) - \tan\delta_2(1 - \exp(-t/\tau_{\tan 2}))$.

Parameters	GDL (n = 2)*	Bacterial culture (n = 3)
G'_0 (Pa)	$102^a \pm 2$	$148^b \pm 6$
G'_1 (Pa)	$36^a \pm 0$	$59^b \pm 3$
$\tau_{G'1}$ (h)	$0.62^a \pm 0.07$	$0.56^a \pm 0.12$
G'_2 (Pa)	$60^a \pm 3$	$117^a \pm 29$
$\tau_{G'2}$ (h)	$11^a \pm 1$	$15^a \pm 3$
G'_∞ (Pa)	198	324
$\tan\delta_0$	$0.314^a \pm 0.004$	$0.342^b \pm 0.03$
$\tan\delta_1$	$0.078^a \pm 0.007$	$0.087^a \pm 0.005$
$\tau_{\tan 1}$ (h)	$0.173^a \pm 0.052$	$0.159^a \pm 0.037$
$\tan\delta_2$	$0.041^a \pm 0.001$	$0.047^b \pm 0.001$
$\tau_{\tan 2}$ (h)	$4.507^a \pm 1.001$	$4.772^a \pm 0.423$
$\tan\delta_\infty$	0.195	0.208

* n: Number of measurements.
[a,b]: Means with different superscripts on the same row differ significantly ($P < 0.05$).

viscoelastic properties were not different between both types of gel, meaning that it took the same length of time to reach the total increase for the 2 kinds of gels, as shown in Figure 5.

The stirred acid gels were subjected to structural breakdown at 64 s^{-1} shear rate for 5 min. Typical curves are shown in Figure 6. The viscosity of the samples decreased within the 5-min shearing and seemed to reach a steady state. Viscosity was measured after 10 s of shearing and the breakdown determined as the loss of viscosity between 10 s and 5 min (Tab. IV). Viscosity just after stirring of the hard bacterial gel was slightly higher as compared with the soft gel (Fig. 6), but the soft gel showed a much greater increase in the viscosity after 28 d of storage. The maximum increase in viscosity occurred between 0 and 7 d of storage (Tab. IV), as did structural breakdown. The higher the viscosity, the higher the breakdown. As shown in Table IV, except for the GDL stirred gel at day 0, the breakdown was

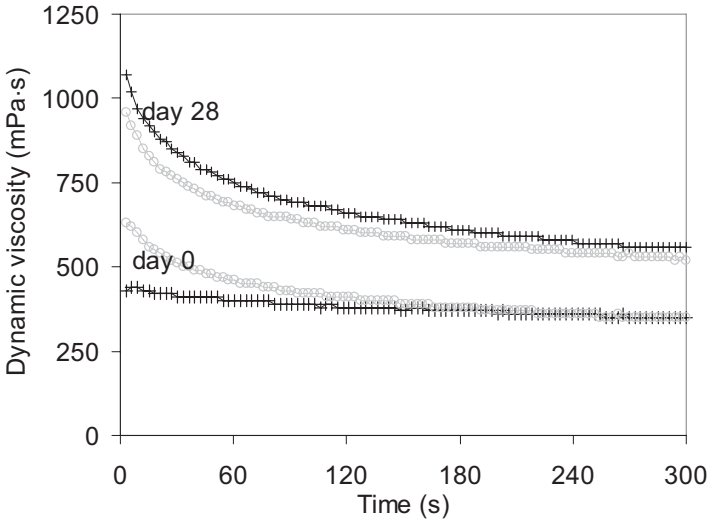

Figure 6. One example of viscosity changes in the stirred gel during 5 min shearing in cone-plate geometry (2° and 5 cm diameter) at 4 °C, measured on the day of stirring (day 0) or after 28 d (day 28): acid gels obtained with GDL (+) or bacterial fermentation (○).

40–43% of the viscosity value. The increase in viscosity during storage might be due to the recovery of low-energy bonds between gel particles, which are broken during shearing.

4. DISCUSSION

This study showed that set gels with different microstructures and rheological properties led to different quality of stirred gels. Just after stirring and over the initial 20 h of storage, the hard acid gel led to a viscoelastic stirred gel with many increased parameters as compared with the soft gel: the instantaneous elastic modulus, the viscosity of the dashpot component, the dynamic viscosity and the short-term recovery of its viscoelastic properties. Interestingly, after 28 d of storage at 4 °C, the dynamic viscosity of the soft stirred gel increased 3.5 times more compared with the hard gel. This suggests that the interactions involved in a set acid gel had significant and opposing effects on short-term and long-term interactions formed during storage. According to Lee and Lucey [14], acid gels obtained at lower incubation temperatures (32 °C compared with 44 °C) increased many parameters of acid gelation: the maximum of tanδ, the structure heterogeneity, as shown by a higher permeability coefficient, the elastic modulus and the yield stress. They also resulted in stirred gels with lower viscosities. These authors assumed that larger pores in the gel and a higher maximum of tanδ, which indicated the presence of weak spots and a greater susceptibility of structural rearrangements, respectively, can lead to local breakage and weaker interactions in the gel.

Many hypotheses could be suggested to explain the effect of the 2 modes of acidification on the rheological properties of the just-stirred gels. First, the hard bacterial gel had a higher gelation pH compared with the soft gel, and hence, micelle particles at the onset of aggregation might be more mineralised, more highly charged and less dissociated as compared with a gel formed at a lower pH. According to Lee

Table IV. Viscosity values (mean ± standard deviation) by flow measurements at 64 s^{-1} in cone-plate geometry of the stirred acid gels stored at 4 °C. Acid gels were obtained either by addition of GDL at 20 °C or bacterial acidification at 38 °C. Measurements were carried out during a 5-min flow. The viscosity (η) is measured at 10 s and breakdown was calculated as the decrease in viscosity between 10 s and 5 min shear.

	GDL (n = 6)*	Bacterial culture (n = 9)
η_0 (mPa·s)†	$509^a \pm 28$	$610^b \pm 24$
η_7 (mPa·s)	$1216^a \pm 345$	$797^b \pm 65$
η_{28} (mPa·s)	$1310^a \pm 541$	$845^a \pm 148$
(η_{28}–η_0) (mPa·s)	$818^a \pm 136$	$235^b \pm 32$
Breakdown$_0$ (mPa·s)	$129^a \pm 32$	$243^b \pm 14$
Breakdown$_7$ (mPa·s)	$525^a \pm 172$	$343^a \pm 34$
Breakdown$_{28}$ (mPa·s)	$571^a \pm 295$	$351^a \pm 77$
Breakdown$_0$/η_0 (%)	$25^a \pm 5$	$40^a \pm 2$
Breakdown$_7$/η_7 (%)	$43^a \pm 2$	$43^a \pm 1$
Breakdown$_{28}$/η_{28} (%)	$42^a \pm 5$	$41^a \pm 2$

*n: Number of measurements.

†: 0 subscript means just after stirring; 7 subscript means for stirred gel stored for 7 d at 4 °C; 28 subscript means for stirred gel stored for 28 d at 4 °C.

[a,b]: Means with different superscripts on the same row differ significantly ($P < 0.05$).

and Lucey [13], when dissociation continues within the gel, it leads to a higher susceptibility to structural rearrangements in the set gel and probably after stirring. This can also lead to a higher increase in G′ and viscosity after stirring. Moreover, in the hard bacterial gel, the production of acid was not as uniform as in a chemical gel, because bacteria are not uniformly dispersed in the milk and grow as macro-colonies, producing protons in a few small localised areas. These protons need to diffuse from the colonies to micellar particles to reduce ionisation, charge and mineralisation of casein particles [9, 10]. Stirring such a gel may accelerate the equilibration of mineral dissociation, casein partition, gel structure and pH, which can lead to greater structural rearrangements, thus enhancing rebodying. Furthermore, a higher temperature during acidification (38 °C compared with 20 °C) may lead to increased hydrophobic interactions inside the particles. This led to a more compact particle structure, with lower contact area, and consequently fewer interactions between casein micelles [13]. In addition, more numerous and stronger hydrophobic interactions at higher temperatures led to a higher stiffness of inter- and intra-particle bonds and to a lower possibility of structural rearrangements. This was confirmed by the lower fractal dimension of acid aggregates at higher temperatures (40 °C versus 10 °C), meaning a more compact structure of particles and a more porous structure of the final network [21]. These last two effects could lower the rheological properties in the set gel and also in the stirred gel. Moreover, stirring may create particles which have altered surface properties (hydrophobic or electrostatic properties), depending on the kinetics and the temperature of acidification, which could lead to different inter-particle interactions. As a higher incubation temperature increases the intra-particle hydrophobicity, this could lead to less hydrophobic surface area of aggregating particles during the texture recovery and to a reduced likelihood of hydrophobic inter-particle bonds being formed. This would probably impede the possibility of rebodying. Finally, when gelation occurs at a higher pH value, the initial interactions formed may further stress the structures and may prevent further bond formation as the pH decreases. Stirring reduced this stress and increased the contact between particles which may facilitate more rearrangements, and increased the formation of new interactions between protein particles. In conclusion, acidification of milk with bacteria led to the higher rebodying of the acid just-stirred gel with the cited effects probably involved.

Just after stirring the soft gel showed a lower dynamic viscosity than the hard gel, but after 28 d of storage at 4 °C, it was the more viscous. In fact, for the soft gel obtained by GDL addition at 20 °C, the viscosity increased more than twofold from the day of stirring to 7 d of storage, while the viscosity of the hard gel obtained by fermentation at 38 °C showed only a very small increase. It would therefore be better manufacturing an acid gel with much lower solid properties, to obtain a highly viscous stirred gel. Strong and weak interactions in the stirred gels were responsible for solid and flow properties of stirred acid gel, respectively. An increase in both of these was observed. As the soft gels were formed at a lower temperature, hydrophobic interactions may be less significant to the gel structure, and therefore other interactions were involved in these gels. The texture recovery could be due to the resetting of interactions already present in the set gel or to the formation of new interactions. Assuming the former hypothesis, stirred gels formed from set gels at 20 °C may be more capable of reorganising at 4 °C, in the absence of hydrophobic interactions, compared with gels formed at a higher temperature, giving higher viscosities after 7 d.

5. CONCLUSIONS

The set acid gel obtained with lactic acid bacteria showed a more heterogeneous structure and a higher viscoelastic modulus, and led to a stirred acid gel with a higher viscosity and higher viscoelastic modulus at day 0, but to a significantly lower dynamic viscosity after 28 d of storage. The stirring of the bacterial gel could lead to structural reorganisation due to the reduction of heterogeneity and to a rapid rebodying, whereas manufacturing the GDL gel at a lower temperature suggests that reorganisation was more likely to occur during the cold storage of the stirred gel for 28 d.

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