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Original article

Development and breakdown of structure in yoghurt studied by oscillatory rheological measurements

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Summary — Yoghurt was produced from heat treated skim milk *in situ* in a rheometer. The development of set yoghurt structure was found to depend on the pH and not on the rate of pH decrease. At pH 4.25, 44°C, 0.1 Hz, within the linear viscoelastic region yoghurt had a storage modulus of ~230 Pa, and a loss angle of ~13°. High strain, up to 0.8, oscillation was used to break down the *in situ* gel structure at varying temperatures. Over a 30-min period, apparent storage modulus decreased by 1–2 orders of magnitude. The decrease in gel storage modulus was found to occur at least on 2 timescales, the proportion of fast and slow breakdown varying with strain and temperature. The estimated relative residual modulus showed little variation in the examined temperature and pH ranges but was strongly dependent on strain and to a lesser extent on frequency of the oscillatory shear.

yoghurt / rheology / breakdown / storage modulus

Résumé — Suivi du développement et de la rupture de la structure du yoghourt. *Des yoghourts sont préparés* in situ *dans le système de mesure d'un rhéomètre à partir de lait traité thermiquement. Le développement et la rupture de la structure sont étudiés par mesures en régime harmonique. On montre que la structure du yoghourt au bout d'un temps donné après l'ensemencement dépend de la valeur du pH atteinte, et non du temps mis à l'atteindre. À pH 4,25 et 44°C, le yoghourt présente un module conservatif et un angle de perte d'environ 230 Pa et 13° respectivement, mesurés à 0,1 Hz dans le domaine linéaire de la viscoélasticité. On utilise une amplitude de déformation élevée (jusqu'à 0,8) pour casser la structure du gel à différentes températures. Le module conservatif chute de 1 à 2 ordres de grandeur sur un laps de temps de 30 min. Cette diminution se fait en 2 phases, rapide puis lente, dont l'importance relative dépend de l'amplitude de déformation et de la température. La valeur relative du modèle résiduel varie peu avec le pH et la température dans les gammes étudiées, mais beaucoup de l'amplitude et de la fréquence de la déformation.*

yoghourt / rhéologie / structure / module conservatif

INTRODUCTION

In yoghurt production, *Lactobacillus delbruckii* subsp *bulgaricus* and *Streptococcus salivarius* subsp *thermophilus* added to milk transform lactose into lactic acid (Zourari *et al,* 1992). The concomitant decrease in pH lowers the net negative charge of casein micelles and dissolves (van Hooydonk *et al,* 1986) the colloidal calcium phosphate associated with the micelles, which is considered essential to micelle integrity (Walstra and Jenness, 1984). At a certain pH which depends on temperature, casein particles which probably differ from the original micelles (Heertje *et al,* 1985; Roefs *et al,* 1985) begin to aggregate. If the aggregation process is allowed to proceed without mechanical disturbance, a casein gel is formed.

In a casein gel, the casein particles are heterogeneously distributed, creating a network with areas devoid of casein, and more or less large casein aggregates in chains and nodes (Kalab and Harwalkar, 1973; Kalab and Emmons, 1975; Kalab *et al,* 1976; Roefs *et al,* 1990a). There is a large variation in length and width of the chains and the size of the pores and the aggregated particles and it has been established that the structure is statistically self-similar at different length scales, *ie* fractal (Bremer *et al,* 1989, 1990). The rheological properties of acid casein gels have been studied extensively by the Wageningen group (van Vliet *et al,* 1989; Roefs and van Vliet, 1990).

In commercial yoghurt production 2 yoghurt types are encountered, the set type and the stirred type. The set type yoghurt is fermented in consumer packages and cooled without disturbance of the gel. In the stirred type yoghurt, milk is fermented in vats, the gel is broken by stirring, pumped through a cooler and packaged at or close to storage temperature. It is well known that viscosity of the resulting yoghurt is to a large degree determined by the mechanical treatment during processing. A series of thorough practical investigations on the subject was performed at NIZO (Steenbergen, 1971a–d, 1972).

Wider availability of rheometers has resulted in a number of studies in well-defined geometries on yoghurt viscosity (Hellinga *et al,* 1986; Steventon *et al,* 1989; Ramaswamy and Basak, 1991, 1992); however, the flow history of the samples has been undefined.

Recently we have proposed (Arshad *et al,* 1993) to study the process-induced structure breakdown by high strain oscillatory shear *in situ,* and thus avoid the sample handling uncertainties; here we report an extension of that study to yoghurt.

MATERIALS AND METHODS

All measurements were carried out using a Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden) with a Couette-type measuring system C 25 HS (bob diam 25 mm, gap 0.31 mm. Torsion bar 4.14 gcm was used during the structure build-up period and for gel characterization, torsion bar 21.55 gcm for structure breakdown. Before the measurements the yoghurt was maintained at the selected temperature for 5 min.

Pasteurized market skim milk was heated from 8°C to 100°C for 22.5 min on a hot plate and kept boiling for 5 min before cooling in a cold water bath to 35°C.

Active starter was produced by adding 50 mg freeze-dried Christian Hansen thermophilic lactic culture B3 to 1 l milk treated as above, fermenting until pH 4.5, cooling to 10°C, and storing at 10°C.

For the experiments proper, 5 or 1% of the previous day's active starter was added to freshly pretreated milk, stirred manually for 2 min and immediately transferred to the rheometer measuring cup maintained at 44°C; the pH was measured in a separately thermostated sample.

Gel build-up at 44°C was followed every 5 min by dynamic oscillating measurement at

0.01–1 Hz, strain 0.0125. After 4 h the yoghurt was subjected to structure breakdown experiments under varying conditions (see table I). With 1 and 5% starter addition, the pH after 4 h was 4.5 and 4.25, respectively.

Commercial low-fat (0.5%) stirred yoghurt was purchased in a local shop and used for some comparisons.

The time-course of storage moduli during breakdown was fitted by the nonlinear least squares routine of Peakfit (Jandel Scientific, Corte Madera, CA).

RESULTS AND DISCUSSION

Preparation of yoghurt

Preliminary experiments were made to establish a suitable milk pretreatment and inoculum size (fig 1). In the milk which had been kept boiling for 5 min, the pH drop was much faster than in milk treated at

Table I. Experimental conditions and 2-exponential fit to the time course of breadown.
Conditions expérimentales et résultats de l'ajustement d'une somme de 2 termes exponentiels à la cinétique de rupture.

Temp (°C)	*Starter (%)*	*Strain (–)*	*Freq (Hz)*	G'_2 *(Pa)*	t_1 *(Min)*	G'_2 *(Pa)*	t_2 *(Min)*	G'_{inf} *(Pa)*	r^2
10	5	0.4	0.1	536	0.04	40.9	18.6	158	1.0000
10	5	0.4	0.1	508	0.05	105	39.4	104	1.0000
10	5	0.4	1,0	692	0.02	144	11.7	79.5	0.9999
10	5	0.8	0.1	706	0.05	23.9	5.95	18.6	1.0000
10	5	0.8	1.0	905	0.05	28.3	5.04	11.7	1.0000
10	5	0.8	1.0	899	0.05	22.5	5.61	11.9	1.0000
30	5	0.4	0.1	171	0.07	113	22.2	73.2	0.9997
30	5	0.4	0.1	164	0.05	144	10.8	56.9	0.9996
30	5	0.4	1.0	384	0.08	152	1.78	43.8	0.9988
30	5	0.8	0.1	331	0.05	8.78	6.41	8.94	1.0000
30	5	0.8	0.1	355	0.06	12.6	5.44	8.46	1.0000
30	5	0.8	1.0	495	0.05	18.3	4.87	9.21	1.0000
30	5	0.8	1.0	512	0.05	18.7	5.83	9.68	1.0000
40	5	0.4	0.1	73.1	0.09	142	4.69	36.2	0.9983
40	5	0.4	0.1	61.8	0.11	128	6.23	63.1	0.9977
40	5	0.4	1.0	176	0.10	128	3.63	33.4	0.9976
40	5	0.8	0.1	249	0.08	11.7	3.98	8.07	1.0000
40	5	0.8	1.0	325	0.06	12.0	4.09	5.77	1.0000
40	5	0.8	1.0	340	0.05	13.5	4.54	6.94	1.0000
50	5	0.4	0.1	75.0	1.62	40.3	10.5	35.2	0.9981
50	5	0.4	0.1	57.1	0.36	79.2	4.88	34.9	0.9975
50	5	0.4	1.0	86.2	0.01	138	2.72	30.3	0.9945
50	5	0.4	1.0	131	0.16	67.0	3.74	26.0	0.9991
50	5	0.8	0.1	131	0.09	11.2	3.17	4.87	0.9999
50	5	0.8	1.0	236	0.05	5.53	4.72	2.89	1.0000
50	5	0.8	1.0	235	0.05	9.95	4.73	4.22	1.0000
50	1	0.4	0.1	69.2	0.11	17.4	2.75	10.4	0.9997
50	1	0.4	0.1	111	0.04	12.9	4.59	31.3	0.9984
50	1	0.4	1.0	113	0.11	26.5	3.07	15.0	0.9995
50	1	0.8	0.1	75.2	0.09	8.90	2.54	4.34	0.9999
50	1	0.8	1.0	130	0.06	6.71	3.35	2.58	1.0000
50	1	0.8	1.0	142	0.07	5.80	3.51	4.05	1.0000

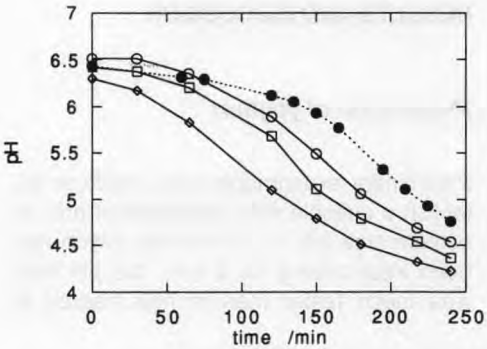

Fig 1. Heat treatment and inoculum size effect on the change of pH during yoghurt fermentation. (●), 3% starter, 90°C/15 min; (O), 1% starter, 100°C/5 min; (❑), 2% starter, 100 °C/5 min; (◊), 5% starter, 100°C/5 min.
Influence du traitement thermique et du taux d'ensemencement sur l'évolution du pH au cours de la fermentation du yoghourt. ● 3% de levain, 90°C/15 min; O : 1% de levain, 100°C/5 min; ❑ : 2% de levain, 100°C/5 min; ◊ : 5% de levain, 100°C/5 min.

90°C for 15 min. This improvement in fermentation was easily inadvertently reversed by over-vigorous stirring when adding the starter. We are of the opinion that in laboratory-scale yoghurt making, due to the large surface area/volume ratio, the effect of oxygen sensitivity of the microbial culture (Driessen *et al,* 1983) is of particular importance. Boiling the sample vastly improves deaeration due to the scrubbing effect of water vapor bubbles.

Yoghurt gel structure development

The reproducibility of gel formation can be estimated from figure 2, where average ± SD values of storage modulus *G'* and phase angle δ are plotted. Measurable storage modulus (≈ 1 Pa) was detected at pH 5.5, in agreement with the onset of gelling of heated milk at 43°C reported by

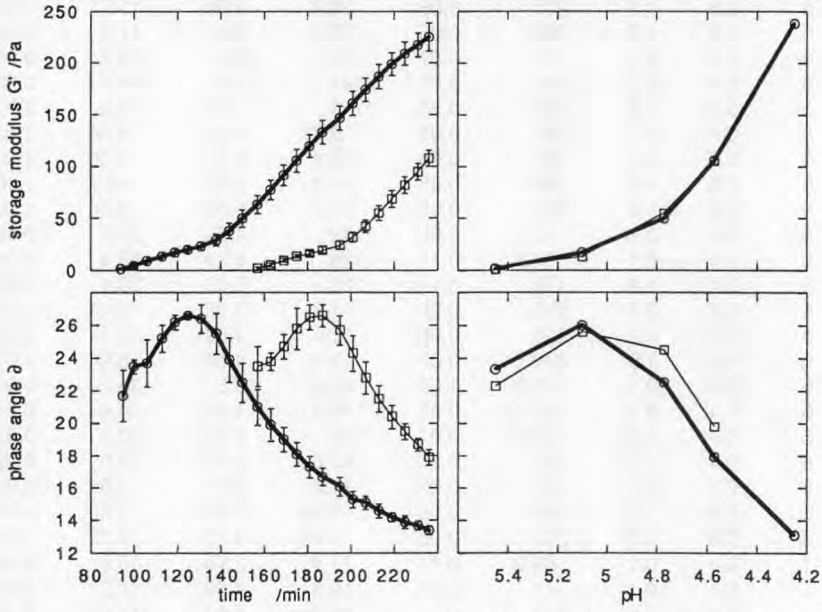

Fig 2. Storage modulus and loss angle *vs* time and *vs* pH during fermentation with 5% (heavy line) and 1% (light line) starter. Vertical lines indicate SD.
Module conservatif et angle de perte en fonction du temps et du pH pendant la fermentation avec 5% (ligne épaisse) et 1% (ligne fine) de levain. Les lignes verticales indiquent l'écart-type.

Heertje *et al* (1985). In the range of acidification rates studied here, it appears that gel properties are only determined by pH; the 1 and 5% curves in figure 2 are virtually identical.

An abrupt change in the rate of gel structure development, *ie* change in the slope of the *G' vs* time curve and a maximum in phase angle δ, was consistently found in our data. This is not common in gel formation. The lactic acid production rate is not expected to change in this pH region (Driessen *et al,* 1977). The change in buffering capacity of milk, while appearing in roughly the correct pH range is not a likely explanation either, as the slope changes by 100% and the buffer index only by 20% (Walstra and Jenness, 1984). The phase angle maximum appears at the same pH as the maximum in T_2 H-NMR correlation time reported by Roefs (1986) in acidified milk. Roefs *et al* (1990b) studied rennet gels produced at different pHs and reported a pronounced minimum in *G'* and maximum in δ for a pH ≈ 5.2. Thus the effect appears not to be of a kinetic nature, and may be related to the charge reversal of β-casein pointed out by Heertje *et al* (1985) and/or the dissolution of micellar calcium phosphate (Walstra, 1990).

The frequency and temperature-dependence of the storage modulus and loss angle were examined for the set yoghurt manufactured at 44°C with 5% starter (fig 3). At all temperatures, the modulus was proportional to $(\text{frequency})^{0.15}$ (data not shown). For comparison, data on a commercial stirred yoghurt are included. The modulus of the stirred yoghurt was smaller by a factor of > 10.

Breakdown of yoghurt gel structure

Stirred yoghurt was reported to be linear up to strain 0.03 at the frequency 0.1 rad/s (Steventon *et al,* 1989). Gross fracture in creep experiments on glucono-δ-lactone gel was observed at 0.5–0.6 strain (Roefs, 1986; van Vliet *et al,* 1989)

We have examined the strain dependence of the storage modulus at 50°C and 0.1 Hz (fig 4). A single reading, corresponding to < 2 oscillation cycles was taken at each strain. At strains of 0.05 and higher, the apparent storage modulus decreased and the decrease was approximately proportional to the strain.

To study structure breakdown, continuous oscillating strain outside the linear region (fig 5, strain 0.4; fig 6, strain 0.8) was applied to the sample and the resulting apparent storage modulus at high strain followed each 50 s (starting at 10 s) for 30 min at different temperatures.

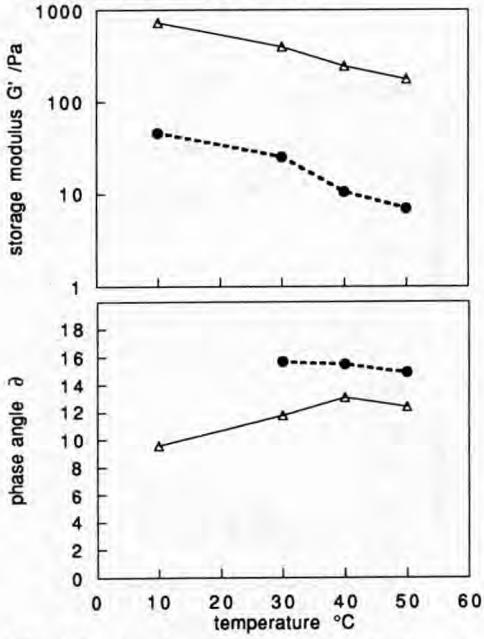

Fig 3. Temperature dependence of storage modulus and loss angle of (Δ) set yoghurt (5% starter) and (●) commercial stirred yoghurt.
Influence de la température sur le module conservatif et l'angle de perte du yoghourt testé (5% de levain) (Δ) et de yoghourt brassé du commerce (●).

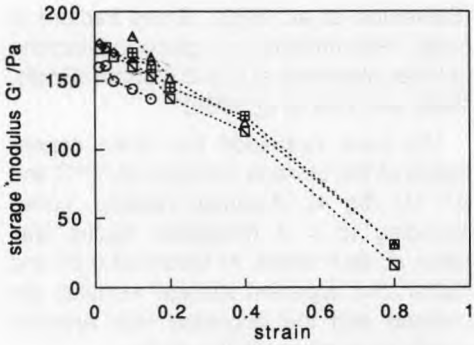

Fig 4. Strain dependence of initial storage modulus of 5% starter yoghurt. Symbols distinguish between individual samples. 50°C, 0.1 Hz.
Influence de l'amplitude de déformation sur le module conservatif initial du yoghurt (5% de levain). Les symboles individualisent les échantillons (50°C, 0,1 Hz).

As expected, the value of the applied strain had the largest effect on breakdown, whereas the differences between 0.1 Hz and 1 Hz frequency were minor. A significant part of the breakdown occurred within a very short time after the breakdown experiment was started. Thus the first measured value at high strain is not well defined, and we prefer to relate the breakdown to the initial low strain modulus, which was in general measured in each individual sample (some missing values were estimated from the sample's measured modulus at fermentation temperature and the temperature dependence of the remaining samples).

At the time-scale studied, the breakdown curves could be empirically well

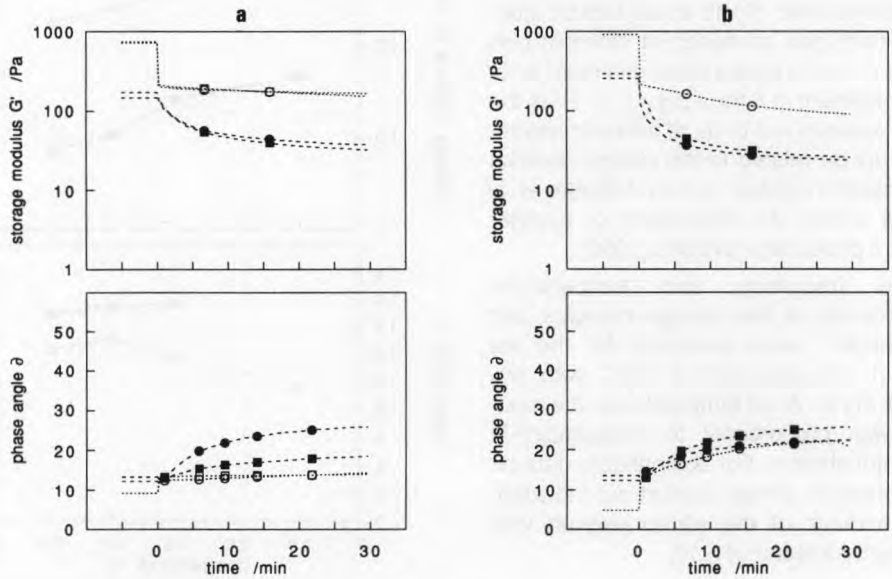

Fig 5. Apparent storage modulus as a function of time after applying stress at 0.4 strain. a) at 0.1 Hz; b) at 1 Hz. Breakdown temperatures : open symbols 10°C; filled symbols 50°C (not all points shown).
Module conservatif en fonction du temps après application d'une amplitude de déformation de 0,4 : a : à 0,1 Hz; b : à 1 Hz; symboles ouverts : à 10°C; symbols pleins : à 50°C. Tous les points ne sont pas représentés.

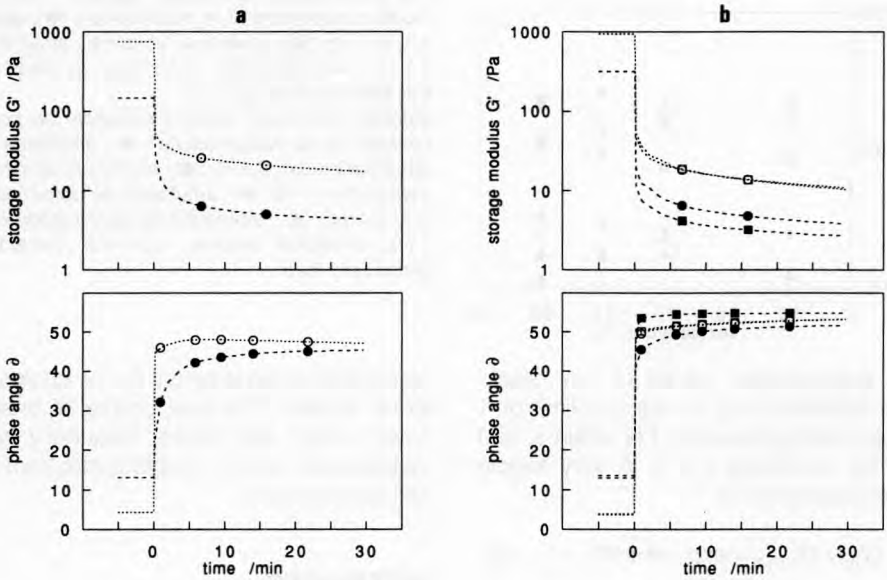

Fig 6. Apparent storage modulus as a function of time after applying stress at 0.8 strain. a) at 0.1 Hz; b) at 1 Hz. Breakdown temperatures: open symbols 10°C; filled symbols 50°C (not all points shown).
Module conservatif en fonction du temps après application d'une amplitude de déformation de 0,8 : a : à 0,1 Hz; b : à 1 Hz; symboles ouverts : à 10°C; symboles pleins : à 50°C. Tous les points ne sont pas représentés.

described ($r^2 > 0.997$, nonlinear least squares) by a sum of 2 exponentials:

$$G' = G'_1 \cdot \exp(-t/\tau_1) + G'_2 \cdot \exp(-t/\tau_2) + G'_{inf} \quad [1]$$

where t: time;

τ_1, τ_2 : characteristic breakdown time constants;

G'_1, G'_2 : amount of fast and slow breakdown;

$G'_{initial}$: initial value of G';

G'_{inf} : estimated G' at infinite time.

The curve-fitting results for all experiments are summarized in table I. The value G'_{inf} can be considered a measure of the residual structure after infinite time. For comparison between different temperatures and measuring frequencies, the residual modulus G'_{inf} is related to the initial modulus in figure 7. The relative residual modulus, $G'_{inf}/G'_{initial}$, tended to be somewhat larger at higher temperatures, thus yoghurt seemed to withstand strain marginally better at higher temperatures. Yoghurt pH did not seem to affect $G'_{inf}/G'_{initial}$, while strain and frequency played a large part.

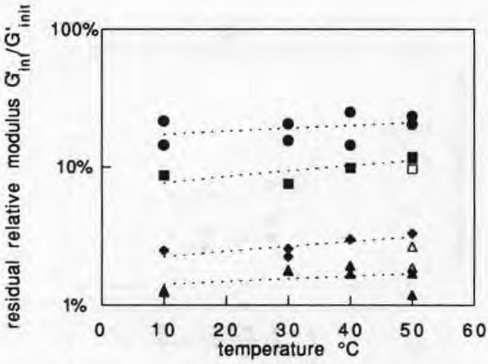

Fig 7. Extrapolated residual apparent storage modulus in percent of its initial value : (●), strain 0.4, 0.1 Hz; (■), strain 0.4, 1 Hz; (◆), strain 0.8, 0.1 Hz; (▲), strain 0.8, 1 Hz. Open symbols pH 4.5; filled symbols pH 4.25.
Module conservatif résiduel extrapolé en pourcentage de sa valeur initiale : ● : amplitude de déformation 0,4, 0,1 Hz; ■ : amplitude de déformation 0,4, 1 Hz; ◆ : amplitude de déformation 0,8, 0,1 Hz; ▲ : amplitude de déformation 0,8, 1 Hz; symboles ouverts : pH 4,5; symboles pleins : pH 4,25.

A mathematical model of the breakdown behaviour has to reach a limit of 1 for decreasing frequency *f* or strain *s*, and of 0 for increasing *f* or *s*. A very simple model would then be :

$$G'_{inf} / G'_{initial} = \exp(-A \cdot s \cdot f^B) \qquad [2]$$

Our results can be fitted with $A = 5.2$, $B = 0.08$ (log transformed, nonlinear least squares, $r^2 = 0.94$). The fit was not significantly improved by including temperature, pH, or by fitting an exponent to the strain.

The decrease of the storage modulus in the experiments can be put into perspective by comparison with the storage modulus of the commercial yoghurt. Comparable values were obtained after 6 min at strain 0.8 and frequency 0.1 Hz, or after 2 min at 0.8 strain and 1 Hz. The strain 0.4 did not reduce the storage modulus to the level of the commercial yoghurt at any temperature, frequency or pH.

CONCLUSIONS

Oscillating shear at high strain makes it possible to follow rheological changes in yoghurt, modelling the transition from a continuous gel to a more particulate structure in production of stirred yoghurt. The amount of structure breakdown can be controlled to a wide extent by the choice of strain applied. The time course of breakdown varied with strain, frequency and temperature, and no simple breakdown kinetics were found.

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