Rheology of Stirred Yogurt as Affected by Added Milk Fat, Protein and Hydrocolloids

M.K. KEOGH and B.T. O’KENNEDY

ABSTRACT

The rheological characterization of stirred yogurt with added milk fat, Na caseinate (or micellar casein) and gelatin (4 Bloom strengths), starch or a xanthan gum/LBG 50:50 mixture was carried out. Dynamic and shear values were measured at 8°C and syneresis at 4°C. Consistency (k* and k) and syneresis were more frequently influenced by the composition variables than the power law factors n* and n and the critical strain \( \gamma_c \). The k* ranged from 15.8 to 576 Pa s\(^{-1}\), n* from 0.038 to 0.220, \( \gamma_c \) from 1.6 to 49.0 \times 10\(^{-3}\), k from 0.37 to 32.47 Pa s\(^{-1}\), n from 0.005 to 0.587, and syneresis from 0.0 to 49.2%.

Key Words: yogurt, composition, rheology, hydrocolloids, syneresis

INTRODUCTION

YOGURT MAY HAVE TWO PRIMARY DEFECTS, variations in viscosity and/or expulsion of serum (syneresis). These changes probably result from seasonal variations in protein ratios and ion values, even when fat and total protein are adjusted to constant levels; but processing, incubation and storage conditions also have an effect. Dairy ingredients and hydrocolloids have sometimes been added to combat such defects (Abrahamsen and Holman, 1980; Tamime and Deeth, 1980; Modler et al., 1983; Klupsch, 1989; Guinee and Mullins, 1993), but reports are not available on interactive effects of Na caseinate and hydrocolloids.

Sodium caseinate increased the consistency k and reduced the power law factor n during shearing from 18.2 to 116.2\(^{-1}\) over 1500s (Guinee and Mullins, 1993). The effects on k and n were less for whey protein concentrates and were intermediate for skim milk and butter milk powders which contained both proteins. Casein and the main whey protein \( \beta\)-lactoglobulin interact chemically on heating. This effectively increases the concentration of gel-forming protein in the yogurt matrix and reduces syneresis through increased entrapment of serum within the interstices of the whey protein molecules attached to the surface of the casein. Dannenberg and Kessler (1988) showed a close relationship between rheological properties of yogurt and the degree of \( \beta\)-lactoglobulin denaturation. Between 60 and 90% denaturation, the firmness of gels increased considerably. The effects of \( \beta\)-lactoglobulin and \( \alpha\)-lactalbumin were investigated by Mortar et al. (1989). Filaments of denatured \( \beta\)-lactoglobulin were shown by electron microscopy to develop on the micelle surface. At higher heating levels, \( \alpha\)-lactalbumin filaments also began to precipitate onto the micelle, which apparently filled the gaps formed by the \( \beta\)-lactoglobulin filaments and formed a smoother micelle surface. According to Rohm (1989), this association of \( \alpha\)-lactalbumin with casein particles appeared to be important for the fusion and hydration of micelles during subsequent fermentation. This affected the rheological properties of the yogurt. Since the focus of our study was on Na caseinate, whey proteins were not used, resulting in an imbalance in the yogurt protein composition and a higher level of defects. However, they could be counterbalanced by the addition of hydrocolloids. Thus, a high tendency to wheying-off and lumpy texture were noted when Na caseinate alone was added to yogurt milk. This was probably due to the large casein particle clusters and robust micellar chains resulting in a more open protein matrix (Tamime et al., 1984).

The hydrocolloids used were gelatin (Bloom strength 225 to 275g), wheat starch and a 50:50 blend of xanthan gum/locust bean gum. Gelatin (0.5% of 240 g Bloom strength) also increased k and decreased n (Guinee and Mullins, 1993), but the changes were less than they were with Na caseinate. The high level of gelatin used (0.5%) reduced syneresis to zero when assessed by centrifugation (g = 284, 1136 and 2556). Starches from various sources have also been used in commercial yogurt manufacture.

In addition to levels of milk protein and hydrocolloid used, milk fat was also varied. Milk is homogenized for yogurt making and the fat becomes coated with casein, effectively causing the homogenized (size-reduced) fat globules to behave as very large casein micelle-coated spheres. Thus, an increase in the consistency k, and a decrease in n and syneresis were expected from increasing either fat or Na caseinate levels. Our objective was to determine the effects of protein-hydrocolloid interactions on stirred yogurt (pH 4.6) and the effects of milk fat.

MATERIALS & METHODS

Materials

One 20-kg bag of Na caseinate (89.9% protein, Code KH18, Kerry Group plc., Lisnowel, Co. Kerry, Ireland), stored at 10°C, was used. Micellar casein was produced by the method of Hewedi et al. (1985). After freeze-drying, the powder had a protein content of 82.5% by Kjeldahl and a Ca:P ratio of 1.6:1. Calcium was determined by atomic absorption spectrophotometry and phosphorus by the IDF method (1990). Gelatin was supplied by ExtracoAB (Klippan, Sweden) with Bloom strengths of 225, 240 and 275 g. Wheat starch was donated by INRA (Nantes, France). Six trials were initially carried out with xanthan gum/locust bean gum mixture, supplied by Kelco (London, UK) was therefore used.

Preparation of yogurt samples

A fresh mixed p.m. + a.m. milk was obtained from the center’s Friesian herd and cream was removed by centrifugal separation at 63°C, using a Westfalia separator (Model MM 1254, Ölde, Germany). After storage at 4°C overnight, the milk batches were warmed to 60°C. The protein and hydrocolloid powders were blended and suspended in a 20-L batch of milk using a mixer (Model AXR with emulsion head, Silverson Machines Ltd., Chesham, U.K.). The milk was heated in a continuous APV Pasilac SSP pilot plant (Silkeborg, Denmark) to 90°C for 120s, homogenized downstream in two stages at 14.5/4.8 MPa, cooled to 42°C and collected in 5-L glass beakers. Milk of specified composition (Table 1a) was inoculated with 1.5% mother culture prepared from 0.1% lyophilized yogurt cultures Redi set CH1 and B3 in the ratio 60:40 (C. Hansen’s Lab, Cork, Ireland), consisting of Streptococcus salivarius subsp. thermophilus and Lactobacillus bulgaricus. Inoculated milk was incubated at 42°C until the

Table 1a—Ingredient levels (% w/w) and gelatin Bloom strengths (g)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Added Na caseinate or micellar caseina</td>
<td>0.0</td>
<td>1.5*</td>
</tr>
<tr>
<td>Gelatin</td>
<td>0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Starch</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Xanthan gum/LBG 50:50</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Gelatin Bloom strength 225</td>
<td>275</td>
<td></td>
</tr>
</tbody>
</table>

*1.33% high level used in the starch trials.

Authors Keogh and O’Kennedy are with the National Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork, Republic of Ireland.
pH was reduced to 4.6, which normally required 4h ± 0.5h. The yogurt was then cooled to 10°C by placing the beakers in a bath of iced water with slow overhead stirring of the yogurt using a gate-type stirrer at 60 rpm. The stirred yogurt was placed in a refrigerator at 4°C for 4h ± 1h before testing.

Rheological measurements

The rheological properties of viscoelastic materials such as yogurt can be determined in dynamic oscillation or shear mode by a controlled strain rheometer such as the Bohlin VOR which we used. Measurements in both modes were carried out at 8°C. Using the C25 cup (dia 27.5mm, nominal capacity 13.0 mL) and bob (dia 25.0mm) geometry, 13.25g of yogurt was added after one and half inversions of the beaker container and subjected to the test sequence:

Frequency sweep. A frequency sweep from 0.03 to 20 Hz was performed without any delay time at a strain of 1 × 10⁻³ to deter-

## Table 1b—Composition variables and number of trials

<table>
<thead>
<tr>
<th>Variables</th>
<th>Designed no. of Trials</th>
<th>Actual no. of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin, gelatin B.s., fat, added Na caseinate</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Starch, fat, added protein in 2 blocks (micellar casein and Na caseinate)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Xanthan gum/LBG, fat, added Na caseinate</td>
<td>15</td>
<td>24*</td>
</tr>
</tbody>
</table>

*Augmented design (see Materials & Methods)

Since the lowest critical strain measured was 1.1 × 10⁻³, the linear viscoelastic region of the yogurts was not exceeded at the strain of 1.0 × 10⁻³ used. Thus, damage to the structure of the yogurts was not caused during these frequency sweeps.

Strain sweep. The frequency sweep was followed immediately by a strain sweep at 8°C to determine the critical strain (γc) of each yogurt sample. The γc was defined as the value of the strain before the value at which a reduction in G of 2% occurred. Dynamic testing was completed at this point due to the structural damage resulting from exceeding the critical strain, but a shear rate sweep was subsequently carried out which resulted in considerably more structure breakdown.

Shear rate sweep.

A shear rate sweep from 18.6 to 116⁻¹ was carried out to determine the relationship

\[ \eta = k' \gamma^{n-1} \]

where k' is the viscosity (Pa sⁿ⁻¹), k' the consistency index (Pa sⁿ), n the shear rate (s⁻¹) and n the power law factor. The power law relationship was the best model fit, expressed as a correlation coefficient, in both the dynamic and shear tests. In the shear test, an increasing shear rate only was carried out since it is known that yogurt is highly thixotropic (Ramaswamy and Basak, 1991). Thus, the n value measured was an assessment of both pseudoplasticity and thixotropy. Ideally, a pre-shear at the highest shear rate (116⁻¹) should be carried out, but this would have largely destroyed the weak gel structure of the yo-
gurt. The calibration of the rheometer was test-
ed weekly using a National Physical Labora-
tory (London, U.K.) certified oil with the fol-
lowing parameters at 25°C: k* = 1.010 Pa sⁿ, n* = 1.00

Syneresis

Yogurt (30-40g) was centrifuged at 222 × g for 10 min at 4°C. The clear supernatant was poured off, weighed and recorded as sy-
neresis (%).

Response surface methodology (RSM)

The ECHIP™ statistical package was used to design the experiment (select the optimum combination of ingredient variable levels used in the trials), analyze the data by regression analysis (relate response variables to levels of control or ingredient variables) and pro-
duce 3-D plots of results (Wheeler, 1989). A curvilinear relationship between control and response variables was anticipated, and a stan-
dard quadratic design was therefore chosen. In order to describe this relationship by a poly-
nomial equation with squared terms, trials at 3 levels of composition variables were re-
quired. The list of composition variables, de-
signed number of trials and replicates and the number carried out are described (Table 1b) for each of the three parts of the study. In the third part, xanthan gum/LBG was first as-
sessed in the range 0.1–0.3%, but when re-
sults suggested that values at 0.0% hydrocol-
loid be included, the experiment was augment-
ed by a further 9 trials, including 5 replicates. Replicate SD gave a measure of accuracy of the replicated tests, while residual SD was a measure of the fit of the model polynomial equation. Some of the responses were expo-
ential. In this case, the Box-Cox transforma-
tion of data was used, where

\[ Y = (y^{λ-1}) / \lambda \]

For λ = 1, the relationship was linear and for λ = 0, the relationship was logarithmic.

RESULTS

The influence of composition vari-
ables (Table 1b) on the response variables k*, n*, γc, k and n at 8°C and syneresis at 4°C of the yogurts was quantified. The main effects and interactions are seen in the analysis of variance or effects tables presented for each part of the study (Tables 2a, b and c). 3-D plots of 5 significant interactions were also compared (Fig. 1 to 5). Results showed that the consistency (k* and n) and syneresis were more influenced by composition variables than the power law factors n* and n the critical strain γc.

Effect of holding time

The yogurts in the trials with gelatin were held in the rheometer at 8°C for 0, 30, or 60 min and assessed dynamically at each time using a frequency sweep. The dynamic consis-
tency index (k*) increased by a mean of 4% (range 0–11) and 8% (range 1–16) after 30 and 60 min, respectively. These increases in k* were accompanied by small corresponding decreases in n*. Since changes were small over this time period, measurements at zero time only were reported.

Dynamic consistency index

The dynamic consistency index (k*) of the yogurts at 8°C ranged from 15.8 to 497 Pa sⁿ for gelatin (maximum at low gelatin, high fat, high Na caseinate), from 34.6 to 576 for starch (all high levels) and from 11.9 to 113 for xanthan gum/LBG (low xanthan gum/ LBG, high fat, medium Na caseinate). The xanthan gum/LBG yogurts had the lowest dynamic consistency index for the range of this hydrocolloid combination used. Starch did not affect the k*, but gelatin and xanthan gum/ LBG decreased k* by 44.11 and 56.97 Pa sⁿ, respectively. The high level of fat increased the k* for each of the three hydrocolloids. The increase was greatest with starch (150.91), intermediate for gelatin (46.04) and least for xanthan gum/LBG (30.44). Added protein also increased k*, by 81.78 Pa sⁿ with starch and by 44.59 with xanthan/LBG. The increase was the same whether micellar casein or Na casein-
ate was used with starch. There was an inter-
action between the gelatin Bloom strength and added Na caseinate (Fig. 1). At the low Bloom strength of 225g, added Na caseinate increased k* from 28.49 to 89.59 Pa sⁿ, but at the high Bloom strength the change in k* due to added Na caseinate was not significant. There was an interaction affecting k* in the starch trials between fat and added protein (Fig. 2). At the high level of fat, added Na caseinate increased the k* from 118.55 to
403.49 Pa s⁻¹, but at the zero level of fat the increase in $k^*$ due to Na caseinate was not significant. The $k^*$ values using micellar casein were only marginally lower. There was an interaction between xanthan gum/LBG and fat on $k^*$ (Fig. 3). At 5% fat, xanthan gum/LBG increased the $k^*$ from 81.78 to 43.63 Pa s⁻¹, but at the zero level of fat the $k^*$ values increased significantly. The $k^*$ values using micellar casein block of trials was 150.91 Pa s⁻¹, high-er than the micellar casein block. Fat increased $k^*$ by the largest amount (10.49 Pa s⁻¹) in the starch trials. There was an interaction between xanthan gum/LBG and fat on $k^*$. At zero levels of fat, xanthan gum/LBG increased the $k^*$ value marginally, but at the high level of fat, xanthan gum/LBG increased the $k^*$ value from near zero to 7.53 Pa s⁻¹.

**Dynamic power law factor and critical strain**

The dynamic power law factor ($n^*$) of the yogurts at 8°C ranged from 0.038 to 0.151 for gelatin, to 0.179 for starch and to 0.220 for xanthan gum/LBG. However, none of the composition variables affected the $n^*$ value significantly. The critical strain ($\gamma_c$) of the yogurts at 8°C ranged from 1.6 to 49.0 × 10⁻³ in the gelatin trials, 3.1 to 10.8 × 10⁻³ for starch and 1.1 to 28.7 × 10⁻³ in the xanthan gum/LBG trials. In the trials where gelatin was used, the high gelatin level increased the $\gamma_c$ by 7.76 × 10⁻³ and fat decreased the $\gamma_c$ by 5.12 × 10⁻³. In contrast, there were no significant effects of starch and xanthan gum/LBG on the $\gamma_c$.

**Consistency index**

The consistency index ($k$) of the yogurts at 8°C ranged from 0.091 to 10.42 for gelatin, from 2.26 to 32.47 for starch and from 1.56 to 13.27 for xanthan gum/LBG (maximum at the high levels). The yogurts made with starch had the highest shear consistency. The higher level of gelatin and xanthan gum/ LBG increased the $k$ value by 1.30 and 4.21 Pa s⁻¹, respectively. Added protein also increased the $k$ value by 1.54 and 3.67 Pa s⁻¹ in the gelatin and xanthan gum/LBG trials. In the starch trials, the mean value of $k$ in the Na caseinate block of trials was 2.88 Pa s⁻¹ higher than the micellar casein block. Fat increased $k$ by the largest amount (10.49 Pa s⁻¹) in the starch trials. There was an interaction between fat and xanthan gum/LBG (Fig. 4). At the low level of fat, xanthan gum/LBG increased the $k$ value marginally, but at the high level of fat, xanthan gum/LBG increased the $k$ value from near zero to 7.53 Pa s⁻¹.
**Power law factor**

The power law factor $n$ of the yogurts ranged from 0.334 to 0.587 for gelatin, 0.067 to 0.394 for starch and 0.005 to 0.427 for xanthan gum/LBG. Gelatin and xanthan gum/LBG decreased the power law factor $n$, that is, the yogurts became more susceptible to shear thinning. Gelatin decreased $n$ by 0.161 and xanthan gum/LBG by 0.184. Starch increased $n$ curvilinearly to a maximum at 0.25–0.30% starch (depending on the level of fat), but $n$ decreased again at higher levels of starch.

In the starch trials also, $n$ decreased the $n$ value by 0.126. In the xanthan gum/LGB trials, added Na caseinate decreased $n$ by 0.126.

**Syneresis**

The syneresis (%) of the yogurts after centrifugation at 4°C ranged from 0.0 to 39.10% for the trials with gelatin, to 23.50% for the trials with starch and to 49.20% for the trials with xanthan gum/LBG. Gelatin reduced syneresis by 14.43%, starch insignificantly, but the effect of xanthan gum/LBG was curvilinear (Table 3). Fat also reduced syneresis in each part of the study; by 10.00, 13.69 and 15.96% for the gelatin, starch and xanthan gum/LBG trials, respectively. Syneresis increased with increasing levels of xanthan gum/LBG up to 0.13–0.16% (depending on level of fat), but above these levels, syneresis decreased again. The interaction between gelatin and fat vs. syneresis (Fig. 5) showed at zero levels of fat and gelatin, syneresis measured 26.78%. With 5% fat alone, syneresis was reduced to 3.46% and with 0.5% gelatin alone, syneresis was reduced to zero. When both components (fat and gelatin) were present at high levels, syneresis (2.45%) was not significantly different.

**DISCUSSION**

Gelatin and xanthan gum/LBG increased the consistency $k$, as expected. However, the effect of wheat starch on consistency was not significant. There were corresponding decreases in $n$ value. Many polymeric materials, and particularly yogurt, display greater shear-thinning (reduced $n$ value) as $k$ increases (Ramaswamy and Basak, 1991). Equivalent changes did not occur for $k^*$, which decreased for the same two hydrocolloids and for $n^*$, for which no changes were found. Thus, the Cox-Merz rule, which states that $k^*$ is near equivalent for polymer melts and solutions was not followed. Departure from the Cox-Merz rule sometimes indicates that the system is shifting from the solution state. It was expected therefore that the rule would not apply for the complex yogurt system. Fat increased the dynamic consistency $k^*$ for each of the hydrocolloids. Fat also increased the $k$ value in the starch trials and interactively at low levels of xanthan gum/LBG, but not in the gelatin trials. Added Na caseinate increased both $k^*$ and $k$, but only at low levels of gelatin B.s. for $k^*$. Note that the effect of Na caseinate was greater than micellar casein on $k$ in the trials with starch. This may be because the addition of casein to yogurt milk in the form of micellar casein or as Na caseinate raised the levels of ionic components in the serum phase after acidification to pH 4.6. However, micellar casein addition then raised the Ca$^{2+}$ and PO$_4$$^{3-}$ levels while Na caseinate raised the level of free Na in the serum phase. This would lead to different ratios of casein-bound inorganic ions. Bringe and Kinsella (1991) suggested that inorganic ions in general can affect the protein-protein interactions between casein micelles during acid gel formation by altering the repulsive hydration forces between protein surfaces. They also showed that Ca$^{2+}$ ions had a greater tendency to inhibit coagulation of casein than Na$^+$ ions. An increase in Ca level decreased the tendency of casein to coagulate near the isoelectric point and could result in a weaker gel. The addition of micellar casein instead of Na caseinate to yogurt milk could therefore result in yogurt with lower $k$ values. This suggests that the final yogurt pH may be determined by the ionic strength and species in addition to the protein concentration and type. As well as the hydrocolloids used, added Na caseinate also reduced $n$, curvilinearly in the starch trials and linearly in the xanthan gum/LBG trials. As mentioned, these reductions in $n$ paralleled increases in $k$ values. Another main point of interest was the relatively large effect of fat on $k^*$ in the starch trials, which was almost 5 times greater than with xanthan gum/LBG. Fat also had a positive effect on brittleness in the gelatin trials. Gelatin increased $k^*$ by $7.76 \times 10^{-3}$ but reduction in brittleness by fat paralleled the decrease in $k^*$ caused by gelatin. It was shown (Marrs et al., 1994) with a 1% Na caseinate/1% gelatin suspension that a coacervate formed on cooling at pH 5.5. Below that pH, precipita-
tion of casein occurred leading to a reduction in $G'$. The wheat starch did not reduce syneresis but both gelatin and xanthan gum/LBG did above $0.15\%$. Fat reduced syneresis by the same order of magnitude in each of the three trials. Since protein was adsorbed onto the surface of the homogenized fat globules, increased levels of fat, in effect, increased the ability of the protein to immobilize water.

**CONCLUSIONS**

In yogurts made under these conditions, a positive effect from the fat, protein and hydrocolloid was found to varying degrees. A wide range of consistencies and brittleness levels could be obtained by varying these component levels. Protein was the most effective component at increasing consistency. Fat was next in effectiveness, since in homogenized yogurt milk, it functioned as a protein-coated fat globule. Fat also reduced syneresis in each trial but any main effects of starch and xanthan gum/LBG were insignificant. When gelatin was present, the effect on dynamic consistency and brittleness was negative, but syneresis was reduced.

**REFERENCES**


Wheeler, B. 1989. ECHIP™ Course Text. ECHIP, Hockessin, Delaware, USA.

Ms received 3/12/97; revised 6/10/97; accepted 9/3/97.

This project was part-funded under the EU AAIR programme as Contract No. RTD PL 92 0245.