Capsule Formation by Nonropy Starter Cultures Affects the Viscoelastic Properties of Yogurt During Structure Formation

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ABSTRACT

The aim of this work was to study the structure formation of yogurt made with cultures containing ropy Lactobacillus delbrueckii ssp. bulgaricus (R), capsule-forming nonropy Streptococcus thermophilus (CNR), and noncapsule-forming nonropy cultures (NCNR). Similar gelation profiles were shown for milk fermented by ropy and nonropy lactic cultures. The gelation point occurred at lower pH values in milk fermented with R or NCNR compared to that of milk fermented with CNR culture. Differences between capsule forming ropy and nonropy cultures were observed in the aggregation behavior of the caseins. Gels made with R culture had the highest maximum in loss tangent (tan δ). However, this maximum occurred at the highest pH value when CNR culture was used. The earlier gelation of milk fermented by the encapsulated nonropy strain of Strep. thermophilus resulted in increased structure rearrangement as the pH dropped, interfering with the formation of a more compact structure.

(Key words: capsule-forming nonropy starter, gelation, viscoelasticity, yogurt)


INTRODUCTION

Yogurt is a three-dimensional protein network consisting of a casein framework in which serum, fat globules, and bacterial cells are entrapped. Because of the relatively weak structure of the casein network, the distribution of the “filling compounds” and their interaction with casein micelles have a significant influence on the rheological behavior of yogurt (Vélez-Ruiz and Barbosa Canovas, 1997). Structure formation of acid milk gels has been extensively studied (Bremer et al., 1990; Vélez-Ruiz and Barbosa Canovas, 1997; Lucey and Singh, 1998; Lucey et al., 1998a,b; Horne, 1999; Lucey et al., 1999). Some of these studies investigated the gelation induced by the hydrolysis of glucono-δ-lactone (GDL), and compared structure development resulting from using GDL and bacterial cultures (van Marle and Zoon, 1995; Lucey et al., 1998a). Other studies demonstrated the role of denatured whey protein on the formation of acid milk gels (Lucey and Singh, 1998; Horne, 1999).

Exopolysaccharide (EPS) producing starter cultures are used to improve the physical properties of yogurt (Valahopoulou and Bell, 1993; Van Marle and Zoon, 1995; Hassan et al., 1996a, 1996b; Hess et al., 1997). To date, most of the studies on viscoelastic properties of yogurt prepared with exopolysaccharide-producing cultures focused their effect on the final product (Rawson and Marshall, 1977; Vlahopoulou and Bell, 1993; Hess et al., 1997; Hassan et al., 2001a). Since properties of the gel network are governed by changes that take place during structure formation, dynamic low shear measurements in situ would provide a better understanding of the role of exopolysaccharide-producing cultures on the viscoelastic properties of yogurt.

Hassan et al. (1995b) followed the coagulation of yogurt made with capsule-forming and noncapsule-forming nonropy starter cultures in real time using confocal scanning laser microscopy (CSLM). They defined the gelation point as the instant when the motion of capsule-forming microorganisms was no longer visible. However, noncapsule-forming cells were obscured by the casein micelles, making the monitoring of their movement difficult. Therefore, it was not possible to define the gelation point based on bacterial movement when such cultures were used, and no conclusions could be drawn between differences in gelation point between yogurt prepared with capsule-forming and noncapsule-forming cultures.

The present study investigated the effect of capsule-forming ropy and nonropy, and noncapsule-forming nonropy cultures on structure development of yogurt by using low shear dynamic measurements.
CAPSULE NONROPY CULTURES IN YOGURT

Table 1. Bacterial strains used in the study (Hassan et al., 1996b).

<table>
<thead>
<tr>
<th>Culture</th>
<th>Composition 1</th>
<th>Designation 2</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule-forming nonropy (CNR)</td>
<td>a. <em>S. thermophilus</em> 3855</td>
<td>Capsule-forming nonropy (5-µm capsules)</td>
<td>Marshall³</td>
</tr>
<tr>
<td></td>
<td>b. <em>Lb. delbrueckii</em> ssp. <em>bulgaricus</em> L4</td>
<td>Capsule-forming nonropy (1.5-µm capsules)</td>
<td>Retail yogurt</td>
</tr>
<tr>
<td>Noncapsule-forming nonropy (NCNR)</td>
<td>a. <em>S. thermophilus</em> 3</td>
<td>Noncapsule-forming nonropy</td>
<td>Retail yogurt</td>
</tr>
<tr>
<td></td>
<td>b. <em>Lb. delbrueckii</em> ssp. <em>bulgaricus</em> L4⁴</td>
<td>Capsule-forming nonropy (1.5-µm capsules)</td>
<td>Retail yogurt</td>
</tr>
<tr>
<td>Capsule-forming ropy (R)</td>
<td>a. <em>S. thermophilus</em> 3</td>
<td>Noncapsule-forming ropy</td>
<td>Retail yogurt</td>
</tr>
<tr>
<td></td>
<td>b. <em>Lb. delbrueckii</em> ssp. <em>bulgaricus</em> RR</td>
<td>Capsule-forming ropy (3-µm capsules)</td>
<td>Minnesota⁵</td>
</tr>
</tbody>
</table>

¹S = *Streptococcus*, Lb = *Lactobacillus*.
²The capsule size produced when cultures are grown in milk is indicated.
³Rhone-Poulenc Dairy Ingredients (Madison, WI).
⁴In NCNR combination, a strain with a small capsule was used because of the unavailability of a noncapsule-forming strain of *Lb. delbrueckii* ssp. *bulgaricus* with similar acidification rates.
⁵Department of Food Science, University of Minnesota, St. Paul.

MATERIALS AND METHODS

Bacterial Cultures

The bacterial strains used in this study are listed in Table 1, previously tested for encapsulation by Hassan et al. (1996a, 1996b). Stock frozen cultures were transferred to Elliker broth (Difco Laboratories, Detroit, MI) and incubated over night at 37°C. These cultures were then subcultured in reconstituted (11% wt/vol), steamed (95°C for 1 h) skim milk (Bi-Lo, Mauldin, SC) and incubated overnight to be used the next day as inocula. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were prepared separately and mixed (1:1, by volume) at time of preparing yogurt. The *Lactobacillus delbrueckii* ssp. *bulgaricus* L4 produced small capsules and was used in both NCNR and CNR strains. Therefore, differences in the viscoelastic properties of yogurt made with CNR and NCNR cultures could be directly related to the effect of the *Streptococcus thermophilus* strain used in each culture.

Rheological Measurements

Reconstituted (11% v/w) skim milk (Bi-Lo) was steamed (95°C for 15 min, cooled, and kept in a refrigerator overnight. Milk was warmed to 37°C and inoculated with 5% of CNR, NCNR, or R cultures. Structure development at 37°C in milk fermented by different cultures was followed using a controlled stress rheometer (SR-5000 Rheometric Sci., Piscataway, NJ) and a couette geometry. The tool temperature was kept at 37°C using a controlled temperature water bath. Approximately 14-ml of the milk/culture mixtures was transferred to the rheometer. Samples were oscillated at a frequency of 0.1 Hz, and strain applied was <0.01, as previously described by Lucey et al. (1999). The pH was monitored in a duplicate sample kept at 37°C in a controlled temperature water bath. Fermentation was terminated at pH 4.5. Deviations of less than 0.05 units were observed between pH values of samples in the rheometer cups and those in the control beakers. The gelation point was defined at the pH value when gel had a $G' > 1$ Pa (Van Marle and Zoon, 1995; Lucey et al., 1998a, 1998b).

Statistical Analysis

Statistical analysis was carried out to determine the effect of CNR, NCNR, or R culture on the viscoelastic properties of yogurt samples by using general linear model procedure, using SAS® (Version 6.1, SAS Institute, Cary, NC) and least square means of three replicate experiments were considered significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Figure 1 describes the average development of acidity in yogurt prepared with CNR, NCNR and R strains. No significant differences could be determined between the three strains. Development of viscoelastic properties of yogurt made with CNR, NCNR and R as a function of pH is shown in Figure 2. The complex shear modulus $G^\ast$ values during structure formation of gels made with different cultures were significantly different (Table 2). Gels
made with CNR culture had higher G* values than did those made with R or NCNR cultures; and significant differences in G* were observed at high pH values (5.3-4.7; except for pH 5.1) between gels made with CNR and those made with either R or NCNR culture. As the pH dropped below 4.7, no significant differences were observed among the different treatments.

Table 3 shows the pH range of gelation for milk fermented by various cultures. The gelation point, which was defined at $G' > 1$ Pa occurred at pH values of 5.52 to 5.58, 5.28 to 5.38, and 5.28 to 5.42 for milk fermented by CNR, NCNR, and R cultures, respectively. The high pH of gelation in milk fermented by CNR culture indicates a faster aggregation of casein micelles. These results confirmed the previous observations of Hassan et al. (1995b) who used CSLM to follow in real time the coagulation of milk fermented by the same capsule-forming strain used in this study. The presence of bacterial capsules seemed to force casein micelles to aggregate and occupy less space. The early aggregation in the presence of encapsulated bacteria would allow a high number of protein contacts at higher pH values (above 5.5) than those normally recorded for R or control strains (Van Marle and Zoon, 1995). This could explain the relatively higher gelation pH observed in this study for milk fermented by CNR culture. Hassan et al. (1995b) also reported that at pH 5.5, which was the observed pH of gelation of milk fermented by this culture, bacterial movement slowed, indicating an increase in resistance and, at pH 5.35, the microbial movement was no longer visible. They considered this point (pH 5.35) the gelation point. However, the rheology data obtained in the present study show that the gelation was initiated at the point where a slower movement of the bacterial cells was observed. Micrographs shown by Hassan et al. (1995b) also showed that at pH range (5.5 to 5.35), casein aggregates in milk fermented by the CNR culture were thicker than those in milk fermented by noncapsule-forming strains. Recently, Hassan et al. (2001b) found that the nonropy, capsule-forming strain of Strep. thermophilus used in this study also produced unattached EPS. The volume exclusion effect of both bacterial capsules and the unattached EPS would explain the differences in gelation point and G* values of milk containing CNR culture, compared...
with those of milk fermented with R or NCNR cultures. Although the ropy strain used in this study was also encapsulated, its effect on structure development was different from that of CNR, and similar to that which resulted from the use of NCNR. The ropy strain used in the R culture was the Lb. delbrueckii ssp. bulgaricus, and the predominance of the Strep. thermophilus at pH higher than 5.5 did not allow production of enough exopolysaccharide to affect the gelation point.

Lucey et al. (1999) reported the pH of gelation of heated milk acidified with GDL to be 5.2. Although the heat treatment they used was different from the one we used in this study, and the acidification rate of milk acidified by GDL is different from that fermented by bacterial cultures, the gelation pH was similar to that observed in this study when NCNR cultures were used. Van Marle and Zoon (1995) observed no difference in gelation pH among gels made with different ropy and nonropy cultures. However, the nonropy cultures were not tested for encapsulation.

As the pH dropped, fewer differences in dynamic moduli were observed between gels made with CNR and gels made with other cultures, indicating a slower development of the elastic characteristics in the CNR gel. This finding is consistent with the observation of Hassan et al. (1995b) who reported less structural differences between gels made with encapsulated and nonencapsulated cultures as the pH dropped, indicating more structure rearrangement in the former type of gel. The earlier aggregation process in milk fermented by capsule-forming culture could allow more rearrangement to take place. A small increase in the viscoelastic modulus has been previously attributed to a rearrangement during structure formation (Lucey et al., 1998a; Van Vliet et al., 1989).

Figure 3 illustrates the average values of tan δ (ratio between the viscous component, G′ and the elastic component, G″) as a function of pH during fermentation. Soon after gel formation, tan δ increased to a maximum before decreasing again. A maximum in tan δ after gel formation was also observed by other authors (Billiad-eris et al., 1992; Van Marle and Zoon, 1995; Lucey and Singh, 1998). Lucey et al. (1998b) hypothesized that the interaction of denatured whey proteins with casein micelles plays a major role in causing the increase in tan δ, and that the maximum tan δ represents a transition from a denatured whey proteins-induced gel at high pH to a network dominated by casein-casein interactions. In our study, significant differences in the pH values of maximum tan δ were found between gels made with CNR (pH 5.2) and those made with the other two cultures (R and NCNR), where the maximum tan δ occurred at pH 4.9. Since milk used in all our treatments was subjected to the same heat treatment, the current interpretation of the maximum in tan δ does not seem to apply. No significant differences were found in the pH interval between the pH at the gelation point and the pH at the maximum tan δ among tested cultures.

Values of tan δ at their maximum and at pH 4.5 are summarized in Table 3. The maximum in tan δ corresponded to a slope change of the G′ versus pH curve. The maximum value of tan δ for samples made with R culture was higher than that of samples made with CNR or NCNR. The higher value of tan δ max for gels made with the R strain demonstrated the presence between the viscous component, G′ and the elastic component, G″) as a function of pH during fermentation. Soon after gel formation, tan δ increased to a maximum before decreasing again. A maximum in tan δ after gel formation was also observed by other authors (Billiad-eris et al., 1992; Van Marle and Zoon, 1995; Lucey and Singh, 1998). Lucey et al. (1998b) hypothesized that the interaction of denatured whey proteins with casein micelles plays a major role in causing the increase in tan δ, and that the maximum tan δ represents a transition from a denatured whey proteins-induced gel at high pH to a network dominated by casein-casein interactions. In our study, significant differences in the pH values of maximum tan δ were found between gels made with CNR (pH 5.2) and those made with the other two cultures (R and NCNR), where the maximum tan δ occurred at pH 4.9. Since milk used in all our treatments was subjected to the same heat treatment, the current interpretation of the maximum in tan δ does not seem to apply. No significant differences were found in the pH interval between the pH at the gelation point and the pH at the maximum tan δ among tested cultures.

Values of tan δ at their maximum and at pH 4.5 are summarized in Table 3. The maximum in tan δ corresponded to a slope change of the G′ versus pH curve. The maximum value of tan δ for samples made with R culture was higher than that of samples made with CNR or NCNR. The higher value of tan δ max for gels made with the R strain demonstrated the presence

### Table 2. Least square means and standard deviations for G′ at selected pH for acidified milk prepared with capsule-forming nonropy (CNR), noncapsule-forming nonropy (NCNR), and ropy (R) cultures.

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>pH</th>
<th>G′ (Pa)</th>
<th>SD</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNR</td>
<td>4.5</td>
<td>46.5 ± 14.3a</td>
<td>67.6 ± 6.6a</td>
<td>106.9 ± 6.7a</td>
<td>150.6 ± 10.7a</td>
</tr>
<tr>
<td>NCNR</td>
<td>4.2</td>
<td>4.2 ± 4.1a</td>
<td>39.7 ± 22.2a</td>
<td>55.15 ± 27.4a</td>
<td>72.1 ± 38.9a</td>
</tr>
<tr>
<td>R</td>
<td>9.2</td>
<td>9.2 ± 9.3a</td>
<td>33.9 ± 9.3a</td>
<td>46.8 ± 11.8a</td>
<td>61.2 ± 12.1a</td>
</tr>
</tbody>
</table>

### Table 3. pH range of gelation point and least square means and standard deviation for tan δ at the maximum peak and at pH 4.5 of gels prepared with capsule-forming nonropy (CNR), noncapsule-forming nonropy (NCNR), ropy (R), and cultures. Gelation point defined as the value of pH when the elastic modulus (G″) > 1 Pa.

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>Gelation pH range</th>
<th>Tan δ Max</th>
<th>Tan δ at pH 4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNR</td>
<td>5.52–5.58</td>
<td>0.481 ± 0.013b</td>
<td>0.264 ± 0.012b</td>
</tr>
<tr>
<td>NCNR</td>
<td>5.28–5.38</td>
<td>0.470 ± 0.023a</td>
<td>0.301 ± 0.014a</td>
</tr>
<tr>
<td>R</td>
<td>5.28–5.42</td>
<td>0.532 ± 0.011b</td>
<td>0.295 ± 0.012a</td>
</tr>
</tbody>
</table>

Values within the same column, followed by different letters are significantly different P < 0.05 as calculated by the general linear model procedure (SAS, version 6.1).
of a greater viscous component. After reaching a maximum, \(\tan \delta\) decreased. Its value at final pH (4.5) was significantly lower in gels made with CNR compared to that in gels made with R or NCNR. The transition from the max \(\tan \delta\) to the value at pH 4.5 occurred at a greater pH range in yogurt containing CNR culture, because of the earlier gelation point.

**CONCLUSIONS**

Similar gelation profiles were shown for milk fermented by ropy or nonropy lactic acid cultures. Production of EPS by CNR strain of *Strep. thermophilus* caused the gelation point of milk to occur at a higher pH value than that resulted from the use of either non-exopolysaccharides producing *Strep. thermophilus* or culture containing encapsulated ropy *Lb. Delbrueckii ssp. bulgaricus*. The earlier gelation of milk fermented by the encapsulated nonropy strain of *Strep. thermophilus* allowed more structure rearrangement to take place as the pH dropped, interfering with the formation of a more compact structure.

**ACKNOWLEDGMENTS**

This research was supported by State and Hatch funds allocated to the Georgia Agricultural Experimental Station and by The University of Georgia Center for Food Safety and Quality Enhancement.

**REFERENCES**


