Rheological and Scanning Electron Microscopic Examination of Skim Milk Gels Obtained by Fermenting With Ropy and Non-Ropy Strains of Lactic Acid Bacteria

S. M. Schellhaass

H. A. Morris

Follow this and additional works at: https://digitalcommons.usu.edu/foodmicrostructure

Recommended Citation
Available at: https://digitalcommons.usu.edu/foodmicrostructure/vol4/iss2/11
Abstract

Physical and rheological parameters of skim milk gels fermented with slime producing (ropy) cultures and non-ropy cultures were compared. The skim milk gels were made from steamed reconstituted nonfat dry milk inoculated with 2% of a single strain starter culture and incubated at 32, 37, and 45°C until pH 4.5 ± 0.05 was attained.

Skim milk gels fermented by slime-producing strains of Streptococcus thermophilus, Streptococcus cremoris, and Lactobacillus bulgaricus exhibited similar rheological and physical characteristics. Electron micrographs of the ropy skim milk cultures showed that slime produced by the organisms was associated with the cell surface as well as the protein matrix of the system.

Skim milk gels fermented by the slime-producing strains exhibited decreased susceptibility to syneresis as compared to skim milk which had been fermented with non-ropy strains at the same temperatures. Excessive slime production (when cultures were incubated for a longer time at a lower temperature) resulted in a coagulum with decreased relative firmness and apparent viscosity. However the skim milk gels fermented by the ropy strains at the higher incubation temperatures exhibited greater viscosity than skim milk fermented by non-ropy strains at the same temperatures.

Introduction

There have been many investigations involving optimization of yogurt texture. These studies have demonstrated that the total solids and fat levels in the milk, heat treatment of the milk prior to inoculation, homogenization, incubation conditions and handling of the ripened coagulum will all affect the body of the final product (Rasic and Kurmann, 1978). Another major way to affect the body of yogurt is through the addition of stabilizers such as gelatin, pectin, or starch. Stabilizers are added to the product to increase viscosity as well as to decrease susceptibility to syneresis.

An alternate way to improve yogurt viscosity is to utilize slime-producing (ropy) bacteria in the starter culture. The use of slime-producing strains to increase the viscosity of yogurt and decrease susceptibility to syneresis has been advocated by many dairy researchers (Davis, 1975; Galesloot and Hassing, 1973; Kosikowska et al., 1979; Rasic and Kurmann, 1978). Ropy cultures have been used extensively in France and The Netherlands because the addition of stabilizers is prohibited in unfruited yogurts (Humphreys and Plunkett, 1969). The manufacture of yogurt without the addition of stabilizers gained popularity in the United States. The increased desire of the consumer for "100% real yogurt" and for the "natural" product in general may have explained the trend (Steinberg, 1979). Also stirred yogurts, yogurt drinks and lowfat yogurt products gained popularity in the United States (Tramer, 1973). The use of viscous cultures in the manufacture of these products has been claimed to give a smooth thick body in unfortified skim milk and to enhance the smoothness of the mouthfeel (Andres, 1982; Vedamuthu, 1982). The use of a slow acid-producing slimy starter culture has been claimed to reduce the mechanical damage (from pumping, blending and filling machines) to the consistency of stirred type yogurt (Tramer, 1973). Also a slimy starter may render the coagulum more resistant to thermal and physical shocks (Robinson, 1981).

Dairy starter cultures that contain strains of Streptococcus cremoris, Streptococcus thermophilus and Lactobacillus bulgaricus capable of producing slime are commercially available in Europe and in the United States. However, little
Table 1. Bacterial Strains

<table>
<thead>
<tr>
<th>Designation</th>
<th>Source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. thermophilus yp</td>
<td>Commercial</td>
<td>Ropy</td>
</tr>
<tr>
<td>S. thermophilus 33</td>
<td>G.A. Somkuti, USDA</td>
<td>Non-ropy</td>
</tr>
<tr>
<td>L. bulgaricus RR</td>
<td>NIZO Netherlands</td>
<td>Ropy</td>
</tr>
<tr>
<td>L. bulgaricus LB</td>
<td>Dept. of Food Science and Nutr., U. of Minnesota</td>
<td>Non-ropy</td>
</tr>
<tr>
<td>S. cremoris 351</td>
<td>Commemronal Direct</td>
<td>Ropy</td>
</tr>
<tr>
<td>S. cremoris 351-U</td>
<td>Mutant from S. cremoris 351</td>
<td>Non-ropy</td>
</tr>
</tbody>
</table>

published information exists regarding the culture conditions which affect the ability of the organisms to produce slime and the resultant rheological properties of fermented milk systems.

The objective of this study was to investigate the physical and rheological properties of a model nonfat yogurt fermented with slime-producing lactic acid bacteria.

Materials and Methods

Lactic Acid Bacteria

The lactic acid bacterial strains were obtained from the collection of H.A. Morris. The source of each strain is listed in Table 1. During the course of the investigation the cultures were routinely propagated in 11% reconstituted nonfat dry milk (NFM). The NFM was steamed for 1 hour and tempered to 37°C prior to inoculation. A 0.5% inoculum was added to the NFM and the culture was allowed to incubate at 37°C overnight (3°C for the Lactococcus cremoris strains).

Surface Structure of Ropy Strains

Appropriate dilutions of overnight ropy milk cultures were spread on 5% skim milk agar plates and incubated for 48-70 h at 32°C (S. cremoris) or 37°C (S. thermophilus and L. bulgaricus). A 2-4 mm cube of the agar with a colony on it was cut from each plate. The samples were fixed at 4°C for 20 h in a 2.0% glutaraldehyde/500 ppm ruthenium red/0.33M sodium cacodylate (pH 7.0) solution; followed by three 10 minute rinses in 0.033 M cacodylate buffer. A 3% agar sol was pipetted into the pores. The samples were fixed in 2% glutaraldehyde in 0.33 M sodium cacodylate buffer (pH 6.0) solution followed by three 10 minute rinses in 0.033 M cacodylate buffer. Post fixation was done in 2% osmium tetroxide in 0.33 M sodium cacodylate buffer solutions. Primary dehydration was carried out at room temperature using an acetone dehydration series: 10 min each in 25%, 50%, 75%, 99% and three changes in 100%. Final dehydration was performed in a Bomar SPX/EX critical point dryer using CO₂ as the transition medium.

The specimens were mounted on aluminum scanning electron microscopy (SEM) stubs with carbon paint and coated with a layer of gold-palladium by a Kinney vacuum evaporator model KSE-2AM. Specimens were viewed on a Philips 500 scanning electron microscope operated at 12 kV accelerating voltage.

Microstructure of Ropy Milk Cultures

A template was made by gluing 4x10mm glass rods to the inside surface of a petri dish cover. A 3% agar sol (60°C) was poured 13 mm deep into the petri dish. The template was then placed into the agar sol. The template was removed after the agar had solidified, which resulted in the formation of cylindrical pores in the agar. The coagulated ropy 11% NFM cultures were then pipetted into the pores. The surface was overlaid with 3% agar which had been tempered to 45°C. After the agar overlay had solidified, 6 mm cubes containing a single cylindrical pore of coagulated milk, were cut out of the agar.

The agar cubes were inoculated with 2.0% glutaraldehyde in 0.33 M sodium cacodylate buffer (pH 6.0) solution followed by three 10 minute rinses in 0.033 M cacodylate buffer. Post fixation was done in 2% osmium tetroxide in 0.33 M sodium cacodylate buffer solutions. Primary dehydration was carried out at room temperature using an acetone dehydration series: 10 min each in 25%, 50%, 75%, 99% and three changes in 100%. Final dehydration was performed in a Bomar SPX/EX critical point dryer using CO₂ as the transition medium.

Susceptibility to Syneresis

The susceptibility of nonfat yogurt to syneresis was evaluated according to the method of Harwalkar and Kwee (1962). The method is based on measuring the amount of whey expelled from a milk gel when the gel is subjected to increasing amounts of g-force. A 2% inoculum of a ropy or a non-ropy strain was added to steamed (1 h) 11% NFM (NFM mixture was tempered to the appropriate incubation temperature prior to inoculation). Ten ml of un inoculated milk was placed in sterile centrifuge tubes, followed by incubation at 32, 37 and 45°C until pH 4.8 was achieved. The gels were then cooled to 4°C in ice water until pH 4.5 ± 0.05 was attained (1-5 h depending on the culture and prior incubation temperature). triplicate tubes, corresponding to each strain and each incubation temperature, were centrifuged in a Beckman J-21C centrifuge with a JA-14 rotary head for 10 minutes each at 500, 1000, 1500, 2000, 2500, and 3000 rpm (i.e., 38.1-1375 x g). The clear supernate was decanted and measured by volume. The extent of susceptibility of the gels to syneresis was estimated from the slope of the regression line of % volume of whey separated on the application of g-force.

Water Activity

Steam (1 h) 11% NFM was inoculated and incubated as described in the previous method. The water activity (a_w) of ropy and non-ropy milk cultures was determined by cryo-osmometry. The instrument used was an advanced Digimatic...
Osmometer 3D II (Advanced Instruments, Inc., Needham Heights, MA). It was equipped with an internal microprocessor which automatically translated freezing point depression into an effective osmotic concentration \((n'_2)\), given as milliosmoles per kg water \((mOsm)\). The determination of \(a_w\) from \(mOsm\) values was obtained by a rearrangement of Raoult's law (equation 1) to equation 2, where \(n'_2\) is a function of \(\gamma\) and \(n_2\):

\[
a_w = \frac{\gamma n_1}{n_1 + n_2} \quad \text{(1)}
\]

\[
a_w = \frac{n_1}{n_1 + n'_2} \quad \text{(2)}
\]

\(\gamma\) = activity coefficient
\(n_1\) = mole fraction of water
\(n'_2\) = effective osmotic concentration; \(mOsm/1000\ g\ of\ H_2O\)

Viscosity

Steamed (1 h) 11% NFM was inoculated and incubated as described in the previous method. The samples were cooled in ice water for 2 h and then stored for approximately 24 h at 5°C. Viscosity analysis was performed using a Haake Rotovisco RV2 rotational viscometer with an MVII sensor system (Haake, Inc., Saddle Brook, NJ). The temperature of the sample was maintained at 8°C by continuously circulating water at 8°C through the jacket surrounding the sensor system by means of an Aquamatic K thermocirculator (Garman-Rupp Industries, Billville, OH). The rotor speeds in the experiment were programmed to reach 30 to 100 rpm in 6 min.

Values of rotor speed (rpm) and the resistance to shear (recorded as “scale units”) were converted to shear rate and shear stress values, respectively, by multiplying by predetermined instrument constants. The constants \((A, M, \& G)\) are dependent upon the characteristic geometry of the sensor system, the electrical specifications of the RV2 and the torque caused by the cylinder end faces. The constants were determined by means of an absolute test of “weighing torques” as outlined by the Haake Manual 105.

Gel Strength

A 2% inoculum of a ropy or non-ropy strain was added to steamed (1 h) 11% NFM. Four hundred ml aliquots of each inoculated milk were then distributed into three 11 x 7.5 cm cylindrical containers followed by incubation as described in the previous method. The gels were then cooled in ice water for 2 h and stored for approximately 24 h at 5°C.

Gel strength was determined from the first peak of the force-distance curve obtained by the Instron Universal Testing Machine 1122 equipped with compression load cell CB. Crosshead speed and chart speed were 50 and 100 mm/min, respectively, and the chart full scale was 20 or 50 grams. A 13/16 inch (2 cm) diameter probe was placed into each sample to a constant depth (4.5 cm below the surface of the gel).

Results and Discussion

Microstructure of the Ropy Cultures

Scanning electron micrographs of the ropy strains (Figures 1A and 2) demonstrate the existence of cell surface appendages not present on the cell surfaces of the non-ropy strains (Figures 1B and 2B). As can be seen in the micrograph of the ropy S. thermophilus strain (Figure 1A) the web-like slime material is associated with the cell surface as well as with the protein matrix of the skim milk gel. This confirms the finding of Kalab et. al. (1983) and Tamime et. al. (1984).

Susceptibility to Syneresis

Whey separation (syneresis) from the coagulum is undesirable in yogurt and many other fermented milk products. The major factors which contribute to syneresis in yogurt include:

a) a product with low acidity (pH < 4.6) or with high acidity (pH < 4.0),

b) high temperatures during incubation or storage,

c) agitation during manufacture or during transportation of the final product,

d) low level of milk solids in the milk,

e) low heat treatment of the milk,

f) milk is not homogenized prior to fermentation.

Several investigators (Rasic and Kurmann, 1978) have observed that the use of slime-producing strains in starter cultures reduces susceptibility to syneresis (Vedamuthu, 1982; Tramer, 1973; Rasic and Kurmann, 1978). The methods used by these workers and others to measure syneresis include measurement of the volume of whey on the surface of the coagulum after a certain length of time in quiescent storage, pouring the coagulum into a funnel lined with filter paper and collecting the exudate, or allowing the product to warm to room temperature for an arbitrary set time and then measuring the amount of whey on the surface of the coagulum.

Harwalker and Kalab (1981) evaluated the susceptibility of acidified gels to syneresis by subjecting the gels to increasing centrifugal force and then measuring the whey syneresed from the gels. The slope of the regression line of volume of whey syneresed on g-force was used as an index of the susceptibility to syneresis. The Harwalker-Kalab method was chosen to compare syneresis of 11% NFM fermented with ropy versus non-ropy strains for several reasons. This method allows for a standardized subjective measurement as opposed to the visual assessment of some methods. Another advantage lies in the dynamic nature of the method, which is more representative of the forces to which fermented milk products are subjected during manufacture and distribution than to measurements made after quiescent storage.

Ropy and non-ropy strains of S. thermophilus, S. cremoris and L. bulgaricus were used to ferment 11% NFM at 3 different incubation temperatures. The plots of % volume of whey syneresed versus g-force are presented in Figures 3-5. The solid and dashed lines represent the calculated regression of the volume of whey syneresed on centrifugal force for ropy and non-ropy cultures respectively. The R² values...
Figure 1. SEM micrograph of *S. thermophilus* grown in nonfat milk (11% solids).
A) ropy strain yp
B) non-ropy strain 33 the arrows on A) indicate exopolymer material

Figure 3. Susceptibility to syneresis of ropy *S. thermophilus* yp and non-ropy *S. thermophilus* 33 milk cultures incubated at 32C, 37C, and 45C.

Figure 2. SEM micrographs of *L. bulgaricus* strains (Surface structure).
A) ropy strain RR
B) non-ropy strain LB

Figure 4. Susceptibility to syneresis of ropy *L. bulgaricus* RR and non-ropy *L. bulgaricus* LB milk cultures incubated at 32C, 37C, and 45C.
Water-binding by the slime produced from the ropy strains. The increased water-holding capacity (WHC) of the milk gels might be due to increased gel stability. However, all of the viscosity measurements were made at a single shear rate. Therefore, shear stress data were collected for the ropy and non-ropy 11% NFM gels at increasing shear rates. The power law (3) is an empirical equation utilized by many investigators (Muller, 1973; Holdsworth, 1969; Rao, 1977) to characterize fluids that demonstrate pseudoplastic behavior.

\[
\log \tau = \log k + n \log \frac{dv}{dr}
\]

where \( \tau \) = shear stress
\( \frac{dv}{dr} \) = shear rate
\( k \) = consistency coefficient
\( n \) = flow behavior index

Two parameters (k and n) can be obtained from this mathematical model. The consistency coefficient (k) has been shown to coincide with the "thickness" of the fluid and varies with temperature. The flow behavior index (n) is a

![Figure 5. Susceptibility to syneresis of ropy S. cremoris 351 and non-ropy S. cremoris 351-U milk cultures incubated at 22°C, 27°C, and 32°C.](image-url)
S. M. Schellhaas and H. A. Morris

Figure 6. Rheological parameters of ropy *S. thermophilus* yp and non-ropy *S. thermophilus* 33 milk cultures incubated at 32C, 37C, and 45C.

Figure 7. Rheological parameters of ropy *L. bulgaricus* RR and non-ropy *L. bulgaricus* LB milk cultures incubated at 32C, 37C, and 45C.

Figure 8. Rheological parameters of ropy *S. cremoris* 351 and non-ropy *S. cremoris* 351-U incubated at 22C, 27C, and 32C.

Figure 9. Effect of time of shearing on the apparent viscosity of ropy *S. thermophilus* yp and non-ropy *S. thermophilus* 33 milk cultures incubated at 32C, 37C, and 45C. Shear rate D: 70 sec⁻¹.
measure of the departure from Newtonian flow, and the temperature effect is usually small. These parameters have been used to predict flow rates in pipes of different diameter, to select pump sizes to give the required flow rate, and to determine mixing and agitation conditions in other food systems (Holdsworth, 1969). Log-log plots of shear stress as a function of shear rate for ropy and non-ropy cultures are presented in Figures 6-8. The plots show that the consistency coefficient (y-intercept) increases as the temperature of incubation increases for both ropy and non-ropy cultures. The ropy cultures incubated at lower temperatures show slightly lower consistency coefficient values than non-ropy cultures incubated at the same temperature. However, the flow behavior index (slope of the line) is much greater for the ropy cultures incubated at the lower temperatures than for non-ropy cultures which were incubated at the same temperatures. The greater flow behavior index values indicate that the ropy cultures exhibit less pseudoplastic behavior than the non-ropy cultures, i.e., the cultures exhibit less of a decrease in viscosity at higher shear rates.

The ropy cultures which were fermented at the highest temperature of incubation had the largest consistency coefficient value. However, the flow behavior index for the ropy cultures fermented at the highest temperature were similar to those of the non-ropy cultures fermented at all three temperatures (i.e., L. bulgaricus RR culture incubated at 45°C had a similar flow behavior index as L. bulgaricus yp culture incubated at 32, 37 and 45°C).

The effect of time of shearing on the apparent viscosity of ropy and non-ropy milk cultures was determined at a constant shear rate of 70 sec⁻¹ (Figures 9, 10 and 11). The viscosity of the nonfat yogurt fermented by the ropy cultures at the higher incubation temperatures decreased with time. This finding agrees with the observation made by Galesloot and Hassing (1973) that yogurt made with a slime-producing starter culture experienced a greater decrease in flow-through time when it was repeatedly passed through a funnel than yogurt made without slime-producing bacteria.

Excessive slime production (at lower incubation temperatures) thus results in a decrease in the consistency of the coagulum, which would be undesirable in the manufacture of most yogurt products. However, at higher incubation temperatures, less slime is produced and the protein structure of the coagulum is more rigid. The lower slime production coupled with the rigid protein structure appears to result in a coagulum with a greater consistency. However, it exhibits greater shear-thinning than a coagulum without slime. The time-dependent shear-thinning behavior would need to be considered when determining the agitation conditions in the manufacture of a stirred yogurt.

Gel Strength

The relative firmness of ropy and non-ropy cultures was estimated by a penetration measurement using an Instron Universal Testing Machine.
Figure 12. Relative firmness of milk cultures which were fermented with:
A) L. bulgaricus RR
B) L. bulgaricus LB
C) S. thermophilus yp
D) S. thermophilus 33
E) S. cremoris 351
F) S. cremoris 351-U

(Figure 12). The results of the analysis indicate that relative firmness of both ropy and non-ropy cultures increases with incubation temperature. As stated previously, the increased rigidity of milk coagulated at higher temperatures was believed to be due to the effect of acid on protein structure at higher temperatures. As stated previously, the increased rigidity of milk coagulated at higher temperatures was believed to be due to the effect of acid on protein structure at higher temperatures (Galesloot, 1958). However, this alone will not explain why 11% NFM which had been coagulated at 22, 27 and 32°C by S. cremoris strains showed similar relative firmness values, respectively, as 11% NFM coagulated at 32, 37 and 45°C by S. thermophilus and L. bulgaricus strains. It appears that the rate of acid production also affects gel firmness.

The relative difference in gel firmness among ropy and non-ropy cultures is greater at the lowest incubation temperature. These results correlate with the results obtained in the viscosity investigations. The ropy cultures that were incubated at the lower and intermediate temperatures had lower consistency coefficient and gel firmness values than the non-ropy cultures. However, the gel firmness results do not correlate with the consistency coefficient obtained for ropy and non-ropy S. thermophilus cultures incubated at 45°C. Galesloot and Hassing (1973) also found a lack of complete correlation between yogurt viscosity and firmness. Both viscosity and firmness must thus be determined to more completely evaluate the texture of yogurt fermented with slime-producing strains in the starter culture.

Acknowledgements

Published as Paper No. 14,338 of the scientific journal series of the Minnesota Agricultural Experiment Station on research conducted under Minnesota Agricultural Experiment Station Project No. 18-75 supported by Hatch Funds.

We wish to thank S. M. Halameck for help on the SEM work.

References


Ropy Lactic Acid Bacteria in Skim Milk Gels


Discussion with Reviewers

A. Y. Tamime: Have the authors isolated and identified the "slimy" material?

Authors: The slimy material has been isolated and identified (Schellhaass, 1983). The slime isolated from S. thermodilus yp and L. bulgaricus RR were similar in composition as indicated by similar 13C-NMR spectra. The slime isolated from each of the ropy strains had absorption patterns indicative of hexoses in the L-cypteine-sulfuric acid reaction. The monosaccharide composition of the exopolymers was determined by a gas chromatographic method and the hexoses were galactose and glucose in a 2:1 ratio.

E. R. Vedamuthu: The authors found that syneresis from ropy gels was less than from non-ropy gels at all three incubation temperatures studied. Is the lower level of syneresis from ropy gels entirely caused by "water-holding" (absorption of water by the slime) capacity of the slime or does mechanical entrapment of water between strands of material also play a part?

Authors: It is likely that the decreased syneresis in the ropy gels is due to: The water-holding capacity of the slime as well as interaction of the slime within the protein network. Perhaps the interaction of the slime with protein helps to prevent excessive protein-protein interactions and allows far better protein hydration.

Kalab et. al. (1983) hypothesized that void spaces surrounding yogurt starter bacteria introduced stress into the casein matrix. Perhaps the exocellular slime extending from the cell surface to the casein aids in reducing stress caused by the void spaces thereby reducing syneresis.

E. R. Vedamuthu: At higher temperatures there was greater syneresis with both ropy and non-ropy gels. The authors suggested that increasing rigidity in the gel structure at higher temperatures leads to increase in syneresis. Could they explain how rigidity affects syneresis? Is this a mechanical or physiochemical phenomenon?

Authors: The rate of acid production was greater at the higher incubation temperatures. Rapid acid formation during incubation favors the formation of a rigid gel. The increased rigidity is due to the formation of a dense aggregation of protein particles with a corresponding decrease in the hydration of the protein network (Rasic and Kurmann, 1978). It is most probably a physiochemical phenomenon (decrease in protein water-holding capacity) which accounts for the greater syneresis.

Additional Reference