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CHANGES IN THE RHEOLOGY AND MICROSTRUCTURE OF ROPY YOGURT DURING SHEARING

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Abstract

Rheological and microstructural changes that occurred in ropy yogurt during shearing were observed. Yogurt made with an exopolymer-producing (ropy) strain of Lactobacillus delbrueckii subsp. bulgaricus and non-ropy strain of Streptococcus thermophilus was subjected to an increasing shear rate from 0-833 s^{-1} using a Haake Rotovisco RV2. Shear stress noticeably increased to a peak value and then decreased to a plateau value as the shear rate continued to increase. Samples taken at eight different shear rates were examined by scanning electron microscopy (SEM). At low shear rates, the exopolysaccharide (EPS) existed as a filamentous network attached to the lactobacilli and casein matrix. At the shear rate where the highest shear stress was recorded, the EPS/bacteria bonds were broken.

SEM micrographs and shear stress curves were used to determine a "bond-strength" of the EPS/lactobacilli interaction. After the interaction was disrupted, the EPS was still incorporated with the casein, where it continued to influence viscosity.

Introduction

Exopolymer (EPS)-producing cultures have been recognized for many years as the cause of slimy or ropy milk (Buchanan and Hammer, 1915; Macy, 1923). Previous researchers investigated the rheological characteristics of fermented milks made with these cultures and found an increase in viscosity and a reduction in susceptibility to syneresis (Schellhaass and Morris, 1985; Giraffa and Berg re, 1987; Cerning et al., 1986).

Many processing problems, such as low viscosity or high syneresis, that occur during yogurt manufacture are often solved by increasing the total solids or adding stabilizers, such as modified starch, carrageenan, guar gum, pectin, gelatin, and sodium caseinate (Winterton and Meiklejohn, 1978; Radema and van Dijk, 1973; Modler et al., 1983; Kessler and Kammerlehner, 1982). However, some feel that these additives adversely affect the true yogurt taste, aroma and mouthfeel (Kroger, 1973; Steinberg, 1979). This suggests that the use of ropy cultures could appeal to those consumers who are looking for a "natural" yogurt (Steinberg, 1979). Ropy cultures are also a potential benefit for yogurt manufacturers in The Netherlands and France, where the addition of stabilizers is prohibited in unfruited yogurt (Humphreys and Plunkett, 1969).

A product's response to an applied stress is determined through rheological measurements. Since yogurt is a non-Newtonian time-dependent fluid (Holdsworth, 1971), rotational viscometers with a concentric cylinder design have been used in recent research at both steady and variable shear rates (Schellhaass and Morris, 1985; Winterton and Meiklejohn, 1978; Macura and Townsley, 1984; Labropoulos et al., 1981; Parnell-Clunies, 1986).

Electron microscopy has often been utilized to study yogurt cultures and yogurt microstructure (Schellhaass and Morris, 1985; Bottazzi and Bianchi, 1986). Variations in heat treatment of the medium, total solids, and thickening agents have all been shown to alter yogurt microstructure (Kalab et al., 1975; Kalab et al, 1976; Davies et al, 1978), but few have integrated this into explanations for rheological behavior. Most studies were also conducted on samples existing in an undisturbed or "static" state and not subjected to any applied stresses.

The objectives of this study were to examine the changes that occur in ropy yogurt when it was subjected to a shear force and to observe what happened to the yogurt microstructure as a result of

shear by using scanning electron microscopy.

Materials and Methods
Yogurt

Yogurt was made from steamed (90°C for 1/2 hour) 11% reconstituted nonfat dry milk (NDM) in 200 ml aliquots contained in 400 ml beakers. The milk was tempered to 32°C and inoculated with 1% each of lactobacilli and streptococci cultures. The cultures for ropy yogurt were Lactobacillus delbrueckii subsp. bulgaricus RR (ropy) and Streptococcus thermophilus C3 (nonropy), while for nonropy yogurt they were Lactobacillus delbrueckii subsp. bulgaricus 880 (nonropy) and Streptococcus thermophilus C3 (nonropy). All of the beakers of yogurt were incubated at 32°C for eleven hours until approximately pH 4.4 was reached and then immediately cooled to 4°C.

The strains of yogurt cultures were obtained from the collection of H.A. Morris (University of Minnesota, St. Paul, MN). They were routinely propagated in steamed (90°C for 1 hour) 11% reconstituted NDM. A 1% inoculum was transferred to the cooled medium and incubated overnight at 37°C.

Rheology of Yogurt

The apparent viscosity of yogurt was measured using a Haake Rotovisco RV2 coaxial cylinder viscometer with a MVII sensor system and 500 measuring head (Haake, Inc., Saddle Brook, NJ). The sample was maintained at 10°C by a circulating waterbath connected to the jacket surrounding the sensor system during testing. The viscometer was programmed so the rotor speed increased from 0 rpm to 925 rpm in three minutes. Scale readings were recorded and calculations for shear stress and shear rate were completed according to Haake Manual 105.

Six samples each of the ropy and nonropy yogurts were sheared in the viscometer and data were plotted as shear stress versus shear rate. In order to observe microstructural changes in the ropy yogurt that were occurring during the various stages of shearing, eight new samples were used and the viscometer was stopped at predetermined times during the programmed cycle so that samples sheared at 0, 139, 167, 194, 222, 250, 278, 416, and 833 sec^{-1} could be removed. These correspond to 0, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, and 3.0 minutes into the shearing cycle. A different sample of yogurt had to be used each time after the cycle was interrupted, but all samples were from the same lot of reconstituted NDM.

Scanning Electron Microscopy of Yogurt

Samples of the sheared yogurt were pipetted into holes (2 mm diameter x 2 mm deep) drilled into aluminum scanning microscopy (SEM) stubs. The stubs were gently dipped into a 3% agar sol (45°C) and allowed to solidify. Primary fixation of the stubs was done with 5.6% glutaraldehyde in 0.033M sodium cacodylate buffer (pH 7.0) and 500 ppm ruthenium red for 48 hours at 4°C. The high concentration of glutaraldehyde was used because it had to penetrate the agar coating on the stubs. Three 10 minute rinses in 0.033M cacodylate buffer were followed by post fixation with 2% osmium tetroxide in 0.033M sodium cacodylate buffer and 500 ppm ruthenium red for 1 hour at 4°C. The stubs were then rinsed three times with distilled water (10 minutes each). Primary dehydration was carried out at room temperature in a graded ethanol series (10 minutes each in 25%, 50%, 75%, 99% and 3 times in

100%). Final dehydration was done in a Ladd critical point dryer (Ladd Research Industries, Inc., Burlington, VT) using CO_2 as the transition medium. The agar layer on top of each stub was gently lifted off and mounted upside down on a clean SEM stub using double-coated tape. Any small samples remaining in the stub holes were mounted on another clean stub with the tape. The samples were surrounded by a coat of carbon paint and coated with a layer of gold-palladium using a Kinney vacuum evaporator (model KSE-2AM). Specimens were viewed in a Philips 500X scanning electron microscope at 6 kV.

Results and Discussion
Rheology

The use of exopolymer-producing cultures in yogurt increases the apparent viscosity (Schellhaass and Morris, 1985; Galesloot and Hassing, 1973). The magnitude of increase varies due to differences in culture strains, yogurt total solids, incubation conditions, and methods for viscosity measurement. Yogurt exhibits pseudoplastic or shear-thinning behavior; therefore, it is more appropriate to measure the shear stress at an increasing shear rate (Schellhaass and Morris, 1985) rather than record single point measurements (Cerning, et al., 1986). In the present study, a shear rate range greater than previously explored was evaluated (Schellhaass and Morris, 1985; Labropoulos, et al., 1981) in order to observe the full rheological history from unsheared yogurt to extremely sheared yogurt. Figure 1 shows the magnitude of difference between ropy and nonropy yogurts and points out the three characteristic parts of the curve for ropy yogurt. The ropy yogurt had a noticeable increase in shear stress as the shear rate was increased from 0 to approximately 220 s^{-1}, as indicated by arrow B. Beyond that shear rate, the shear stress decreased to a plateau level where it remained even though the shear rate continued to increase. This unusual "hump" in the shear stress curve was less noticeable in the nonropy yogurt, but its small presence suggests that protein-protein interactions in the yogurt gel structure are broken during the initial period of shearing. This could also happen in the ropy yogurt, but there is obviously another factor involved.

The physical nature of pseudoplastic fluids makes it difficult to apply an empirical equation, such as the power law (1) over a wide shear rate range (Van Wazer, et al., 1963). Figure 2 is a log-log plot of Figure 1 and it exhibits a similar shape with the hump in the center of the curve. Consequently, this research was directed towards using SEM to try to explain why such curves were obtained when using the ropy yogurt.

Scanning Electron Microscopy

Scanning electron micrographs have visually demonstrated that ropy cultures have web-like filaments attached to the cell surface, while nonropy cultures are void of such attachments (Schellhaass and Morris, 1985; Kalab et al., 1983). Figure 3A confirms these findings in yogurt made with ropy L. delbrueckii subsp. bulgaricus and nonropy S. thermophilus. The sample has not been sheared, so the EPS is still attached to the rods. There is no obvious change in the yogurt microstructure as it is subjected to a shear rate of 139 s^{-1} (Figure 3B). Micrographs of yogurt subjected to shear rates up to 167 s^{-1} and 194 s^{-1} are not shown because there is
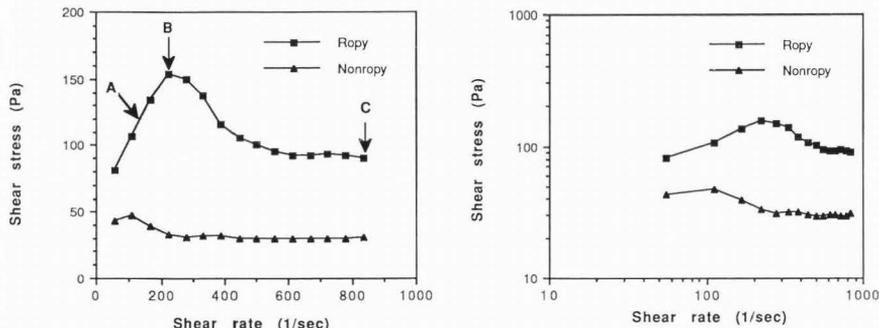
Figure 1 (at left). Shear stress versus shear rate curves of ropy and nonropy yogurt made from reconstituted nonfat milk (11% solids). Yogurt was made by incubating ropy and nonropy strains of Lactobacillus delbrueckii subsp. bulgaricus with nonropy strains of Streptococcus thermophilus at 32°C for 11 hours to pH 4.4. Each line represents the average of six samples. Arrows indicate yogurt sheared at: **A)** 139 s^{-1}; **B)** 222 s^{-1}; and **C)** 833 s^{-1}.

Figure 2 (at right). Log shear stress versus shear rate curves of ropy and nonropy yogurt made from reconstituted nonfat milk (11% solids). Yogurt was made by incubating ropy and nonropy strains of Lactobacillus delbrueckii subsp. bulgaricus with nonropy strains of Streptococcus thermophilus at 32°C for 11 hours to pH 4.4.

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little visible difference in the progression.

Several investigators have suggested that the EPS is not only attached to the cell surface, but also to the protein matrix (Schellhaass and Morris, 1985; Tamime et al., 1984). In Figure 3A, the casein is visible as distinct micelles clumped together. It appears that the EPS could be attached to the casein, but this remains to be verified.

There are marked differences in the yogurt microstructure once a shear rate of 222 s^{-1} has been reached (Figure 3C). At this point, less EPS is visibly present and the casein can be described as a more undefined, fluffy mass. Figures 3D, 3E, and 3F show the progression of changes in the microstructure when exposed to shear rates of 250 s^{-1}, 416 s^{-1}, and 833 s^{-1}, respectively. The micrograph of yogurt at a shear rate of 278 s^{-1} is not shown because there is little significant change. The EPS is no longer attached to the rods; therefore, it appears to become aggregated with the casein, though not necessarily attached to the protein. The rough surface of the rods could be casein fragments which were dislodged during shearing and adhered to the bacteria. The casein matrix also continues to appear fluffy at the higher shear rates and less defined in appearance.

Rationalization of Rheological Patterns by Electron Microscopy

The scanning electron micrographs of yogurt under stress might provide a feasible explanation for the unusual rheological behavior exhibited. The initial rapid increase in shear stress or resistance to stretching could be attributed to the bonds between EPS and the rods. At the peak of the stress, the bonds have reached their maximum limit and consequently, they break. This results in a decreased shear stress even though the shear rate continues to increase. One could quantify the breaking point by comparing the SEM micrographs and shear stress curves to determine the "bond-strength" between the EPS and rods. From Figure 1, this appears to be approximately 150 Pa, but could range between 140-160 Pa depending on the sample observed.

Based on our observations, the EPS is actually attached to the casein in addition to the bacteria. The EPS/casein interaction could be weaker in strength than the EPS/bacteria interaction and not have a visible influence on the measured shear stress of the yogurt or the EPS/casein interaction might be very strong and require a much stronger force before it is broken.

Once the EPS is separated from the bacterial cell surface as a result of the shearing, it remains incorporated with the casein in some manner, where it continues to influence the viscosity. This is most likely due to a continued interaction of EPS with casein, which is evidenced in Figure 1, where the ropy yogurt exhibits a greater shear stress than nonropy yogurt even at high shear rates.

Conclusion

The application of SEM to explain rheological behavior when studying EPS-producing cultures was used to help understand the mechanism by which the EPS interacts with its surroundings and influences viscosity. In ropy cultures, the EPS is attached to the bacterial cell surface and also interacts with the casein. The EPS/bacteria interaction is disrupted when the yogurt is sheared at an increasing shear rate to 220 s^{-1}, which is at the peak of the hump in the shear stress versus shear rate curve. The peak shear stress could be defined as the "bond-strength" between the EPS and bacteria and is approximately
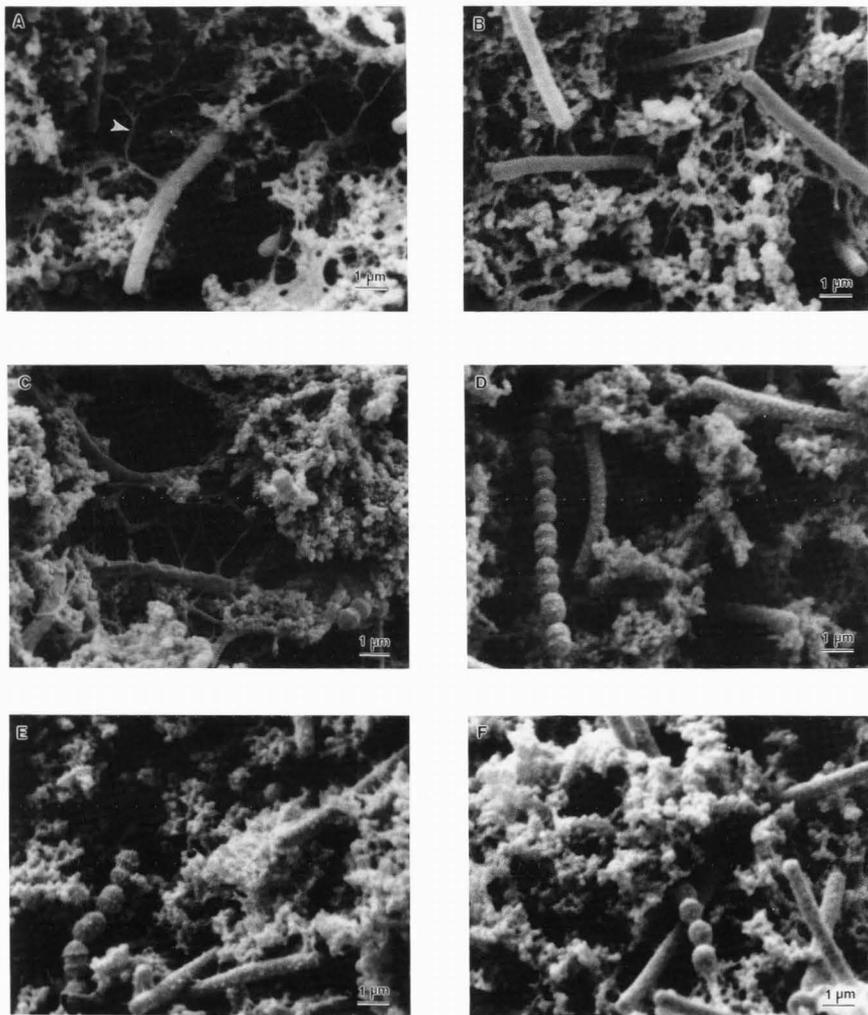
Figure 3. Microstructure (SEM) of ropy yogurt made from reconstituted nonfat milk (11% solids), ropy L. delbrueckii subsp. bulgaricus and nonropy S. thermophilus. Arrow in Fig. 3A indicates filaments of EPS. Casein is present as micelles associated with the bacteria and EPS. Yogurt was sheared to the following shear rates: **A)** 0 sec⁻¹;   **B)** 139 sec⁻¹;   **C)** 222 sec⁻¹;
                            D) 250 sec⁻¹;   **E)** 416 sec⁻¹; and   **F)** 833 sec⁻¹.

150 Pa. After the EPS is separated from the cell surface, it continues to interact with the casein and influence the viscosity of the yogurt.

Acknowledgement

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Discussion with Reviewers

S.M. Schellhaass: Many investigators have observed that incubating at lower than optimum growth temperatures results in increased EPS production. Have you investigated the impact of slower growth conditions (i.e., lower incubation temperatures) on the resistance of the EPS to detachment from the cell surface?

Authors: Rheological measurements and scanning electron microscopy have been conducted on yogurt incubated at higher temperatures (45°C) for comparison to lower temperature incubation (32°C). The higher incubation temperature gave shear stress values that were lower over the entire shear rate range. The micrographs did not clearly indicate that less EPS was present; therefore, this would suggest that lowered resistance to detachment did exist. However, we have not determined the amount recovered when EPS is isolated from yogurt incubated at 45°C versus 32°C.

B.E. Brooker: This study is critically dependent on the ability to image EPS using scanning electron microscopy. Although the so called filaments of EPS are found only in EPS producing strains, what evidence do the authors have that this appearance accurately depicts the polysaccharide in life? If there is no evidence that the polysaccharide is preserved in a natural or near natural state, does this not make the observations in the present paper very difficult to interpret and of doubtful value?

Authors: It is true that we do not know the full effects of electron microscopy preparation techniques, especially the critical point drying, on the exopolysaccharide. However, this conventional SEM method has been used previously on ropy yogurt to obtain similar results (Schellhaass and Morris, 1985).

It has been argued that critical point drying promotes artifacts because the organic solvents may extract gelatinized starch or polysaccharides that are present (Schmidt, 1982; Kalab, 1981). In this research, there are obviously differences occurring in the micrographs during the progression of shear rates. Even if the filaments are not depicted exactly as in their natural state, they will be modified similarly and there is a significant enough change to warrant the stated observations and conclusions. An alternative method for sample preparation could utilize cryofixation and freeze-fracturing, which would avoid the use of any chemicals (Schmidt, 1982).

R.W. Martin: It remains very difficult to correlate rheology of dairy systems to microstructural characteristics. Are any biochemical or microstructural studies planned to further investigate this correlation?

Authors: No further biochemical or microstructural studies are planned at this time. However, the viscoelastic properties of ropy and nonropy yogurts are being investigated.

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