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Rheological characterization of exopolysaccharides produced by two strains of *Streptococcus thermophilus*

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Abstract: Two strains of *Streptococcus thermophilus* producing capsular and capsular-ropy exopolysaccharides (EPS) were cultivated in M17 medium supplemented with lactose at 37°C for 24 hours. At the end of fermentation, EPS were extracted and their flow behavior was assessed as a function of concentration, pH and temperature. Apparent viscosity of capsular EPS dispersions was higher than that of the capsular-ropy EPS, while activation energy was lower. A pH increase amplified activation energy in both types of EPS. Flow behavior of both EPS dispersions was fitted well by the Cross model, with capsular-ropy EPS showing greater zero shear rate viscosity, relaxation time and limiting slope. These parameters were greatly affected by the strain type, pH and temperature.

Keywords: ***Streptococcus thermophilus,*** **exopolysaccharides, growth conditions, rheological properties**

Introduction

Considerable attention has been given to bacterial exopolysaccharides (EPS) in foods arising from their ability to provide potential health benefits to consumers (Looijesteijn *et al.*, 2001; De Vuyst and Degeest, 1999) and their application as thickening agents in processed products (Cerning, 1990). Although certain bacterial species can produce considerable amounts of EPS, yoghurts prepared with strains of *Streptococcus thermophilus* result in less than 0.1% EPS in the final product (Cerning, 1990). Nevertheless, EPS play an important role in the development of yoghurt texture, with the type of EPS exerting a greater effect than their concentration (Vaningelgem *et al.*, 2004). In general, two strain-dependent types of EPS (Mozzi *et al.*, 2006) have been frequently assessed for their effects on yoghurt texture, namely 'ropy' and 'capsular'. Certain bacterial strains may even produce a mix of these two types in various proportions (Zisu and Shah, 2003; 2005). Furthermore, combinations of the two types of EPS producing cultures improved not only the total EPS production but also yoghurt texture (Marshall and Rawson, 1999).

Numerous studies have been conducted in order to enhance our understanding of the factors governing EPS production and the mechanisms by which they affect the yoghurt texture. Most of the EPS produced during yoghurt fermentation are heteropolysaccharides, and their production may be unpredictable due to a plasmid-related instability (Boels *et al.*, 2001). EPS production, thickening properties, molecular mass and structural conformation are greatly affected by environmental factors (Ruas-Madiedo *et al.*, 2005). In some species, these affected only EPS yield, because their monosaccharide composition remained unchanged (Looijesteijn and Hugenholtz, 1999). Depending on the strain and growth conditions, maximum yield of EPS may be achieved in the exponential (Duenas *et al.*, 2003) or stationary growth phase (Gancel and Novel, 1994). At the end of growth phase, there are some indications that EPS undergoes undesirable enzymatic degradation (Degeest *et al.*, 2002; Pham *et al.*, 2000).

Although the type of EPS has been reported to influence yoghurt texture, and some mechanisms on their interactions with milk proteins has been proposed, there is a lack of information on the rheological properties of EPS produced by yoghurt cultures. Such information can be useful for predicting possible interactions of EPS with milk component(s) that would influence the texture of yoghurt. More

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importantly these complex carbohydrates could also be applied in other food systems as thickeners. Therefore, this study aimed to examine rheological properties of two distinctly different EPS produced by selected strains of *S. thermophilus* which produced capsular-ropy and capsular EPS.

Materials and methods

Bacterial Cultures

The two strains of *S. thermophilus* examined in the study were kindly provided by Australian Starter Culture Research Centre (now Dairy Innovation Australia Ltd, Werribee, Australia). *S. thermophilus* ST 1275 produces mainly ropy with a smaller portion of capsular EPS, while ST 285 produces only capsular EPS (Zisu and Shah, 2003, 2005). The frozen (-80 °C) cultures in 300 mL/L glycerol were activated by growing them twice in glucose containing M17 medium (Amyl Media, Merck Pty Ltd., Kilsyth, Victoria, Australia) at 37°C for at least 24 hours.

Culture growth and EPS isolation

The medium used for the primary culture cultivation and EPS production was basal M17 medium supplemented with lactose at 20 g/L. Sugar was added to the basal medium at the required concentration post-sterilization through a 0.22µm microfilter. Prior to this, the activated cultures were grown in glucose containing M17 (Amyl Media) at 37°C for 8 hrs prior to inoculation. The cultures were then inoculated at 1% (w/w) level into 30 mL of the sterile medium in 50 mL Falcon tubes (Falcon, Blue Max, Becton Dickinson and Company, Franklin Lakes, N.J., USA) and incubated at 37°C for 24 hours under aerobic conditions.

EPS assessed in the rheological studies were extracted by precipitation with ethanol (Van Geel-Schutten *et al.*, 1998) at the end of the fermentation period. Initially, fermentation was stopped by adding 25 mL/L of trichloroacetic acid (80 g/100 g) into the suspension and keeping the container in a cold room (4°C) overnight. This was followed by protein removal by centrifugation at 11000*xg* and 4°C for 10 min (Model J2-HS, Beckman, Fullerton, CA, USA). The supernatant was collected and mixed with two volumes of cold ethanol. The mixture was allowed to stand for twelve hours for complete precipitation of EPS at 4°C. This procedure was repeated twice. At the end of this procedure, ethanol was carefully decanted and remaining EPS pellets were freeze-dried (Dynavac freeze drier, Dynavac Eng. Pty. Ltd., Melbourne, Australia).

Rheological characterization of EPS

Prior to analysis, the freeze-dried crude EPS was dissolved in 0.1M citric acid-phosphate buffer at pH 3 and also at 6.5 achieving concentrations of 0.1, 0.25, 0.5, 0.75 or 1 g/100 g. Samples were subjected to a controlled shear rate ramp from 0.01 to 100/s in a cone and plate geometry (50 mm diameter, 2°) of a rheometer (Physica MCR 301, Anton Paar, GmbH, Germany) at a selected temperature (4, 20, 37 or 42°C). Temperature regulation was achieved with a Viscotherm VT 2 circulating bath and controlled with a Peltier system (Anton Paar) to an accuracy of ± 0.1°C. The data were analyzed with proprietary software (Rheoplus/32 v2.81, Anton Paar). The flow curves were fitted to rheological models; Ostwald (for overall shear rate curve 0-100/s), and Cross (for non-Newtonian flow at a shear rate below 10/s). These models are presented by the following equations:

Ostwald model: $$\eta = K\dot{\gamma}^{(n-1)} \quad (1)$$

Cross model: $$\eta = \frac{\eta_0}{1+(\tau\dot{\gamma})^m} \quad (2)$$

with K, n, η, $\dot{\gamma}$ as consistency index (mPa.s^n), flow behaviour index (dimensionless), apparent viscosity (mPa.s) and shear rate (1/s), respectively; η_o presents the zero shear rate viscosity (mPa.s), τ relaxation time (s), and m is the limiting slope (dimensionless). The effect of concentration on viscosity was studied by applying the following equation (Speers and Tung, 1986):

$$\eta = a \cdot C^b \quad (3)$$

where a and b are parameters derived from the intercept (mPa.s g/100g^b) and the slope of a logarithmic plot (dimensionless), respectively. C presents EPS concentration (g/100 g). The influence of temperature on apparent viscosity was also assessed using the Arrhenius model (Macosko, 1994; Speers and Tung, 1986):

$$\eta = A \cdot e^{\frac{\Delta E}{RT}} \quad (4)$$

where η is the apparent viscosity (mPa.s) at shear rate of 100/s, A the frequency factor (mPa.s), ΔE the activation energy (J/mol), R the gas constant (8.314 J/mol K), and T is the absolute temperature (K).

Statistical Analysis

A randomized split plot block design was applied to the design of the experiments. This was set up with three main effects: strain (two levels), temperatures (three levels), pH (two levels) and EPS concentration (5 levels). This design was replicated twice with at least two sub samplings. Results were analyzed using a General Linear Model procedure (SAS, 1996). The level of significance was set at P=0.05. The best fit correlational analysis for rheological data was carried out using the Rheoplus software (v2.81, Anton Paar).

Results and Discussion

Flow curves of dispersions containing both EPS types at pH 6.5 were fitted to the Ostwald model with a high degree of correlation (between 0.8-0.9). At very low shear rates (below 10/s), a small overshoot as well as thixotropy was observed (figure not shown). In the Ostwald model, consistency index (K) denotes the shear resistance of material, with a high value indicating greater resistance. The other parameter, flow behaviour index or n designates deviation from the Newtonian flow (n = 1) (Rao, 1999). The values of both K and n were affected significantly (P<0.05) by strain, pH, and temperature (Table 1a,b). The effect of interaction between strain and pH was only significant (P<0.05) for K value, whilst, the n value was affected significantly by the interaction between strain and temperature. K values were in general low, apparently a sign of a low shear-resistance (Speers and Tung, 1986). Capsular EPS showed 10 fold higher K values than capsular-ropy EPS at pH 6.5, indicating a more shear-resistant nature. The flow behaviour indices (n) of the capsular-ropy EPS dispersions were close to that of a Newtonian fluid. In contrast, n values of the capsular EPS dispersions were low, with considerable deviation from Newtonian flow and thus exhibiting greater pseudoplastic behaviour. In most samples, lower EPS concentrations and higher temperatures had a negative effect on K values, with no apparent trend for n values. However, EPS dispersions at pH 3 did not show any observable difference of Ostwald constants between the two types of EPS. The low pH may diminish any rheological differences between the two.

An increase of EPS concentration caused a concomitant increase in the apparent viscosity. The parameter 'a' was slightly greater for capsular than capsular-ropy EPS, which denoted a more temperature-sensitive nature of the capsular EPS (Table 2). The Arrhenius plot showed that temperature decreased apparent viscosity of both colloids (Fig. 1A,B). In the same manner, the activation energy (ΔE) also decreased with increasing temperature (Table 3). Activation energy is the energy required to facilitate the motion of a liquid during flow, and is smaller when kinetic energy of the material increases due to rise in temperature (Speers and Tung, 1986). This phenomenon was also evident in our work, with the ΔE values for the capsular-ropy EPS were slightly higher than those of the capsular EPS (Table 3). Apparently, the capsular-ropy EPS molecules were slightly more restricted in movement than those of the capsular EPS. Activation energy was correlated with cohesion and stickiness of the sugar dispersions (Chen, 2007). Nevertheless, the relatively low values of ΔE obtained in the present study were somewhat comparable to earlier reports (Kwon et al., 1996; Speers and Tung, 1986), thus emphasizing a negligible influence of temperature on apparent viscosity (Fig. 1A,B). On the other hand, pH had an apparent effect on the viscosity and ΔE which tended to be lower than at higher pH (Table 2 and 3).

Polymer characteristics are often studied by assessing flow behaviour at very low shear rate, such as below 10/s (Gorret *et al.*, 2003). Some polysaccharides exhibit non-Newtonian flow, obeying the power law only in the intermediate shear-rate region, but not at low and high shear rates (Rao, 1999). In our work, the data were fitted to the Cross model with a relatively high degree of correlation (Table 4). The Cross model is commonly used to describe the flow characteristics of materials possessing first and second Newtonian plateaus at low and high shear rates and the power law flow in between (Macosko, 1994). Zero-shear viscosity (η_o) was affected significantly (P<0.05) by strain and pH, but not by concentration (P>0.05). Any interaction between two factors involving concentration was not significant (P>0.05). In an acidic environment (pH 3), dispersions of the capsular-ropy EPS showed higher η_o than those of the capsular EPS (Table 4). However, the oppsite occured at pH 6.5. Higher η_o may relate to lower mobility due to water-binding and enlargement of EPS molecules (Ravi and Bhattacharya, 2004), and may indicate more extensive coil overlap (Gorret *et al.*, 2003). Similarly, the values of τ (structural relaxation time) were significantly (P<0.05) affected by all factors examined except concentration. A greater structural relaxation time (τ) was a sign of more extensive entanglement leading to a less mobile EPS chain, and consequently a longer time to develop new entanglement after disruption during shearing (Gorret *et al.*, 2003). Greater values of τ at increasing polymer concentration (Table 4) as shown in our work might be ascribed to non-gelling properties (Gorret

Table 1a. Concentration and temperature dependency of consistency (K) and flow behaviour index (n) of EPS dispersions at pH 3

EPS (g/100 g)	Temp. (°C)	Strain					
		Capsular-ropy			Capsular		
		K (mPa s^n)	n -	r^2	K (mPa s^n)	n -	r^2
1.00	4	7.5	0.79	0.91	14.8	0.57	0.75
	20	5.3	0.80	0.98	7.9	0.63	0.77
	37	9.9	0.44	0.87	7.6	0.55	0.77
	42	7.7	0.62	0.95	8.1	0.62	0.80
0.75	4	7.8	0.70	0.79	7.3	0.73	0.77
	20	6.5	0.88	0.89	5.7	0.67	0.78
	37	8.7	0.47	0.77	7.6	0.51	0.79
	42	3.8	0.75	0.92	3.3	0.79	0.96
0.50	4	5.8	0.79	0.87	5.6	0.78	0.84
	20	2.8	0.89	0.93	4.1	0.68	0.76
	37	7.6	0.48	0.82	5.6	0.55	0.75
	42	3.6	0.71	0.96	3.4	0.72	0.95
0.25	4	5.1	0.74	0.83	4.2	0.82	0.84
	20	1.9	0.98	0.92	3.0	0.7	0.86
	37	5.0	0.62	0.85	7.5	0.44	0.74
	42	4.6	0.63	0.94	2.5	0.78	0.95

Table 1b. Concentration and temperature dependency of consistency (K) and flow behaviour (n) index of EPS dispersions at pH 6.5

EPS (g/100 g)	Temp (°C)	Strain					
		Capsular-ropy			Capsular		
		K (mPa s^n)	n -	r^2	K (mPa s^n)	n -	r^2
1.00	4	4.2	0.84	0.93	19.4	0.37	0.86
	20	2.7	0.87	0.97	20.5	0.32	0.95
	37	1.8	0.90	0.97	21.6	0.23	0.73
	42	3.8	0.62	0.80	12.1	0.38	0.76
0.75	4	5.9	0.79	0.87	19.2	0.36	0.75
	20	3.4	0.77	0.93	17.9	0.31	0.95
	37	5.7	0.44	0.79	13.4	0.25	0.82
	42	1.5	0.90	0.98	7.3	0.45	0.73
0.50	4	3.8	0.85	0.92	16.3	0.36	0.42
	20	2.8	0.73	0.89	19.5	0.22	0.82
	37	2.6	0.65	0.78	10.5	0.34	0.92
	42	1.7	0.82	0.97	6.0	0.45	0.75
0.25	4	3.2	0.85	0.89	12.3	0.49	0.66
	20	1.8	0.81	0.85	18.3	0.19	0.92
	37	2.3	0.66	0.78	6.1	0.14	0.89
	42	1.7	0.78	0.98	5.4	0.45	0.69

Table 2. Apparent viscosity (at 100/s shear rate) as a function of concentration for capsular-ropy and capsular EPS dispersion from 4 to 42°C

Temperature (°C)	Capsular-ropy			Capsular		
	a (mPa.s g/100g^b)	b -	r^2	a (mPa.s g/100g^b)	b -	r^2
			pH 3.0			
4	9.96	0.21	0.89	13.96	0.27	0.93
20	5.23	0.18	0.83	7.42	0.24	0.91
37	3.27	0.17	0.76	5.75	0.27	0.96
42	2.76	0.16	0.70	6.31	0.32	0.96
			pH 6.5			
4	3.00	0.12	0.61	3.07	0.23	0.91
20	1.83	0.10	0.59	1.88	0.21	0.91
37	1.15	0.11	0.58	1.21	0.21	0.91
42	0.91	0.11	0.61	0.99	0.22	0.90

Table 3. Arrhenius-like temperature dependency of apparent viscosity of capsular-ropy and capsular EPS dispersion determined at 100/s shear rate and pH 3 and 6.5

Concentration (g/100 g)	Capsular-ropy			Capsular		
	ΔE (kJ/mol)	A (mPa s)	r^2	ΔE (kJ/mol)	A (mPa s)	r^2
			pH 3.0			
1.00	8.31	0.05	0.99	8.32	0.05	0.99
0.75	8.82	0.04	0.99	8.26	0.05	0.99
0.50	8.77	0.04	0.99	8.83	0.04	0.99
0.25	8.22	0.04	0.99	8.43	0.04	0.99
0.10	7.72	0.05	0.99	9.13	0.03	0.99
			pH 6.5			
1.00	9.82	2.35	0.99	9.52	0.03	0.99
0.75	9.22	2.20	0.99	9.18	0.03	0.99
0.50	9.47	2.26	0.99	9.48	0.02	0.99
0.25	9.52	2.27	0.99	9.11	0.03	0.98
0.10	9.41	2.25	0.99	9.20	0.03	0.98

Table 4. Cross model parameters describing the dependence of the apparent viscosity of capsular-ropy and capsular EPS dispersions on concentration at pH 3 and 6.5

EPS (g/100 g)	Capsular-ropy				Capsular			
	η_o (mPa s)	τ (s)	m	r^2	η_o (mPa s)	τ (s)	m	r^2
				pH 3.0				
1.00	150.82	15.82	0.47	0.92	93.72	9.36	0.61	0.85
0.75	30.49	3.96	0.45	0.78	27.09	5.11	0.74	0.74
0.50	19.59	2.92	0.45	0.77	13.81	1.77	0.94	0.76
0.25	15.91	1.84	0.64	0.72	8.79	0.72	1.04	0.94
0.1	14.36	0.43	1.21	0.73	9.24	1.05	0.97	0.83
				pH 6.5				
1.00	7.80	0.08	2.15	0.98	376.81	8.19	1.88	0.88
0.75	8.01	0.05	2.26	0.94	352.56	14.59	1.10	0.92
0.50	6.96	0.15	1.88	0.88	218.79	15.44	1.65	0.84
0.25	5.88	0.14	1.88	0.88	113.76	19.62	3.49	0.93
0.1	5.45	0.14	1.99	0.88	90.83	27.20	5.92	0.90

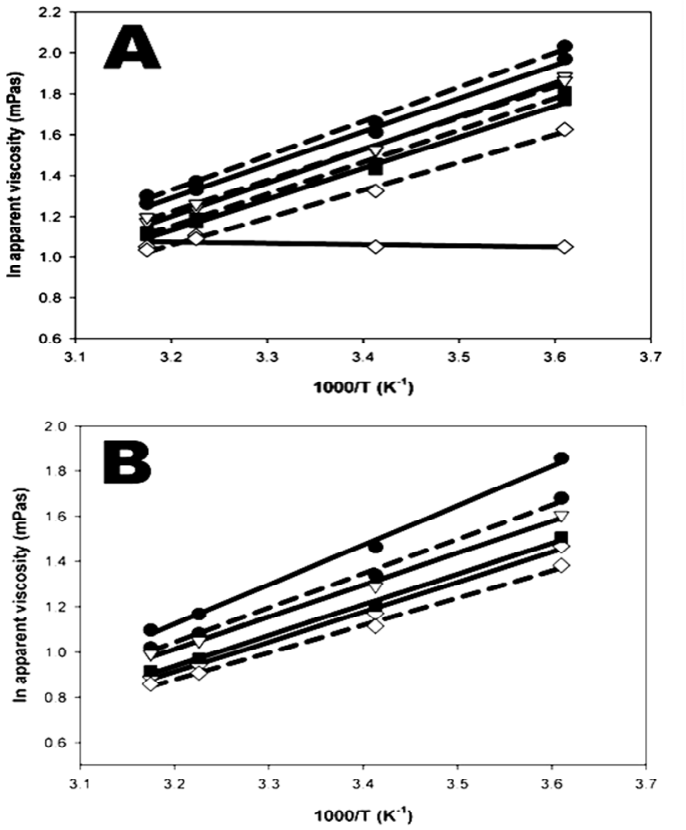

Figure 1. The Arrhenius plot of the capsular-ropy (solid line) and capsular (dashed line) EPS as a function of different temperatures and concentrations (0.25 - ◇; 0.5 - ■; 0.75 - ▽; 1 g EPS/100g - ●) at pH 3.0 (A) and pH 6.0 (B) at constant shear rate of 100/s

et al., 2003). However, we observed that higher concentrations of capsular EPS at pH 6.5 exhibited lower τ (Table 4) indicating a gelling character under these conditions. The value of m is equal to (1-n) where n is flow behaviour index in the power law model (Ravi and Bhattacharya, 2004). Therefore, the greater the m values, the more the material deviates from the Newtonian behaviour. The m values were not affected (P>0.05) by strain and interaction between strain and pH. They were affected by concentration, pH, and temperature, and the combinations of two factors among them. The insignificant role of concentration in our study may be due to the low concentrations used. Considerably higher concentration was required to enable examination of viscoelastic properties, eg. 6 g/L in the case of the EPS from *Propionibacterium acidipropionici* (Gorret *et al.*, 2003). Although it was apparent from our work that the two types of EPS exhibited different rheological characteristics, their interactions with milk components during fermentation may be more dominant in governing the final texture of yoghurt (Rohm and Kovac, 1994).

Conclusions

The viscosity of capsular EPS was predominantly influenced by temperature. Compared to capsular EPS, capsular-ropy EPS exhibited greater resistance to flow and was less able to structurally rearrange after shear-induced disruption. The activation energy of capsular-ropy EPS appeared to be higher than that of the capsular EPS. Origin, temperature and pH were significant factors in governing the flow properties of the EPS dispersions. Further studies are needed to understand the relation between the rheological properties of EPS and their role in different model and food systems, especially their function in creation of texture of dairy products.

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