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Full Length Research Paper

Effects of stabilizers and exopolysaccharides on physiochemical properties of fermented skim milk by *Streptococcus thermophilus* ST1

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In this study, the effects of exopolysaccharide (EPS) produced by *Streptococcus thermophilus* ST1 and stabilizers on the textural and microstructural properties attributes of fermented skim milk were investigated. ST1 had a high capacity to produce acids and EPS. Within 4.5 h, the pH dropped to 4.5, the acidity reached 0.8%, and EPS reached 65.27 mg/L. ST1 was more effective at increasing viscosity as well as water holding capacity and reducing spontaneous whey separation compared with control sample (ST0). ST1 combined with carrageenan had the highest viscosity and the best water holding capacity as well as the lowest spontaneous whey separation. ST1 combined with whey protein concentrates showed better results with xanthan, but worse with carrageenan. The microstructure of the fermented milk was examined using scanning electron microscopy. ST1 combined with carrageenan resulted in compact and uniform structure, which is agreed with quality improvement of fermented skim milk that used ST1 combine carrageenan as fat substitute.

Key words: *Streptococcus thermophilus* ST1, exopolysaccharide, fermented skim milk, texture.

INTRODUCTION

Yogurt is a popular fermented milk product and has been a major part of people's diet around the world. Low-fat yogurts are increasingly popular due to their nutritional and potentially therapeutic characteristics (Aguirre-Mandujano et al., 2009). Fat reduction can cause some defects in yogurt such as lack of flavour, weak body and poor texture (Guven et al., 2005). Several studies have discussed the improvement of physical, textural, flavour and rheological properties of low-fat yogurts by incorporating the stabilizers into the milk. This increases the viscosity and reduces syneresis (Lucey, 2002). Xanthan gum has been used to improve the texture, increase the firmness and prevent syneresis in yogurt (El-Sayed et al., 2002). Carrageenan, synergistic with some other gums, could increase the gel strength and water-binding capabilities as well as modifying the gel texture (Ertan et al., 2009). Whey protein concentrate (WPC) affects the textural and physical properties of yogurts (Sodini et al., 2005). Sandoval et al. (2004) found that reduced-fat yogurt with WPC showed similar texture (tension, firmness, adhesiveness, cohesiveness, and springiness) to full-fat yogurt.

The exopolysaccharide (EPS) produced by low-fat yogurt starter cultures affect the textural and physical properties of yogurt and improve the sensory characteristics such as mouthfeel, shininess, clean cut, ropiness and creaminess (Folkenberg et al., 2006; Hassan et al., 2002; Ruas-Madiedo et al., 2005; Ramchandran and Shah, 2009). Both capsular and ropy EPS possess high water binding ability, resulting in increased water retention in yogurt (Hassan et al., 1996b). EPS has been reported to provide physiological benefits such as lowering of

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Abbreviations: ST, *Streptococcus thermophilus*; **EPS,** exopolysaccharide; **WPC,** whey protein concentrate; **LAB,** lactic acid bacteria; **WHC,** water-holding capacity.

cholesterol, immunomodulation, and antitumor activity (Welman and Maddox 2003). Therefore, using EPS producing cultures when developing a low-fat yogurt with physiological functions is necessary.

The objective of this study was to investigate the effect of EPS-producing culture and stabilizers on the textural and physical characteristic of fermented skim milk, and to reveal the mechanisms of these changes by micro-structure of the fermented skim milk.

MATERIALS AND METHODS

Cultures

EPS-nonproducing strains *Streptococcus thermophilus* ST0 and ropy exopolysaccharide-producing strains *S. thermophilus* ST1 obtained from Culture Collection of Northeast Agricultural Research Center of China (NARCC) were used throughout the study. The strains were stored at -80°C in 10% (w/v) skim milk (Fonterra, New Zealand) containing 30% (v/v) glycerol. *S. thermophilus* ST1 and ST0 were propagated three times consecutively using a 3% (v/v) inoculum in 10% skim milk at 42°C before use.

Determination of pH, titratable acidity and viable counts

The pH values were determined at 42°C. The titratable acidity was determined according to the method of Seo (2010). 10 ml of bacterial cultures and 20 ml of deionized water were mixed, and 0.5 ml of phenolphthalein was added into the mixture. This mixture was titrated with 0.1 M NaOH. The acidity of the bacterial cultures was calculated as percent (%) lactic acid. Viable counts in fermented skim milk were enumerated ST1 and ST0, respectively. *S. thermophilus* was estimated by plating M17 agar for incubation at 42°C for 48 h (Li, 2006).

Isolation and quantification of EPS

EPS was isolated from the fermented milk samples using a modified procedure previously described by Elin et al. (2010). Briefly, trichloroacetic acid was added to the sample culture to a final concentration of 4% (w/v) and allowed to rest for 2 h at room temperature. After centrifugation (10000×g for 30 min at 4°C) to remove the precipitated proteins and bacterial cells, the supernatant was mixed with a double volume of cold ethanol and then stored at 4°C for 24 h. The precipitated EPS was collected by centrifugation (10000×g for 30 min at 4°C), dissolved in deionized water and mixed with a double volume of cold ethanol and stored at 4°C for 24 h. EPS was precipitated with ethanol. It was recovered by centrifugation at 4°C 10000×g for 30 min. Total EPS (expressed as mg/L) was estimated in each sample by phenol–sulphuric method (Dubois et al., 1956) using glucose as a standard (Torino et al., 2001).

Fermented skim milk preparation

Several samples of fermented skim milk were manufactured using 1.0% low fat milk, with various stabilizers: 0.01% (w/v) xanthan, 0.05% (w/v) carrageenan (kappa) and 0.1% (w/v) WPC-80, dissolved at appropriate temperatures, pasteurized at 90°C for 10 min and cooled to 42°C. The samples were inoculated with 3% starter cultures (ST0 or ST1) and fermented at 42°C for 4.5 h. The samples were stored at 4°C for 24 h for analysis.

Texture analysis

Fermented skim milk texture analysis was carried out with TA-XT2i (Texture Technologies Corp., Scarsdale, NY) using a 5 kg load cell. A 35 mm diameter solid rod (A/BE35) was thrust into the test sample, a sample depth of 30 mm with crosshead speed of 1 mms^{-1} (Sandoval-Castilla et al., 2004).

Viscosity analysis

The viscosity determination was based on Rawson and Marshall (1997) method, with some modifications. The gel was broken by stirring with a glass rod (10 times clockwise; 10 times anticlockwise). Rotational viscosity measurements were made using a Brookfield Viscometer (Model DV-Ⅲ; Brookfield Engineering Laboratories, Stoughton, MA). N_0 3 spindle was used, with about one third of the spindle immersed. Each measurement was made at room temperature at 100 rpm for 1 min.

Water-holding capacity analysis

The water-holding capacity (WHC) of fermented skim milk was determined as described by Doleyres et al. (2005). Briefly, 10 g of fermented skim milk was centrifuged at 5000×g for 10 min at 5°C. The resulting supernatant was carefully weighed to determine the amount of excluded water (% wt/wt).

Spontaneous whey separation analysis

Spontaneous whey separation was determined according to the procedure described by Amatayakul et al. (2006). A cup of set fermented skim milk was removed from refrigerator at 4°C, weighed and kept at approximately 45°C to allow the whey on the surface to be collected on the side of the cup. A needle connected to a syringe was used to withdraw the liquid whey from the surface of the sample, and the cup of fermented skim milk was weighed again. The process lasted for less than 10 s to avoid further leakage of whey from the curd. The syneresis was expressed as the percentage weight of the whey over the initial weight of the fermented skim milk sample.

Microstructure analysis

Fermented skim milk microstructures were examined as described by Sandoval-Castilla et al. (2004). Fermented skim milk samples (0.3 g) were excised from approximately 1 cm below the surface and mixed with 0.3 g of 3% aqueous agar solution at 45°C and the mixtures were solidified by cooling at 20°C. The gelled samples were cut into 1 mm cubes and fixed in a 2% glutaraldehyde solution in phosphate buffer (0.1 M, pH 7.2) at room temperature for 2 h, then held for 24 h at 4°C. The fixed samples were washed with phosphate buffer and dehydrated in a graded ethanol series (30, 50, 70, 80 and 90%, 1 h in each) and afterwards in absolute ethanol for 24 h. These pieces were dried to critical point, and coated with gold for 2 min by ion sputter (JFC-1600, JEOL, Japan). Samples were viewed by a SEM (SEM, JEOL, Japan).

Statistical analysis

All analyses were done in triplicate. Statistical analyses were

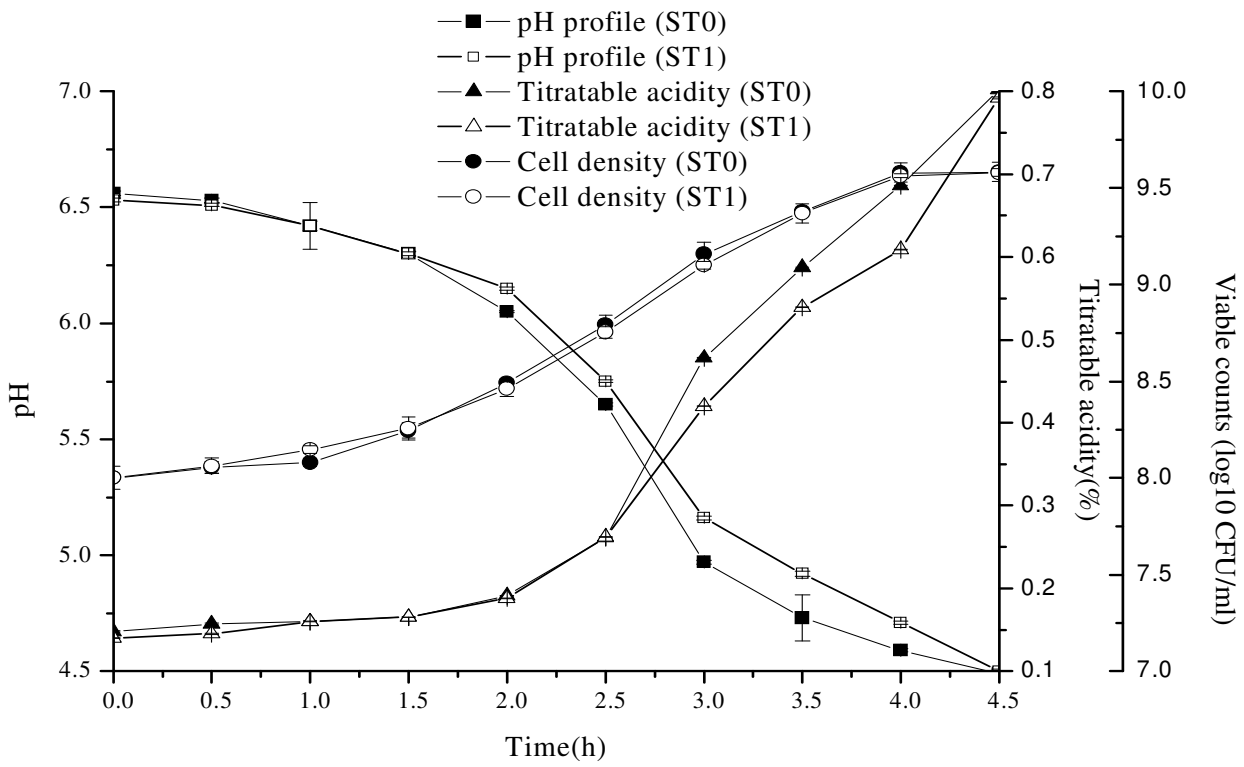

Figure 1. Changes in pH, titratable acidity and viable counts of fermented skim milk by ST0 and ST1 at 42℃.

Table 1. pH, EPS and viable counts of the culture at the end of fermentation

Culture	pH	EPS (mg/L)	Viable counts (log_{10} CFU/ml)
ST0	4.49±0.01	6.64±0.12	9.58±0.05
ST1	4.50±0.01	65.27±0.09	9.58±0.02

performed with SPSS 16.0. Significant differences between treatments were tested by ANOVA.

RESULTS AND DISCUSSION

Characteristics of culture

Results in Figure 1 and Table 1 show that the ST1 and ST0 strains produced acids fast and had the same acidification rate. There was not a significant difference in viable counts, and the only difference was that ST1 produced EPS. In 4.5 h, the pH dropped to 4.5, the acidity reached 80°T. ST1 had not only high acidification rate but produced EPS. At 4.5 h, the amount of EPS produced by ST1 reached 65.27 mg/L.

Level of stabilizers

Three different stabilizers were used as fat substitutes: xanthan, carrageenan and WPC. The amounts of each stabilizer added are shown in Table 2. The results (Table 2) show the amounts of the stabilizers had a significant impact on the texture of low-fat fermented skim milk. In this study, firmness, consistency, cohesiveness and resistance were the major indexes. As shown in Table 2, samples with 0.01% xanthan, 0.05% carrageenan and 0.10% WPC had the highest firmness, as well as the highest cohesiveness and resistance, respectively.

Textural characteristics

Fermented skim milk made using ropy EPS-producing ST1 culture had the lowest firmness. Similar results were reported by others. Amatayakul et al. (2006) found that the firmness of fermented skim milk made using capsular EPS-producing or ropy EPS-producing cultures was lower than that of fermented skim milk made with non-EPS-producing starter cultures. The results (Table 3) also show that the consistency and cohesiveness of fermented skim milk made using ST1 were lower than those

Table 2. Effects of different levels of stabilizers on texture of fermented skim milk by ST0

	Firmness (g)	Consistency (g.s)	Cohesiveness (g)	Resistance (g.s)
ST0	88^{e}	2140^{g}	-39f^{g}	-60^{e}
ST0-xanthan gum 0.01%	168^{h}	3948^{m}	-97^{a}	-126^{a}
ST0-xanthan gum 0.02%	105^{g}	2302^{j}	-78^{b}	-82^{c}
ST0-xanthan gum 0.05%	62^{b}	699^{b}	-25^{h}	-14^{h}
ST0-xanthan gum 0.10%	88^{e}	2082^{f}	-36^{g}	-60^{e}
ST0-carrageenan 0.01%	12^{a}	255^{a}	-7^{i}	-2^{i}
ST0-carrageenan 0.02%	97^{f}	2336^{k}	-38f^{g}	-57^{ef}
ST0-carrageenan 0.05%	102^{g}	2165^{h}	-45^{cd}	-76^{d}
ST0-carrageenan 0.10%	69^{c}	1713^{c}	-40^{ef}	-73^{d}
ST0-WPC 0.05%	81^{d}	1899^{d}	-43de	-54^{fg}
ST0-WPC 0.10%	105^{g}	2660^{l}	-48^{c}	-86^{b}
ST0-WPC 0.15%	83^{d}	2075^{e}	-39^{fg}	-53^{g}
ST0-WPC 0.2%	90^{e}	2198^{i}	-41^{ef}	-59^{e}

$^{a-m}$Means within the same column with different subscriptions are significantly different ($P < 0.05$).

Table 3. Effects of different stabilizers on texture of fermented skim milk by ST1

	Firmness (g)	Consistency(g.s)	Cohesiveness (g)	Resistance(g.s)
ST1	54^{a}	1391^{b}	-42^{e}	-67^{f}
ST1-xanthan gum 0.01%	103^{cd}	2483^{f}	-82^{b}	-151^{a}
ST1-carrageenan 0.05%	89^{b}	1716^{c}	-50^{c}	-102^{c}
ST1-WPC 0.10%	52^{a}	1169^{a}	-26^{g}	-40^{h}

$^{a–h}$Means within the same column with different subscriptions are significantly different ($P < 0.05$).

made using ST0 (Table 2). Fermented skim milk structure appears to have strong protein–protein interactions as a result of the fermentation process (acid development and protein interaction). The fermented skim milk gel formation process was accompanied by EPS secretion, and the EPS interfered with protein-protein interactions, which resulted in a soft curd (Hassan et al., 2003; Ayala-Hernández et al., 2009). The added xanthan gum or carrageenan increased the firmness, consistency and cohesiveness of the low-fat fermented skim milk ST0 or ST1. Fermented skim milk supplemented with WPC combined with ST1 had lower firmness, consistency and cohesiveness than the control fermented skim milk ST1.

Viscosity

The viscosity of fermented skim milk is presented in Figure 2. The viscosity of sample from EPS-producing culture ST1 was higher ($P < 0.05$) than that of ST0. Several studies have found that fermented skim milk made with ropy EPS-producing starter cultures have higher viscosity values than those made with non- EPS-producing starter cultures (Ayala-Hernández et al., 2009; Hassan et al., 1996a, 2003). These results may be due to interaction between EPS and milk proteins resulting in the increase in viscosity (Ayala-Hernández et al., 2008). Figure 2 shows the improvement of viscosity for the three stabilizers indicating that each has a significant effect compared to the control. Carrageenan leads to the maximum fermented skim milk viscosity, carrageenan binds more water than other stabilizers, the protein and sugar components of the milk interacted to form a network. Free water in the system was reduced, which significantly increased the viscosity of fermented skim milk.

Water-holding capacity

Fermented skim milk made with ST1 exhibited higher water holding capacity, and fermented skim milk made with ST0 exhibited the lowest (Figure 3). These results confirm the ability of EPS to bind sufficient water to significantly affect fermented skim milk consistency. Hassan et al. (1996b) found similar results. With carrageenan in the system, fermented skim milk had the highest water holding capacity. The xanthan gum or WPC did not show good water holding capacity.

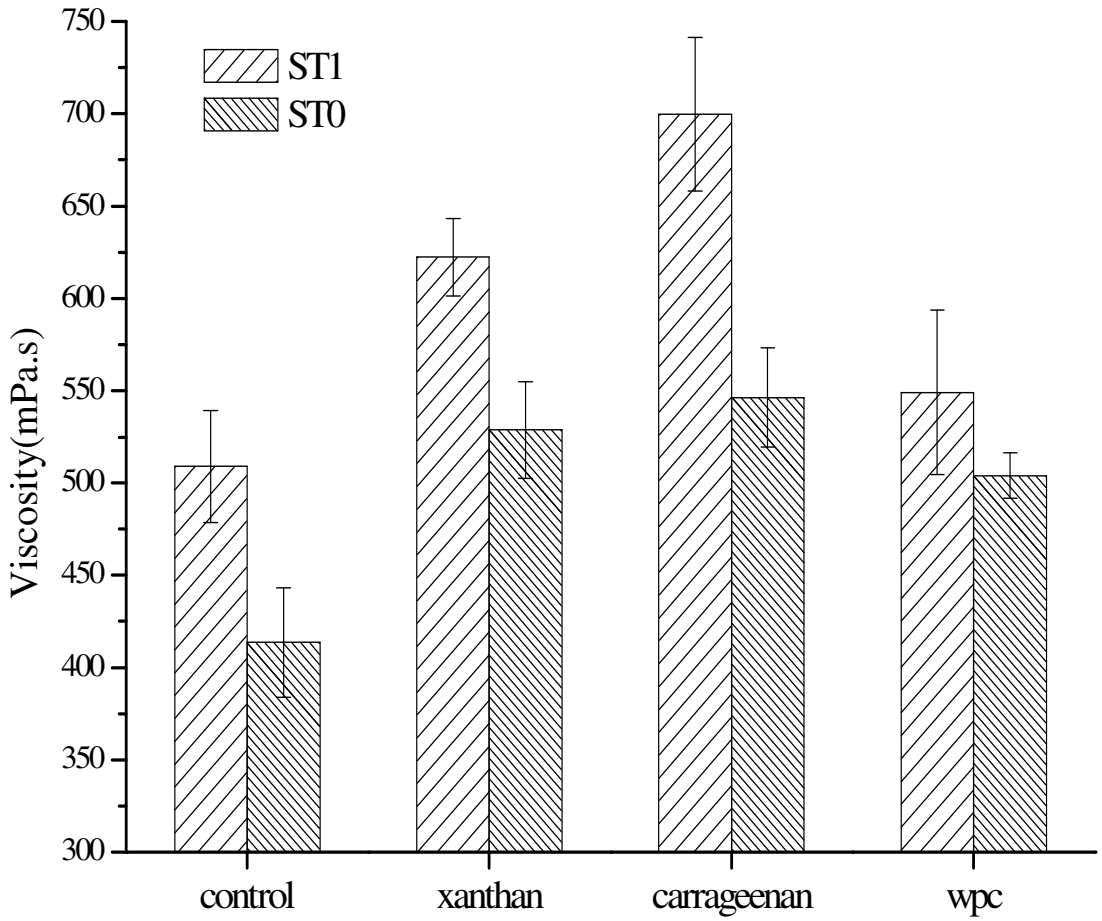

Figure 2. Effects of different cultures and stabilizers on the viscosity of the fermented skim milk.

Spontaneous Whey separation

Figure 4 shows the changes in spontaneous whey separation of fermented skim milk. The use of ropy EPS-producing starter culture ST1 reduced the level of syneresis in fermented skim milk significantly, especially in the products made from ST1 combined with carrageenan. Only fermented skim milk made with xanthan gum showed a significant level of syneresis compared to others. Similar results about the effect of EPS on the reduction in syneresis of fermented skim milk have been reported by others (Marshall and Rawson, 1999; Amatayakul et al., 2006). This may be due to high water-binding capacity of EPS and reduce permeability of serum through skim milk gel (Amatayakul et al., 2006). Figure 4 shows that the control, samples with carrageenan and WPC had no difference in the levels of spontaneous whey separation.

Microstructure

Figure 5 shows the microstructure of fermented skim milk made with the different stabilizers. The fermented skim milk made using EPS-producing culture ST1 had small and evenly distributed pores, and the protein network structure was thin (Figure 5A). The results of Figure 5 were associated with the presence of EPS, and EPS led to this network structure. The fermented skim milk made with ST1 had high viscosity and good water holding capacity as well as a low level of spontaneous whey separation. Figure 5B shows the fermented skim milk by ST1 and carrageenan had closer and more uniform network structure, and appeared to have higher water holding capacity and viscosity. ST1 combined with xanthan gum showed many flaps, and these flaps twist with casein and lactic acid bacteria, and did not integrate into the typical protein network (Figure 5C). This might be because carrageenan has a much smaller molecular weight compared with xanthan. The interaction between the negative ions on the surface of carrageenan and the positive ions on the surface of casein resulted in the tiny net structure (Figure 5B). It binds more water preventing whey separation (Soukoulis et al., 2007), but the large molecular weight of xanthan gum formed a network with large gaps and limited the number of caseins in the larger

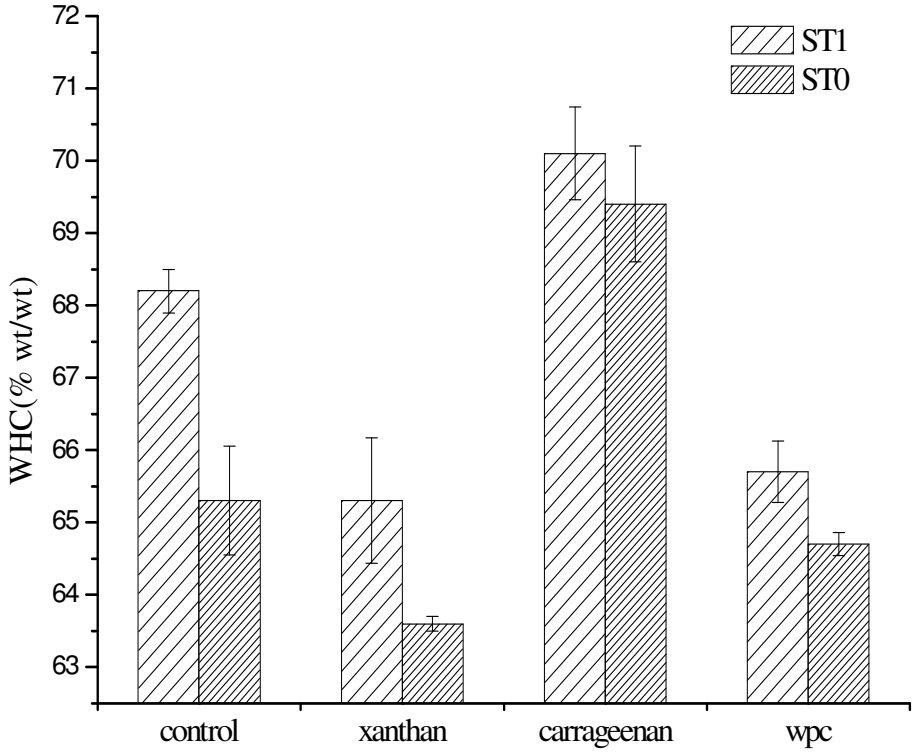

Figure 3. Effects of different cultures and stabilizers on WHC of the fermented skim milk. WHC, Water-holding capacity.

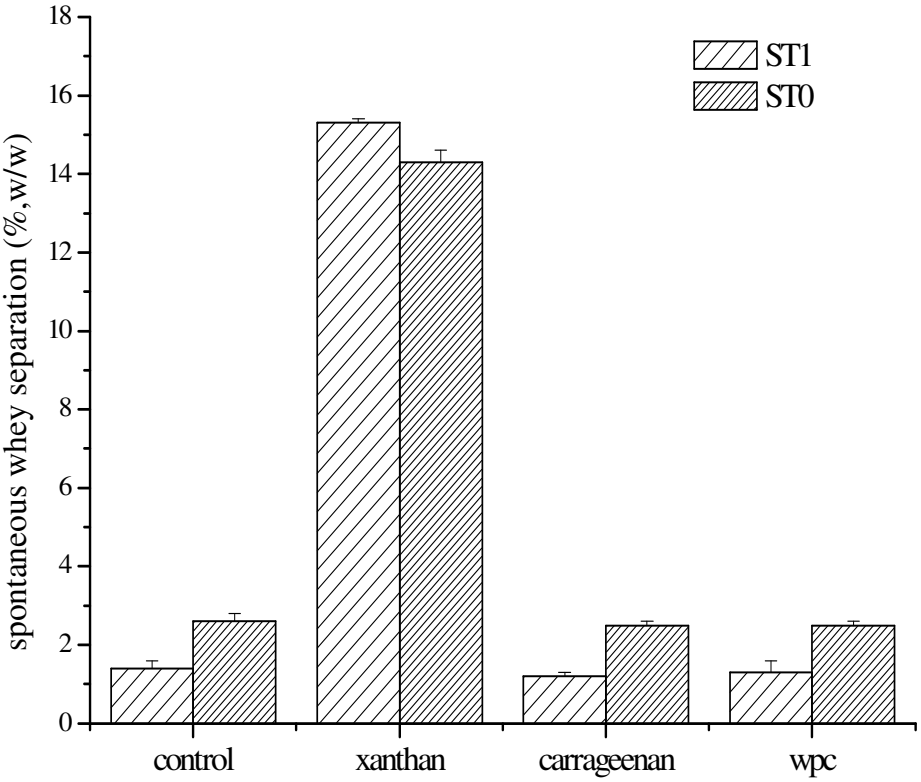

Figure 4. Effects of different cultures and stabilizers on spontaneous whey separation of the fermented skim milk.

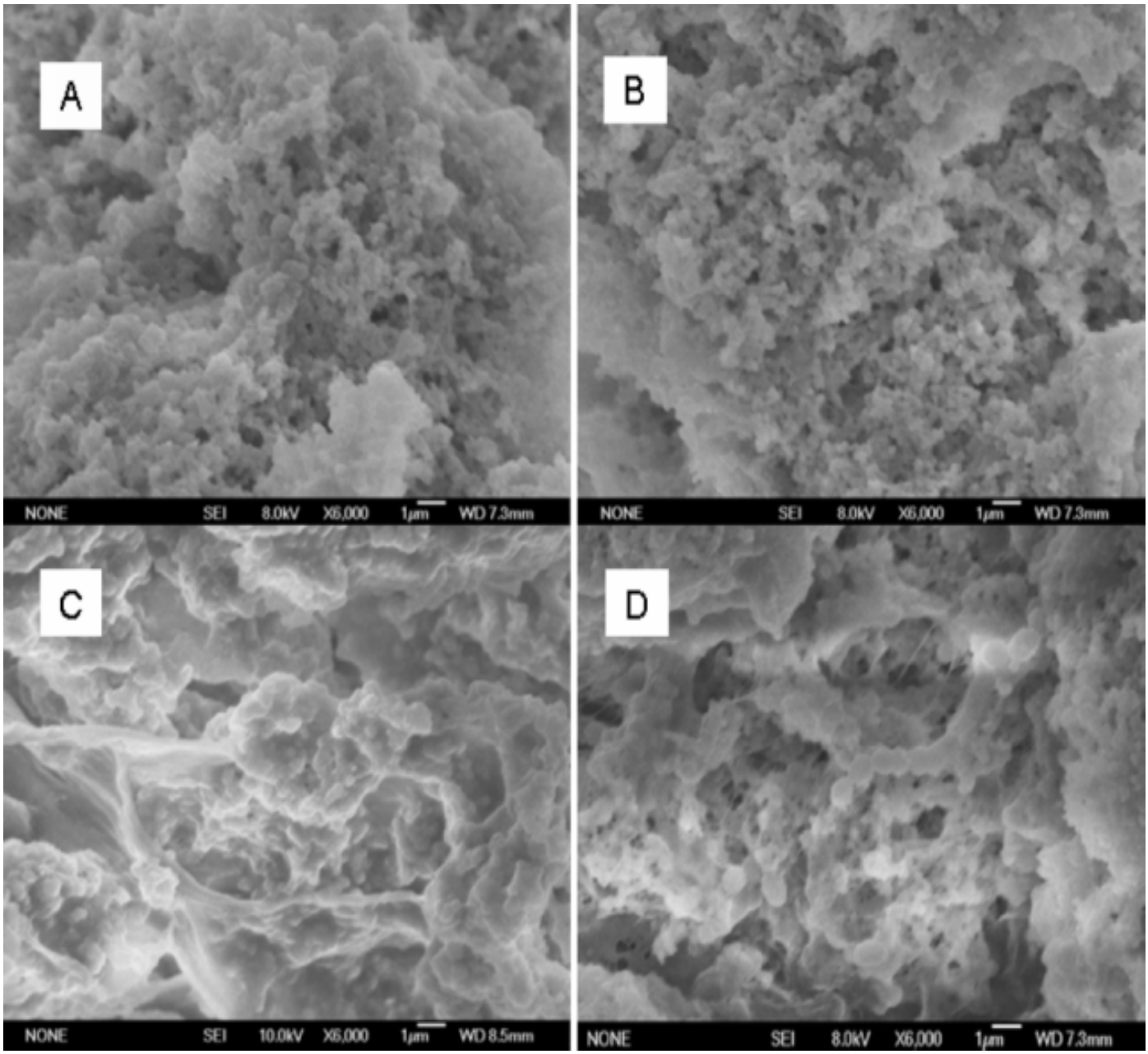

Figure 5. Microstructure of the fermented skim milk (A) ST1; (B) ST1+carrageenan; (C) ST1+xanthan; (D) ST1+WPC. WPC, Whey protein concentrate.

isolated intervals (Soukoulis et al., 2007). Lactic acid bacteria fermentation resulted in increased acidity and casein aggregation. Larger particles precipitated, which caused lower water-holding capacity as well as more marked whey separation. Fermented skim milk made with ST1 combined with WPC (Figure 5D) was observed, and it is clear that casein particles did not form large aggregates, but the particles interconnected to form filaments with the bigger interspace. This had a great impact on the water-holding capacity of the network and its viscosity. The whey separation was also influenced to some extent.

Conclusions

Results indicate that exopolysccharides produced by ST1 combined with exogenous stabilizers have a considerable effect on the physiochemical properties and the microstructure of fermented skim milk by ST1 compared with the samples using ST0. EPS-producing culture ST1 may be effective in improving the viscosity and the water holding capacity as well as reducing spontaneous whey separation. The microstructure of the fermented skim milk also showed the interactions between casein micelles

and other components.

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