Pilot-scale production of exopolysaccharide from *Leuconostoc pseudomesenteroides* XG5 and its application in set yogurt

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ABSTRACT

Exopolysaccharide from *Leuconostoc pseudomesenteroides* XG5 (XG5 EPS) is a linear dextran that is built by glucose units via α -1,6 glycosidic bond. The primary objective of this study was to investigate the yield of XG5 EPS and its application in set yogurt. In laboratory scale, the culture conditions of XG5 EPS production were optimized using the L9 (3^3) orthogonal test. Here, the optimized yield of XG5 EPS was 26.02 g/L under the conditions of 100 g/L sucrose, initial pH 7.0, 25°C incubation, and 100 rpm for 36 h in a shaking flask. Based on the optimized parameters of laboratory scale, a pilot fed-batch fermentation was performed in a 50-L bioreactor with an adjusted agitation speed of 20 rpm. The XG5 EPS yield reached 40.07 g/L in fed-batch fermentation, which was 54% higher than that achieved in laboratory scale. In addition, XG5 EPS was added into set yogurt to investigate its effect on the stability of set yogurt. Our data demonstrated that the XG5 EPS improved the water-holding capacity, texture profile, and viscosity of set yogurt during cold storage compared with the controls. In particular, addition of 0.5% XG5 EPS increased the structure of 3-dimensional network of set yogurt, which eventually improved the physical stability of the set yogurt. Overall, this study provided new insights for exploring the pilot scale production and application of dextran.

Key words: *Leuconostoc pseudomesenteroides* XG5, exopolysaccharide, dextran, fed-batch fermentation, set yogurt

INTRODUCTION

Leuconostoc pseudomesenteroides XG5 is gram-positive bacteria that is isolated from homemade wine.

There is ample indication that *L. pseudomesenteroides* is the most common lactic acid bacteria in naturally fermented fruits and vegetables (Fessard and Remize, 2019). Lactic acid bacteria have been closely associated with humans, and some of them are classified as generally recognized as safe microorganisms. It has been demonstrated that *L. pseudomesenteroides* P1 exhibited antibacterial activities and probiotic potential, and the safety of *L. pseudomesenteroides* P1 were assessed in vivo and in vitro (Wang et al., 2018). Some studies have shown that *L. pseudomesenteroides* exhibit an anti-obesity function in obese mice with high-fat diets (Sun et al., 2020). Moreover, *L. pseudomesenteroides* is also considered to be an important producer of microbial exopolysaccharide (Xu et al., 2018; Farinazzo et al., 2020).

Microbial exopolysaccharide is an important and abundant compound that can be secreted by bacteria, fungi, and algae. Generally, microbial exopolysaccharide is divided into 2 groups: homopolysaccharide and heteropolysaccharide. Homopolysaccharide contains only one type of monosaccharide (e.g., dextran), whereas heteropolysaccharide consists of several monosaccharides (e.g., xanthan; Barcelos et al., 2020). In recent decades, microbial exopolysaccharide has gained attention of the food industry because it played an important role in fermented dairy product and bakery product, and it has the advantages of a low cost and short cycle (Freitas et al., 2017; Lynch et al., 2018). Meanwhile, microbial exopolysaccharide has great potential for applications, based on its chemical structure and biological activities. It has been reported that *Lactobacillus plantarum* RJF4 exopolysaccharide exerted antiproliferative effects on MiaPaCa2-pancreatic cancer cell line in an in vitro study (Dilna et al., 2015). Zhou et al. (2019) found that exopolysaccharide from *Lactobacillus plantarum* NCU116 modulated colonic mucosal homeostasis. We recently reported the chemical structures and functional properties of the exopolysaccharide from *L. pseudomesenteroides* XG5 (XG5 EPS, a linear dextran) (Zhou et al., 2018; Pan et al., 2020).

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Dextran is an important exopolysaccharide produced by bacteria, and it is a good choice for drug carriers, which can effectively control drug release (Chen et al., 2020). At present, the production of dextran generally includes conventional fermentation and dextransucrase biosynthesis (Yang et al., 2015; Vuillemin et al., 2018). It has been reported that the industry still employs the conventional fermentation to produce the commercial dextran, which may be due to the high cost and technical difficulty of dextran biosynthesis by dextransucrase (Li et al., 2020b). Until now, most studies have focused on the optimization of dextran production at the shake flask level (Kanimozhi et al., 2017; Xing et al., 2018). To the best of our knowledge, studies on the preparation of dextran in pilot scale were still rare. Considering the sustainable and cost strategy of optimizing XG5 EPS in pilot scale, the present work first employed orthogonal experimental design to optimize the yield of XG5 EPS in laboratory scale. Then, the XG5 EPS production was verified by batch fermentation in a 50-L bioreactor containing a 35-L working volume, and the fed-batch fermentation was used to further increase the production of XG5 EPS. In this study, the beneficial parameters were obtained by XG5 EPS pilot production, which provides theoretical basis for industrial dextran production in the future. Moreover, antioxidant activity and application in set yogurt of XG5 EPS were investigated to further explore the applications of XG5 EPS in food industry.

MATERIALS AND METHODS

XG5 EPS Extraction

Leuconostoc pseudomesenteroides XG5 was isolated from homemade wine, and the stock culture of *L. pseudomesenteroides* XG5 was cultured in de Man, Rogosa, and Sharpe (MRS) broth with 20% (vol/vol) glycerol for long time preservation. The XG5 EPS was extracted by the method of Zhou et al. (2018). Briefly, *L. pseudomesenteroides* XG5 was grown in MRS medium supplemented with 12.5% (wt/vol) sucrose (MRS-S) at 30°C for 48 h unless otherwise specified. After that, the medium was boiled in a water bath at 100°C for

15 min and centrifuged at $10,000 \times g$ for 30 min at 4°C. Three volumes of cold ethanol (95%, vol/vol) were added to the supernatant and incubated overnight at 4°C to precipitate XG5 EPS. The precipitate was dissolved in deionized water, and trichloroacetic acid (10%, wt/vol) was added to the EPS solution to remove the proteins at 4°C for 12 h. Finally, 3 volumes of cold ethanol (95%, vol/vol) were added to the supernatant and incubated overnight at 4°C to precipitate XG5 EPS. The precipitate was lyophilized for the further study.

Optimization of Culture Conditions

Leuconostoc pseudomesenteroides XG5 was activated by transfer to MRS broth medium and cultivated at 30°C for 24 h in 5 mL of MRS broth. First, the culture conditions of exopolysaccharide from *L. pseudomesenteroides* XG5 was investigated by the one-factor-at-a-time method. *Leuconostoc pseudomesenteroides* XG5 (2%, vol/vol) was cultured in 50-mL flasks with 15 mL of MRS-S; the MRS-S medium contains (per L) 5.5 g of glucose·H₂O, 125 g of sucrose, 10 g of tryptone, 10 g of beef extract powder, 5 g of yeast extract powder, 2.62 g of K₂HPO₄·3H₂O, 5 g of sodium acetate anhydrous, 2 g of ammonium citrate tribasic, 0.58 g of MgSO₄·7H₂O, 0.25 g of MnSO₄·H₂O, and 1 mL of Tween 80, and was incubated at 30°C for 48 h. In this work, the initial pH (5.0, 6.0, 7.0, 8.0, and 9.0), fermentation temperature (25, 30, and 37°C), rotary speed (0, 50, 100, 150, and 200 rpm), fermentation time (12, 24, 36, 48, and 60 h), and sucrose concentration (50, 75, 100, 125, and 150 g/L) were optimized to improve the production of exopolysaccharide. Finally, L9 (3³) orthogonal test (Table 1) was applied to optimize the culture conditions, including initial pH, fermentation temperature, and fermentation time. The biomass of *L. pseudomesenteroides* XG5 was measured at 600 nm by spectrophotometer (V-1600, MAPADA).

Fed-Batch Fermentations

Based on the optimized parameters of the 50-mL flasks with a 15-mL working volume, the working vol-

Table 1. Factors and levels for orthogonal test to optimize the culture conditions for production of exopolysaccharide from *Leuconostoc pseudomesenteroides* XG5

Factor	Item	Level		
		1	2	3
X ₁	Initial pH	6.0	7.0	8.0
X ₂	Fermentation temperature (°C)	25	30	37
X ₃	Fermentation time (h)	24	36	48

ume of fed-batch fermentation was upscaled to 35 L. First, *L. pseudomesenteroides* XG5 was activated by transfer to MRS broth medium and cultivated at 30°C for 24 h in 700 mL of MRS broth, and the fermentation process was cultivated in the 50-L bioreactor (Yixing Pharmaceutical Equipment Factory) containing 2% (vol/vol) inoculum. The parameters of bioreactor were set according to the following: fermentation temperature 25°C, agitation speed 20 rpm, and fermentation time 60 h. The fermentation medium contains (per L) 5.5 g of glucose·H₂O, 100 g of sucrose, 10 g of tryptone, 10 g of beef extract powder, 5 g of yeast extract powder, 2.62 g of K₂HPO₄·3H₂O, 5 g of sodium acetate anhydrous, 2 g of ammonium citrate tribasic, 0.58 g of MgSO₄·7H₂O, 0.25 g of MnSO₄·H₂O, and 1 mL of Tween 80, with the initial pH value of 7.0. Notably, the pH of fermentation broth was adjusted to 6.5 during each fed-batch fermentation. Here, the fermentation broth was periodically sampled to determine dry cell weight, residual sucrose, glucose and fructose concentration, and XG5 EPS production.

Antioxidant Activity In Vitro

2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of XG5 EPS was determined according to Bomfim et al. (2020) with minor modifications. In brief, the XG5 EPS solution (0.5 mL, 0.2–1.0 mg/mL) was mixed with the DPPH (1 mL, 0.05 mM, dissolved in ethanol). Subsequently, the mixture was shaken and incubated at 25°C for 30 min in the dark, and centrifuged at 5,000 × *g* for 5 min at 25°C. Absorbance of the supernatant was measured at 517 nm by spectrophotometer (V-1600, MAPADA), ascorbic acid (Vc) was used as the positive control. The DPPH radical scavenging activity was calculated as

$$\text{Scavenging activity (\%)} = [1 - (A_2 - A_1)/A_0] \times 100,$$

where A₀ represents the absorbance of the control (DPPH and water), A₁ represents the absorbance of the blank (XG5 EPS and ethanol), and A₂ represents the absorbance of the sample (XG5 EPS and DPPH).

2,2'-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) Radical Scavenging Activity. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of XG5 EPS was conducted according to Liu et al. (2016) with modifications. Briefly, the ABTS radical was produced by reacting ABTS solution (7 mM, dissolved in water) with potassium persulfate (final concentration 2.45 mM) at room temperature for 16 h in the dark. The

stock solution of ABTS radical was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and stocked before use. Then, the XG5 EPS solution (50 µL, 0.2–1.0 mg/mL) was mixed with 1 mL of the ABTS diluent. Moreover, the mixture was shaken and incubated at 25°C for 6 min in the dark, and centrifuged at 5,000 × *g* for 5 min. Absorbance of the supernatant was measured at 734 nm by spectrophotometer (V-1600, MAPADA), and Vc was used as the positive control. The ABTS radical scavenging activity was calculated as

$$\text{Scavenging activity (\%)} = [1 - (A_2 - A_1)/A_0] \times 100,$$

where A₀ represents the absorbance of the control (ABTS and water), A₁ represents the absorbance of the blank (XG5 EPS and ethanol), and A₂ represents the absorbance of the sample (XG5 EPS and ABTS).

Ferrous Ion Chelating Ability. Ferrous ion chelating ability was evaluated as previously described (Li et al., 2020a). In brief, the reaction system contained XG5 EPS solution (0.5 mL, 0.2–1.0 mg/mL), ferrozine (0.15 mL, 5 mM), FeCl₂ (0.1 mL, 2 mM), and 0.55 mL of methanol; the mixture was shaken and incubated at 25°C for 10 min. Absorbance of the mixture was measured at 562 nm by spectrophotometer (V-1600, MAPADA), Ethylene diamine tetra acetic acid was used as the positive control. Ferrous ion chelating ability was calculated as

$$\text{Ferrous ion chelating ability (\%)} = (1 - A/A_0) \times 100,$$

where A₀ represents the absorbance of the control (water instead of XG5 EPS) and A represents the absorbance of the sample (XG5 EPS).

Preparation of Set Yogurt. Commercial milk (100 g of milk contained 3.2 g of protein and 3.8 g of fat) was transferred into sterilized glass jars and heated to 70°C. The sucrose (7%, wt/wt) was added into milk, the mixture was homogenized at 30 MPa and subsequently heat treated at 95°C for 300 s. Afterward, XG5 EPS were added to the mixture and mixed well, and then cooled down to 43°C to add the yogurt starter (YO-PROX 699, Bioproducts). The final concentration of XG5 EPS was 0 (control), 0.1% (LXE), and 0.5% (HXE), respectively. Thereafter, the set yogurt sample was poured into 100-mL plastic cups and incubated at 43°C until pH reached 4.5 ± 0.1. Last, the set yogurt was refrigerated at 4°C for 1, 7, and 14 d.

Water-Holding Capacity. Water-holding capacity (WHC) of set yogurt was determined according to the method of Fu et al. (2018) with some modification. Briefly, 10 g of the set yogurt was centrifuged at 3,000

$\times g$ for 30 min. The WHC was evaluated using following equation:

$$\text{WHC (\%)} = (1 - W_2/W_1) \times 100,$$

where W_1 is the weight of set yogurt before centrifugation (g), and W_2 is the weight of supernatant after centrifugation (g). The WHC of set yogurt sample was evaluated 3 times during cold storage (1, 7, and 14 d).

Texture Profile Analysis. Texture profile analysis of the set yogurt sample was assessed by a texture analyzer (TA-XT plus, Stable Micro Systems Ltd.) equipped with a 36-mm (P/36R) cylindrical probe. The set yogurt sample was tested at 1 mm/s. Trigger force was 5.0 g and the compression ratio was 30%. The hardness, cohesiveness, chewiness, and resilience of set yogurt sample was collected and analyzed by the Texture Exponent 32 software (Stable Micro Systems, version 6.1.18.0). The texture profile of set yogurt sample was evaluated 3 times during cold storage (1, 7, and 14 d).

Sensory Evaluation. The sensory evaluation of set yogurt samples was assessed by the modified method of Hovjecki et al. (2021). Briefly, sensory evaluation was conducted by 6 panelists (3 women and 3 men), aged 25 to 50, recruited from the laboratory. For all samples, odor was the first property assessed, and then the appearance, texture properties and consumer acceptance were evaluated, with the scores ranging from minimum (0) to maximum (10). The sensory evaluation of set yogurt sample was performed 3 times during cold storage (1, 7, and 14 d).

Rheological Analysis. Rheological testing was performed according to the previous method with modification (Zhao et al., 2020). In brief, rheological properties of set yogurt sample was analyzed by rheometer (Physica MCR301, Anton Paar Ltd.) with a measuring plate (PP50, 1.000-mm gap) at 4°C. The oscillation strain results indicated that the value of 0.5% was suitable for all samples. Viscosity was measured at shear rate from 0.1 s⁻¹ to 50 s⁻¹. Three-interval thixotropy test was performed to obtain the antishear properties of set yogurts; the sample was oscillated at 1 Hz with 0.5% strain until steady state reached. Then high shear rate (1,000 s⁻¹) was loaded in very short period (1 s) to induce the structure deformation. The storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta$, $\tan \delta = G''/G'$) were monitored until steady state recovered. The rheological testing of set yogurt sample was performed 3 times during cold storage (1, 7, and 14 d).

Microstructure. Microstructure of set yogurt sample was observed by scanning electron microscopy (Regulus 8100, Hitachi) according to the previous

method with modification (Khubber et al., 2021). In brief, the set yogurt sample was diluted (1:6) in deionized water and lyophilized. Afterward, the set yogurt samples were coated with a gold-palladium layer and scanned. The electron accelerating voltage was 3.0 kV and the magnification was 5,000 $\times$.

Statistical Analysis

Data are presented as mean $\pm$ standard deviation in triplicate, and statistical analysis was performed using SPSS Statistics 28 (IBM Corp.). One-way ANOVA was used to determine the significant differences among the groups followed by Duncan's multiple comparison test.

RESULTS AND DISCUSSION

Optimization of Culture Conditions for Improving Production of XG5 EPS

The production of XG5 EPS was closely related to culture conditions (e.g., initial pH, fermentation temperature, rotary speed, fermentation time, and substrate concentration), which may affect the growth of *L. pseudomesenteroides* XG5. The maximum level of XG5 EPS was observed at pH 7.0 with 28.86 ± 0.75 g/L (Figure 1a). Previously, Xing et al. (2018) found that the optimal value of initial pH was 7.18 for exopolysaccharide production of *Leuconostoc mesenteroides* strain. The effect of fermentation temperature on XG5 EPS production is shown in Figure 1b. The XG5 EPS production reached the maximum of 31.05 ± 0.72 g/L at 25°C, and rapidly decreased thereafter. The fermentation temperature may have an effect on the enzyme activities and further effect the production of XG5 EPS. The result was consistent with the optimal fermentation temperature of *Leuconostoc mesenteroides* KIBGE-IB22 (Siddiqui et al., 2014). Considering the cost-effectiveness, fermentation temperature lower than room temperature was not used in this study. As shown in Figure 1c, the production of XG5 EPS showed a steady increase (from 0 to 100 rpm). Remarkably, the production of XG5 EPS was decreased when the rotary speed was higher than 100 rpm. The result might be due that the high rotary speed increased shear stress and oxygen supply, which affected the production of XG5 EPS in the shake flask fermentation (Zhang et al., 2019). In addition, the production of XG5 EPS reached 32.69 ± 0.93 g/L until 36 h of fermentation, and then slightly decreased (Figure 1d). Similar to previous findings, the preferable fermentation time of levan from the *Tanticharoenia sakaeratensis* was 35 h (Aramsangtienchai et al., 2020). The production of

Table 2. The L9 (3³) orthogonal test applied for optimizing production of *Leuconostoc pseudomesenteroides* XG5 exopolysaccharide (EPS)

No.	Factor			XG5 EPS (g/L)
	X ₁ (pH)	X ₂ (°C)	X ₃ (h)	
1	6.0	25	24	18.55
2	6.0	30	36	19.67
3	6.0	37	48	9.36
4	7.0	25	36	26.02
5	7.0	30	48	21.98
6	7.0	37	24	13.26
7	8.0	25	48	19.64
8	8.0	30	24	18.10
9	8.0	37	36	11.02
K ₁	47.57	64.21	49.90	
K ₂	61.26	59.74	56.71	
K ₃	48.76	33.64	50.98	
R	4.56	10.19	2.27	

¹K = sum of the experimental indices of this level in each factor; R = difference between the average of K_{max} and K_{min} values.

XG5 EPS might be influenced by the sucrose concentration. As shown in Figure 1e, the percent yield of XG5 EPS increased from $43.71 \pm 1.03\%$ to $53.33 \pm 0.81\%$ with increasing the sucrose concentration from

50 to 100 g/L, and the production of XG5 EPS was 26.67 ± 0.41 g/L in MRS-S medium containing 100 g/L sucrose. Here, we integrated the results from the one-factor-at-a-time method, an orthogonal test was applied to optimize the culture conditions to further improve production of XG5 EPS. The optimal culture condition was obtained with the initial pH 7.0 of MRS broth at 25°C for 36 h, and the optimal yield of XG5 EPS reached 26.02 ± 0.82 g/L (Table 2). As shown in Table 3, initial pH and fermentation temperature had significant effects on the production of XG5 EPS. According to above-described results, the optimal culture conditions for XG5 EPS production were initial pH 7.0, fermentation temperature 25°C, 100 RPM, fermentation time 36 h, and sucrose concentration 100 g/L at the laboratory level.

Fed-Batch Fermentation of XG5 EPS in 50 L Bioreactor

To further explore the yield of XG5 EPS, the 50-L bioreactor was used to conduct batch fermentation. The

Figure 1. Optimization of culture conditions to enhance the production of exopolysaccharide from *Leuconostoc pseudomesenteroides* XG5 (XG5 EPS). The *L. pseudomesenteroides* XG5 was cultivated in de Man, Rogosa, and Sharpe medium supplemented with 12.5% (wt/vol) sucrose with different (a) initial pH, (b) fermentation temperature, (c) rotary speed, (d) fermentation time, and (e) sucrose concentration. Biomass was measured by optical density at 600 nm (OD₆₀₀). Data are presented as mean $\pm$ SD in triplicate.

yield of XG5 EPS reached a maximum of 29.63 g/L in 24 h of batch fermentation, and declined slightly thereafter (Figure 2a). The XG5 EPS decrease may be due to the use of XG5 EPS by *L. pseudomesenteroides* XG5 after 24 h of fermentation. Similarly, Abdeslahian et al. (2020) found that β -glucan accumulated in the initial 72 h of fermentation time and later consumed. It is remarkable that the higher dry cell weight was achieved in 24 h of batch fermentation, and the maximum yield of XG5 EPS was harvested at this time. In addition, we found that the glucose concentration of fermentation broth was slow to decrease during fermentation. The fructose content of fermentation broth increased to 13 g/L at 24 h and then decreased; we speculate that fructose may be used by *L. pseudomesenteroides* XG5 for its own growth.

As shown in Figure 2b, sucrose concentration declined to 67 g/L after 12 h of fermentation. To attain higher XG5 EPS production, sucrose was added once at 12 h during the fed-batch fermentation. Subsequently, the sucrose concentration increased to 103 g/L. Remarkably, the yield of XG5 EPS reached a maximum of 40.07 g/L after 42 h of fermentation, which was 35.24% higher than that of batch fermentation. A previous study has shown that the yield of microbial polysaccharide enhanced 19.79% by fed-batch fermentation (Mummaleti et al., 2021). Indeed, the sucrose supplied at 18 h during the fed-batch fermentation, but the viscosity of the fermentation broth was too high, and the density of the fermentation broth was lower than that of sucrose at the 18 h of fermentation, which meant that the sucrose could not be mixed with the fermentation broth after feeding. Finally, the sucrose was fed at 12 h during the fed-batch fermentation. Currently, many researchers have attempted to extract the dextran by the fermentation of different bacteria, most of which have the relatively low yields of dextran. For instance, it was reported that *L. pseudomesenteroides* YF32 only yielded the 12.5 g/L of dextran in MRS medium supplemented with sucrose (Yang et al., 2018). In addition, Castro-Rodriguez et al. (2019) found that the yield of dextran from *L. mesenteroides* SF3 was 20 g/L. Unfortunately, these studies on the yield of dextran were only at the laboratory level, and the pilot production of dextran have not been given enough at-

tention, whereas the yield of dextran reached 40.07 g/L in a 50-L bioreactor in our study. Therefore, this study provides a feasible strategy for industrial production of dextran.

Antioxidant Activity of XG5 EPS In Vitro

The DPPH and ABTS radical scavenging activity and ferrous ion chelating ability were used to evaluate the antioxidant activity of XG5 EPS. The antioxidant activity of XG5 EPS and ascorbic acid or EDTA are shown in Figure 3, the DPPH and ABTS radical scavenging activity of XG5 EPS at all concentrations were much lower than ascorbic acid (Figure 3a,b). Previously, similar results were observed that the highest DPPH radical scavenging activity of exopolysaccharide from *Streptococcus thermophilus* GST-6 and *Enterococcus faecium* WEFA23 were about 10% (Zhang et al., 2016, Jia et al., 2019). As shown in Figure 3c, the ferrous ion chelating ability of XG5 EPS slightly increased with the rising of XG5 EPS concentration, and it reached to $7.63 \pm 1.61\%$ at the concentration of 1.0 mg/mL. It has been reported that the antioxidant activity of polysaccharides might be related to molecular weight, composition and glycosidic bonds (Li et al., 2019). Overall, the results showed that XG5 EPS might slightly have antioxidant activity in vitro.

Water-Holding Capacity, Texture Profile Analysis, and Sensory Evaluation of Set Yogurt During Cold Storage

Water-holding capacity was investigated to evaluate the stability of the set yogurt. As shown in Table 4, the WHC of set yogurt significantly increased after adding of XG5 EPS, especially in the HXE group. This phenomenon was probably caused by the fact that XG5 EPS bind water into the milk base, thereby improving the stability of set yogurt. Moreover, XG5 EPS reacts with the milk constituents, mainly the proteins, to increase the level of water hydration (Tamime and Robinson, 1999). There was evidence that the WHC of set yogurt significantly increased after addition of β -glucan (Zhao et al., 2020). Additionally, the change in pH values of yogurts during cold storage is shown in

Table 3. Significance analysis of the L9 (3^3) orthogonal test

Factor ¹	Sum of deviation square	Mean of square	F-value	P-value
X ₁	38.309	19.155	61.641	0.016
X ₂	181.794	90.897	292.514	0.003
X ₃	8.913	4.457	14.342	0.065
Error	0.621	0.311		

¹See Table 1 for factors.

Figure 2. Fed-batch fermentation of *Leuconostoc pseudomesenteroides* XG5 for the production of XG5 exopolysaccharide (EPS). (a) Batch fermentation. (b) Sucrose was fed at 12 h during the fed-batch fermentation. (■, XG5 EPS; ▼, sucrose; ◆, glucose; ▲, fructose; ●, dry cell weight).

Table 4. Compared with control yogurts, the set yogurt containing 0.5% XG5 EPS showed significantly lower pH values. A similar result was also found in the previous study that low-methoxyl pectin added set yogurts showed lower pH compared with controls (Khubber et al., 2021).

Texture profile analysis of set yogurts containing different concentrations of XG5 EPS are shown in Table 4. The hardness (force 1) of set yogurt containing 0.5% XG5 EPS was remarkably lower than that of the controls during cold storage period of 7 and 14 d. The lower hardness of set yogurt containing 0.5% XG5 EPS could be attributed to its higher WHC (Table 4) and 3-dimensional network structure (Figure 5c, f, and i). Similar observations have been reported for set yogurt containing date palm spikelet extracts (Almusallam et al., 2021). The chewiness values indicated that the addition of 0.5% XG5 EPS resulted in set yogurts with considerably lower chewiness during cold storage period of 7 and 14 d, which was similar to the previous study (Kaur and Riar, 2020). Remarkably, the resilience of set yogurt containing 0.5% XG5 EPS expressed observ-

Figure 3. Antioxidant activity of *Leuconostoc pseudomesenteroides* XG5 exopolysaccharide (XG5 EPS) in vitro. (a) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, (b) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, and (c) ferrous ion chelating ability. Data are presented as mean $\pm$ SD in triplicate.

Table 4. Water-holding capacity (WHC), pH, texture profile analysis, and sensory evaluation of set yogurt during storage¹

Storage period	Addition dose ²	Texture profile analysis ³							Sensory evaluation
		WHC (%)	pH	Hardness (force 1)/N	Hardness (force 2)/N	Cohesiveness	Chewiness	Resilience	
Day 1	Control	75.3 ± 2.0 ^{b,B}	4.37 ± 0.03 ^{a,A}	2.431 ± 0.848 ^{a,A}	1.430 ± 0.394 ^{a,A}	0.444 ± 0.042 ^{a,A}	100.799 ± 30.243 ^{a,A}	0.048 ± 0.007 ^{a,A}	9.57 ± 0.09 ^{a,B}
	LXE	77.7 ± 1.0 ^{ab,B}	4.32 ± 0.02 ^{ab,A}	2.807 ± 0.293 ^{a,A}	1.509 ± 0.080 ^{a,A}	0.401 ± 0.021 ^{a,A}	105.290 ± 3.636 ^{a,A}	0.042 ± 0.006 ^{a,A}	9.60 ± 0.17 ^{a,B}
	HXE	80.3 ± 1.0 ^{a,A}	4.29 ± 0.03 ^{a,A}	2.455 ± 0.248 ^{a,A}	1.414 ± 0.084 ^{a,A}	0.427 ± 0.009 ^{a,A}	100.64 ± 8.249 ^{a,A}	0.044 ± 0.005 ^{a,B}	9.59 ± 0.08 ^{a,B}
Day 7	Control	78.3 ± 1.0 ^{b,A}	4.32 ± 0.04 ^{a,AB}	2.415 ± 0.117 ^{a,A}	1.257 ± 0.027 ^{a,A}	0.403 ± 0.012 ^{a,A}	93.694 ± 2.966 ^{a,A}	0.039 ± 0.003 ^{a,AB}	9.79 ± 0.07 ^{a,A}
	LXE	79.5 ± 0.3 ^{ab,A}	4.23 ± 0.02 ^{b,B}	2.341 ± 0.098 ^{a,B}	1.181 ± 0.033 ^{b,B}	0.388 ± 0.014 ^{a,A}	87.643 ± 0.703 ^{b,A}	0.042 ± 0.009 ^{a,A}	9.80 ± 0.05 ^{a,A}
	HXE	80.2 ± 0.6 ^{a,A}	4.19 ± 0.01 ^{b,B}	2.004 ± 0.072 ^{b,B}	1.075 ± 0.032 ^{c,B}	0.398 ± 0.006 ^{a,B}	76.141 ± 1.619 ^{c,B}	0.055 ± 0.005 ^{b,A}	9.77 ± 0.09 ^{a,A}
Day 14	Control	76.1 ± 0.7 ^{c,AB}	4.28 ± 0.03 ^{a,B}	3.014 ± 0.165 ^{a,A}	1.604 ± 0.173 ^{a,A}	0.401 ± 0.014 ^{a,A}	112.187 ± 6.156 ^{a,A}	0.035 ± 0.003 ^{a,B}	9.37 ± 0.11 ^{c,C}
	LXE	78.0 ± 0.4 ^{b,B}	4.23 ± 0.01 ^{b,B}	2.975 ± 0.171 ^{a,A}	1.653 ± 0.201 ^{a,A}	0.403 ± 0.008 ^{a,B}	111.942 ± 7.145 ^{a,A}	0.042 ± 0.005 ^{a,A}	9.53 ± 0.12 ^{b,B}
	HXE	79.5 ± 0.6 ^{a,A}	4.20 ± 0.03 ^{b,B}	2.553 ± 0.175 ^{b,A}	1.376 ± 0.070 ^{b,A}	0.387 ± 0.005 ^{a,B}	94.563 ± 5.764 ^{b,A}	0.040 ± 0.005 ^{a,B}	9.70 ± 0.13 ^{ab,AB}

^{a-c}Values with different lowercase letters within a column indicate significant differences ($P < 0.05$) among treatments.^{A-C}Values with different uppercase letters within a column indicate significant differences ($P < 0.05$) for the same sample during cold storage.¹Values are presented as mean ± SD in triplicate.²Control = 0 added *Leuconostoc pseudomesenteroides* XG5 exopolysaccharide (XG5 EPS); LXE = 0.1% added XG5 EPS; HXE = 0.5% added XG5 EPS.³Force 1 = the hardness at the first compression; force 2 = the hardness at the second compression.

able improvement at the cold storage period of 7 d in comparison with the controls. Moreover, XG5 EPS enhanced the sensory evaluation of set yogurt with the cold storage period of 14 d. The sensory results are related to WHC, which may be due to the observation of whey in the controls at the cold storage period of 14 d.

Rheological Properties of the Set Yogurt During Cold Storage

The viscosity of set yogurts decreased with the increasing of shear rate, indicating that all the set yogurts exhibited shear-thinning behavior (Figure 4a, d, g). It is remarkable that the viscosity of set yogurt added with 0.5% XG5 EPS is significantly higher than that of the control group at shear rate from 0.1 to 50 s⁻¹. The possible explanation was that exopolysaccharide interact with casein micelles to form a strong network, which contributes to increase the viscosity of the set yogurt (Nguyen et al., 2017). According to Xu et al. (2019) the viscosity of set yogurt increased when okra polysaccharide was added to set yogurt. In addition, a 3-interval thixotropy test was performed to obtain the antishrink ability of set yogurts during cold storage. Thixotropy results showed that transient shear stress changed the structure of all set yogurts. Meanwhile, the storage modulus (G') and loss modulus (G'') of set yogurt containing XG5 EPS were higher than that of the controls during cold storage period of 7 and 14 d (Figure 4e, h). The loss tangent ($\tan \delta$, $\tan \delta = G''/G'$) of all samples are shown in Figure 4c, f, and i, it is useful to evaluate the solid or liquid behavior of set yogurts during loading a transient shear stress (Belsito et al., 2017). When $\tan \delta < 1$, the sample exhibits a solids-like behavior, whereas $\tan \delta > 1$ indicates that the sample has liquid-like behavior (Silva et al., 2016). In this study, all samples presented low values of $\tan \delta$ than 1, indicating that all set yogurts maintained a solids-like behavior. However, there was no difference in $\tan \delta$ values for all set yogurt samples during cold storage.

Microstructure

Microstructure difference among the set yogurt samples is shown in Figure 5, the structure of 3-dimensional network was observed in the set yogurt containing XG5 EPS during cold storage (Figure 5c, f and i), and the structure of 3-dimensional network improved when the concentration of XG5 EPS increased. In contrast, no similar structure was visualized in the set yogurt only containing sucrose. A previous study found that adding Salecan, a macromolecular glucan, to set yogurt formed an extra string-like structure, which strengthened the

Figure 4. Rheological properties of set yogurts. Panels a, d, and g: Viscosity of set yogurts containing different concentrations of *Leuconostoc pseudomesenteroides* XG5 (XG5 EPS) at different shear rates [strain (γ) = 0.5%]. Panels b, e, and h: storage modulus (G' ; solid symbols) and loss modulus (G'' ; open symbols), and panels c, f, and i: $\tan \delta$ (G''/G') of set yogurt under strain sweep (shear rate: $1,000 \text{ s}^{-1} \times 1 \text{ s}$, $\gamma = 0.5\%$, frequency = 1 Hz) during cold storage (panels a, b, c = d 1; panels d, e, f = d 7; and panels g, h, i = d 14). Black squares = control (0 added XG5 EPS); green triangles = LXE (0.1% added XG5 EPS); and red triangles = HXE (0.5% added XG5 EPS).

stability of set yogurt (Fu et al., 2018). In addition, Zhao et al. (2020) found that curdlan, a glucan, increased the string-like additional structure and porous structure of the set yogurt. This finding suggested that addition of 0.5% XG5 EPS improved the stability of the set yogurt, consistent with the data in the WHC, texture profile and viscosity. Accordingly, XG5 EPS is a stabilizer for application in set yogurt production.

CONCLUSIONS

Leuconostoc pseudomesenteroides is a promising producer of dextran. In this study, the yield of XG5 EPS

up to 26.02 g/L under the conditions of initial pH 7.0, fermentation temperature of 25°C, 100 rpm, fermentation time of 36 h, and sucrose concentration of 100 g/L at the laboratory level, and the yield of XG5 EPS was further enhanced to 40.07 g/L in a 50-L bioreactor by the fed-batch fermentation. Moreover, the addition of 0.5% XG5 EPS improved the stability of the set yogurt, including the WHC, texture profile, viscosity, and microstructure during cold storage. These results indicate that XG5 EPS exhibited an industrial production prospect, and XG5 EPS could be considered as a potential stabilizer for industrial production of set yogurt.

Figure 5. Scanning electron micrographs of set yogurt containing different concentrations of *Leuconostoc pseudomesenteroides* XG5 exopolysaccharide (XG5 EPS) with magnification of 5,000 $\times$. Panels a, d, g: Control (0 added XG5 EPS); panels b, e, h: LXE (0.1% added XG5 EPS); panels c, f, i: HXE (0.5% added XG5 EPS) during cold storage (panels a, b, c = d 1; panels d, e, f = d 7; and panels g, h, i = d 14). Scale bar = 10 μ m.

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