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Research Article

Partial Purification, Characterization, and Application of Exopolysaccharides Produced by *Lactobacillus plantarum* NS1905E in Yogurt

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Exopolysaccharides (EPS) of lactic acid bacteria (LAB) has gained special interests in the dairy industry due to their health-promoting properties and contribution to the rheology of fermentation milk products. In the present study, an EPS designated as EPS-NS1905E was partially purified and characterized secreted from *L. plantarum* NS1905E. The EPS-NS1905E had an average molecular weight of 2.658×10^5 Da and was composed of glucose, arabinose, rhamnose, mannose, and galactose in an approximate molar ratio of 58.83 : 17.4 : 6.31 : 5.86 : 2.74. The FTIR spectroscopy showed the prevalence of carboxyl and hydroxyl groups. The EPS-NS1905E had a thermal stability and exhibited higher viscosity in the NaCl solution and low acidity conditions (pH 2–4). Furthermore, the EPS-NS1905E possessed strong scavenging abilities against DPPH radicals (96%). The addition of EPS-NS1905E in skim milk significantly shortened the fermentation time of yogurt, and the water-holding capacity (WHC) and viscosity of yogurt were improved during storage. The results indicated that the EPS-NS1905E has great potential for use as a food additive in the food industry.

1. Introduction

Microbial exopolysaccharides (EPS) are produced by a wide variety of bacteria in the natural ecological environments, and they played a critical role in the prevention of desiccation and protection against environmental stresses and adherence to surfaces [1–3]. Several natural EPS produced by bacteria have been characterized and used as bioflocculants, bioabsorbents, and drug delivery agents in the food, cosmetic, and pharmaceutical industries [4]. In the last decades, the EPS produced by lactic acid bacteria (LAB) have received increasing attention due to their food-grade status [5,6]. EPS-producing LAB has been widely employed in yogurt and cheese for improving the rheology, texture, and mouth feel [7,8]. In addition, there has been an increasing interest in characterizing and exploiting the EPS produced by LAB for their potential biological activities including antioxidant, antitumor, immune stimulatory, and cholesterol lowering [9–11].

L. plantarum is one of the most studied species of LAB and frequently isolated from many food products. The differences in characteristic and functional properties of EPS produced by *L. plantarum* strains were observed. Due to the biological activity and health benefits, more EPS produced by novel *L. plantarum* strains attracted a great deal of interest. Liu et al. [12] reported that EPS produced by *L. plantarum* HY exhibited considerable antioxidant and α-amylase inhibitory activities. EPS produced by *L. plantarum* C70 exhibited potential anticancer, antidiabetic, and antioxidant bioactivities [10]. EPS derived from *L. plantarum* WLPL09 inhibited the proliferation of HepG-2 and HCT-8 cells [13].

Raw milk is a complex matrix that facilitates the development of microorganisms. *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, often presented in raw milk, are associated with the manufacturing of dairy products. In the previous study, we have evaluated the EPS-

producing abilities of LAB strains through the EPS content determination, and the isolated strain *L. plantarum* NS1905E (which was isolated from fresh milk in Nanshan Pasture, Nanshan Animal Husbandry Co., Ltd.) exhibited an excellent EPS-producing ability [14]. In the present study, we aim to characterize the partial purified EPS produced by *L. plantarum* NS1905E, and its *in vitro* antioxidant activity and application in yogurt were evaluated.

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions. *L. plantarum* NS1905E was cultured in MRS broth (Guangdong Huankai Microbial Technology Co., Ltd., Guangzhou, Guangdong, China) and incubated at 37°C for 20 h.

2.2. Isolation of EPS-NS1905E. *L. plantarum* NS1905E was cultured in MRS broth at 37°C overnight. After incubation, culture medium was centrifuged (9000 × *g*, 20 min, 4°C) to remove cells. Three volumes of cold ethanol were added to the supernatant at 4°C for 48 h for extracting the EPS. The precipitate was collected by centrifugation (9000 × *g*, 15 min at 4°C) and dissolved in distilled water (ca. 1 g/L). The protein was precipitated by adding Sevag agent (chloroform : *n*-butylalcohol = 4 : 1, v/v) and removed by centrifugation (9000 × *g*, 15 min at 4°C). Furthermore, the solution was dialyzed against distilled water for 72 h. The polysaccharide aqueous solution after dialysis was lyophilized for advanced study.

2.3. Basic Components Analysis of EPS-NS1905E. Total sugar content of EPS was determined according to the method described by Dubois et al. [15] with glucose as standard. The total protein content was assessed by the Bradford method [16].

2.4. Molecular Weight (Mw) and Monosaccharide Composition Analysis of EPS-NS1905E. The Mw of partially purified EPS-NS1905E was determined according to the method described by Zhao et al. [17] via gel permeation chromatography-multiangle laser light scattering (GPC-MALLS).

Monosaccharide composition of EPS-NS1905E was determined by high-performance anion-exchange chromatograph coupled with pulsed amperometry detection (HPAEC-PAD) equipped with a CarboPac^{TM} PA10 guard column and a CarboPac^{TM} PA10 analytical column (4 mm × 250 mm). Monosaccharides (D-Glc, D-Gal, L-Rha, D-Ara, D-Fru, D-Man, and L-Fuc) were used as standards.

2.5. Fourier-Transform Infrared (FTIR) Spectroscopy of EPS-NS1905E. The analysis of functional groups presented in EPS-NS1905E was conducted by Fourier-transform infrared spectroscopy. The spectra from 400 to 4000 cm^{-1} were recorded by a FTIR-8400S spectrophotometer (Shimadzu, Japan).

2.6. Rheological Characterization of EPS-NS1905E. The aqueous solutions of EPS-NS1905E were prepared by dissolved lyophilized EPS in 2 M Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} solutions and distilled water at a concentration of 1% (w/w), respectively. The aqueous solution of EPS-NS1905E was adjusted to different pH values (2, 4, 7, 10, and 12) by 1 N HCl and NaOH solutions. To evaluate the effect of temperature on the viscosity of the EPS-NS1905E, the aqueous solution of EPS-NS1905E was exposed to different temperatures (−20, 25, and 90°C), respectively. The rheological properties of EPS-NS1905E aqueous solution were measured by a HAAKE RS6000 rheometer (Thermo Fisher Scientific Inc., MA, USA) with a cone-plate geometry (35 mm diameter) at 25°C.

2.7. Biological Activity of EPS-NS1905E. The DPPH radical scavenging activity of EPS-NS1905E was detected according to the method described by Wang et al. [9]. DPPH scavenging activity was expressed as a percentage and calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{absorbance of sample}}{\text{Absorbance of blank}} \times 100. \quad (1)$$

The antitumor activity of EPS-NS1905E was evaluated according to the protocol described by Ayyash et al. [10]. The inhibition effect of EPS-NS1905E on Caco-2 was calculated via the following formula:

$$\text{Inhibitory (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}}\right) \times 100. \quad (2)$$

2.8. Preparation of Set-Type Yogurt. Skim milk powder was reconstituted to 14% (w/v) with distilled water. EPS-NS1905E was mixed at concentration levels of 0.01%, 0.05, and 0.1% (w/w), respectively, with milk without EPS as the control group. The reconstituted milk was pasteurised at 90°C for 5 min. After cooling to 43°C in waterbath, samples were inoculated with 3% of yogurt starter (Danisco YO-MIX 883) and distributed in

yogurt cups. The incubation was carried out at 42°C until the pH reached 4.6. The fermentation was stopped in a cold room and stored at 4°C for 21 days.

2.9. pH and Titratable Acidity Determination. The change in pH of yogurt samples during fermentation and storage was measured with a pH meter (FE-20 FiveEasy Plus, Mettler Toledo). The change in titratable acidity of yogurt samples was investigated. Yogurt samples were added in distilled water at 50% (w/v) and titrated with 0.1 M sodium hydroxide; 0.5% phenol-phthalein was used as an indicator. Titratable acidity was expressed as °T.

2.10. Water-Holding Capacity Analysis. Yogurt samples were centrifuged at $3250 \times g$, 4°C for 10 min. After removing the whey expelled, the precipitate was weighed. The water-holding capacity (WHC) expressed in % was defined as follows:

$$\text{WHC (\%)} = \frac{M_1}{M_2} \times 100, \quad (3)$$

where M_1 and M_2 are mass of precipitate (g) and yogurt sample (g), respectively.

2.11. Rheological Properties' Analysis. The change in viscosity of yogurt samples during storage was measured by HAAKE RS6000 rheometer (Thermo Fisher Scientific Inc., MA, USA) at 25°C. In brief, the fixture model is P35TiL and the shear rate range is 0.01–150 s^{-1}.

2.12. Statistical Analysis. Each measurement was conducted in triplicate. The significant differences of results were evaluated by one-way ANOVA of the Tukey method (SPSS 16.0).

3. Results and Discussion

3.1. Basic Components' Analysis of EPS-NS1905E. Carbohydrates and proteins in the purified EPS not only affect the formation of EPS three-position gel network but also the physical properties and chemical activity of EPS [18,19]. In the present study, the composition of the partially purified EPS was determined. The total sugar content in EPS-NS1905E was estimated to be 84.44 ± 0.01%, and a low total protein content (1.35 ± 0.03%) was observed (data not shown). Similar results were reported in previous studies [20].

3.2. Molecular Weight (Mw) of EPS-NS1905E. The average molecular weight (Mw) of partially purified EPS-1905E was 2.658×10^5 Da, which is consistent with related reports that the Mw of EPS produced by *L. plantarum* ranges from 10^5 to 10^6 Da (data not shown). The Mw of EPS-1905E was lower than the Mw of EPS produced by *L. plantarum* C70 and *L. plantarum* C88 [10, 21]. The lower Mw of EPS may exhibit better water solubility and relatively extended chain conformation, leading to a better biological activity [9, 21, 22].

3.3. Monosaccharide Composition Analysis of EPS-NS1905E. Sugar analysis of EPS-NS1905E was conducted by HPAEC-PAD and showed that EPS-NS1905E was a heteropolysaccharide (Figure 1), which was composed of glucose, arabinose, rhamnose, mannose, and galactose in a ratio of 58.83 : 17.4 : 6.31 : 5.86 : 2.74, in accordance to the literature in which glucose, mannose, and rhamnose are the most common identified EPS structures from *Lactobacillus* spp. [18, 23, 24]. The EPS containing arabinose were also reported before [25]. However, the composition of EPS-NS1905E was different from the EPS from *L. plantarum* 70810 (glucose) [9], *L. plantarum* WLPL09 (mannose and glucose) [13], and *L. plantarum* C70 (glucose, arabinose, mannose, and galactose) [10]. The diversity of the monosaccharide composition is related to bacterial species' specificity, culture conditions, and medium composition.

3.4. Structure Analysis of EPS-NS1905E. The FTIR spectrum of EPS-NS1905E was distinguished in the region between 400 cm^{-1} and 4000 cm^{-1} (Figure 2). The absorption at 3391.80 cm^{-1} was assigned to the O-H stretching vibration of the hydroxyl group [26]. The peak at 2941.53 cm^{-1} could be attributed to C-H groups [27]. The absorption at 1649.59 cm^{-1} may correspond to the vibration of C=O bonds [28, 29].

The region below 1500 cm^{-1} corresponding to the fingerprint region was known as the polysaccharide [18]. The absorption peak at 1449.92 cm^{-1} was attributable to the stretching vibration of the C=O bond. The peaks between 1403.30 and 1242.74 cm^{-1} may correspond to C-O stretch groups, C-H bond, and S=O stretching vibrations [28]. The bands observed within 900–1150 cm^{-1} may correspond to the vibration of the C-O-C bond [30]. The peaks at 1000–1200 cm^{-1} were assigned to the pyranose ring [9], which should be further confirmed by the NMR analysis.

3.5. Rheological Characterization of EPS-NS1905E. The rheological properties of the EPS-NS1905E were investigated in different salt solutions (KCl, NaCl, $MgCl_2$, and $CaCl_2$), pH (2, 4, 7, 10, and 12), and temperatures (−20, 25, and 90°C). Along with the increasing shear rates, a decreased viscosity of EPS-NS1905E solutions was observed, which indicated that the EPS-NS1905E had a shear thinning behavior.

The EPS-NS1905E in different salt solutions exhibited less viscosity than in water (Figure 3(a)). The EPS-NS1905E in the NaCl solution has shown more viscosity than in other salt solutions over the lower shear rate range. This result may be related to the binding strength of salt ions on negative EPS sites. The salt resistance of EPS is a benefit of its application in the dairy industry [31]. A higher viscosity of the EPS-NS1905E solution at acidic pH (4) was observed (Figure 3(b)), which would be beneficial for application of this EPS to improve the texture of the yogurt. Similarity, Wang et al. [23] observed that the EPS from *L. plantarum* YW11 exhibited higher viscosity at acidic pH (4 and 6). The rheological characteristics of EPS-NS1905E at different temperatures is shown in Figure 3(c); a stable rheological property of EPS-NS1905E was observed with the increasing temperature from −20 to 90°C. Wang et al. [23] and

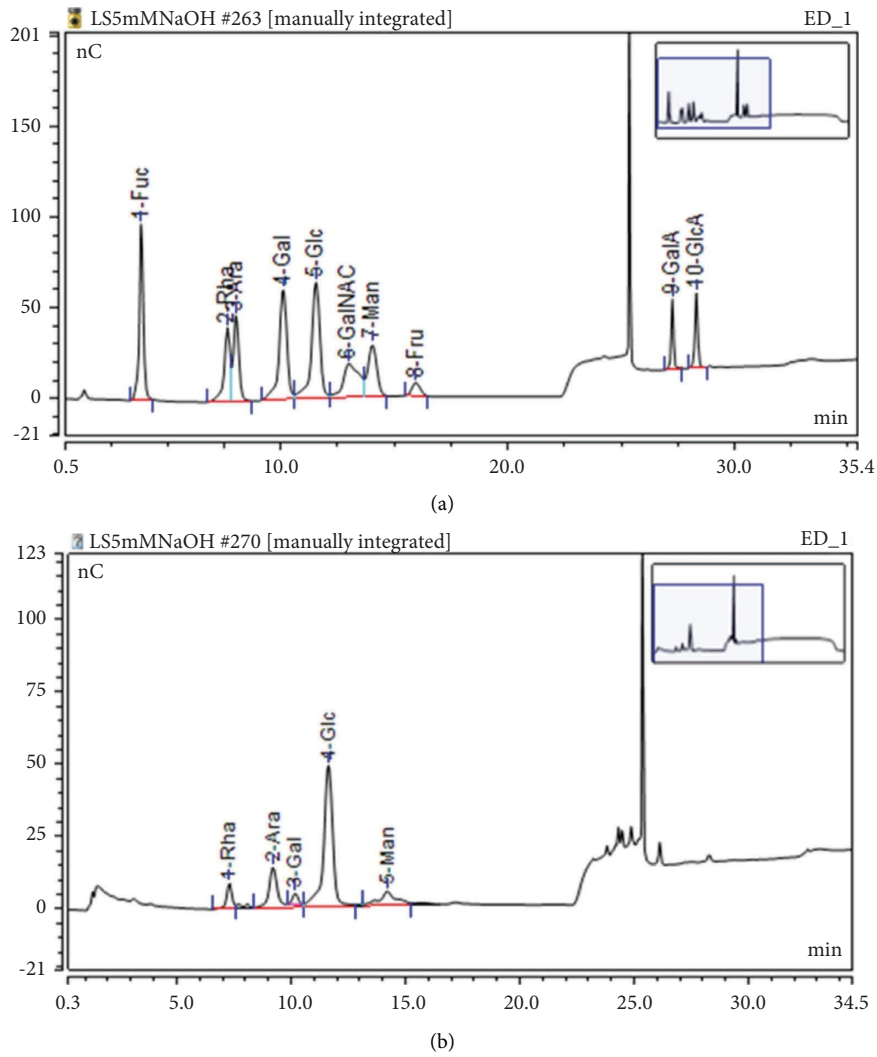

Figure 1: HPAEC-PAD profiles of standard monosaccharides (a) and partially purified EPS-NS1905E (b).

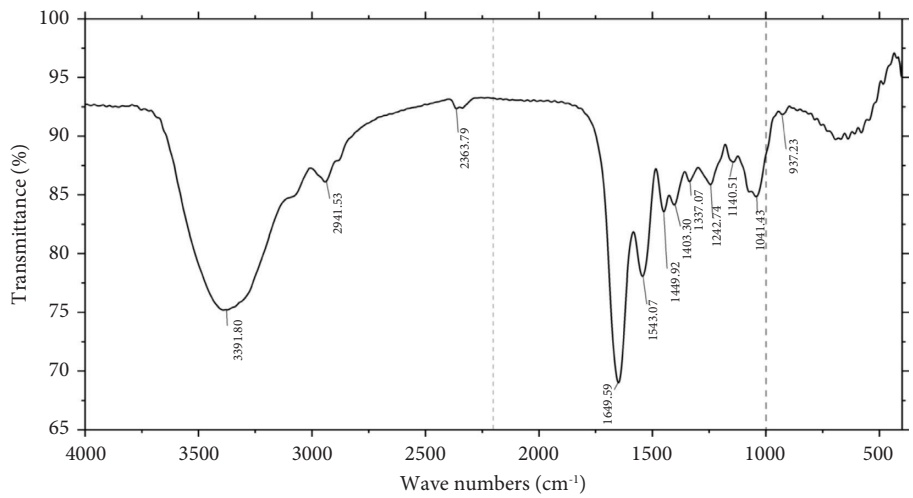

Figure 2: FTIR spectra of EPS-NS1905E fractions.

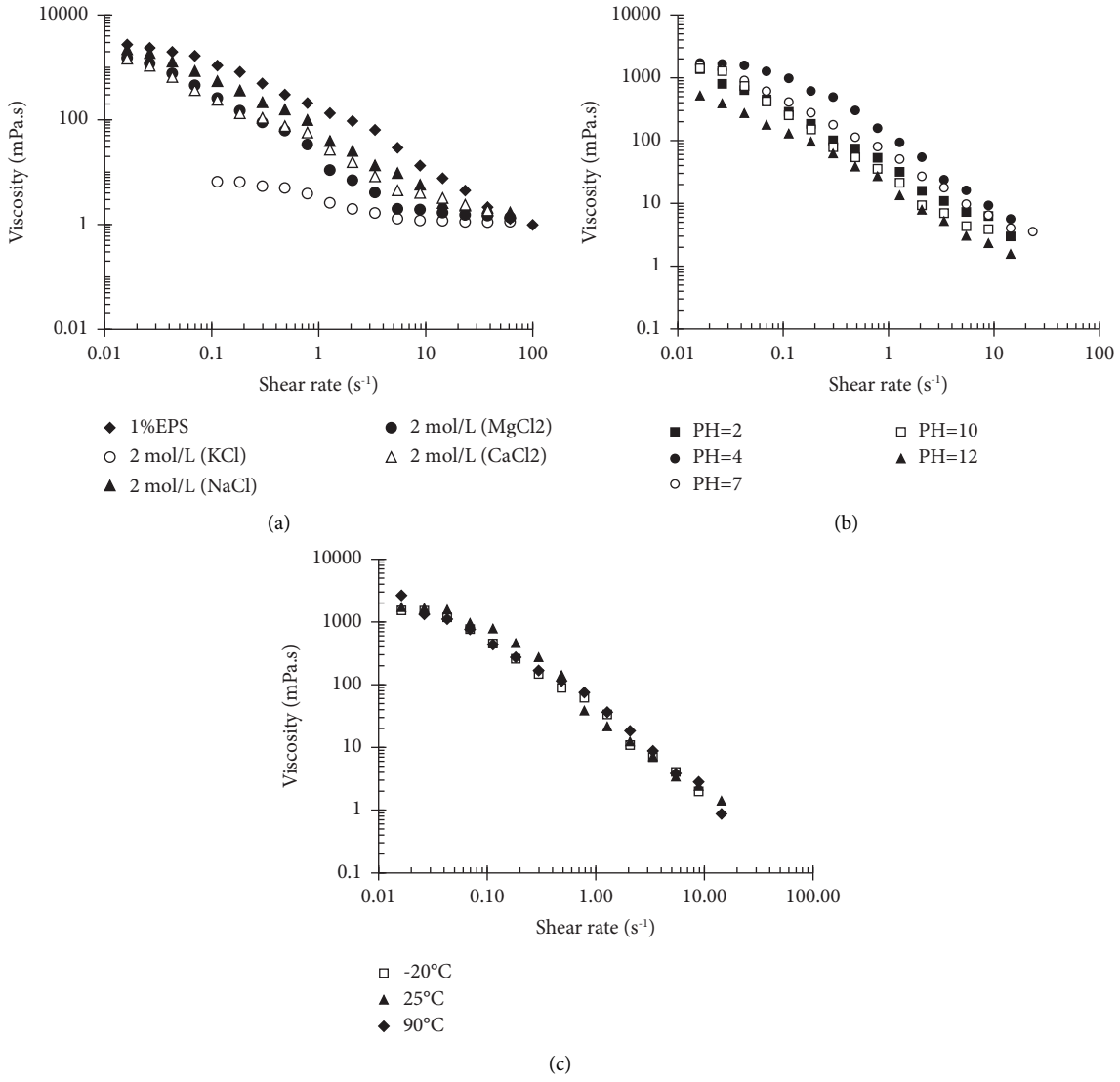

Figure 3: Rheological characteristics of EPS-NS1905E in different salt solutions (a), at different pH values (b), and at different temperature (c).

Kanmani et al. [32] stated that the EPS exhibited decreased viscosity with increasing temperatures. The thermal properties of EPS-NS1905E make it suitable as a potential additive used in food processing. These differences in rheological properties of EPS under different treatment conditions may be caused by changes in Mw, type of glycosidic bond, monosaccharide composition, functional groups, and substitutions [10, 33].

3.6. Biological Activity of EPS. The scavenging ability of EPS-NS1905E on DPPH free radicals was measured, and the result is shown in Figure 4(a). A strong scavenging activity of EPS-NS1905E towards DPPH free radicals was observed. At a concentration of 1% (m/m), EPS-NS1905E showed 96% of DPPH radical scavenging activity. Wang et al. [23] reported that a lower DPPH elimination activity (30%) of EPS is produced by *L. plantarum* YW32. The antioxidant activity of EPS may depend on its molecular weight, monosaccharide composition, and the purification methods used [34].

The antitumor activities of EPS-NS1905E against Caco-2 cells were investigated, and the results showed that the exhibited inhibition activities were observed in a concentration-dependent manner (Figure 4(b)). The inhibition ratio of EPS-NS1905E (1%, m/m) against Caco-2 cells was 21%. Wang et al. [9] reported that the inhibition ratios of r-EPS1 and r-EPS2 (600 μg/mL) produced by *L. plantarum* 70810 against Caco-2 were 25.94% and 35.04%, respectively. The differences in the antitumor activities of these EPS might result from molecular weight, monosaccharide composition, and specific structure [35, 36].

3.7. Effects of EPS-NS1905E on Storage of Yogurt. The changes of pH and acidity of EPS-NS1905E fortified and control yogurt samples were investigated. As shown in Figure 5, as predicted, the titratable acidity was negatively correlated with pH values. The skim milk fortified with 0.1% (m/m) EPS-NS1905E showed the lowest pH reduction and shorter fermentation time during fermentation (data not shown).

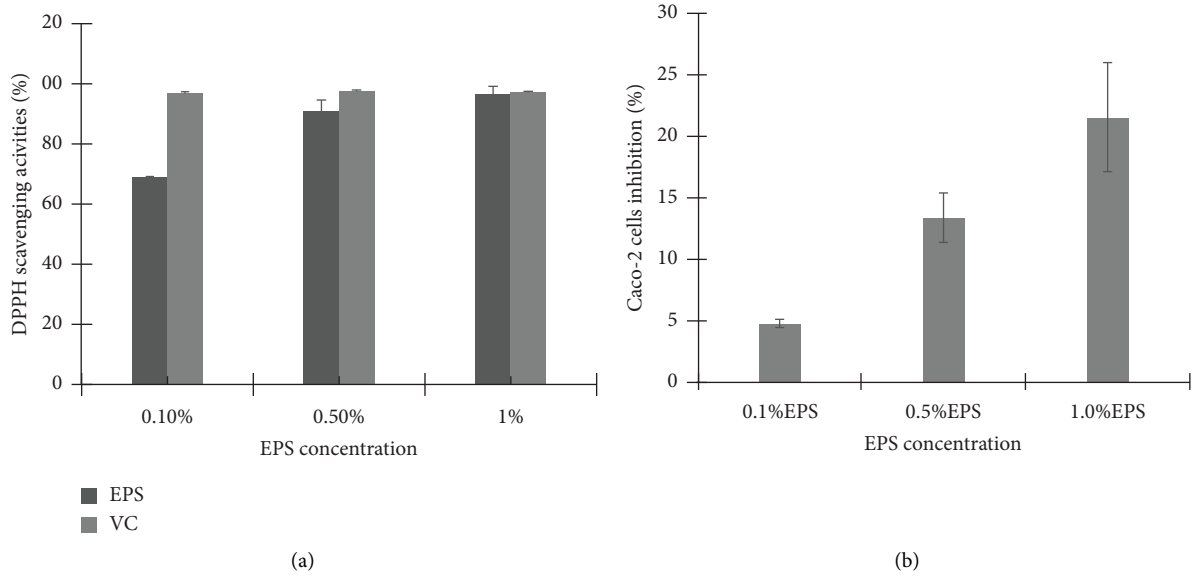

Figure 4: Biological activity of EPS-NS1905E: (a) DPPH radical scavenging activity and (b) antitumor activities against Caco-2 cells.

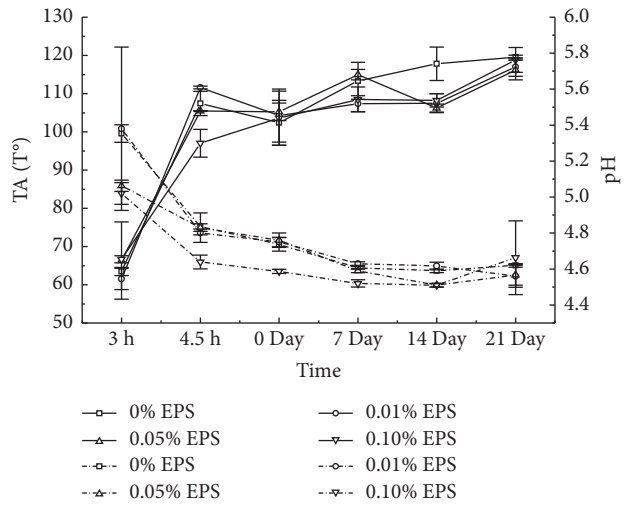

Figure 5: Changes in pH and acidity of set-type yogurt with and without EPS-NS1905E during cold storage.

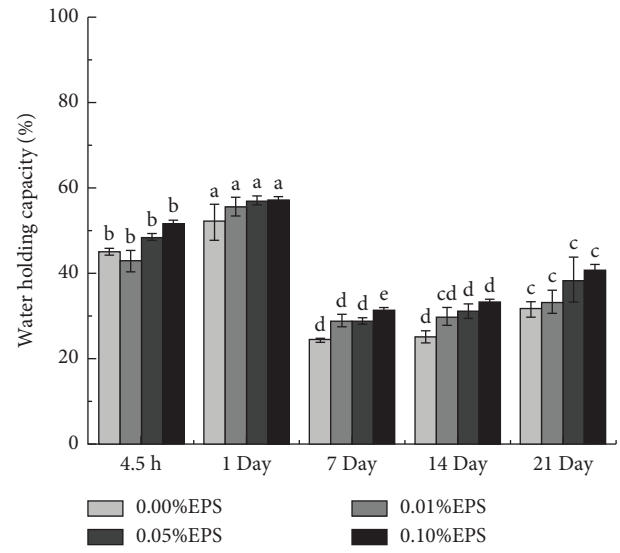

Figure 6: Changes in water-holding capacity of yogurt with EPS-NS1905E during cold storage.

The EPS-NS1905E fortified yogurt exhibited lightly higher acidity than the non-EPS yogurt result from the fact that the added EPS acts as an additional carbon source in the lactic acid fermentation [37]. The EPS-NS1905E fortified and control yogurt samples showed a significant decrease in pH during the storage and was in accordance with the results reported by Prasanna et al. [38].

3.8. Effects of EPS-NS1905E on Yogurt Water-Holding Capacity. The water-holding capacity (WHC) of yogurts fortified with EPS-NS1905E during storage was studied, and results are shown in Figure 6. During the first 7 days of storage, the WHC of yogurt decreased significantly. The WHC of yogurts fortified with EPS-NS1905E were increased in a concentration-dependent manner, and the yogurt with 0.1% EPS-NS1905E showed a significant ($p \leq 0.05$) increase. There was a consensus that EPS could improve WHC of the yogurt by interacting with biological macromolecule of milk, such as proteins and micelles [37, 39]. However, no significant difference ($p > 0.05$) of WHC was found between EPS-NS1905E fortified and control yogurt samples along with the storage.

3.9. Effects of EPS-NS1905E on Yogurt Viscosity during Storage. The viscosity of all yogurt samples during storage is shown in Figure 7. At the beginning of storage (day 1), the yogurt with 0.1% EPS-NS1905E has shown more viscosity

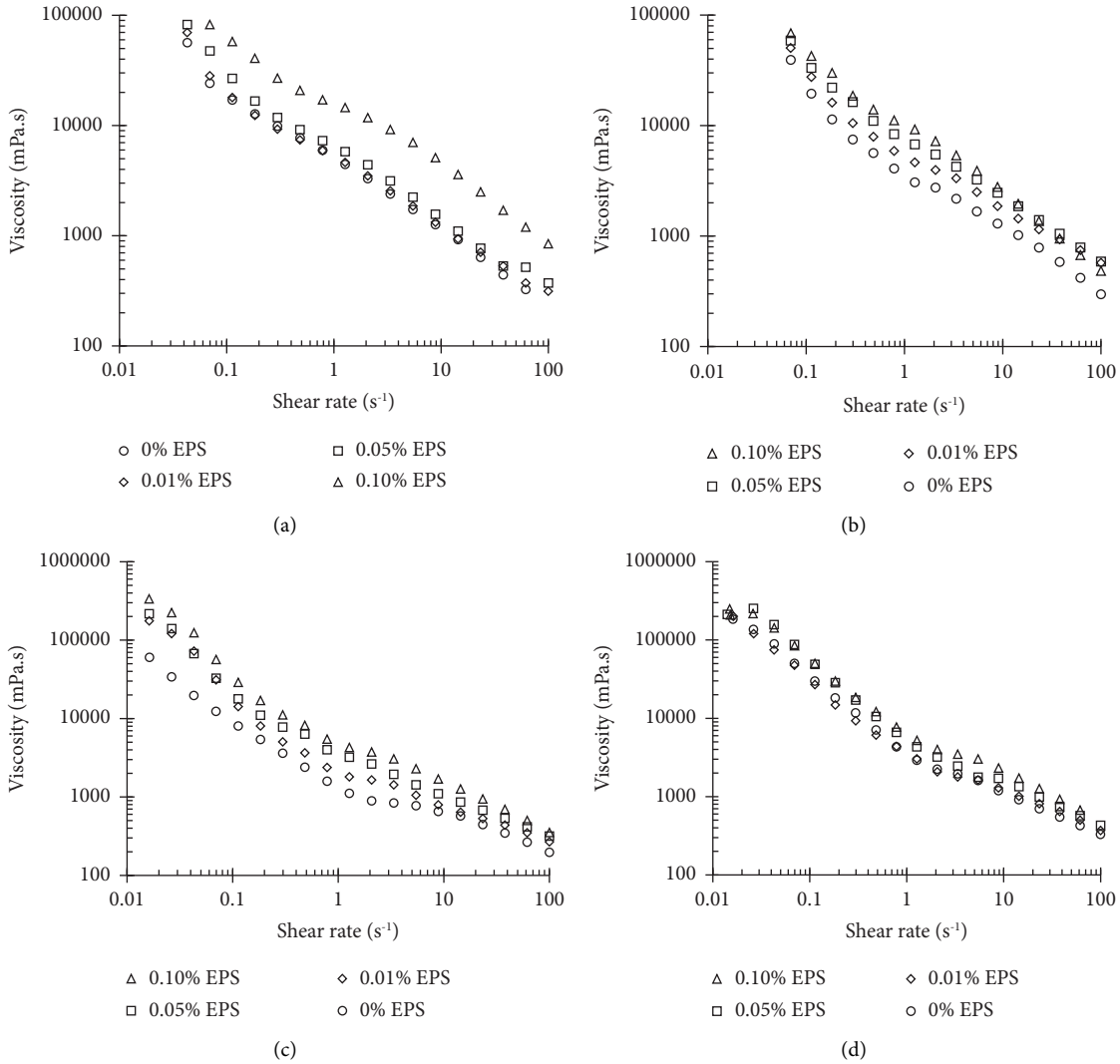

Figure 7: Changes in apparent viscosity of yogurt containing different levels of EPS-NS1905E during cold storage. Samples were taken at day 1 (a), day 7 (b), day 14 (c), and day 21 (d) of storage.

over lower shear rate range, and no obvious difference in viscosity was observed in yogurts with 0.01% and 0.05% (m/m) EPS-NS1905E and control samples (Figure 7(a)). The viscosity of the yogurt-added EPS increased gradually in the next two weeks of storage in a concentration-dependent manner (Figures 7(b) and 7(c)). Similar results were reported by Ayala-Hernández et al. [40] and Zhang et al. [41]. EPS can improve the structure of yogurt by binding with the serum phase of yogurt [37].

4. Conclusions

In this study, the EPS produced by *L. plantarum* NS1905E had shown to consist of glucose, arabinose, rhamnose, mannose, and galactose in an approximate molar ratio of 58.83 : 17.4 : 6.31 : 5.86 : 2.74 and had a molecular weight of 2.658×10^5 Da. The EPS was thermally stable and had a higher viscosity in acidic pH. Furthermore, the EPS had good DPPH scavenging antioxidant activity. The addition of EPS in skim milk shortened the fermentation time and improved the WHC and viscosity of yogurt during storage. Therefore, the EPS produced by *L. plantarum* NS1905E could be exploited as a food additive for application in functional foods.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Wenping Lei, Qi Chen, and Yan Liu are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Wenping Lei, Qi Chen, and Yan Liu contributed equally to the work.

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References

[1] L. De Vuyst, F. De Vin, F. Vaningelgem, and B. Degeest, "Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria," *International Dairy Journal*, vol. 11, no. 9, pp. 687–707, 2001.

[2] L. Jolly, S. J. F. Vincent, P. Duboc, and J. R. Neeser, "Exploiting expolysaccharides from lactic acid bacteria," *Antonie Van Leeuwenhoek*, vol. 82, no. 1-4, pp. 367–374, 2002.

[3] I. W. Sutherland, "Polysaccharases for microbial exopolysaccharides," *Carbohydrate Polymers*, vol. 38, no. 4, pp. 319–328, 1999.

[4] Y. P. Wang, Z. Ahmed, W. Feng, C. Li, and S. Y. Song, "Physicochemical properties of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir," *International Journal of Biological Macromolecules*, vol. 43, no. 3, pp. 283–288, 2008.

[5] L. Axelsson, "Lactic acid bacteria: classification and physiology," in *Lactic Acid Bacteria Microbiological and Functional Aspects*, S. Salminen, A. V. Wright, and A. Ouwehand, Eds., pp. 1–66, Marcel Dekker, New York, NY, USA, 2004.

[6] H. Charchoghlyan and H. Park, "Characteristics of a novel bacterial polysaccharide consisted of glucose and mannose as major components," *Food Hydrocolloids*, vol. 30, no. 2, pp. 512–518, 2013.

[7] S. Chiang and T. Pan, "Beneficial effects of Lactobacillus paracasei subsp. paracasei NTU 101 and its fermented products," *Applied Microbiology and Biotechnology*, vol. 93, no. 3, pp. 903–916, 2012.

[8] R. Coda, A. Lanera, A. Trani, M. Gobbetti, and R. Di Cagno, "Yogurt-like beverages made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties," *International Journal of Food Microbiology*, vol. 155, no. 3, pp. 120–127, 2012.

[9] K. Wang, W. Li, X. Rui, X. Chen, M. Jiang, and M. Dong, "Structural characterization and bioactivity of released exopolysaccharides from Lactobacillus plantarum 70810," *International Journal of Biological Macromolecules*, vol. 67, pp. 71–78, 2014.

[10] M. Ayyash, B. Abu-Jdayil, P. Itsaranuwat et al., "Characterization, bioactivities, and rheological properties of exopolysaccharide produced by novel probiotic Lactobacillus plantarum C70 isolated from camel milk," *International Journal of Biological Macromolecules*, vol. 144, pp. 938–946, 2020.

[11] Z. Liu, L. Dong, K. Jia et al., "Sulfonation of Lactobacillus plantarum WLPL04 exopolysaccharide amplifies its antioxidant activities in vitro and in a Caco-2 cell model," *Journal of Dairy Science*, vol. 102, no. 7, pp. 5922–5932, 2019.

[12] T. Liu, K. Zhou, S. Yin et al., "Purification and characterization of an exopolysaccharide produced by Lactobacillus plantarum HY isolated from home-made Sichuan Pickle," *International Journal of Biological Macromolecules*, vol. 134, pp. 516–526, 2019.

[13] B. Jiang, L. Tian, X. Huang et al., "Characterization and antitumor activity of novel exopolysaccharide APS of Lactobacillus plantarum WLPL09 from human breast milk," *International Journal of Biological Macromolecules*, vol. 163, pp. 985–995, 2020.

[14] Q. Chen, W. Lei, Q. Xiao et al., "Optimization of fermentation conditions for exopolysaccharide production by Lactobacillus plantarum XN1904E," *Journal of dairy science and technology*, vol. 43, no. 3, pp. 1–5, 2020.

[15] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350–356, 1956.

[16] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[17] M. Zhao, N. Cui, F. Qu et al., "Novel nano-particulated exopolysaccharide produced by Klebsiella sp. PHRC1.001," *Carbohydrate Polymers*, vol. 171, pp. 252–258, 2017.

[18] V. B. Bomfim, J. H. Pereira Lopes Neto, K. S. Leite et al., "Partial characterization and antioxidant activity of exopolysaccharides produced by Lactobacillus plantarum CNPC003," *LWT--Food Science and Technology*, vol. 127, Article ID 109349, 2020.

[19] Y. Tian, L. Zheng, and D. Sun, "Functions and behaviors of activated sludge extracellular polymeric substances (EPS): a promising environmental interest," *Journal of Environmental Sciences*, vol. 18, no. 3, pp. 420–427, 2006.

[20] A. T. Adesulu-Dahunsi, A. I. Sanni, K. Jeyaram, J. O. Ojediran, and A. O. Ogunsakin, "Extracellular polysaccharide from Weissella confusa OF126: production, optimization, and characterization," *International Journal of Biological Macromolecules*, vol. 111, pp. 514–525, 2018.

[21] L. Zhang, C. Liu, D. Li et al., "Antioxidant activity of an exopolysaccharide isolated from Lactobacillus plantarum C88," *International Journal of Biological Macromolecules*, vol. 54, pp. 270–275, 2013.

[22] C. Marieta, I. Ibarburu, M. Dueñas, and A. Irastorza, "Supramolecular structure and conformation of a (1 ⟶ 3) (1 ⟶ 2)-β-d-glucan from Lactobacillus suebicus CUPV221 as observed by tapping mode atomic force microscopy," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 14, pp. 6183–6188, 2009.

[23] J. Wang, X. Zhao, Y. Yang, A. Zhao, and Z. Yang, "Characterization and bioactivities of an exopolysaccharide produced by Lactobacillus plantarum YW32," *International Journal of Biological Macromolecules*, vol. 74, pp. 119–126, 2015.

[24] B. Jiang, L. Wang, M. Zhu et al., "Separation, structural characteristics and biological activity of lactic acid bacteria exopolysaccharides separated by aqueous two-phase system," *LWT--Food Science and Technology*, vol. 147, Article ID 111617, 2021.

[25] Y. Zhu, X. Wang, W. Pan et al., "Exopolysaccharides produced by yogurt-texture improving Lactobacillus plantarum RS20D and the immunoregulatory activity," *International Journal of Biological Macromolecules*, vol. 121, pp. 342–349, 2019.

[26] Y. Wang, C. Li, P. Liu, Z. Ahmed, P. Xiao, and X. Bai, "Physical characterization of exopolysaccharide produced by Lactobacillus plantarum KF5 isolated from Tibet Kefir," *Carbohydrate Polymers*, vol. 82, no. 3, pp. 895–903, 2010.

[27] M. R. S. Melo, J. P. A. Feitosa, A. L. P. Freitas, and R. C. M. de Paula, "Isolation and characterization of soluble sulfated polysaccharide from the red seaweed Gracilaria cornea," *Carbohydrate Polymers*, vol. 49, no. 4, pp. 491–498, 2002.

[28] K. Zhou, Y. Zeng, M. Yang et al., "Production, purification and structural study of an exopolysaccharide from Lactobacillus plantarum BC-25," *Carbohydrate Polymers*, vol. 144, pp. 205–214, 2016.

[29] X. Ji, J. Guo, D. Ding et al., "Structural characterization and antioxidant activity of a novel high-molecular-weight polysaccharide from Ziziphus Jujuba cv. Muzao," *Journal of Food Measurement and Characterization*, vol. 16, no. 3, pp. 2191–2200, 2022.

[30] Y. Shuhong, Z. Meiping, Y. Hong et al., "Biosorption of Cu2+, Pb2+, and Cr6+ by a novel exopolysaccharide from Arthrobacter ps-5," *Carbohydrate Polymers*, vol. 101, pp. 50–56, 2014.

[31] M. Han, C. Du, Z. Y. Xu, H. Qian, and W. G. Zhang, "Rheological properties of phosphorylated exopolysaccharide produced by Sporidiobolus pararoseus JD-2," *International Journal of Biological Macromolecules*, vol. 88, pp. 603–613, 2016.

[32] P. Kanmani, R. Satish kumar, N. Yuvaraj, K. Paari, and V. Pattukumar, "Production and purification of a novel exopolysaccharide from lactic acid bacterium Streptococcus phocae PI80 and its functional characteristics activity in vitro," *Bioresource Technology*, vol. 102, no. 7, pp. 4827–4833, 2011.

[33] M. Ayyash, B. Abu-Jdayil, A. Olaimat et al., "Physicochemical, bioactive and rheological properties of an exopolysaccharide produced by a probiotic Pediococcus pentosaceus M41," *Carbohydrate Polymers*, vol. 229, Article ID 115462, 2020.

[34] J. Cao, H. Zhang, and C. Xu, "Culture characterization of exopolysaccharides with antioxidant activity produced by Pycnoporus sanguineus in stirred-tank and airlift reactors," *Journal of the Taiwan Institute of Chemical Engineers*, vol. 45, no. 5, pp. 2075–2080, 2014.

[35] Z. Wu, J. Lu, X. Wang et al., "Optimization for production of exopolysaccharides with antitumor activity in vitro from Paecilomyces hepiali," *Carbohydrate Polymers*, vol. 99, pp. 226–234, 2014.

[36] X. Ji, Y. Cheng, J. Tian, S. Zhang, Y. Jing, and M. Shi, "Structural characterization of polysaccharide from jujube (Ziziphus jujuba Mill.) fruit," *Chemical and Biological Technologies in Agriculture*, vol. 8, no. 1, p. 54, 2021.

[37] S. Tiwari, D. Kavitake, P. B. Devi, and P. Halady Shetty, "Bacterial exopolysaccharides for improvement of technological, functional and rheological properties of yoghurt," *International Journal of Biological Macromolecules*, vol. 183, pp. 1585–1595, 2021.

[38] P. H. P. Prasanna, A. S. Grandison, and D. Charalampopoulos, "Microbiological, chemical and rheological properties of low fat set yoghurt produced with exopolysaccharide (EPS) producing Bifidobacterium strains," *Food Research International*, vol. 51, no. 1, pp. 15–22, 2013.

[39] A. N. Hassan, "ADSA foundation scholar award: possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods," *Journal of Dairy Science*, vol. 91, no. 4, pp. 1282–1298, 2008.

[40] I. Ayala-Hernández, A. N. Hassan, H. D. Goff, and M. Corredig, "Effect of protein supplementation on the rheological characteristics of milk permeates fermented with exopolysaccharide-producing Lactococcus lactis subsp. cremoris," *Food Hydrocolloids*, vol. 23, no. 5, pp. 1299–1304, 2009.

[41] T. Zhang, Z. Zhang, H. Yan, D. Li, Z. Yang, and M. Guo, "Effects of stabilizers and exopolysaccharides on physiochemical properties of fermented skim milk by Streptococcus thermophilus ST1," *African Journal of Biotechnology*, vol. 11, pp. 6123–6128, 2012.