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Structure characterization and antioxidant activities of exopolysaccharide from soybean whey fermented by *Lacticaseibacillus plantarum* 70810

Juanjuan Tian[1], Qingyan Mao[1], Mingsheng Dong[1], Xiaomeng Wang[1], Xin Rui[1], Qiuqin Zhang[1], Xiaohong Chen[1], Wei Li[1]*

[1] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, P.R. China.; 2018208012@njau.edu.cn (J. T.); qingyanmao@njau.edu.cn (Q. M.); dongms@njau.edu.cn (M. D.); 2019208028@njau.edu.cn(X. W.); ruix@njau.edu.cn (X. R.); zqq@njau.edu.cn(Q. Z.); xhchen@njau.edu.cn(X. C.)

* Correspondence: lw1981@njau.edu; Tel.: +86 25 84396989

Abstract: Soybean whey is high-yield but low-utilization agricultural by products in China. In this study, soybean whey was used as substrates of fermentation by *Lacticaseibacillus plantarum* 70810 strains. An exopolysaccharide (LPEPS-1) was isolated from soybean whey fermentation by *L. plantarum* 70810 and purified with an ion-exchange chromatography. Its preliminary structural characteristic and antioxidant activities were investigated. Results showed that LPEPS-1 was composed of mannose, glucose and galactose with molar ratios of 1.49: 1.67: 1.00. The chemical structure of LPEPS-1 was consisted of →4)-α-D-Glc*p*-(1→, →3)-α-D-Gal*p*-(1→ and →2)-α-D-Man*p*-(1→. Scanning electron microscopy (SEM) revealed that LPEPS-1 had relatively rough surface. In addition, LPPES-1 exhibited strong scavenging activity against DPPH, superoxide radicals and chelating ability on ferrous ion. This study demonstrated that soybean whey was feasible fermentation substrates for the production of polysaccharide from *L. plantarum* 70810, and the polysaccharide could be used as a promising ingredient for health-beneficial functional foods.

Keywords: *Lacticaseibacillus plantarum* 70810; fermentation; soybean whey; polysaccharide; structure characterization; antioxidant activity

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1. Introduction

Lacticaseibacillus are the largest genus of Lactic acid bacteria (LAB) and more than 50 different species of LAB belong to the genus *Lacticaseibacillus*. The *Lacticaseibacillus* mainly exist in the human gut and traditional fermented foods [1]. People have isolated different *Lacticaseibacillus plantarum* from olives, pickles, fermented cucumbers and fermented dairy products [2-4]. Studies have shown that *L. plantarum* are safe and can produce a variety of secondary metabolites that were beneficial to human health such as lactic acid, acetic acid, exopolysaccharide, specific proteases and bacteriocins by fermentation. Polysaccharide is biopolymer and it is widely distributed in all animals, plants, fungi and bacteria. Bacterial polysaccharides, especially those exopolysaccharide (EPS) produced by LAB have been widely used in the food industry [5,6]. EPS from LAB has been reported to exhibit several biological activities such as immunomodulatory, antioxidant and antitumor activities [7-9]. It has been reported that the activities of EPS were related to their structural characteristics such as monosaccharide compositions, molecular weight, types of glycosidic linkages as well as their chain conformation. Hence, research on the relationship between structure and activity on polysaccharide has become a hot topic.

Soybean products is a traditional food in china. By-product soybean whey would be generated from the preparation of soybean products [10]. For a long time, soybean whey has been considered as a waste product by the food industry and its random discharge causes an environmental and industrial problem [11]. In fact, soybean whey contains a

rich source of proteins, carbohydrates, oligosaccharides and some trace elements (calcium, iron, magnesium, etc.), and it should not be classified as waste [12,13]. Nowadays, some experts are developing new ideas on how to turn soybean whey into treasure. Researchers found that soybean whey was rich in nutrients and it could be used as a substitute for microbial culture medium. Marazza et al [14] used *L. rhamnosus* to ferment soybean whey, which can increase the content of isoflavone aglycone and improve the antioxidant capacity. Others have reported that soybean whey could be converted into soybean alcoholic beverage by fermentation of some commercial strains [15]. Microbial fermentation is a useful biotechnological strategy, which can turn soybean whey into bioactive ingredients. At present, ~~the~~ *L. plantarumn* 70810 was isolated from traditional Chinese Sichuan pickles by our laboratory. In our previous study, polysaccharide from *L. plantarumn* 70810 fermentation by a synthetic liquid medium has been studied, and the polysaccharide exhibits potent antioxidant and antitumor activities [16, 17]. However, polysaccharide from *L. plantarumn* 70810 fermented soybean whey has not attracted attention. It was reported that the production of microbial EPS was affected by the composition of the culture medium, growth conditions, the age of the strain and the amount of inoculation [18]. Among them, the composition of the medium, especially the carbon source and nitrogen source, has a greater impact on the growth of microbial and the production of EPS [18]. Therefore, it was meaningful to study the production of polysaccharide by *L. plantarum* 70810 on the soybean whey medium.

Therefore, in this study, the EPS from soybean whey fermented by *L. plantarum* 70810 was prepared and characterized by ultraviolet (UV) spectrophotometer, fourier transform infrared (FI-IR) spectrophotometer, gas chromatography (GC), scanning electron microscope (SEM) and nuclear magnetic resonance (NMR). In addition, the *in vitro* antioxidant activities of EPS against DPPH, superoxide anion and chelating activity on ferrous ion were evaluated.

2. Materials and Methods

2.1 Materials and chemicals

The strain *L. plantarum* 70810 was isolated from traditional Chinese Sichuan pickles by our laboratory [19]. The strain was activated by the De Man Rogosa Sharpe (MRS) liquid medium, which (1 L) contained 20 g of glucose, 10 g of beef cream, 10 g of peptone, 5 g of anhydrous sodium acetate, 5 g of yeast extract, 2.62 g of potassium dipotassium phosphate, 2 g of triammonium citrate, 0.58 g of magnesium sulfate heptahydrate, 0.198 g of manganese sulfate and 1.0 mL of tween 80. The soybean whey was obtained from Nanjing Fruit and Fruit Food Co., Ltd. The soybean whey contained a total solid content of 18.2 ± 1.3 g/L, including 4.5 g/L proteins, 8.5 ± 0.4 g/L carbohydrates (predominantly stachyose and sucrose) and 3.4 ± 0.3 g/L total ashes.

DEAE-cellulose-52 was purchased from Waterman Co. Ltd. (Springfield Mill, UK). Dialysis membrane with a molecular weight of 8,000-10,000 Da was purchased from Solarbio Co., Ltd. (Beijing, China). D-galactose, flupromazine, 2,4,6-tripyridazine (TPTZ), nitrotetrazolium chloride (NBT), phenazine methyl sulfate (PMS) and reducing coenzyme I (NADH) were purchased from Sigma Chemical Co., Ltd. (St. Louis, Mo, USA). All other reagents were analytical grade and purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Isolation and purification of EPS

The isolation of EPS referred to the pervious method [20]. After fermentation at 37℃ for 24 h in the soybean whey, the fermentation supernatant was obtained by centrifugation (4℃, 12,000 rpm, 10 min). 80% (w/v) TCA was added to the supernatant, resulting in a final concentration of 4% TCA in the supernatant. This step was to remove the protein from the supernatant. Then, the solution was centrifuged, concentrated and precipitated with 95% ethanol at 4 ℃ for 12 h. The precipitate was dissolved in the deionized water

and dialyzed for 3 days in the running water. The crude EPS was obtained after lyophilization.

The crude EPS was further separated and purified by DEAE-52 cellulose column (2.6 × 30 cm). The polysaccharide solution was eluted with deionized water, 0.1 and 0.3 M NaCl at a flow rate of 1.0 mL/min. 10 mL of elution was collected in each tube and the total sugar content was measured by phenol-sulfuric acid method [20]. The collected two fractions were named LPEPS-1and LPEPS-2.

2.3 Analysis of monosaccharide composition

Monosaccharide composition was detected by GC according to our previous method [21]. Firstly, the polysaccharide was hydrolyzed with trifluoroacetic acid(TFA). 5 mg of sample was hydrolyzed with 2 mL TFA (2 M) at oil bath (120℃) for 2 h. The hydrolyzed solution was evaporated to dryness under reduced pressure. Then, the hydrolysates were derivatized with aldononitrile acetate. GC analysis was on an Agilent 6890N GC equipped with a flame ionization detector (FID). An HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm) was used. The column temperature program was as follows: the initial temperature was 120 °C and maintained for 3 min, and then the temperature was increased to 210 °C for 4 min at a rate of 15 °C /min. The temperature of the injector and detector were 250 °C and 280 °C, respectively. The nitrogen flow rate was 1 mL/min. The composition and molar ratio of monosaccharides in the sample were determined by comparing the retention time and chromatographic peak area of the sample and the standard sample

2.4 UV and FT-IR spectra analysis

1.0 mg/mL sample was prepared for UV (U-4100, Hitachi Ltd., Japan) measurement in a wavelength range of 190-500 nm. 1.0 mg of dried sample was mixed with 120.0 mg KBr, and then pressed into pellet for FT-IR spectrum analysis (Bruker Co, Ettlingen, Gremany) from 500 to 4000 cm^{-1}.

2.5 Nuclear magnetic resonance (NMR) analysis

60 mg of the sample was dissolved in 1.0 mL D_2O (99.9% D, Aladdin Co., Ltd. Shanghai, China). The one-dimensional (1D) (^1H ,^{13}C) and two-dimensional (2D) (^1H-^1H COSY, ^1H-^{13}C HSQC) NMR experiments were performed on a Bruker AVANCE AV-500 spectrometer (Bruker Group, Fällanden, Switzerland). The residual solvent (D_2O) signal used as internal standard. The chemical shifts (δ) were described in parts per million (ppm).

2.6 Scanning electron microscopy (SEM) analysis

The surface structural characteristic of sample was observed using an S-3000 scanning electron microscope (FESEM, S-4800, Hitachi, Japan). The dried sample was sputter-coated with gold layer before observation, and then the images were recorded by using the SEM.

2.7 Particle size and zeta-potential

The EPS solution (0.2 mg/mL) was used for zeta-potential and hydrodynamic diameter (Z-average) determination. The experiments were carried out using a Malvern Zeta-size (Malvern Instruments, UK) with the measurement temperature at 25℃.

2.8 Antioxidant activities in vitro

2.8.1 Scavenging activity of DPPH radicals

DPPH free radical scavenging activity was determined by referring to Qiao et al [22] method with slightly modifications. Different concentrations (0.125, 0.25, 0.5, 1, 2 and 4

mg/mL) of LPEPS-1 were prepared. The 0.4 Mm DPPH in ethanol was prepared. 1.0 mL of the polysaccharide solution, 0.2 mL DPPH solution and 1.8 mL deionized water were mixed. Then, it was incubated for 30 min at room temperature. The absorbance at 517 nm was measured by using a spectrophotometer. Ascorbic acid (Vc) was used as positive controls. The scavenging activity on DPPH radical was calculated by the following equation:

$$\text{Scavenging activity (\%)} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \times 100 \quad (1)$$

2.8.2 Scavenging activity of superoxide anion radical

The scavenging activity of superoxide anion radical was carried out by the method described by Qi et al [23]. The reaction mechanism is that PMS reacts with NADH to produce superoxide anions. The generated superoxide anion can react color with NBT. The colored substance has the maximum absorption wavelength at 560 nm. If the polysaccharide solution has the ability to clear superoxide anions, its reaction color will become lighter. The lower the absorbance, the stronger the removal ability of superoxide anions. Briefly, 72 μM NBT, 30 μM PMS and 338 μM NADH prepared with 0.1 M phosphoric acid buffer (pH 7.4). Different concentrations (0.125, 0.25, 0.5, 1, 2 and 4 mg/mL) of LPEPS-1 were prepared. The samples solution was added to NBT, PMS and NADH at ratios of 1: 1: 1: 1, and the mixture solution was incubated for 5 min at room temperature. The absorbance was measured at 560 nm. The scavenging activity on superoxide anion radical was calculated as follows:

$$\text{Scavenging activity on superoxide anion radical (\%)} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}} \times 100 \quad (2)$$

2.8.3 Chelating metal ion capacity

The ability of chelating metal ion was determined by Liu et al [24]. $FeCl_2$ can chelate with Felozine to form Felozine-Fe^{2+}, however, when polysaccharide solution is added, the polysaccharide can chelate with Fe^{2+}, thereby inhibiting Felozine-Fe^{2+} formation. The chelating rate was calculated by measuring the absorbance change before and after adding the polysaccharide by a UV spectrophotometer. Different concentrations (0.125, 0.25, 0.5, 1, 2 and 4 mg/mL) of LPEPS-1 were prepared. 1.0 mL sample, 0.05 mL $FeCl_2$ (2 mM) and 0.2 mL ferrozine (0.2 mM) were mixed together. Total volume was up to 4 mL with distilled water. Then, the mixture solution was shaken evenly and reacted for 10 min at room temperature. Absorbance was measured at 562 nm. The chelating ability on ferrous ion was given bellow:

$$\text{Metal chelating effect (\%)} = A_{\text{blank}} - A_{\text{sample}} / A_{\text{sample}} \times 100 \quad (3)$$

2.9 Statistical analysis

All the experiments were carried out in triplicate. The results were expressed as means ± standard deviation (SD). The experiment data analysis was conducted using SPSS 23.0 software and origin 2018 software.

3. Results and discussion

3.1. Isolation and purification of EPS

The crude EPS was isolated and purified from the soybean whey fermented by *L. plantarum* 70810. In order to obtain the purified EPS fraction, DEAE-52 cellulose column was used to separate and purify polysaccharide. As shown in Figure 1a, two fractions LPEPS-1 and LPEPS-2 were obtained with the yield of 78.7% and 21.3%, respectively.

Considering that the yield of LPEPS-2 was relatively low, so we mainly studied on the structure and antioxidant activities of LPEPS-1.

3.2. UV and FT-IR spectra analysis

The characteristic UV spectrum of LPEPS-1 was observed from 190 nm to 500 nm. Figure 1b implied that LPEPS-1 did not contain nucleic acid and protein, because it was no absorption peaks at 260 nm or 280 nm. In the wavelength range of 4,000-500 cm^{-1}(Figure 1c), LPEPS-1 exhibited typical absorption peaks of polysaccharide. The wide and strong absorption peaks at 3395 cm^{-1} was the stretching vibration of the hydroxyl group (-OH) [25]. The strong absorption peak at 2936cm^{-1} was the stretching vibration of the C-H bond group. The signal strong absorption peak at 1632 cm^{-1} was related to the asymmetric stretching vibration of the C=O bond. The weak absorption peak at 1437 cm^{-1} was the angular vibration of C-H bond [26]. The wave numbers between 1200 and 1000 cm^{-1} were dominated by the glycosidic linkage C-O-H and C–O–C stretching vibration contribution [27].

3.3. Monosaccharide composition of LPEPS-1

The monosaccharide composition analysis result of LPEPS-1 obtained by acid hydrolysis was shown in Figure 1d. Compared the retention time with standard sugars, the results indicated that LPEPS-1 was composed of mannose, glucose and galactose with molar ratios of 1.49: 1.67: 1.00. This monosaccharide composition result was consistent with that reported by Wang et al [16]. Meanwhile, Wang et al [17] cultured *L. plantarum* 70810 in a semi-defined medium and the EPS from *L. plantarum* 70810 was only contained glucose. Ismail et al [28] reported the EPS from *L. plantarum* was composed of glucose and mannose with a molar ratio of approximately 2: 1. These different results indicated that strains, culture conditions and medium composition would affect the production of EPS by *L. plantarum*.

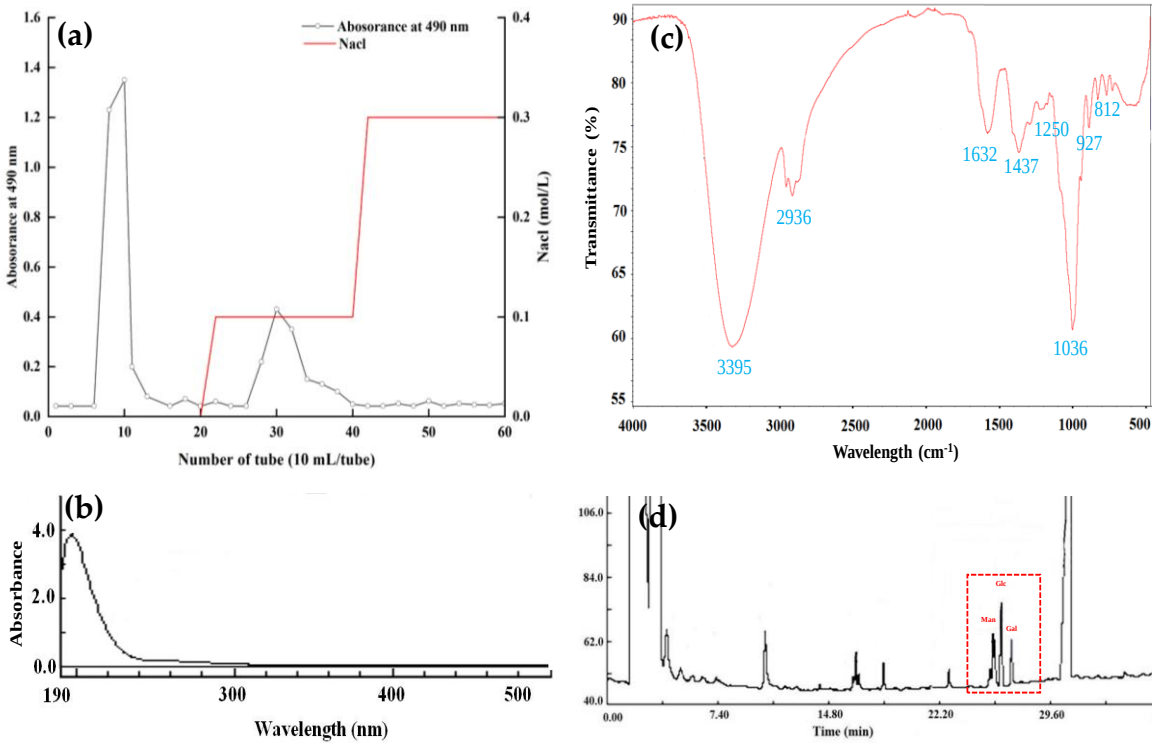

Figure 1. Stepwise elution curve of EPS produced by *L. plantarum* 70810 on DEAE Cellulose-52 chromatography column (a); UV spectrum (b), FT-IR spectrum (c), GC chromatogram of monosaccharide composition of LPEPS-1 (d).

3.4. NMR characterization of EPS

The anomeric region signals of δ 4.5-5.5 ppm were analyzed in the ^{1}H NMR spectrum. Normally, there were three proton signals in this region, which means that three kinds of glycoside bonds were existed. As shown in the Figure 2a, three proton signals occurred at δ 5.37, 5.32, 4.94 ppm and they were labeled as **A**, **B** and **C**, respectively. The ^{13}C spectrum of LPEPS-1 was distributed in the range of δ 60-110 ppm (Figure 2b). The anomeric C-1 signals were detectable at δ 102.57, 102.19 and 101.10 ppm. According to the chemical shift (δ) and coupling constant value (J) of the anomeric proton, it could be judged that **A**, **B** and **C** were all α-configurations. The complete of ^{1}H and ^{13}C for were assigned by 2D COSY and HSQC. From the ^{1}H-^{1}H COSY spectrum (Figure 2d), the chemical shifts of H-2 to H-6 might be determined. These signals cross linked to the proton signals at δ 5.37/3.58, 3.58/3.93, 3.93/3.67, 3.67/3.78 and 3.78/3.82. Taking together with the ^{1}H-^{13}C spectrum of LPEPS-1, the relevance between protons and carbons could be appointed. The signals of C-1 at δ102.57, 102.19 and 101.10 ppm might be assigned to **B**, **A** and **C**, respectively. The C2-C6 resonance signals of residue **A** were assigned to δ 73.88, 75.13, 79.52, 73.88 and 63.11 ppm, respectively. The C-4 signal of residue **A** had moved downfield to 79.52 ppm, which indicated that C-4 of residue **A** was substituted [29]. In addition, DEPT-135 spectrum also showed chemical shifts with ^{13}C spectrum. However, DEPT-135 spectrum exhibited positive CH_3 and CH signals and negative CH_2 signals. In the Figure 2c, negative peaks in DEPT-135 showed a signal CH_2 at δ 63.19 ppm, which was attributed to the C-6 of unsubstituted carbon of **A**, **B** and **C** residues. Combining with the monosaccharide composition, NMR analysis and literature data, residue **A** was identified as →4)-α-Glc$p$ (1→ [30, 31]. In the same way, residues **B** and **C** were identified as →2)-α-Man$p$ (1→ and →3)-α-Gal$p$ (1→, respectively [32-35]. The ^{1}H NMR and ^{13}C NMR chemical shifts of residues **A**- **C** were summarized in Table 1.

Table 1. Chemical shifts (ppm) of ^{1}H and ^{13}C signals for the LPEPS-1 recorded in D_2O at 313 K

Residues	Sugar linkages	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-6
A	→ 4)-α-Glc$p$ (1→	5.37 102.19	3.58 73.88	3.93 75.13	3.67 79.52	3.78 73.88	3.82 63.11
B	→ 2)-α-Man$p$ (1→	5.32 102.57	3.60 79.24	3.90 70.88	3.69 71.94	3.89 69.53	3.74 62.40
C	→ 3)-α-Gal$p$ (1→	4.94 101.10	3.58 74.38	3.97 76.10	4.00 72.37	3.72 75.50	3.80 63.16

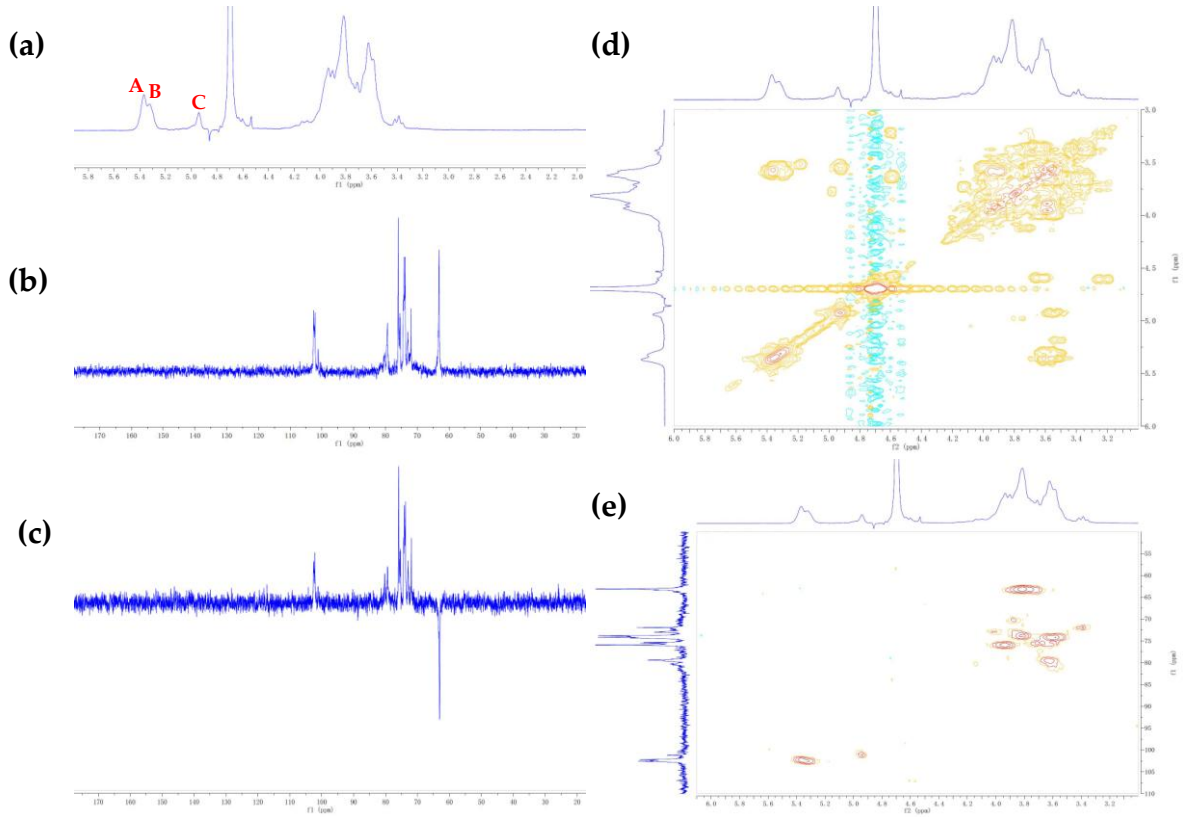

Figure 2. NMR spectra of LPEPS-1. ^{1}H NMR (a), ^{13}C NMR (b), DEPT-135 ^{13}C NMR (c), ^{1}H-^{1}H COSY (d) and ^{1}H-^{13}C HSQC of LPEPS-1 (e).

3.5. SEM analysis

SEM is an important tool for characterizing the advanced structure of polysaccharide, which is a good technique to analyze the structural morphology, including size, shape and porosity [36]. The morphology of polysaccharide surface can be observed intuitively by magnification different times. From the Figure 3, the surface of LPEPS-1displayed a sheet-like appearance, and its surface was slightly rough with holes of different sizes, showing a compact and uneven distribution under the microscope. The structure micrographs were different from the SEM images of EPS from *L. plantarum* WLPL04 [29], which showed an irregular structure resembling sheets. In addition, EPS from *L. plantarum* KX041 presented flake shapes structure with rough surface [37]. The differences of microstructure and surface morphology of EPS may be related to the physicochemical properties, the selective type of extraction, the method of drying and the structure of polysaccharide [38, 39].

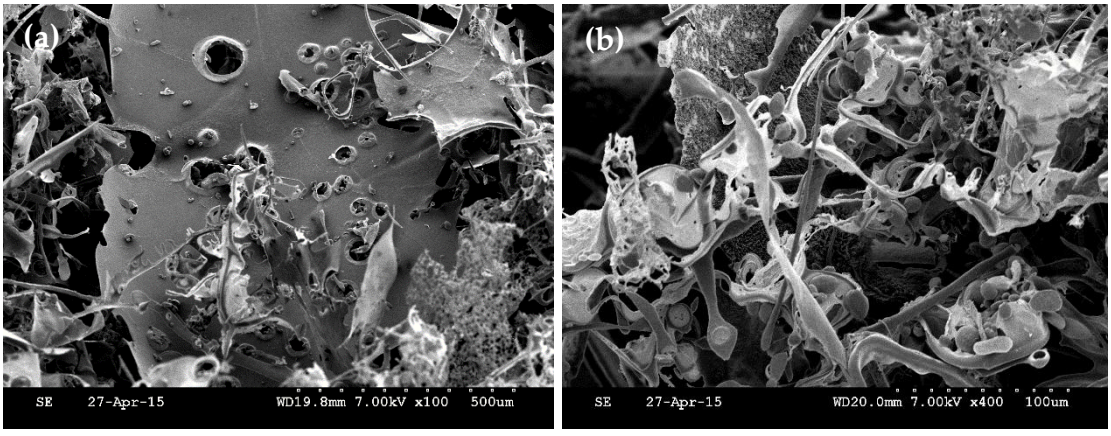

Figure 2. SEM images of LPEPS-1 from *L. plantarum* 70810. 100 × LPEPS-1 (a), 400 × LPEPS-1 (b).

3.6. Particle size and zeta-potential analysis

The particle size is important parameters of a substance in a solution. It can reflect the stability of the solution and decide the substance's potential application prospect [40]. In different environments, polysaccharide always shows different conformations, which can be expressed by apparent particle size on a laser particle size analyzer. Han Q et al [41] reported that tea (*Camellia sinensis*) flower polysaccharide solution could produce bigger size particles by heat treatment, compared to before heating. In this study, the particle size distribution of LPEPS-1 with concentration of 0.2 mg/mL in aqueous solution was determined by laser particle size analyzer. The results were shown in Figure 4a & 4b. The mean diameter of LPEPS-1 was 34.22 nm. The size distribution of LPEPS-1 was narrow and uniform, indicating that LPEPS-1 was homogeneous polysaccharide. The zeta potential was also an important parameter that reflects the repulsive force between polysaccharide molecules in solution. In general, large repulsive force can prevent particles from aggregation [42]. LPEPS-1 was negatively charged, and the zeta potential was -2.93 ± 0.13 mv. Due to the charge polarities, the particles were not massively aggregated. Taken together, LPEPS-1 might be all had good stability.

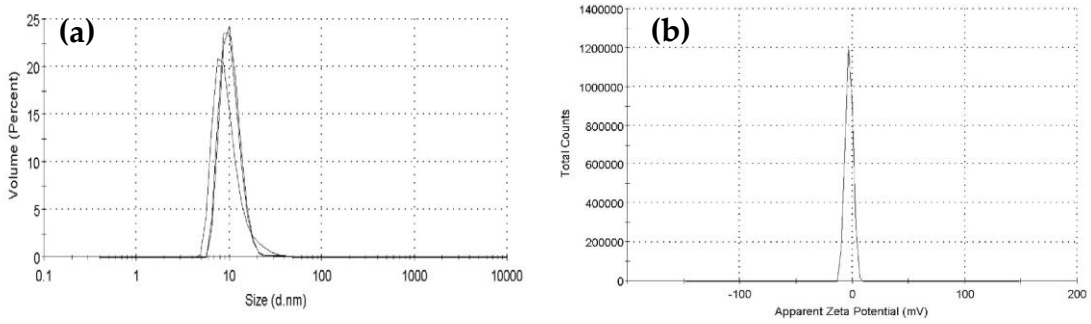

Figure 2. Particle size distribution and apparent zeta potential of LPEPS-1. Particle size distribution (a), apparent zeta potential (b)

3.7. Analysis of antioxidant activities in vitro

3.7.1 DPPH radical scavenging activity

DPPH free radical has become a commonly used free radical to evaluate the antioxidant activity of various polysaccharides. The antioxidant mechanism of DDPH free radicals is that it can accept hydrogen protons to become non- radical DPPH-H, which leads to the disappearance of DPPH purple. According to the change of purple color before and after adding polysaccharide samples, the DPPH scavenging rate can be calculated [43]. The antioxidant property of LPEPS-1 was evaluated with DPPH, which compared with those of Vc. In the Figure 5a, there was no significant changes in the scavenging rate of LPEPS-1 on DPPH did not change significantly at the selected concentrations range (0.125 mg/mL- 4.0 mg/mL). However, it can be seen that the scavenging ability of DPPH still had a dose-dependent relationship. The scavenging activity of LPEPS-1 was 42.6%, which was lower half than that of Vc at 4.0 mg/mL, indicating that LPEPS-1 had moderate scavenging activity on DPPH. The scavenging activity was similar to Wang et al [16] reported the DPPH radical scavenging activities of r-EPS1 and r-EPS2 isolated from *L. plantarum* 70810 were 27.98% and 48.43% respectively. The DPPH free radical scavenging rate was mainly affected by the monosaccharide composition. In addition, it has been reported that high molecular weight polysaccharide may affect its solubility in water-ethanol systems, thus affecting its scavenging ability [37].

3.7.2 Superoxide anion free radical scavenging activity of EPS

Superoxide anion is a precursor of active free radicals and it is a relatively weak oxidant [44]. When it interacts with singlet oxygen or hydroxyl radicals, it can generate strong reactive oxidative species and cause oxidative damage and various diseases [45]. Therefore, the superoxide radical scavenging activity of LPEPS-1 was evaluated. As shown in Figure 5b, the scavenging ability of LPEPS-1 against the superoxide anion showed low scavenging ability at the low concentration range (0.125 mg/mL- 0.5 mg/mL). Their scavenging abilities were less than 30%. As the concentration of polysaccharides increased, the scavenging activity gradually improved. When the test concentration was up to 4.0 mg/mL, the highest scavenging activity was 40.13%. Compared with Vc, the scavenging activity of LPEPS-1 was relatively low. However, LPEPS still exhibited similar antioxidant activity to those reported in the literature[21].

3.7.3 Metal ion-chelating activities

Metal ions, such as ferrous ions and copper ions, are powerful oxidants. It is reported that they can induce the production of free radicals and reactive oxygen species, which can lead to lipid peroxidation and DNA damage[46]. The Fe^{2+}-chelating activity of LPEPS-1 was evaluated (Figure 5c). For chelating activity on ferrous ion, LPEPS-1 showed excellent chelating ability. As the concentration of polysaccharides increased, the chelating ability remarably increased. The chelating ability of LPEPS-1 on Fe^{2+} at 4.0 mg/mL was up to 62.8%, which was closed to the result of EDTA-2Na. Additionly, previuos studies have shown that compounds containing -OH, C=O, -SH, -O-, C=O and so on functional groups had good metal chelation [47]. These results indicated that the -OH, -O- and C=O functional groups in LPEPS-1 played a role in metal chelation.

The antioxidant property of polysaccharide has been a hot topic. Recent studies have reported that the antioxidant property of polysaccharide was related to many factors such as molecular weight, monosaccharide compositions, molar ratio of monosaccharides, glycoside bonds and others [48, 49]. Lo et al [50] reported that the composition of monosaccharides affects the antioxidant capacity of EPS, and its monosaccharides containing rhamnose and galactose have good antioxidant capacity. In our studies, LPEPS-1 contained the galactose composition, so it exhibited outstanding antioxidant activity. Although there are many researches on the antioxidant activity of polysaccharides, there are few researches on the antioxidant mechanism, which needs further exploration.

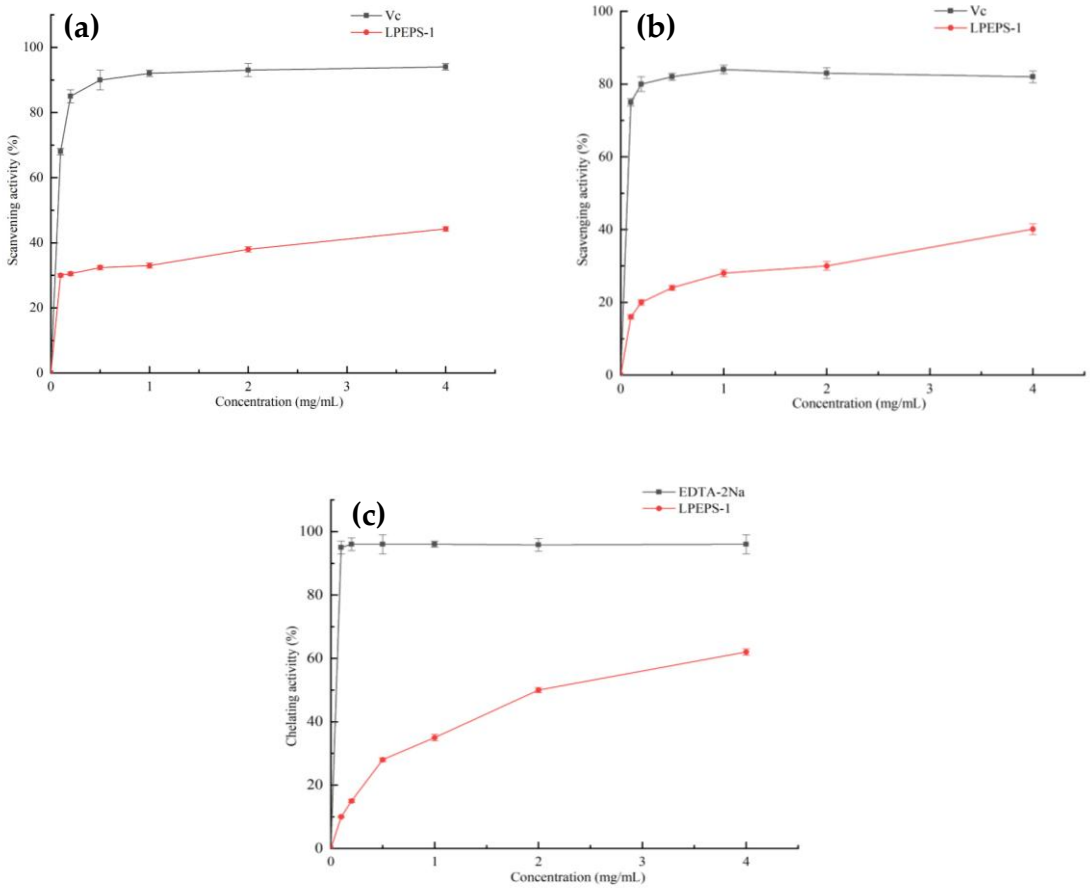

Figure 5. Antioxidant activities *in vitro* of LPEPS-1. Scavenging activity on DPPH radical (a), scavenging activity on superoxide radical (b), chelating activity on metal ion (c), Vc and EDTA-2Na as positive control. Data presented as means ± SD triplicates.

4. Conclusions

In this sutdy, one purified EPS fraction (LPEPS-1) was extracted from soybean whey fermented by *L. plantarum* 70810, and its structural characteristics and antioxidant activities of LPEPS-1 were investigated. Results showed that it was consisted of mannose, glucose and galactose with molar ratios of 1.49: 1.67: 1.00. Particle size analysis showed that LPEPS-1 had small particle sizes, and it was negatively charged. Finally, we also evaluated its antioxidant activities *in vitro*. LPEPS-1 exhibited better antioxidant bioactivities against the DPPH radical scavenging activity, superoxide anion radical scavenging activity and chelating activity on ferrous ion. On the basis of these results, the *L. plantarum* 70810 can be used soybean whey to produce polysaccharide, and the EPS has a variety of antioxidant activities, which has further research value.

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Conflicts of Interest: The authors declare no conflict of interest.

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