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Syneresis rate, water distribution, and microstructure of wheat starch gel during freeze-thaw process: Role of a high molecular weight dextran produced by *Weissella confusa* QS813 from traditional sourdough

Xiaojuan Tang[1] | Na Liu[2] | Weining Huang[1] | Xiaoyan Cheng[2] | Feng Wang[2] | Binle Zhang[1] | Jiafang Chen[1] | Hui Jiang[1] | Jacob O. Omedi[1] | Zhibin Li[3]

[1]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China

[2]MagiBake International Inc., Wuxi, China

[3]Fujian Wheat City Food Development Co., Ltd., Jinjiang, Quanzhou, China

Correspondence
Weining Huang, State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, China.
Email: wnhuang@jiangnan.edu.cn

Background and objectives: Microbial exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) can enhance technological properties of frozen goods. In this study, 120 LAB strains isolated from Chinese traditional sourdough were screened for EPS production ability. The effect of the produced EPS on the syneresis rate, water distribution, and microstructure of a wheat starch gel during freeze-thaw cycle (FTC) was investigated using centrifuge methods, low field-nuclear magnetic resonance (LF-NMR), scanning electron microscopy (SEM), and a frozen section-optical microscope technique.

Findings: Of all screened isolates, *Weissella confusa* QS813 was identified and found to produce large amounts (up to 18.9 g/L) of high molecular mass, low-branched dextran with 97% α-(1,6) linkages in the presence of 5% sucrose at 30°C after 24 h of incubation. When added in wheat starch (WS) at different concentrations, the dextran at 1% significantly ($p < .05$) reduced the syneresis rate among all samples, thus the highest stability during free-thaw cycles. The LF-NMR results showed that the addition of EPS at high concentration to WS appeared to alter the distribution and mobility of water in the starch gel. The microstructure of the wheat starch gel with 1% dextran was denser and more uniform compared to that of the wheat starch without dextran.

Conclusion: The dextran produced by *W. confusa* QS813 significantly improved the freeze-thaw stability of wheat starch gel.

Significance and novelty: A novel dextran from *W. confusa* fermentation was appropriately used as a stabilizer in wheat starch based on frozen dough, thus adding new knowledge and its application potential in the frozen dough and food industry trending with consumers' demand for clean label products.

KEYWORDS
dextran, exopolysaccharide, freeze-thaw stability, frozen section-optical microscope technique, low field-nuclear magnetic resonance, *Weissella confusa*, wheat starch gel

1 | INTRODUCTION

Frozen dough technology is known to extend the shelf life of baked goods, a necessary advantage both at small-scale production and at large-scale production. However, thermal fluctuations and phase changes during freezing processing and storage lead to product quality deterioration (Huang, Kim, Li, & Rayas-Duarte, 2008; Kim, Huang, Du, Pan, & Chung, 2008). This has partly been attributed to water distribution, starch and protein structural changes during frozen dough process (Jia et al., 2014; Kim, Huang, et al., 2008; Kim, Seo, et al., 2008). Starch is the most abundant constituent in wheat flour (Goesaert et al., 2005). Studies have shown that the native starch granule thermal and structural properties in frozen dough can be influenced by freeze processing and storage (Ribotta, León, & Añón, 2003; Silvas-García et al., 2016; Tao, Wang, Wu, Jin, & Xu, 2016). Also, starch-based frozen products as observed in frozen dough products undergo textural changes related to retrogradation and show syneresis after freeze-thaw cycles (Li, Kim, Huang, Jia, & Xu, 2010). The gelatinized starch separates into starch-rich and starch-deficient regions during the freezing and frozen storage processes, a phenomenon associated with formation of ice crystals, hence leading to a sponge-like structure with increased retrogradation in the starch-rich region (Lorenzo, Zaritzky, & Califano, 2009; Zeleznak & Hoseney, 1996; Zhang & Simsek, 2009). The stability of gelatinized wheat starch, an important factor in prebaked frozen bread, as it interacts with water molecules in the presence of additives such as dextrins and hydrocolloids may offer improved stability during freezing process (Bárcenas, Haros, & Rosell, 2003). However, little information is available regarding the additives' effectiveness during freeze-thaw cycles.

Hydrocolloids are commonly used to improve the texture, rheological, and the stability of starch-based frozen foods (Barcenas, Benedito, & Rosell, 2004). In starch gels, hydrocolloids enhance stability during freeze-thaw cycles through decrease in starch retrogradation rate, increase in viscosity, and lowering the rate of syneresis (Charoenrein, Tatirat, Rengsutthi, & Thongngam, 2011; Chen, Fu, & Luo, 2015; Ferrero, Martino, & Zaritzky, 1994; Muadklay & Charoenrein, 2008; Pongsawatmanit & Srijunthongsiri, 2008; Saekang & Suphantharika, 2006). Most of the hydrocolloids used in the food industry are currently derived from plants or seaweed (Galle & Arendt, 2014).

Microbial exopolysaccharides (EPS), produced by lactic acid bacteria (LAB) reportedly act as hydrocolloid alternatives especially those of high molecular weight (Dubey & Jeevaratnam, 2015). Based on their composition, microbial EPS are divided into two groups: hetero- (HePS) and homopolysaccharides (HoPS), with the former consisting of small amounts of two to eight monosaccharides and the latter consisting of one monosaccharide (either fructose or glucose) produced in large amounts (Badel, Bernardi, & Michaud, 2011). Microbial EPS have a high application potential as hydrocolloids alternative due to their high water retention capacity and ability to positively affect the rheological and pasting properties of starch while stabilizing freeze/thaw processes of stored bread. The LAB genera of *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Weissella* are known to synthesize extracellular EPS (Katina et al., 2009; Kim, Huang, et al., 2008; Kim, Seo, et al., 2008; Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008; Malang, Maina, Schwab, Tenkanen, & Lacroix, 2015; Shukla et al., 2011), all of which have been successfully isolated from traditionally prepared sourdoughs. The LAB synthesized EPS have been reported to be composed of a variety of structures (Bounaix et al., 2009; Galle, Schwab, Arendt, & Gänzle, 2011; Maina et al., 2008).

Throughout its history, sourdough has been used in the preparation of Chinese steamed bread (CSB), a popular cereal food for most of the Chinese people (Kim, Huang, Zhu, & Rayas-duarte, 2009; Wu et al., 2012). Microbiologically, Chinese traditional sourdoughs collected from different geographical regions have a diverse and unique microbial community (Liu, 2014; Huang, Cheng, & Li, 2015; Cheng, 2015). Despite this diversity, exopolysaccharide LAB producers can be found in all sourdough types if judiciously examined. Therefore, in response to consumer demand for clean labels, this study screened, isolated, and identified LAB strains with EPS synthesizing potential from Chinese traditional sourdoughs. Most studies have focused on the structural and physical properties of EPS produced from different strains, with few focusing on the potential applications of EPS in wheat starch-based frozen foods.

In our previous frozen process and freezing process-related studies, ice structuring proteins (Li et al., 2010) and an enzyme system (Li et al., 2011) were used to attain stability in the frozen systems. Traditional Chinese sourdough starters were collected from different wheat cultivation and steamed bread-consuming areas of China (Liu, 2014). The collected samples were stored at 4°C and transported to the laboratory for analysis. The objective of this study was to screen, isolate, and identify EPS-producing lactic acid bacteria from Chinese traditional sourdoughs starters. The EPS produced by the selected strain (s) were characterized. The effect of the characterized EPS on % syneresis, water distribution, and microstructure of wheat starch gels during freeze-thaw cycles (FTC) was also investigated.

2 | MATERIALS AND METHODS

2.1 | Screening and identification of EPS-producing strains

In total, 120 LAB strains isolated from four Chinese traditional sourdough starters were screened for the production of EPS. Agar plates containing modified lactobacilli MRS (mMRS) medium (50 g/L sucrose instead of 20 g/L glucose) were used as selection medium.

The TIANamp Bacteria DNA Kit (TianGen Biotech (Beijing) CO., Ltd.) was used for DNA extraction as described by the manufacturer. Fragments of the 16S rRNA gene were amplified using universal primers 27F and 1492R (Lane, 1991). Each 50 μl PCR reaction contained final concentrations of the following reagents: 2 μl of DNA template, 5 μl 10 × PCR Buffer, 4 μl dNTPs (2.5 mM each), 1.5 μl of each of the primers, and 0.5 μl Easy Taq DNA Polymerase (5 U/μl) (TransGene Biotech, Beijing). The PCR reaction was performed under the following conditions: 94°C for 4 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min; final extension at 72°C for 10 min. The PCR products from LABs were sequenced by Shanghai Sunny Biotechnology Co., Ltd. The sequences were manually aligned using SeqMan software (version 7.1), and the searches were performed in the EzTaxon database (http://www.ezbiocloud.net/eztaxon/identify) to determine the closest relatives of the partial 16S rRNA sequences. MEGA (version 5.0) software was used to construct the phylogenetic tree by the Neighbor-Joining distance method.

2.2 | Isolation and purification of EPS

The production of EPS by *Weissella confusa* QS813 was performed in mMRS broth. The strain was cultivated at 37°C for 24 h in MRS medium. It was subcultured at least two times prior to experimental use. For EPS production, the strain was cultured in mMRS and incubated at 30°C for 24 h. Cells were removed by centrifugation at 12,000 × g for 50 min at 4°C. A final concentration of 4% (w/v) trichloroacetic acid (TCA) was used for protein precipitation and kept at 4°C overnight. Proteins were removed by centrifugation at 12,000 × g for 30 min at 4°C. Three volumes of 95% (v/v) cold ethyl alcohol was then added to the supernatant, followed by centrifugation at 12,000 × g for 30 min at 4°C. The precipitate was resuspended in ultrapure water and dialyzed (Mw cutoff 8,000–10,000 Da) for 48 h at 4°C with changes in water every 8 hrs. Finally, the dialyzed EPS preparations were freeze-dried and stored at −18°C.

2.3 | Monomer composition analysis of EPS

The monomer composition of the EPS was measured by HPLC (Waters HPLC, Model 1525) after complete acid hydrolysis. Approximately 10 mg of purified EPS was hydrolyzed with 2.5 ml 1.75 M perchloric acid. The mixtures were incubated at 80°C for 16 h, and then, 5 M potassium hydroxide was added as described by Bounaix et al. (2009). Precipitate was removed by centrifugation (6,000 × g, 5 min, 4°C), and the supernatants were passed through a 0.45 μm filter membrane and subjected to monosaccharide determination by HPLC using an XBridge BEH Amide column (4.6 × 250 mm, Waters, USA) and refractive index (RI) detector under the following conditions: column temperature at 30°C and mobile phase 70% aqueous acetonitrile (v/v) at a flow rate of 0.8 mL/min.

2.4 | Molecular mass analysis of EPS

Size of the purified EPS was estimated using SEC-MALLS-RI (Wyatt Technology, Santa Barbara, CA). The system was equipped with a Shodex OHpak SB-806HQ column (8 × 300 mm). The eluent was monitored using an RI detector and multiangle laser light scattering (MALLS) detector. The EPS (1 mg/ml) were filtered through a 0.45 μm cellulose acetate filter and injected into a 200 μl loop. The flow rate was 0.5 ml/min, and the eluent was 0.1 M NaNO$_3$ solution containing 0.02% sodium azide.

2.5 | FTIR and NMR analysis of EPS

The FTIR spectrum of the EPS from *W. confusa* QS813 was recorded by a FTIR spectrometer (Thermo Electron Co., Waltham, MA). 2 mg purified EPS was ground with 200 mg dry potassium bromide powder for 2 mins under an infrared lamp and pressed into pellet form for FTIR measurement. Spectra were acquired in the absorbance mode from 400 to 4,000 cm^{-1} with 32 scans. The data were processed with Omnic spectra software (version 7.0).

The ^{1}H spectra of EPS were recorded with an AVANCE III 400 MHz NMR spectrometer (Brucker Co., Billerica, MA, USA). Prior to determination, 50 mg of purified polysaccharide sample was dissolved in 0.50 ml deuterium oxide. Chemical shifts were expressed in ppm, and the spectrum was referenced internally with acetone [δH 2.225 ppm].

2.6 | Scanning electron microscopy of EPS

0.1 mg/ml and 0.5 ml/ml EPS were vacuum freeze-dried for microstructure observation. The samples were coated with a layer of gold (10 nm thick) and then observed by

SEM (Model JSM6380-LV, JEOL, Tokyo, Japan) at an accelerating voltage of 5.0 kV.

2.7 | Preparation of fresh and freeze-thaw wheat starch gel

Purified EPS (0.25, 0.5, 0.75, and 0.1 g) were sprinkled into 100 ml deionized water and continuously stirred for complete dissolution. Eight gram of unmodified wheat starch (Sigma-Aldrich Chemical Co., USA) was then dispersed in EPS solution and stirred for 30 min at room temperature. The mixed suspension was heated in a water bath at 90°C for 30 min with continuous stirring. After heating, the hot slurry was cooled to 30°C in a water bath. For freeze-thaw stability analysis, the cooled starch gels were transferred into plastic containers and frozen at −20°C for 22 h in the refrigerator and then thawed at 30°C for 2 h in a water bath. Five freeze-thaw cycles (FTC) were repeated.

2.8 | Syneresis measurement of starch gels

The % syneresis of wheat starch gel during five FTC was determined according to a method described by Pongsawatmanit, Temsiripong, Ikeda, and Nishinari (2006) with minor modifications. Briefly, 3 g fresh wheat starch gel was added to a 5 ml disposable syringe without the tip and plunger. A little pledget was placed at the bottom of the syringe. The syringe was placed in a weighed (W_0) 10 ml centrifuge tube. The tube was then centrifuged at 2100 g for 10 min. The syringe with cover was pulled from the centrifuge tube. The centrifuge tube with water separated from the wheat starch gel was weighed (W_1) after the syringe with cover was pulled from the centrifuge tube. The % syneresis was calculated as follows:

$$\%\text{syneresis} = 100\% \times (W_1 - W_0)/3.00$$

2.9 | LF-NMR measurements of starch gels

Determination of proton distribution in fresh and freeze-thawed starch gel was performed with an NMR spectrometer-MesoMR23-060 V-I (Niumag Electric Corporation, Shanghai, China) at a resonance frequency of 23 MHz for ^{1}H. The fresh and freeze-thawed starch samples prepared as described above (20.00 g) were transferred into the NMR probe (60 mm external diameter). Spin−spin (T_2) relaxation time was acquired using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. The pulse parameters were as follows: TW=5000 ms, NECH=10000, SW=100 KHz, PRG=1, NS=16. Niumag NMR analysis software (Niumag Electric Corporation, Shanghai, China) was used to invert the collected signals to the T_2 relaxation time curves.

2.10 | Microstructure observation of starch gels

Fresh and freeze-thawed gel samples were prepared as described above and cut to roughly 5 × 5×5 mm with a sharp blade, embedded in tissue freezing medium (Leica Biosystems, Richmond, USA) and kept under −20°C for 30 mins. Ten-micrometer sections were cut with a cryostat microtome (CM1950, Leica Biosystems, Wetzlar, Germany) at −20°C. Macro photographs of the frozen sections were taken with a microscope (ZEISS Axio vert. A1, Germany) equipped with a computer. Images were recorded at 200 × magnification.

Fresh and freeze-thawed gel samples were processed with vacuum freeze-drying. Microstructures were further observed using SEM (S-4800, Hitachi Science Systems, Ltd., Japan). Micrographs were taken at magnifications 200 × at 20 kV.

3 | RESULTS AND DISCUSSION

3.1 | Screening, isolation, and identification of EPS-producing bacterial strains

A phenotypic screening is often the method of choice for identification of EPS-producing bacterial strains (Rühmann, Schmid, & Sieber, 2015). In this study, 120 LAB strains originated from traditional Chinese sourdoughs were screened for EPS production by cultivating strains on mMRS agar at 30°C for 48 h. Among the tested strains, LAB strain QS813 exhibited a highly viscous slimy phenotype on mMRS. Using morphological and physicochemical tests as well as 16S rDNA (data not shown), the EPS positive strain was identified as *W. confusa*. A confirmation of sourdough LABs EPS-producing ability was reviewed by Gänzle (2014).

The yield of the purified EPS achieved was 18.9 g/L (dry weight) in mMRS broth at 30°C in 24 h of incubation. Fermentation substrate, for instance sucrose instead of glucose used in this study, might have influenced the EPS yield achieved. This is in agreement with several studies that found that the production of homopolysaccharides from sucrose is a frequent metabolic trait of sourdough LABs (Gänzle, 2014). However, the total yield of EPS produced by LAB may also depend on the incubation conditions and is strain-specific (Degeest, Vaningelgem, & Vuyst, 2001; Dubey & Jeevaratnam, 2015).

3.2 | Molecular mass and monosaccharide composition of EPS

The molecular weight of the EPS was evaluated by SEC-MALLS-RI (Figure 1). The analysis showed a single peak,
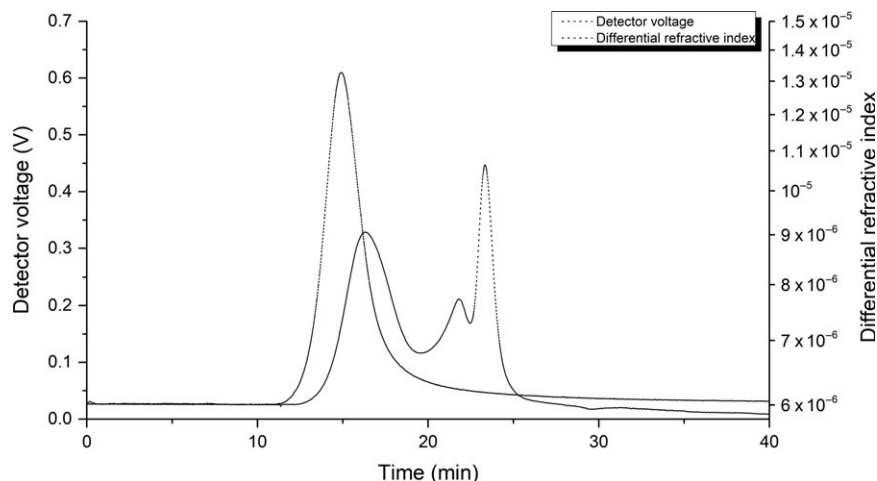
FIGURE 1 HPSEC-MALLS-RI chromatogram of exopolysaccharide from *Weissella confusa* QS813

confirming the homogeneity of the purified EPS sample. The results showed that the average molecular weight (M_w) for the EPS produced by *W. confusa* QS813 was 1.6×10^8 Da (error 3%). High molecular weight EPS from *Weissella* spp. strains have been reported in the ranges of 8×10^5 Da to $>2 \times 10^7$ Da (Ahmed, Siddiqui, Arman, & Ahmed, 2012; Malang et al., 2015; Tingirikari, Kothari, Shukla, & Goyal, 2014).To analyze the monosaccharide composition of the purified EPS produced by *W. confusa* QS813, the EPS were submitted to acid hydrolysis. Subsequent HPLC results showed that the EPS were exclusively composed of glucose, thus indicating that a glucan was produced by *W. confusa* QS813.

3.3 | FTIR and NMR analysis of EPS

The FTIR spectrum of EPS from *W. confusa* QS813 is shown in Figure 2. The broad absorption peak at 3326 cm^{-1} corresponds to the stretch vibration of the hydroxyl groups of carbohydrates, and the band approximately 2940 cm^{-1} might be attributed to C-H stretching vibration. The high
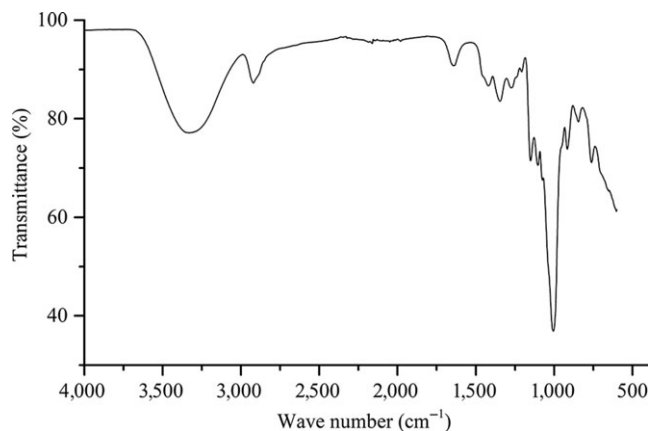
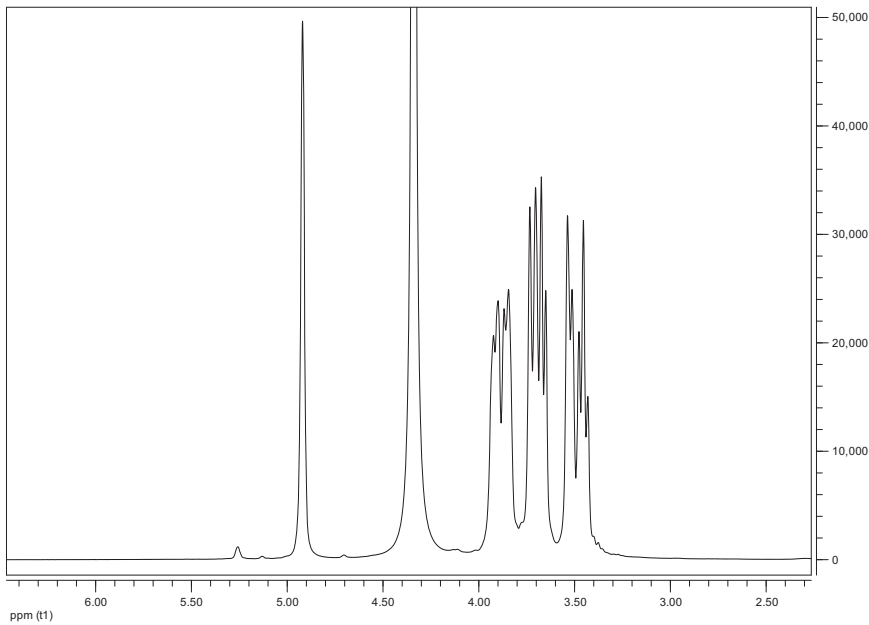**FIGURE 2** FTIR spectrum of purified exopolysaccharide from *Weissella confusa* QS813

absorbance between 1200 and 950 cm^{-1} is considered the fingerprint region and can be used to characterize structure differences (Park, Ahn, Kim, & Chung, 2013). The band at 1155 cm^{-1} is caused by covalent vibrations of the C-O-C bond and glycosidic bridge. The α-glycosidic bond is also confirmed by the peak at 917 cm^{-1}, and the absence of a peak at 870–890 cm^{-1} indicates no β-configuration in the EPS.

The ^{1}H NMR spectrum of the EPS from *W. confusa* QS813 in D$_2$O is shown in Figure 3. The dominant H1 signal at δ4.96 ppm indicates typical anomeric protons of α-(1, 6) linked dextran, and the low signal at δ5.261 ppm is due to the branching of α-(1, 3) glucose units (Seymour 1979). The relative intensity of these anomeric protons was found to be 97% and 3%, respectively.

The FTIR and ^{1}H NMR spectral analyses confirmed that the EPS produced by *W. confusa* QS813 are a low-branched dextran with 3% α-(1, 3) branch linkages and 97% α-(1, 6) linkages. Production of high molar mass dextran with few branches has been suggested to be a common feature of *Weissella* spp. (Ahmed et al., 2012). This is in agreement with the structure analysis of EPS produced by *W. confusa* QS813 in this study.

3.4 | SEM of EPS

Two different concentrations of EPS were processed with vacuum freeze-drying. The scanning electron micrographs of EPS are illustrated in Figure 4. EPS from *W. confusa* QS813 had a slender fiber-shaped and sheet-like structure at low (1 mg/ml) and high (5 mg/ml) concentration. In a separate study by Tingirikari et al. (2014), SEM analysis of dextrans from *W. cibaria* JAG8 showed a porous surface morphology. Research has shown that high molecular weight linear dextran is highly soluble and imparts high viscosity (Maina et al., 2008). Therefore, in this study, the high levels of EPS (1.6×10^8 Da) might have enhanced

FIGURE 3 ¹H NMR spectra of purified exopolysaccharide from *Weissella confusa* QS813
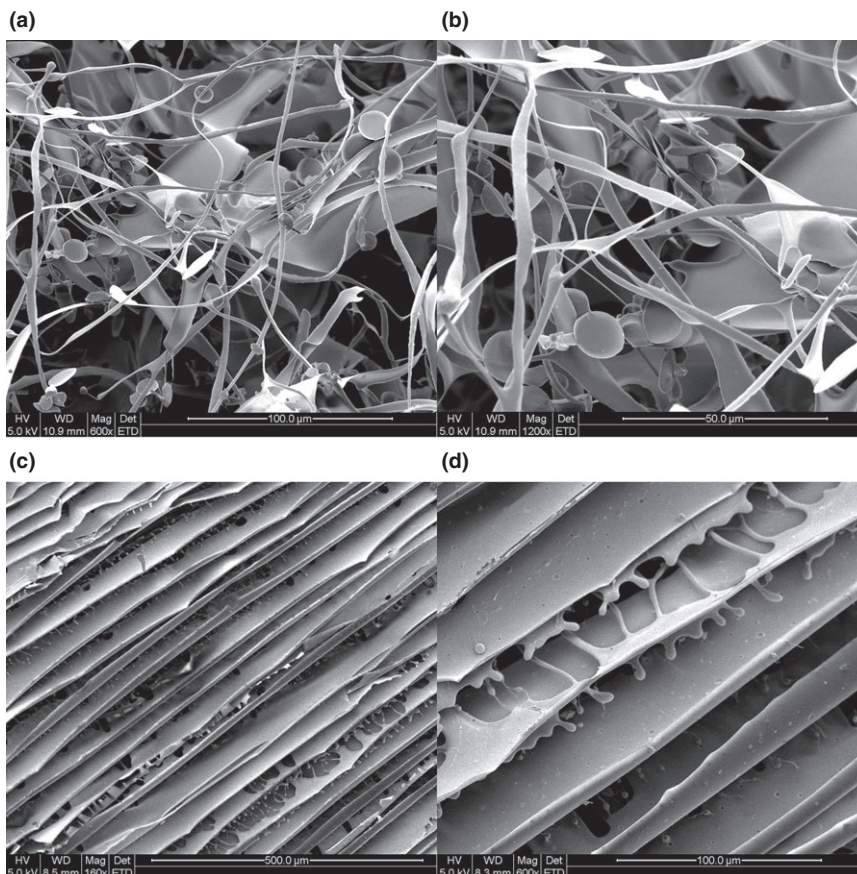
FIGURE 4 Scanning electron microscopic analysis of exopolysaccharide (EPS) from *W.confusa* QS813. (a, b 1 mg/ml of EPS before freeze-drying; c, d 5 mg/ml of EPS before freeze-drying)

association reaction of hydrogen bonds leading to an increase in polymer aggregation and thus the formation of sheet-like structure. The unique molecular structures and microstructure characteristics of EPS produced in this study make it a potential texturing agent in the food industry.

3.5 | Syneresis measurements of gels after five freeze-thaw cycles

The determination of % syneresis from freeze-thawed starch gel is used to evaluate the ability of starch to withstand the undesirable physical changes that occur during

freezing and thawing (Muadklay & Charoenrein, 2008). The % syneresis of wheat starch gel after 5 FTC is presented in Table 1. As the number of FTC increased, the % syneresis of the starch gel increased for all samples. From the first to fifth FTC, EPS addition significantly reduced the % syneresis. Under the experimental conditions, the % syneresis of wheat starch gel without EPS increased from 18.3% to 72.8% after five FTC, while for 8% WS+1%EPS, it was 63.3% after 5FTC. Overall, 1% EPS showed the highest freeze-thaw stability among samples. Addition of EPS at different concentration to the wheat starch gel delayed the % syneresis of the starch gel after five FTC. This shows that EPS have the potential use in improving the freeze-thaw stability of wheat starch gel.

3.6 | Water distribution of gels after five freeze-thaw cycles

Low-resolution (LR) proton nuclear magnetic resonance (1H NMR), a rapid, reliable, and nondestructive technology that detects the proton distribution in complex systems (Li et al., 2016), has been used to determine the spin−spin relaxation times (T_2) in various starch granules and starch gels (Bosmans et al., 2012; Chen, Tian, Tong, Zhang, & Jin, 2017; Tang, Godward, & Hills, 2000; Yamazaki et al., 2017). In our study, the proton distribution in the WS and WS-EPS gel after five FTC was investigated, and the relaxation curves are shown in Figure 5. For fresh starch gel samples, the presence of three CPMG proton populations was distinguished from the relaxation signals: one fraction with the T_2 less than 4.69 ms (population A in Figure 5), one with T_2 range of 9.33–35.20 ms (population B in Figure 5), and another fraction with higher T_2 range of 388.69–838.70 ms (population C in Figure 5), which could be assigned to the lower mobility (populations A and B) and higher mobility water (population C) in the paste system, respectively. After freeze-thaw processing, a new population D appeared, which represented free water separated from the starch gel.

For the fresh gel, the water distribution of the WS gel with different concentrations of EPS was similar to that for WS gel without EPS (Figure 5a). However, remarkable changes were seen after five FTC. The changes in populations C and D were predominant (Table 2). Population C is the most abundant proton population in the CPMG curve. The area% of 8% WS gel decreased with the repeat time of freeze-thaw cycles, whereas the area% of wheat starch gel with EPS addition remained unchanged. T_2 of population B remained immobile after FTC in all experimental groups. The most significant changes in population C were the initial and end times of T_2 (Table 3). Freeze-thaw processing induced a reduction in initial T_2 and an increase in end T_2, which indicated that the heterogeneity of the proton mobility of population C increased. Moreover, the heterogeneity increased as the FTC increased. The formation of ice crystals during freezing causes phase separation of starch gel into one starch-rich and one starch-deficient phase (Eliasson & Kim, 1992). In the starch-rich phase, water molecules are likely to re-orient more slowly as they are more extensively hydrogen bonded to relatively large and immobile starch polymers (Tananuwong & Reid, 2004). We therefore suggest that such interaction between the starch chains induced the reduction in initial T_2 in the starch-rich phase, while the increase in end T_2 was due to the formation of the starch-deficient phase. The addition of EPS delayed increase in heterogeneity increase and with more addition, the lower the heterogeneity. For instance, the initial T_2 and end T_2 of population C changed little even after five FTC with 1% EPS addition (Figure 5 and Table 3), indicating that EPS addition could delay ice crystal formation and phase separation of wheat starch gel during FTC.

After one FT cycle, the appearance of the new population D in 8% WS and 8% WS+0.25% EPS indicated that the structure of the wheat starch gel was damaged by crystals accompanying the water separation. Wheat starch gel with higher concentration than 0.25% showed no signal in population D even after being subjected to five FTC.

Based on the changes in T_2 distribution, the addition of a high concentration of EPS to WS appeared to alter the water distribution and mobility in the starch gel and generate a gel with good freeze-thaw stability.

TABLE 1 Syneresis of wheat starch (WS) containing increasing amounts of exopolysaccharide (EPS) from *Weissella confusa* QS813 after freeze-thaw cycles (FTC)[z]

Sample	Syneresis (%)					
	0 FTC	1 FTCs	2 FTCs	3 FTCs	4 FTCs	5 FTCs
8% WS	18.3 ± 0.7c	55.9 ± 3.1d	64.9 ± 1.7d	66.5 ± 2.6c	69.7 ± 2.9c	72.8 ± 1.3d
8% WS + 0.25% EPS	19.0 ± 1.1c	49.3 ± 0.5c	60.7 ± 1.6c	65.9 ± 1.7bc	67.8 ± 1.2bc	70.9 ± 0.2c
8% WS + 0.50% EPS	18.8 ± 1.3c	44.1 ± 3.6b	57.6 ± 1.5b	61.1 ± 4.0ab	66.3 ± 1.8b	66.7 ± 1.7b
8% WS + 0.75% EPS	15.8 ± 1.1b	40.9 ± 3.1b	56.8 ± 2.1b	60.4 ± 1.6a	64.5 ± 0.7ab	68.3 ± 0.6b
8% WS + 1.00% EPS	10.2 ± 3.1a	29.3 ± 0.7a	51.0 ± 1.1a	56.6 ± 3.4a	61.6 ± 1.5a	63.3 ± 0.7a

Means ± *SD* (*n* = 3) with different letters within a column are significantly different (*p* < .05).
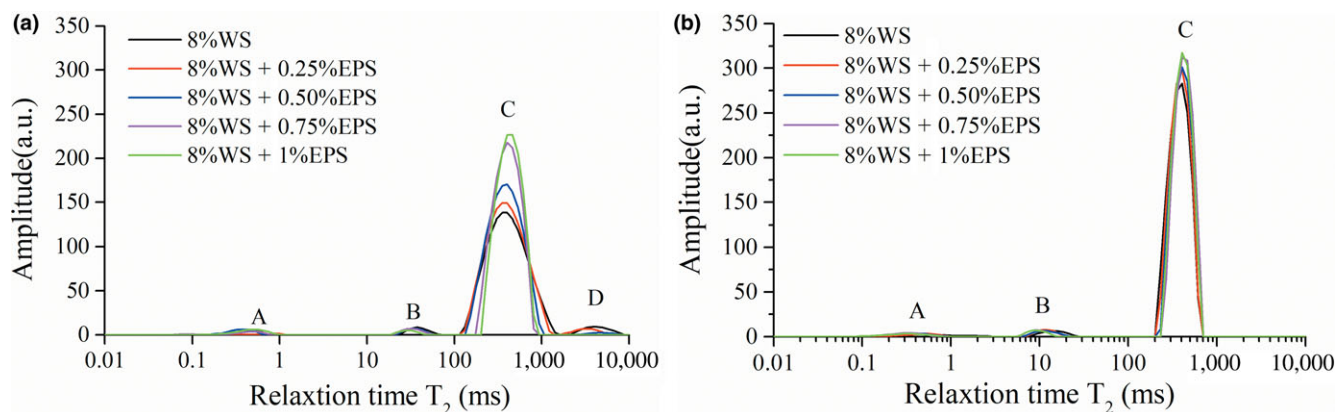
FIGURE 5 Carr−Purcell−Meiboom−Gill (CPMG) proton distributions of wheat starch containing increasing amounts of exopolysaccharide from *Weissella confusa* QS813, fresh (a) and after five freeze-thaw cycles (b). Amplitudes are given in arbitrary units (au). The different proton populations are indicated with capital letters in order of their increasing mobility

TABLE 2 T_2 relaxation times (ms) and areas (au) of the Carr–Purcell–Meiboom–Gill (CPMG) populations A, B, C, and D of wheat starch (WS) containing increasing amounts of exopolysaccharide (EPS) from *Weissella confusa* QS813 after freeze-thaw cycles (FTC)

Sample	Population A		Population B		Population C		Population D	
	T2 (ms)	Area (%)	T2 (ms)	Area (%)	T2 (ms)	Area (%)	T2 (ms)	Area (%)
Fresh								
8% WS	0.40	1.56ab	11.26a	2.51	386.17ab	95.57c	nd	nd
8%WS + 0.25% EPS	0.47	1.39a	10.73a	2.48	403.70b	96.12c	nd	nd
8%WS + 0.50% EPS	0.47	1.83abc	10.73a	2.49	403.70b	95.89c	nd	nd
8%WS + 0.75% EPS	0.38	1.90abc	11.26a	2.50	403.70b	95.37c	nd	nd
8%WS + 1.00% EPS	0.38	1.88abc	9.79a	2.69	406.33b	94.83c	nd	nd
1FTC								
8% WS	0.47	2.53c	18.74c	2.17	351.12a	93.21b	3,764.94a	2.46b
8%WS + 0.25% EPS	0.43	2.30abc	18.74c	2.64	351.12a	94.69c	3,764.94a	0.93a
8%WS + 0.50% EPS	0.43	2.12abc	18.74c	2.52	403.70b	96.06c	nd	nd
8%WS + 0.75% EPS	0.40	2.08abc	17.93c	2.65	403.70b	96.24c	nd	nd
8%WS + 1.00% EPS	0.43	2.07abc	14.88b	2.48	386.17ab	96.40c	nd	nd
5FTCs								
8% WS	0.47	2.02abc	34.38de	2.53	368.65ab	92.99a	3,764.94a	3.97c
8%WS + 0.25% EPS	0.43	2.36bc	31.32d	3.02	368.6ab	93.15b	3,764.94a	2.61b
8%WS + 0.50% EPS	0.43	2.15abc	36.01e	2.67	403.00bd	94.84c	nd	nd
8%WS + 0.75% EPS	0.40	1.78abc	32.75de	2.39	385.67ab	95.20c	nd	nd
8%WS + 1.00% EPS	0.43	2.14abc	32.96de	2.26	403.70b	0.9556c	nd	nd

nd, not detectable; Means $\pm$ *SD* (n = 3) with different letters within a column are significantly different ($p < .05$).

3.7 | Microstructure of gels after five freeze-thaw cycles

The frozen section technique has been widely used for rapid microscopic analysis of a specimen. It is most commonly used in surgical procedures to confirm diagnoses. This research puts forward the combination of frozen section procedure and optical microscope technique for the observation of microstructure in the starch gel. The morphology change in wheat starch with or without EPS after five FTC was analyzed by this technique. Results showed that the microstructural variation of the starch gel with or without EPS after FTC was clearly observed.

Figure 6a,b shows that the network structure of fresh wheat starch gel with or without EPS was compact and homogeneous. However, significant differences were

TABLE 3 The initial and end T2 relaxation times (ms) of Carr−Purcell−Meiboom−Gill (CPMG) population C of wheat starch (WS) containing increasing amounts of exopolysaccharide (EPS) from *Weissella confusa* QS813 after freeze-thaw cycles (FTC)

Sample	Fresh		1FTC		5FTCs	
	Initial T2 (ms)	End T2 (ms)	Initial T2 (ms)	End T2 (ms)	Initial T2 (ms)	End T2 (ms)
8% WS	231.01	705.48	132.19a	811.13b	132.19a	1417.47d
8%WS + 0.25% EPS	231.01	705.48	151.99b	811.13b	132.19a	1232.85c
8%WS + 0.50% EPS	231.01	705.48	174.75c	811.13b	151.99b	932.60b
8%WS + 0.75% EPS	231.01	705.48	174.75c	811.13b	200.92c	811.13a
8%WS + 1.00% EPS	231.01	705.48	174.75c	705.48a	231.01d	811.13a

Means $\pm$ *SD* (*n* = 3) with different letters within a column are significantly different (*p* < .05).
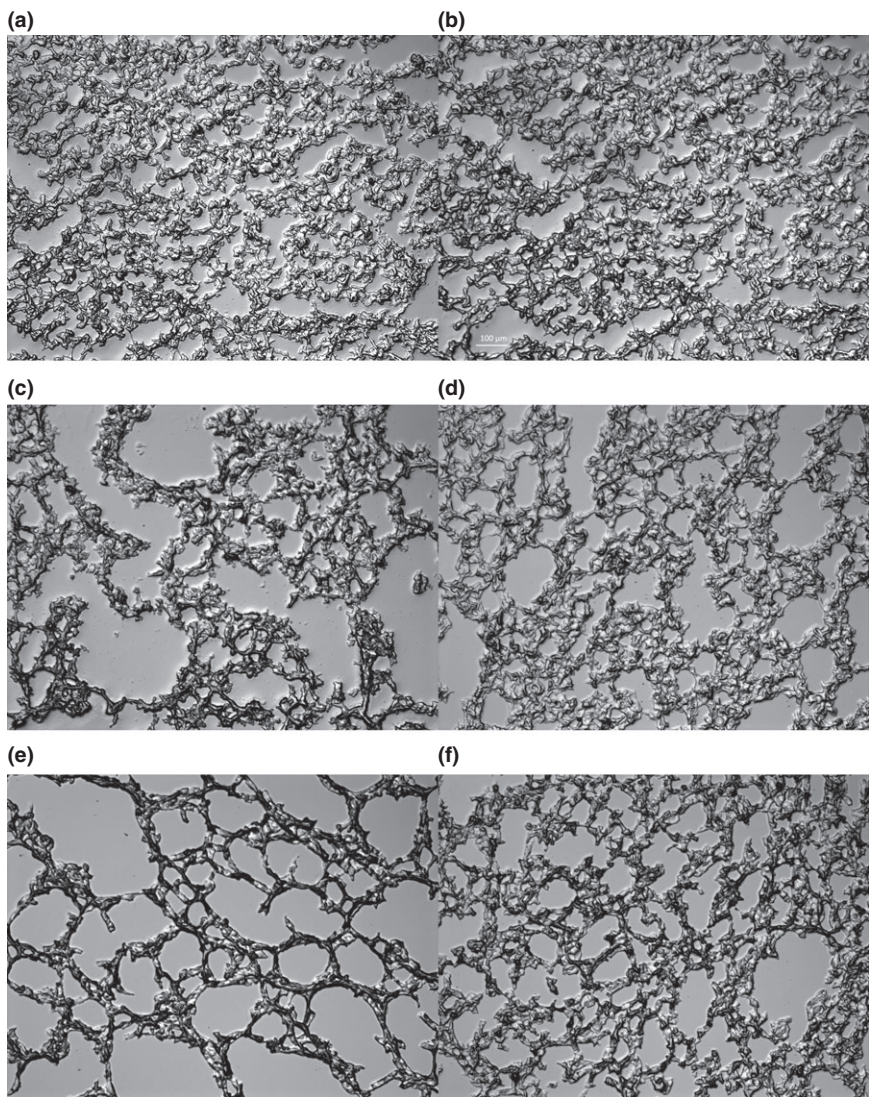
FIGURE 6 Optical microscope observations of wheat starch containing increasing amounts of exopolysaccharide (EPS) from *Weissella confusa* QS813. (a) Fresh wheat starch without EPS addition, (b) fresh wheat starch with 1% EPS addition, (c) wheat starch without EPS addition after one freeze-thaw cycle, (d) wheat starch with 1% EPS addition after one freeze-thaw cycle, (e) wheat starch without EPS addition after five freeze-thaw cycles, (f) wheat starch with 1% EPS addition after five freeze-thaw cycles

observed after the 1st FT cycle. In the wheat starch sample without EPS addition, fibrous concentrated starch chains could be observed, and the cavities in the wheat starch gel sample became larger and more irregular (Figure 6c), which was caused by ice crystal formation and phase separation (Eliasson & Kim, 1992; Lee, Baek, Cha, Park, & Lim, 2002). The starch gel with EPS addition was more connected and uniformly distributed than that in Figure 6c. After five FTC, some large and sparse holes and sparse network structure were observed (Figure 6e), whereas a uniform well-structured gel with a stable network was formed with 1% EPS addition (Figure 6f). The structure

variations of starch gels during FTC correlated well with the finding in % syneresis and T_2 relaxation times of CPMG population D with EPS addition. Consequently, these findings confirm that EPS addition effectively improved the structural stability of the wheat starch gel during FTC.

Morphology changes in wheat starch gels could be successfully observed by frozen section-optical microscope technique. The results were compared to SEM, which is commonly used to visualize starch gels microstructure. More detailed information about the impact of EPS and FTC treatment on wheat starch gel structures could be further observed by SEM technique. Figure 7 shows that the porosity size of fresh gel decreases with added EPS. The EPS were filled in the fragment of starch granule and fused to form the lamellar structure, which induce a denser gel. Obvious microstructural differences could be observed between the wheat starch with and without EPS addition subjected to different times of FTC. It is consistent with the results obtained from frozen section-optical microscope analysis.

4 | CONCLUSIONS

In this study, we isolated a bacterium from a Chinese traditional sourdough starter and identified it using 16S rDNA
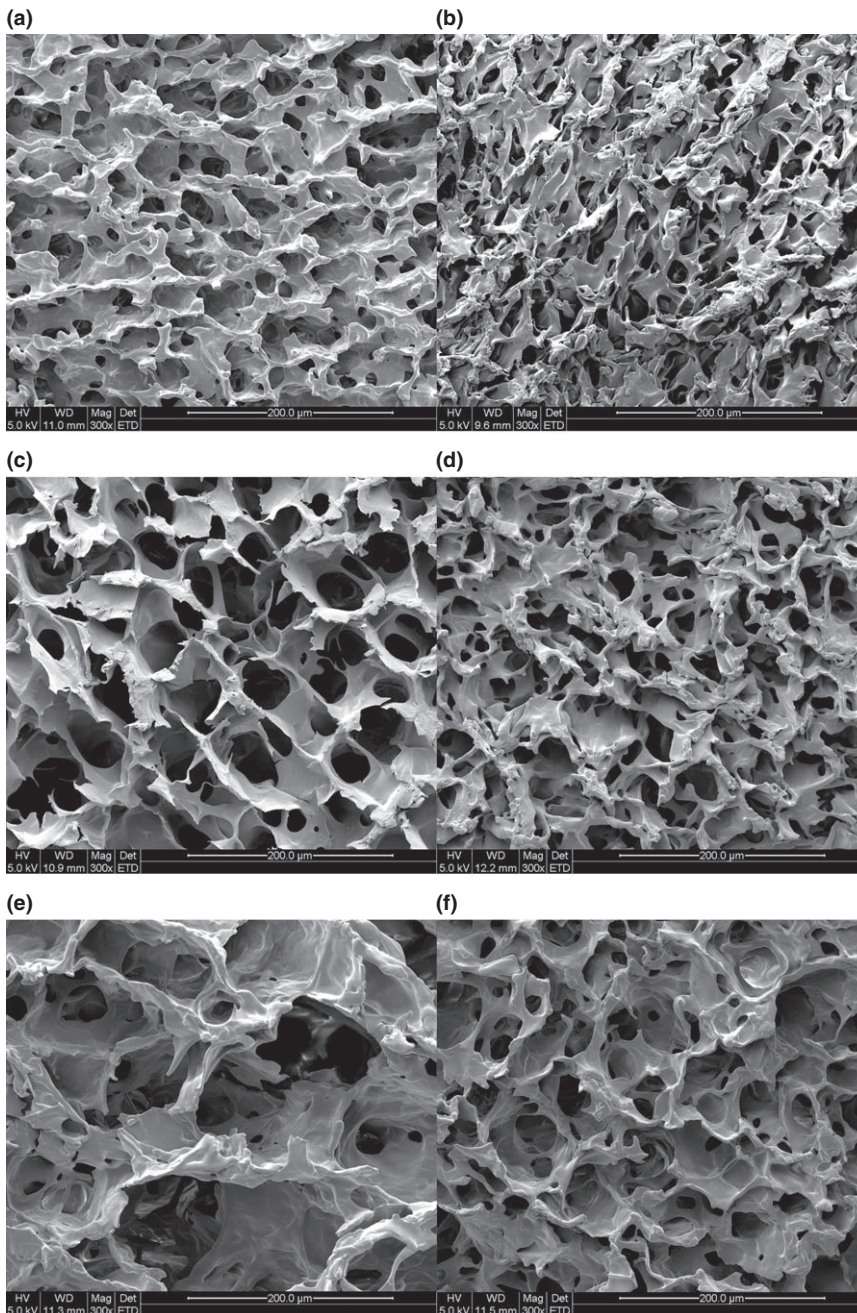
FIGURE 7 Scanning electron micrographs of wheat starch containing increasing amounts of exopolysaccharide (EPS) from *Weissella confusa* QS813. (a) Fresh wheat starch without EPS addition, (b) fresh wheat starch with 1% EPS addition, (c) wheat starch without EPS addition after one freeze-thaw cycle, (d) wheat starch with 1% EPS addition after one freeze-thaw cycle, (e) wheat starch without EPS addition after five freeze-thaw cycles, (f) wheat starch with 1% EPS addition after five freeze-thaw cycles

sequence analysis and biochemical characterization as *W. confusa* QS813. The results of SEC-MALLS-RI, FTIR, and 1H NMR analyses confirmed that dextran from *W. confusa* QS813 is a high molecular weight linear dextran with predominant α-(1, 6) linkages and a few (3%) α-(1, 3) linked branches. The addition of dextran reduced the % syneresis of the wheat starch gel during FTC. The LF-NMR results showed that the dextran delayed the heterogeneity increase in population C in CPMG curves and significantly reduced the water mobility of the wheat starch gel after FTC. The microstructure of a wheat starch gel with 1% dextran was denser and more uniform compared to that of a wheat starch gel without dextran. Dextran produced by *W. confusa* QS813 was found to be an effective agent for improving the freeze-thaw stability of wheat starch gel. These results suggested that this novel dextran from *W. confusa* was appropriate for use as a stabilizer in the wheat starch-based frozen food industry.

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