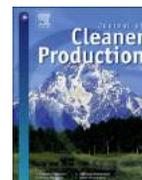




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## Green recovery of pectic polysaccharides from citrus canning processing water



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### ABSTRACT

Canned citrus segments are popularly consumed worldwide with a large global market. However, the production needs chemical treatment to remove segments membranes, producing a large amount of acidic and basic effluents with very high chemical oxygen demand (COD), causing serious environmental problems. This research is focused on the plant scale recovery of pectic polysaccharides from the above acidic and basic processing water to decrease COD and develop a new product, through steps including water reuse (alternative), two-step filtration, concentration, ethanol precipitation and ethanol recovery. The yields of polysaccharides were 0.30% and 0.45% (w/v) from reused acidic and basic water, respectively, dramatically decreasing the COD by ~75% from ~10,000 mg/L, and having a great estimated output value of ~\$1.8 million annually for each factory. Polysaccharides from acidic water mainly contains homogalacturonan regions with ~50% degree of esterification, whereas that from basic water was dominated with rhamnogalacturonan I regions with almost no esterification. The high viscosity of these branching polysaccharides, combined with gelling experiment results, suggested that both polysaccharides might be potential food thickening and gelling agents with large benefits to both the environment and the economy.

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### 1. Introduction

Canned citrus segments, a delicious and convenient fruit product, are popular around the world with an annual trade value of nearly \$900 million (source: UN Comtrade). China is the largest citrus planting and harvesting country in the world (Ministry of Agriculture, 2013), providing most of the mandarin oranges for citrus segments canning, and accounting for nearly 70% canned citrus segments on the international market (Wu et al., 2016).

The process of mandarin oranges canning includes peeling, segment separation, segment membrane removal, washing and syrup addition. With easy-to-eat properties of the citrus segment

products, the canning production requires the segments membrane removal as an important step. Although enzymatic hydrolysis for membrane removal is emerging as an alternative method (Moliner et al., 2008; Shu et al., 2014), chemical treatment with acidic and basic hydrolysis is still commonly used until now as it is low cost and rapidly performed. Unfortunately, chemical hydrolysis produces a large quantity of acidic and basic water, about 600 m<sup>3</sup> per day, from a single factory among the nearly 100 factories in China. The effluent water, as segment membrane solution in fact, contains a high level of organic substances (polysaccharides principally), leading to a very high chemical oxygen demand (COD) representing an environment challenge.

Many articles have been published on the treatment of food industry wastewater containing organic materials. The easy approach requiring little treatment is to use the wastewater as soil fertilizer to promote fruit yield and quality (Qaryouti et al., 2015). Nevertheless, not all the wastewater can be used directly. Ryan et al. (2008) applied coagulation method for decoloration and COD

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removal of molasses process water, and coagulation has also been used for treating high COD organic effluent from a fertilizer plant (Akyol et al., 2013). Unfortunately, many organic substances from other food processes are difficult to be coagulated. A summary shows aerobic and anaerobic processing may be a more appropriate method for organic matter removal (Gupta et al., 2012). For the common saline wastewater in food processing, a combined system of biological/physico-chemical treatment with salt-adapted microorganisms and membrane technology has been proposed (Lefebvre and Moletta, 2006). A membrane bioreactor (MBR) system with anaerobic and aerobic treatment for fruit juice process wastewater has been implemented (Tai et al., 2013) to limit the sludge space and automate the treatment process. However, because of the cost, time consumption and the insufficient treatment of pectin by microorganisms, biological treatment is not an optimal choice for citrus canning wastewater with high pectic polysaccharides content, leaving the citrus canning effluent treatment still a challenging problem. Recovery of pectic polysaccharides from the citrus canning water might represent an alternative approach. A lab-scale of dietary fibers recovery from olive mill wastewater has been reported (Galanakis et al., 2010). But there are few reports on pectic polysaccharides or pectin recovery from fruit processes effluents (much different from the olive mill wastewater), particularly in the citrus canning industry on a plant scale level.

Pectin is a popular ingredient worldwide for the promotion of health (Wicker et al., 2014), and today is in short supply despite having a global market value of more than \$850 million at 2013 with an annual growth rate  $\gg 5\%$  (Ciriminna et al., 2015). Thus, broadening new raw materials as pectin source is needed to supplement the commonly used materials of citrus peels and apple pomace. Citrus segment membrane, similar to citrus peels with polysaccharides as structural component, contains high galacturonic acid (GalA), and can be conveniently obtained as a form of segment membrane suspension liquid from citrus canning acidic and basic procedure. This also means that no extra hot chemical extraction, which is applied in commercial pectin production is needed. However, these suspension liquids are usually discharged directly and cause environmental pollution. There are few publications or industrial pilot studies for the recovery of pectic polysaccharides from the processing water and reducing the COD value. Most research on food waste utilization in citrus industry is focused on citrus peels (Pfaltzgraff et al., 2013; Ravindran and Jaiswal, 2016) rather than the processing water.

This study makes an effort to establish a pilot plant scale pectic polysaccharide recovery system for effluent treatment in the citrus fruit canning industry. The effluent water quality was evaluated and the processing water was reused (prior to recovery procedure) for enriching the polysaccharides and meanwhile for water saving. The process of polysaccharides recovery was optimized, and the products were evaluated by chemical and physical methods. A comparison of the polysaccharides from singly used and reused water was conducted to evaluate product stability on water reuse. The recovered pectic polysaccharides might be useful as healthy food additives for gelling, thickening and emulsifying, creating economic value and meanwhile protecting the environment.

## 2. Materials and methods

### 2.1. Resource for polysaccharides extraction

The acidic and basic water, discharged from citrus canning factories during the segment membrane removal process, was collected from citrus fruit canning factories in China. The acidic or basic water was divided into singly used and reused water, sorted

by an adjustable water reuse system, and samples were abbreviated as PAS, PAR, PBS and PBR for polysaccharides recovered from acidic singly used and reused water, basic singly used and reused water, respectively (also indicated visually in Fig. 1.), and the polysaccharides recovered from acidic and basic water, whether under single use or reuse, were collectively called PA and PB, respectively. The 95% (v/v) ethanol used as polysaccharides precipitant is food grade, as well as other agent or materials used in the polysaccharides extraction.

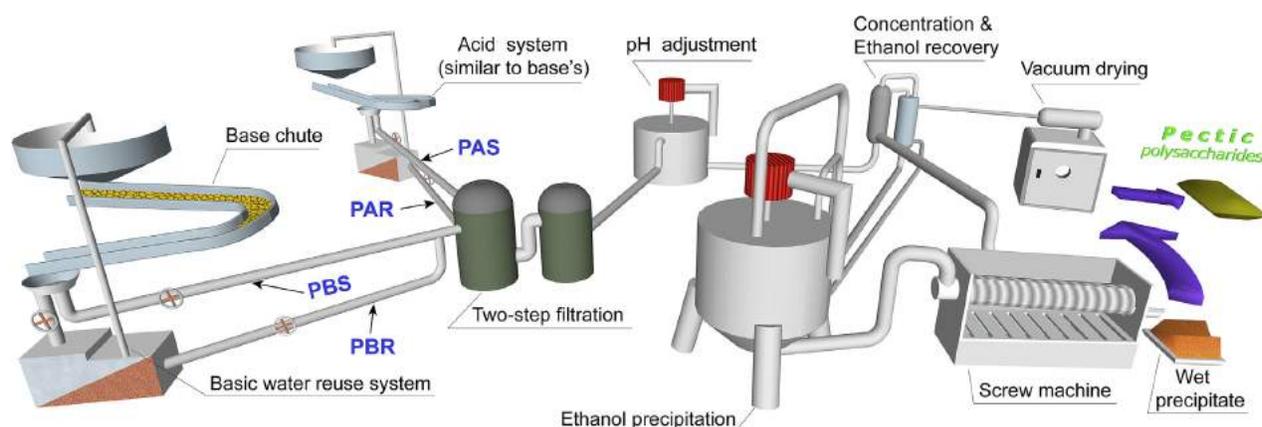
### 2.2. Analysis of effluent water quality

The COD value of effluent water from segment membrane removal process was determined by COD test tube assay on Spectroquant<sup>®</sup> NOVA60 (Merck, Germany), and all the water samples were representatively collected in a volume of 1 L and shaken sufficiently before sampling 1 mL into the test tube. The same sampling method was applied in the following test. The biochemical oxygen demand (BOD<sub>5</sub>) was determined by OxiTop BOD equipment (WTW, Germany). Ammonia nitrogen was determined by test tube assay on Spectroquant<sup>®</sup> NOVA60 (Merck, Germany). The microbial status including total viable counts and total coliform counts of a water sample were determined after adjusting to neutral pH by standard microbial methods. Total polysaccharides content and GalA content of the water samples were determined after dilution by the method of phenol-sulfuric acid assay (Dubois et al., 1956) and m-phenylphenol assay (Blumenkrantz and Asboe-Hansen, 1973), respectively. Other water indexes including pH, turbidity, chroma, total solids and soluble solids content were determined according to the standard methods (Chinese Environmental Protection Administration, 2002). All the determinations were conducted in triplicate.

### 2.3. Polysaccharides recovery

The plant scale process of the polysaccharides recovery is visually shown in Fig. 1. All the equipment is made of 316 stainless steel for chemical and heat resistance. The water from acid (hydrochloric acid) or base (sodium hydroxide) chute represents the suspended extract liquid of citrus segments membrane. The water designed for either singly using or reusing, and that can be selected by switching valves, to collect different samples easily. At the beginning of polysaccharides recovery, a two-step filtration with 200 and 400 meshes filters (size:  $\Phi \times h = 1 \text{ m} \times 2 \text{ m}$ , 0.3 kw\*2) were used to eliminate the suspended solid particles, and then the filtrate was pumped (13 m<sup>3</sup>/h, 11 kw) to the pH adjustment reactor (volume: 8 m<sup>3</sup>, stirring power: 4 kw) for neutralization, followed by vacuum concentration (size: 5 m × 6 m × 9 m, 40 kw) at 70 °C. Precipitation (volume: 4 m<sup>3</sup>, stirring power: 2 kw) by ethanol at final ethanol concentration of 50% was performed with gentle stirring. After standing for 30 min, precipitation was completed and a screw machine (size: 3 m × 0.6 m × 2 m, 0.75 kw) was applied to recover the precipitates, which were the polysaccharides (insoluble in ethanol solution), and the filtrate was then transported to the alcohol recovery unit (integrated with the concentration unit, 12 kw). The precipitate was washed once with 95% ethanol and again ethanol recovered. Subsequently, vacuum drying (size: 1.5 m × 1.5 m × 1.7 m, 5 kw) was conducted on precipitate also with ethanol recovery. The dry polysaccharides were ground into powder to obtain potential pectin product. The yield of polysaccharides is calculated using the formula of  $y = w/v \times 100$ , here y is the yield in % (w/v); w is the weight of dry product powder in kg; v is the volume of acidic or basic water for polysaccharides recovery in L.

During the process of pectic polysaccharides recovery, the acidic



**Fig. 1.** Schematic diagram of the plant scale process of pectic polysaccharides recovery. Polysaccharides sample names are marked on the lines of the corresponding raw material water, and PAS, PAR, PBS, PBR are short for the polysaccharides recovered from acidic singly used and reused water, basic singly used and reused water, respectively.

and basic water was concentrated before precipitation. Thus, a concentration ratio from 1-fold to 4-fold was optimized using an industrial concentrator system to obtain an ideal concentrated solution. Since the difficulties of end volume control during industrial scale concentration, the total solids content divided by the original total solids content, was used to calculate the concentration ratio. Liquids after concentration with four ratios were sampled to determine the Bostwick consistency, which is expressed by the distance (cm) of the sample flowing in a Bostwick consistometer over 30s, determined in triplicates.

Polysaccharides recovery involves another unit as alcohol precipitation. The economical use of ethanol is essential to control production cost. Five gradient values of ethanol percentage (calculated based on the final suspension, v:v) used in precipitation were designed. Under each ethanol concentration, the production yield and process economics were examined to obtain an optimal process.

#### 2.4. Chemical composition determination

The monosaccharide composition was determined by a modified 1-phenyl-3-methyl-5-pyrazolone (PMP) – high performance liquid chromatography (HPLC) method (Strydom, 1994). In brief, pectic polysaccharides samples (typically 2–3 mg) were hydrolyzed with 2 M trifluoroacetic acid at 110 °C for 8 h, then dried under a stream of nitrogen and neutralized with 0.1 M sodium hydroxide. The hydrolyzates with internal standard (lactose) were derived with 450  $\mu$ L PMP solution (0.5 M, in methanol) and 450  $\mu$ L of 0.3 M sodium hydroxide at 70 °C for 30 min. The reaction was stopped by neutralization by 0.3 M hydrochloric acid, and excess reagent was extracted with chloroform (3  $\times$  1 mL). HPLC analysis was performed by Waters e2695 (Waters, US) on a Zorbax Eclipse XDB-C18 column (250 mm  $\times$  4.6 mm, 5  $\mu$ m, Agilent, USA) at 25 °C with UV detection (2489 UV/Vis Detector, Waters, US) at 250 nm. The mobile phases were: solvent A, 15% acetonitrile with potassium phosphate buffer (0.05 M, pH 6.9), solvent B, 40% acetonitrile with the same buffer. Elution relied on a gradient of B from 0% to 15% in 10min, then from 15% to 25% in the next 20 min at the flow rate of 1 mL/min.

The degree of methylation (DM) and the degree of acetylation (Dac) were calculated from methanol and acetic acid content with the GalA% determined by the m-phenylphenol method. The methanol and acetic acid were determined following saponification of polysaccharides by HPLC (Waters 1525, US) on a C18 column

(SinoChrom ODS-BP 250 mm  $\times$  4.6 mm, 5  $\mu$ m, Elite, China) with refractive index (RI) detection (Waters 2414, US) using isopropanol as internal standard and eluting with 4 mM sulfuric acid (Levigne et al., 2002).

#### 2.5. IR spectral analysis

The Fourier transform (FT) infrared (IR) analysis was applied to obtain IR spectra of the polysaccharides using a Nicolet Avatar 370 instrument. Samples (~1 mg) were ground together with 200 mg KBr, pressed into pellets for IR scanning from 400 to 4000  $\text{cm}^{-1}$ . The degree of esterification and other functional groups were determined.

#### 2.6. SEC-MALLS analysis

Pectic polysaccharides was dissolved in 0.2 M NaCl solution to the concentration of 1 mg/mL, filtered through a syringe-filter (membrane pore size, 0.45  $\mu$ m) prior to injection using a 100  $\mu$ L sample loop. The molar mass and root mean square (RMS) radius of gyration were determined through high-performance (HP) size-exclusion chromatography (SEC) equipped with multi-angle laser-light scattering (MALLS) (Wyatt Dawn Heleos-II, USA) and RI detector at 25 °C. Isocratic elution with 0.2 M NaCl solution at a flow rate of 0.75 mL/min was performed on a series of columns including Shodex OH SB-G (pre-column), Shodex SB-806 HQ and Shodex SB-804 HQ (Showa Denko KK, Japan). The molar mass was calculated using the  $dn/dc$  value of 0.1355 and 0.0880 mL/g for polysaccharides recovered from acidic and basic water, respectively.

All the results above with multiple determinations were reported as means  $\pm$  standard deviations.

#### 2.7. Rheological measurements

The rheology properties were determined using a plate (50 mm) and plate geometry rheometer (Anton Paar MCR 302, Austria) at 25 °C with 1 mm sample gap. The relationship between viscosity and shear rate (0.01–100  $\text{s}^{-1}$ ) of the samples aqueous solution with 1% and 2% (w/v) concentrations were studied to evaluate the flow behavior. Before frequency sweep tests, the linear viscoelastic range was determined by strain sweep from 0.01% to 100% at a constant angular frequency of 10 rad/s. A strain of 1% (smaller than the maximum value of linear viscoelastic range) was recommended by the Rheoplus for the subsequent experiment. Thus, the angular

**Table 1**

The quality of the acidic and basic water was examined, including singly used and reused water.

Singly used water						
	pH	COD (mg/L)	BOD <sub>5</sub> (mg/L) <sup>a</sup>	Ammonia nitrogen (mg/L)	Total bacterial count (CFU/mL)	The coliform group (CFU/mL)
Acidic	1.17 ± 0.3	6600 ± 736	2180 ± 350	0.7 ± 0.1	0	0
Basic	13.11 ± 0.2	6530 ± 582		1.2 ± 0.2	0	0
	Turbidity (NTU)	Chroma (HU, platinum-cobalt color)	Total solids content (mg/L)	Soluble solid content (mg/L)	Total sugar (mg/L)	GalA content (mg/L)
Acidic	410 ± 32	62 ± 6	4935 ± 424	3785 ± 219	3394 ± 103	1720 ± 55
Basic	297 ± 17	1950 ± 21	9040 ± 728	7970 ± 380	3750 ± 114	1792 ± 67
Reused water						
	pH	COD (mg/L)	BOD <sub>5</sub> (mg/L) <sup>a</sup>	Ammonia nitrogen (mg/L)	Total bacterial count (CFU/mL)	The coliform group (CFU/mL)
Acidic	1.15 ± 0.2	9980 ± 891	3220 ± 510	0.9 ± 0.2	0	0
Basic	13.14 ± 0.2	9430 ± 680		1.5 ± 0.2	0	0
	Turbidity (NTU)	Chroma (HU, platinum-cobalt color)	Total solids content (mg/L)	Soluble solid content (mg/L)	Total sugar (mg/L)	GalA content (mg/L)
Acidic	530 ± 60	75 ± 9	7425 ± 620	5634 ± 369	5190 ± 132	2571 ± 75
Basic	375 ± 22	2750 ± 41	13,050 ± 980	11,500 ± 430	5432 ± 145	2610 ± 86

<sup>a</sup> BOD<sub>5</sub> as a Biochemical Oxygen Demand, mixing acidic and basic water to offer a wastewater with neutral pH was conducted.

frequency sweep was conducted from 0.1 to 100 rad/s at 1% stain to monitor the changing of storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of the samples.

### 3. Results and discussion

#### 3.1. Effluent water quality

The water quality is presented in Table 1. The high COD values obviously show the requirement to treat the effluent water from citrus canning industry even for the singly used water. The water reuse system increased the COD concentration and further increased the need for water treatment. Since the BOD<sub>5</sub> was much smaller than COD, a bio-contact oxidation process may not be effective for improving water quality.

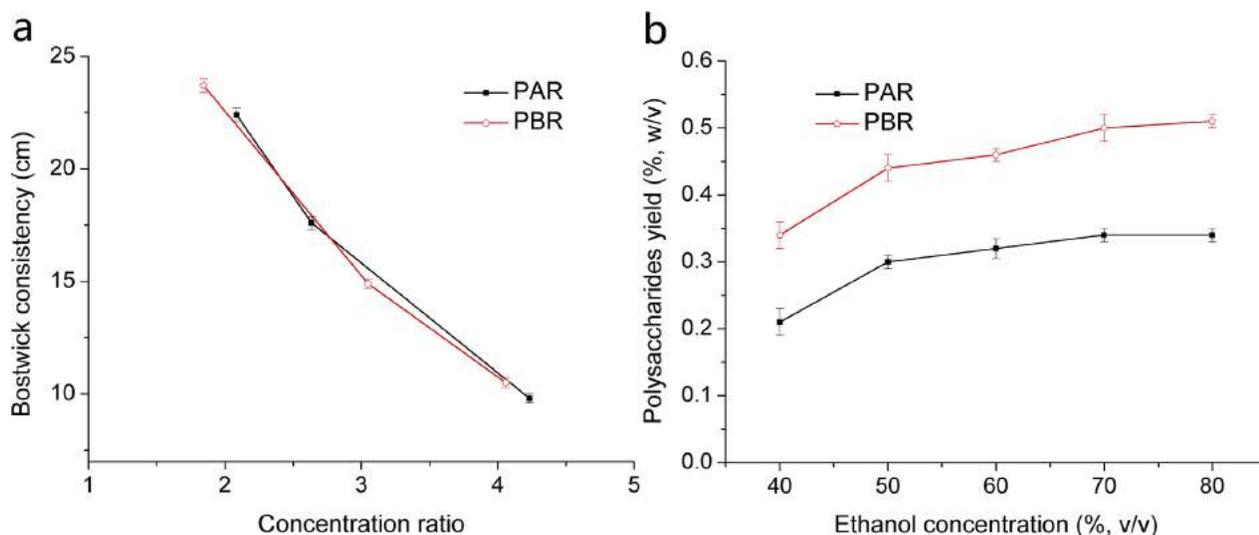
The soluble solids, total sugars and GalA content of both acidic

and basic water are relatively high and increased after water reuse, which indicates the nutrients is inside and can be condensed for prospective recovery and utilizing. The high GalA content in both processing water indicates the organic substances are mainly contributed by pectic polysaccharides. The microbial status suggested the safety of the recovered polysaccharides from the processing water for developing as food additives or nutrients. Other water indexes, such as pH and turbidity, will also be improved after polysaccharides recovery.

#### 3.2. Recovery of pectic polysaccharides

##### 3.2.1. Optimization of polysaccharides recovery process

Although the polysaccharides content have been increased with water reuse system, it is still not sufficiently economic for the direct recovery of the pectic polysaccharides by ethanol precipitation, as a



**Fig. 2.** (a) Concentration leads to consistency increasing of the raw material water containing PAR and PBR, the consistency of ratio "1" were not shown as the value is out of the measurement range (bigger than 25 cm); (b) yields of polysaccharides vs. different ethanol concentrations applied in precipitation.

**Table 2**  
Economic calculations of ethanol-related costs and polysaccharides yields (based on 2.5 m<sup>3</sup> concentrated acidic or basic water during alcohol precipitation).

Sample	Final alcohol concentration during precipitation (% v/v)	95% ethanol additive amount (m <sup>3</sup> ) <sup>a</sup>	Cost of power consumption for ethanol recovery (\$ A) <sup>b</sup>	Cost of ethanol loss (\$ B) <sup>c</sup>	Polysaccharides yields (% w/v)	Estimated output value (\$ C) <sup>d</sup>	P=C-A-B (\$ P) <sup>e</sup>
PAR (from acidic water)	40	1.82	~21	~33	~0.21	~189	~135
	50	2.78	~26	~50	~0.3	~270	~194
	60	4.29	~33	~78	~0.32	~288	~177
	70	7.00	~46	~127	~0.34	~306	~133
	80	13.33	~76	~242	~0.34	~306	~12
PBR (from basic water)	40	1.82	~21	~33	~0.34	~306	~252
	50	2.78	~26	~50	~0.44	~396	~320
	60	4.29	~33	~78	~0.46	~414	~303
	70	7.00	~46	~127	~0.5	~450	~277
	80	13.33	~76	~242	~0.51	~459	~141

<sup>a</sup> The volume of water/ethanol mixture was simply calculated by water volume plus ethanol volume.

<sup>b</sup> Power cost contains electricity (\$0.14/kwh in China, 1 m<sup>3</sup> water/ethanol mixture needs 12kw\*0.4 h recovering) and superheated steam (\$26/ton in China, 1 m<sup>3</sup> water/ethanol mixture needs 0.16 ton steam for ethanol recovery).

<sup>c</sup> The price of 95% ethanol is \$766/ton in China. The ethanol loss rate is estimated at 3% (v/v).

<sup>d</sup> The price of pectic polysaccharides is estimated at \$12/kg on average.

<sup>e</sup> "P", calculated by the formula "P = C-A-B" (A,B and C are labeled on Table 2), can reflect the cost efficiency.

large amount of ethanol would be consumed in an industrial-scale recovery process. Thus, an industrial concentrator system was utilized to concentrate of the solution. Bostwick consistency (the greater the Bostwick consistency value the lower the fluid viscosity), as a function of concentration ratio of the PAR and PBR samples, is presented in Fig. 2a. (these two samples are expected to be finally applied). We find that the ratio of about four is almost the limit value for concentration giving excessive viscosity, preventing fluid flow in industrial concentrator pipes. A concentration ratio of around three is the best, with acceptable flow behavior and energy consuming, maximally reducing the water volume (subsequently limiting the cost of ethanol).

The polysaccharide in the concentrated acidic and basic water was further precipitated by a series of different ethanol percentages. The yields of different precipitation with several final alcohol concentrations are shown in Fig. 2b. We found that the more the ethanol used, the better the recovery yield obtained. The polysaccharides yield increased greatly from 40% ethanol to 50% ethanol, and then increased slowly in a range of 50%–80%. A final ethanol concentration of 70% almost resulted in the greatest yield, while 80% ethanol afforded an insignificant improvement in yield but significantly increased the consumption of ethanol. Further economic calculations with overall consideration on the yields and costs are shown in Table 2 to optimize the final alcohol concentration. The 50% alcohol concentration group with a maximum value of "P = C-A-B" is the optimized concentration for both PAR and PBR precipitation. That is, a 50% final ethanol concentration in an industrial precipitation can obtain an acceptable yield at relatively low cost.

### 3.2.2. Positive effect of polysaccharides recovery

The water-reuse system, designed for polysaccharides enriching, resulted in a lower cost for concentration and ethanol precipitation, and also reduced both the water consumption and water fee (Table 3). Based on the ~100 days per year running for citrus canning factories, the reduction of water fee would reach to \$15,000 annually for a single factory. The yield of polysaccharides recovered by the optimized process was increased by water reuse from 0.19 to 0.29% for PA and from 0.30 to 0.45% for PB, resulting in a large estimated output value of polysaccharides product. Based on the limited cost of optimized production, the estimated profit is great (Table 3), suggesting that it is economically feasible to recover polysaccharides from the processing water. Meanwhile, the COD concentration is dramatically decreased during the polysaccharides recovery with other organic substances removed at the same time. The COD emissions can be reduced up to ~30,000 ton annually by citrus canning industry in China, having large environmental benefit.

### 3.3. Chemical composition analysis

The chemical composition of the saccharide components, the DM, and the DAC were determined to investigate the potential application of recovered polysaccharides (Table 4). HPLC analysis shows GalA (in mole%) is a principle component of PAS and PAR, while arabinose (Ara) is the main saccharide present in PBS and PBR. The small content of other monosaccharides present in all four polysaccharides, indicates possible chain branching.

The DM assay of PAS or PAR shows a relatively high (~50%) DM,

**Table 3**  
Environmental and economic effects (one day, one factory) of the four polysaccharides (PAS, PBS, PAR and PBR) recovery processes.

	Acidic/basic water consumption (m <sup>3</sup> )	Water cost (\$) <sup>a</sup>	Yields (% w/v)	Estimated output value (\$) <sup>b</sup>	Cost of power consumption (\$) <sup>c</sup>	Cost of ethanol loss (\$) <sup>d</sup>	Cost of equipment depreciation (\$) <sup>e</sup>	Estimated COD removal profit (\$) <sup>f</sup>	rate (%)	Reduction of COD emissions (ton)
PAS line	~320	~250	0.19 ± 0.04	~7296	~4160	~2152	~226	~758	63 ± 3	~1.33
PBS line	~290	~226	0.30 ± 0.05	~10,440	~3770	~1950	~226	~4494	82 ± 4	~1.56
PAR line	~210	~164	0.29 ± 0.05	~7308	~2730	~1412	~226	~2940	67 ± 2	~1.41
PBR line	~200	~156	0.45 ± 0.07	~10,800	~2600	~1345	~226	~6629	86 ± 3	~1.63

<sup>a</sup> The industrial water cost is \$0.78/m<sup>3</sup> in China.

<sup>b</sup> The price of pectic polysaccharides is estimated at \$12/kg on average.

<sup>c</sup> Power cost contains electricity (\$0.14/kwh in China) and superheated steam (\$26/ton in China). It needs 26 kwh and 0.36 ton steam to recover polysaccharides from 1 m<sup>3</sup> acidic/basic water.

<sup>d</sup> The price of 95% ethanol is \$766/ton in China. The ethanol loss rate is estimated at 3% (v/v).

<sup>e</sup> The depreciation rate is 10% per year.

<sup>f</sup> The profit is calculated by "Estimated output value" minus "the costs of power, ethanol and depreciation".

**Table 4**  
Chemical properties of recovered polysaccharides.

	Monosaccharides (mol%)									DM <sup>a</sup> (%)	Dac <sup>b</sup> (%)
	Man	Rha	GlcA	GalA	Glc	Gal	Xyl	Ara	Fuc		
PAS	1.32 ± 0.02	3.05 ± 0.01	0.31 ± 0.01	46.92 ± 0.01	7.39 ± 0.03	11.83 ± 0.02	1.60 ± 0.03	23.47 ± 0.02	4.11 ± 0.03	51.08 ± 1.25	1.03 ± 0.12
PBS	0.35 ± 0.06	6.32 ± 0.12	0.14 ± 0.01	16.42 ± 0.96	2.20 ± 0.61	15.91 ± 0.93	2.72 ± 0.05	50.95 ± 1.55	4.99 ± 1.1	5.73 ± 0.67	0.58 ± 0.09
PAR	1.14 ± 0.01	3.69 ± 0.01	0.3 ± 0.07	44.95 ± 1.45	3.15 ± 0.31	8.91 ± 0.13	2.08 ± 0.03	22.87 ± 0.25	12.91 ± 0.78	48.84 ± 0.92	0.94 ± 0.15
PBR	0.48 ± 0.03	4.78 ± 0.08	0.33 ± 0.01	21.72 ± 0.09	1.23 ± 0.05	14.36 ± 0.15	3.77 ± 0.05	42.65 ± 0.65	10.68 ± 0.66	4.38 ± 0.35	0.34 ± 0.11

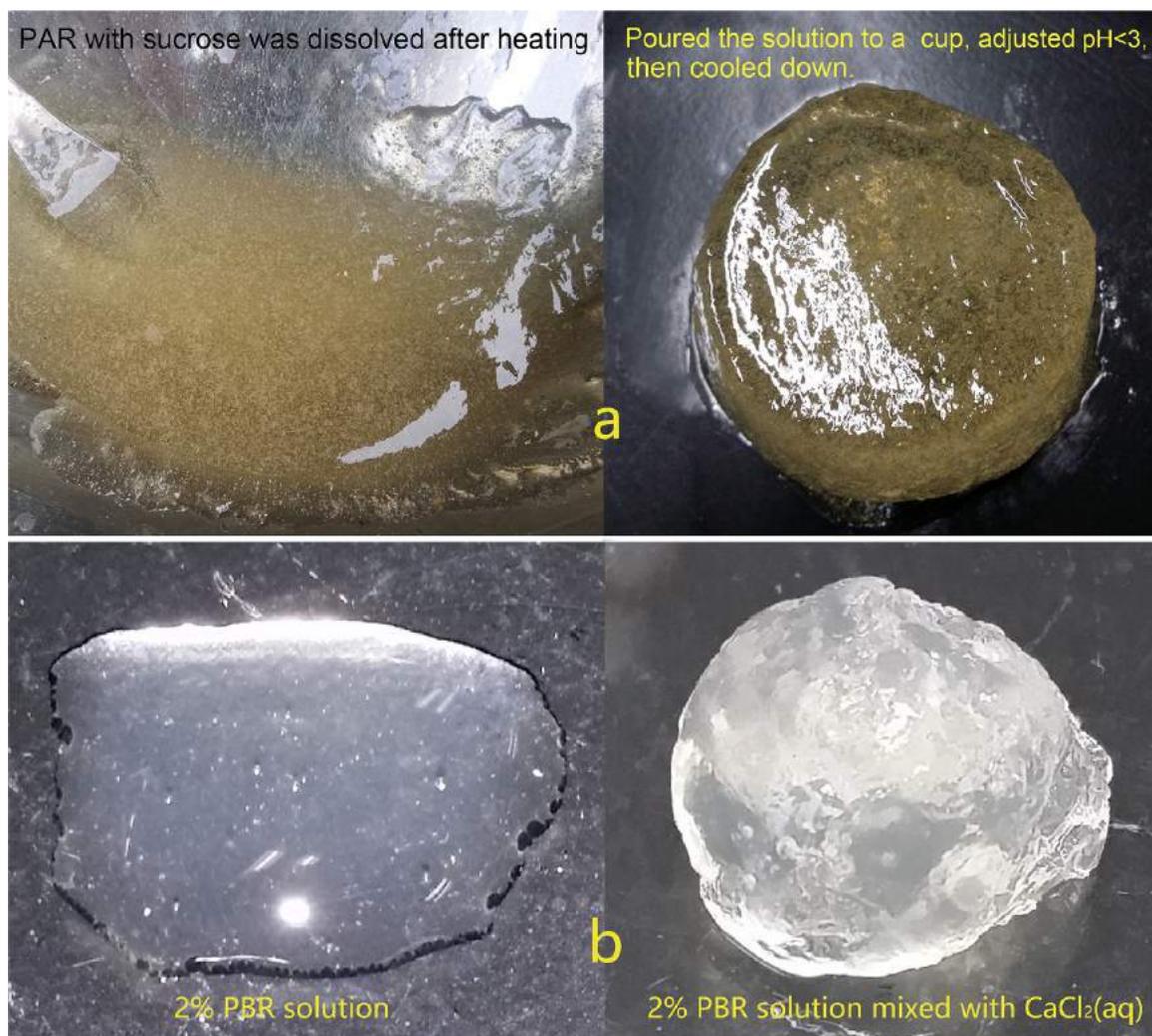
<sup>a</sup> DM: degree of methylation.

<sup>b</sup> DAc: degree of acetylation.

and they almost correspond to high-methoxyl pectins (50% < DM < 100%, HMP). A verification test was conducted for PAR by the HMP gelling procedure (Kastner et al., 2014) and the results are shown in Fig. 3a. Based on the medium methoxyl content of PAR compared with HMP and low-methoxyl pectin (LMP, 0% < DM < 50%), PAR gelling under the HMP procedure combined with LMP gelling process with Ca<sup>2+</sup> resulted in higher gel strength (data not shown).

PBS and PBR have low methoxyl content. Although they contain

relative low GalA, a verification test, mixing PBR and CaCl<sub>2</sub> with Ca<sup>2+</sup> concentration of 12.5 mM (Moreira et al., 2014), still indicates that PBR can be applied as a potential LMP (Fig. 3b). However, more detailed optimization of the gelling needs to be done. Moreover, Ara is a major saccharide in pectin side chain of the rhamnogalacturonan I region (Willats et al., 2006), the key region of pectin accounting for the health benefits (Maxwell et al., 2012). Thus, PBS and PBR represent a healthier food additive.



**Fig. 3.** Verification test of (a) PAR as high-methoxyl pectin and (b) PBR as low-methoxyl pectin.

### 3.4. FTIR spectra analysis

The FTIR spectra of these four different samples (PAS, PAB, PBS and PBR) are shown in Fig. 4. Typical pectic polysaccharides are presented, and signals at 1740 and 1630  $\text{cm}^{-1}$ , correspond to the carboxylic ester and carboxylic acid groups of pectin, respectively (Chatjigakis et al., 1998). The degree of methylation (DM) of pectin was estimated from dividing the signal at 1740  $\text{cm}^{-1}$  by the sum of the signal at 1740 and 1630  $\text{cm}^{-1}$  (Fellah et al., 2009; Gnanasambandam and Proctor, 2000). The results show that only the samples recovered from acidic water (PAR and PAS) gave an absorbance at 1740  $\text{cm}^{-1}$ , with an estimated DM value of about 50%, consistent with the results from HPLC determination. The polysaccharides from basic water (PBR and PBS) show an obvious peak at 1630  $\text{cm}^{-1}$ , indicating almost non-esterified pectic polysaccharide. This is probably due to alkaline saponification during the process.

Therefore, IR spectra of the polysaccharides from acidic (PAS and PAR) and basic water (PBS and PBR) confirmed them should be an almost HMP and potential LMP, respectively. In addition, the similar DM value between polysaccharides from singly used and reused water indicates good product stability under the process of water reuse with respect to esterification degree.

### 3.5. SEC-MALLS-RI analysis

Molecular size, evaluated by molar mass and root mean square (RMS) radius of gyration (Rg), is an important index for polymer characterization and influences polymer behavior. SEC-MALLS-RI system gives more accurate information for molecular size of the recovered polysaccharides (Table 5). The weight average of molar mass (Mw) of PA and PB are both larger than commercial pectin (~120 kDa) (Corredig and Wicker, 2001), implying the potential of high viscosity of PA and PB. The Mw of PA was significantly smaller than that of PB. However the z-average root mean square radius of gyration (Rz) of PA is larger than that of PB. This may be due to different chemical compositions of the two kinds of

**Table 5**

Average values of molecular weight and radius of pectic polysaccharides recovered from acidic water (PAS, PAR) and basic water (PBS and PBR).

	Mw <sup>a</sup> (kDa)	Mn <sup>b</sup> (kDa)	Polydispersity (Mw/Mn)	Rz <sup>c</sup> (nm)
PAS	483.5 ± 0.6%	233.0 ± 1.0%	2.075 ± 1.182%	52.2 ± 1.0%
PBS	1888.1 ± 0.30%	391.5 ± 0.4%	4.823 ± 0.49%	37.9 ± 0.90%
PAR	537.7 ± 0.6%	243.7 ± 0.7%	2.207 ± 0.929%	49.8 ± 1.1%
PBR	1766.4 ± 0.3%	363.0 ± 0.4%	4.866 ± 0.516%	36.4 ± 1.1%

<sup>a</sup> Mw: weight-average of Molar mass.

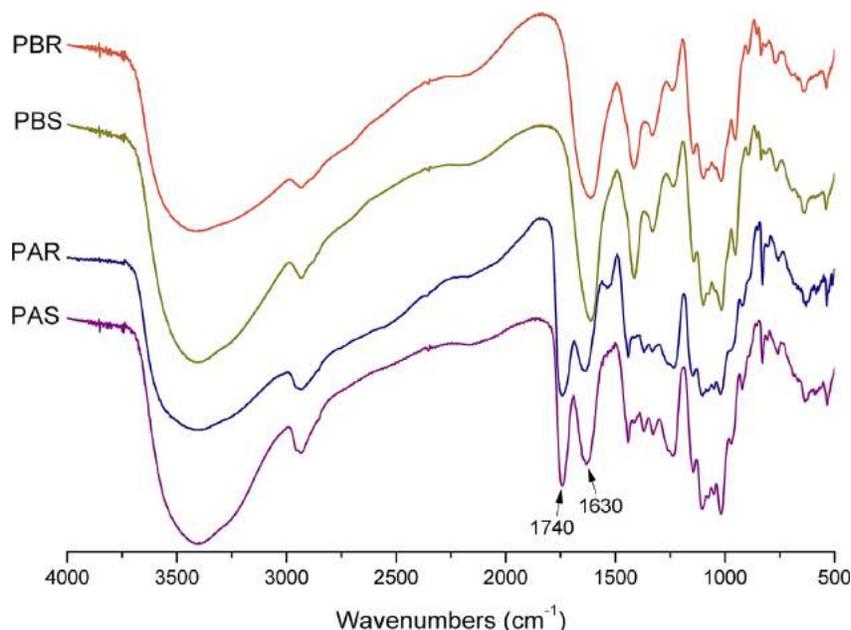
<sup>b</sup> Mn: number-average of molar mass.

<sup>c</sup> Rz: z-average of root mean square radius of gyration.

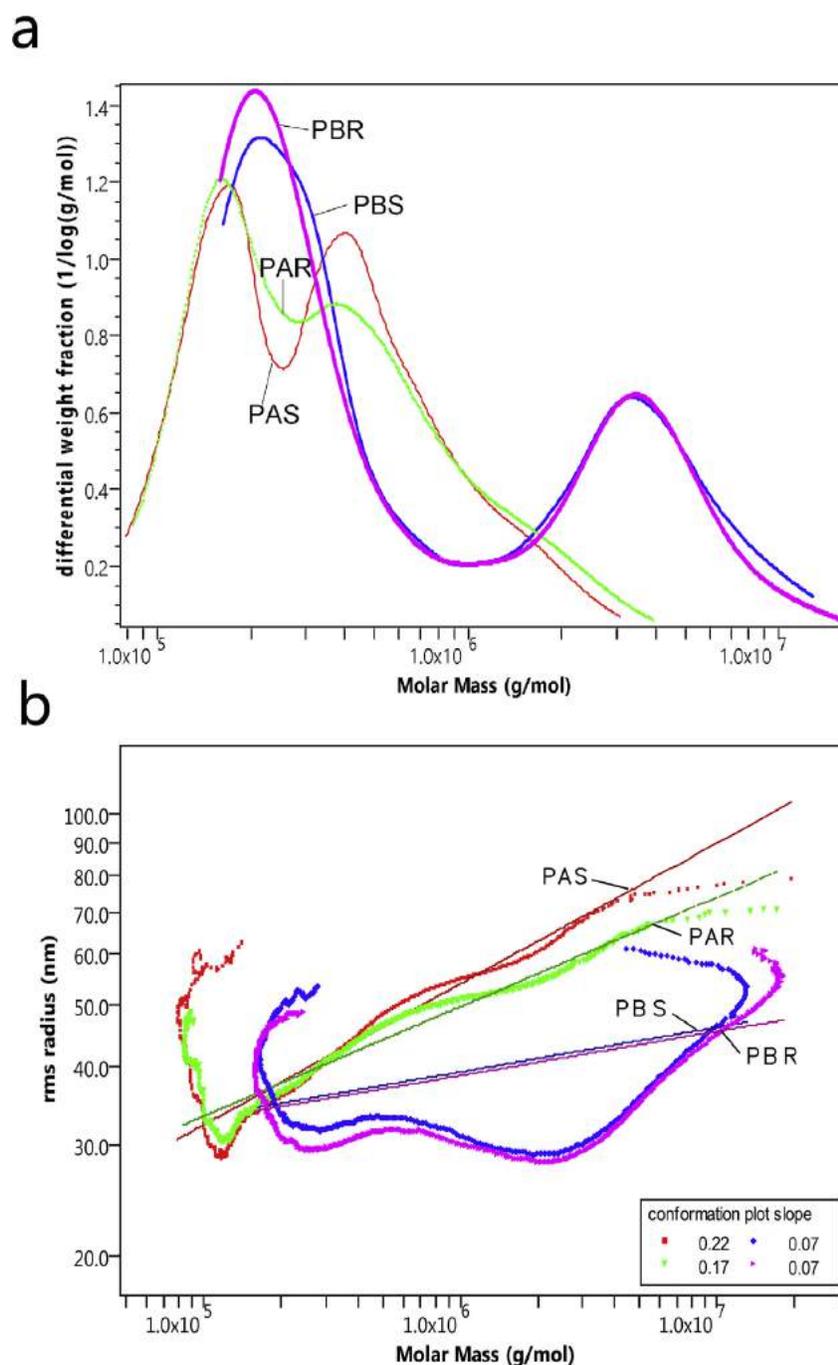
polysaccharides which subsequently has different molecular pattern. The polysaccharides containing higher Ara have a larger molecular weight but smaller Rz than those containing higher GalA (Nakauma et al., 2008). This indicates the molecular structure of PB is “tighter” than PA, and this “tight” structure is additionally due to PB being highly branched (illustrated in Fig. 5b.). Only a slight difference in the molecular size was observed between samples from singly used water and reused water, indicating water reuse is feasible on recovered product stability.

The differential weight fraction plots clearly indicate that PA and PB have different distributions of molar mass although both of them have two major peaks (Fig. 5a). PB presents two larger molar mass peaks, and the bigger peak even centers at about  $3.5 \times 10^6$  g/mol, while the smaller peak is only around  $2.5 \times 10^5$  g/mol, indicating PB was a larger molecule with higher polydispersity (Table 5). The log-log plots of RMS radius vs. molar mass (Fig. 5b.) indicate all polysaccharides were branched especially for PBS and PBR by the theory of the slope value of linear fitting that isn't 0.333, 0.5–0.6 or 1.0 (Gnanasambandam and Proctor, 2000; Wyatt, 1993) and the abnormal SEC phenomenon with the plots shifting upward at the low molar mass region (Podzimek et al., 2001).

After water reuse, an increasing proportion of smaller molecules and decreasing proportion of larger molecules were observed in PAR and PBR (region from  $2 \times 10^5$  to  $7 \times 10^5$  g/mol), indicating slight degradation had occurred. However, the level of degradation



**Fig. 4.** FTIR spectra of recovered pectic polysaccharides.



**Fig. 5.** (a) Molar mass distribution by differential weight fraction. (b) Conformation plot of RMS radius vs. molar mass, determining with PAS, PAR, PBS and PBR in 0.2 M NaCl solution.

estimated by proportion changing was tolerable for both samples, suggesting the influence of water reuse on the stability of polysaccharides product can be ignored.

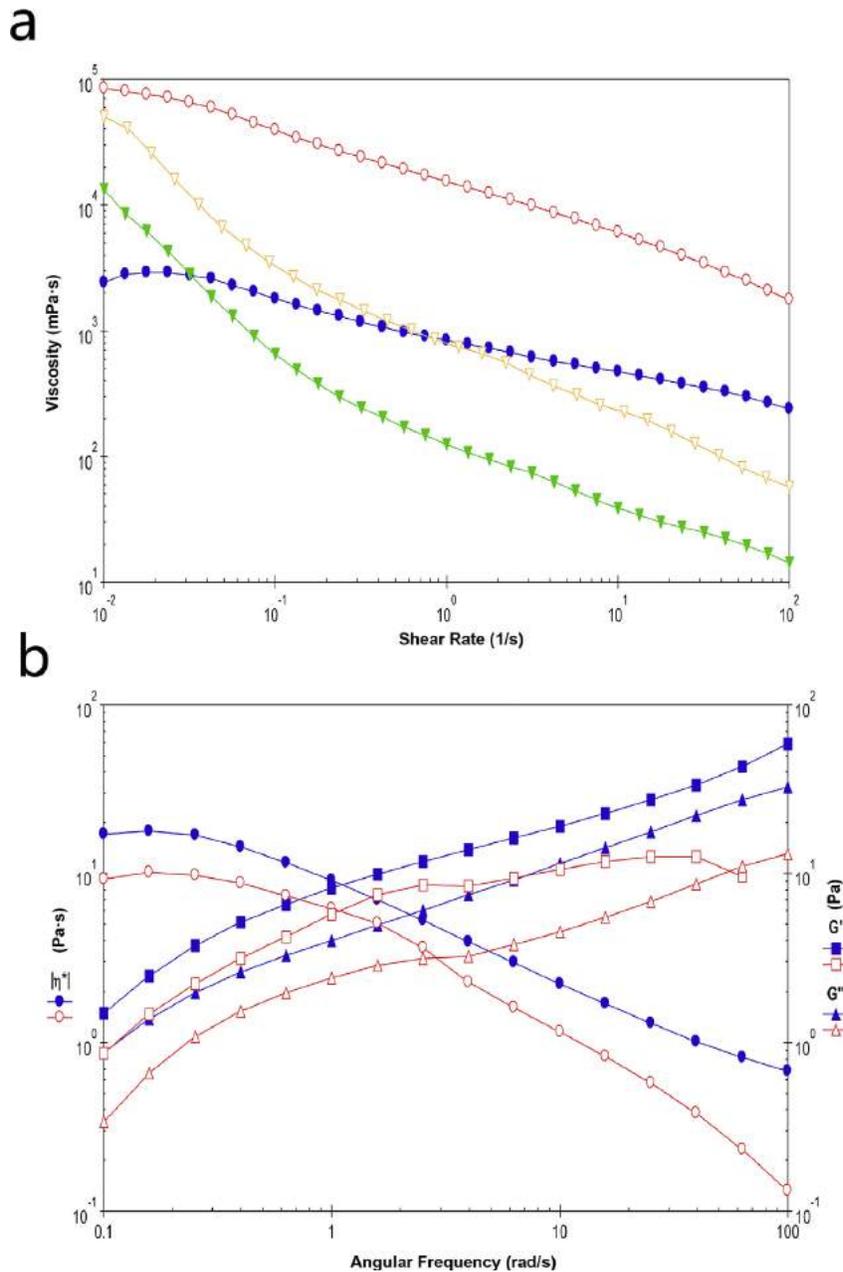
### 3.6. Rheology properties

PAR and PBR were chemically stable after water reuse according to the above analysis. Thus, their rheological properties were

analyzed to further evaluate the feasibility of application as food thickening or gelling agents.

#### 3.6.1. Steady shear flow behavior

The flow properties of PAR and PBR solution show viscosities that changed with increasing shear rate (Fig. 6a), demonstrating that they were typical pseudoplastic fluids, with shear-thinning fluid behavior (Bao et al., 2016). Compared with other



**Fig. 6.** (a) The flow behaviors of (○) PAR 2%, (●) PAR 1%, (▽) PBR 2% and (▼) PBR 1%. (b) Angular frequency sweep for PAR 1% (filled symbols) and PBR 2% (open symbols). The storage modulus ( $G'$ , ■ or □), loss modulus ( $G''$ , ▲ or △) and complex viscosity ( $|\eta^*|$ , ● or ○) are presented.

polysaccharides, PBR samples had the greater shear-thinning property, indicating the orientation of PBR molecular chain can be more easily obtained by shearing (Xu et al., 2016). This property may be due to the low GalA content and low degree of methylation of PBR, which resulted in low viscosity and little interactions between molecular chains (Wang and Lü, 2014). Higher concentrations of PAR or PBR solution display increased viscosity (Fig. 6a), suggesting the concentration dependence of viscosity and can be utilized to prepare a desired viscosity for an application partly by polysaccharides concentration control.

Both of PAR and PBR have a higher viscosity (~50 Pa s) than the commercial pectins (~2 Pa s) at near zero shear-rate (Wang and Lü,

2014), indicating their potential as food thickeners and the market value of PAR and PBR. Moreover, PAR and PBR with different shear-thinning ability can be used in different food processes with special demands, such as milk beverage (can easily flow when they are shaken) and pasty (need shear-thinning mouthfeel).

### 3.6.2. Dynamic viscoelastic properties

The steady shear results show the PAR 1% and PBR 2% will have excellent viscosities for further applications. The oscillatory measurements of these two samples were performed to determine  $G'$  (elastic response) and  $G''$  (viscous response) as functions of angular frequency, under a strain of 1% (Fig. 6b). The  $G'$  and  $G''$  moduli of

PAR were larger than those of PBR, indicating that more molecule entanglement occurred in PAR leading to increased viscoelastic properties (Wang and Lü, 2014). The larger  $G'$  than  $G''$  in the selected frequency range indicates polymer chain interactions inside PAR solution can promote gel formation, and because  $G'$  can reflect the degree of elasticity, thus, PAR solution can be inferred weak gel was formed (Geresh et al., 2002; Xu et al., 2016).

PBR solution also exhibits weak gel formation properties. Larger  $G'$  than  $G''$  is presented at most angular frequency. However, the gel properties of PBR would be gradually destroyed starting at 40 rad/s, and reached to viscous fluid property at 55 rad/s, where  $G'$  and  $G''$  crossed.

In conclusion, PAR and PBR solution both exhibit gel-like properties under a general angular frequency and PAR had greater gelling potential even at low (1%) concentration, indicating that both polysaccharides probably had good performance as food thickeners or gelling agents.

#### 4. Conclusions

Environmental protection is becoming increasingly important. The citrus canning industry is economically challenged and has serious problems associated with process water treatment. The water during chemical hydrolysis process with very high COD (~10,000 mg/L) will decrease the average quality of total effluent. Thus, treating the acidic and basic process water produced in segment membrane removal is the critical solution.

We established a plant scale processing line to recovery of pectic polysaccharides from acidic and basic singly used or reused (enriched in polysaccharides) water with the yield up to 0.45% (w/v), eliminating COD for ~65%–85%, that is, could reducing ~30,000 ton COD emissions annually in China, and highly decreasing process costs and creating large product income.

The chemical analysis shows the different saccharides composition and methoxyl content of polysaccharides from acidic and basic water, indicating the two products could be applied in different ways and areas. The product from acidic water, with higher GalA content (~46 mol%) and higher viscosity based on rheology testing, may have greater potential in gel applications. The product from basic water with ~45 mol% Ara would be a healthier additive for thickening or emulsifying, for example, using as a fat replacer (like bread spread) represents a promising application. The recovered polysaccharides have relatively high molecular weight (500–1800 kDa), and the basic water polysaccharides are highly branched. Water reuse has little influence on polysaccharides' characteristic, so the product from reused water could be regarded as stable.

The present study with the aim of water treatment and cost control is attempting to achieve an environmental benefit by COD removal, a social benefit by increasing citrus resource efficiency, broadening new raw material for pectin production, and an economic benefit from the income of pectic polysaccharides.

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