**Using the high temperature resistant pH electrode to auxiliarily study the sugar
beet pectin extraction under different extraction conditions**

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Abstract: A full two-level factorial experiment design was employed to investigate the effects of temperature (75 and 85 °C), time (2 and 3h) and pH(1.5 and 2.0) on the molecular composition of sugar beet pectin extracted by citric acid. And the high temperature resistant pH electrode was used to monitor the pH variation in the process of sugar beet pectin extraction. The results showed that the pH of pectin extraction solution varied greatly with the temperature during pectin extraction, and the pH of extraction solution fluctuated more widely at 85°C than at 75°C. In the experiment design, the pH was the most significant factor on pectin composition such as Ara, NS, and DA, while the temperature became the most important factor for yield, GalA, Rha, Gal, and Mw. The DM of extracted samples was significantly affected by extraction time. It was observed that the emulsifying property of the extracted pectin samples was significantly affected by the extraction conditions. All the results revealed that 1.) the pH value of extraction solution is directly related to the yield and the degradation of the extracted pectin during sugar beet pectin extraction, 2.) the extraction temperature could significantly affect the pH fluctuation of extraction liquid, 3.) the most significant effect factor for a given pectin composition is dominated by the pH fluctuation of the extracting liquid and acid stability of this discussional pectin composition, 4.) beet pectin extracted by citric acid is an effective emulsifier.

Key words: Sugar beet pectin; pH; Citric acid; Extraction; Characterisation; Emulsifying property

1. Introduction

Sugar beet pulp is a valuable by-product from beet sugar industry. It is an abundant renewable resource in China, USA, and Europe (Suhartini, Heaven, & Banks, 2013; Bellido et al., 2015; Ma et al., 2013). Large amounts of sugar beet pulps could cause some disposal problems. Generally, sugar beet pulp is used as low-value animal feed (Mihajlovski, Radovanović, Veljović, Šiler-Marinković, & Dimitrijević-Branković, 2016; Hamleybennett & Lye, 2016). Therefore, finding a new way to increase the economic benefit of sugar beet pulp has become a pressing problem for the sugar industry. Sugar beet pulp, containing about 15-30% pectin (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007), presents a potential raw material source for pectin extraction. Sugar beet pulp pectin is unlike citrus peel pectin and apple pomace pectin which are extensively used as gelling agents or stabilizers in the food industry (May, 1990; Yapo et al., 2007). But pectin extracted from sugar beet pulp has been widely investigated as a emulsifier of the oil in water emulsion system due its characteristic molecular composition such as a higher degree of acetylation, a higher content of neutral sugar and a lower molar mass (Yapo et al., 2007; Guo, et al., 2015a; Chen, Fu, & Luo, 2016).

Pectin, a kind of complex polysaccharide, exists in primary cell walls of higher plants (Agoda-Tandjawa, Durand, Gaillard, Garnier, & Doublier, 2012). Pectin molecules are mainly composed of homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) regions, which have been widely believed in previous reports (Yapo, 2009b; Voragen, Coenen, Verhoef, & Schols, 2009; Williams, Aurélie, Anna, & Marie-Christine, 2009; Gómez, Gullón, Yáñez, Parajó, & Alonso, 2013; Denman and Morris, 2015). The HG region which is generally called "smooth region" consists of partly methyl-esterified and acetyl-esterified

$\alpha(1,4)$ -linked D-galacturonic acid (GalA) residues (Yapo et al., 2007; Voragen et al., 2009; Xu et al., 2014; Guo et al., 2015a). The region of RG-I, generally called "hairy region", is constituted by the $[\rightarrow 2)\text{-}\alpha\text{-L-Rhap-(1}\rightarrow 4)\text{-}\alpha\text{-D-GalAp-(1}\rightarrow]$ repeats (Voragen et al., 2009; Renard, Crepeau, & Thibault, 1999). The rhamnose residues of RG-I region can bear various side chains which are mainly composed of arabinogalactan, arabinan and galactan (Fishman, Chau, & Cooke, 2008; Voragen et al., 2009). The RG II region, a complex minor component of pectin, is composed of 12 different monomers (Yapo & Koffi, 2008).

The physicochemical properties of pectin could be influenced by different extraction conditions. Extractants such as mineral acid, organic acid, enzymes as well as calcium-ion chelators have generally been used for pectin extraction (Panouillé, Thibault, & Bonnin, 2006; Lv, Wang, Wang, Li, & Adhikari, 2013; Li et al., 2015; Lefsih et al., 2016). Although various extraction methods have been investigated for the pectin extraction in recent years, commercial pectin is usually extracted by hot acid extraction due its high extraction yield and low degradation of HG region of pectin (Yapo, 2009b; Ma et al., 2013). The commonly used acid extractants are hydrochloric acid, nitric acid and sulfuric acid (Koubala et al., 2008; Yapo, 2009b; Ma et al., 2013). However, the main drawbacks of those mineral acids mentioned above are their toxicity and the environmentally unfriendly effluents (corrosive) they generate (Minjares-Fuentes et al., 2014). Citric acid, a kind of natural and non-toxic organic acid, could effectively overcome those drawbacks. Therefore, the exploration of citric acid extracting pectin technology can effectively enrich the methods of pectin extraction. Recently the citric acid has been widely used to extract pectin from various materials (Canteri-Schemin, Waszczynskyj, & Wosiacki, 2005; Yapo, 2009a; Yapo, 2009b; Oliveira et al., 2016; Pereira et al., 2016; Li et al., 2015; Chan & Choo, 2013).

The pH value, a variable during pectin extraction, is significantly affected the chemical composition of pectin. But little work has yet been done for revealing the changing of pH in the process of pectin extraction. Therefore, the purposes of this work was to investigate the changing tendency of pH value during sugar beet pectin extraction, and then using the obtained pH value changing curves to analyze the effects of extraction parameters (pH, time and temperature) on the yield and chemical composition of extracted pectin, at last the emulsifying property of the extracted pectin was also investigated.

2. Materials and methods

2.1 Materials

Sugar beet pulp was obtained from Lvxiang Sugar Company (Xinjiang, China). The beet pulp was thoroughly washed with distilled water three times at room temperature to remove impurities, oven-dried at 45°C for 24h to constant weight, and then pulverized into powder using a grinder. The powder was stored at ambient temperature for further experiments. Unless otherwise stated, all reagents used in this experiment were of analytical grade.

2.2 Experimental design

Full factorial design, a powerful statistical approach, can effectively reduce the total number of experiment times in order to obtain the best overall optimization of the experiment system (Brasil et al., 2005; Christelle, Thierry, Bernard, Jean-Claude, & Michel, 2006; Pavan, Gushikem, Mazzocato, Dias, & Lima, 2007; Denman & Morris, 2015). Therefore, we built a two-level full factorial design (Table 1) for the three important extraction parameters (pH, time, and temperature) in order to research the effect of the extraction variables on the characteristics of the extracted pectin. The factor levels were standardised and coded as (- 1, + 1). Eight experimental points

were designed by Minitab version 15 (Minitab Inc., Philadelphia, U.S.A.) and each extraction was carried out three times.

2.3 Pectin extraction and pH value monitoring

The extraction conditions were carried out according to the factorial experiment design (Table 1). Sugar beet pectin was extracted in a glass reactor with a mechanical agitator. The temperature of the extraction was regulated using a water bath. Dried sugar beet pulp powder was dispersed in distilled water (solid-liquid ratio 1:20, w/v), and then the pH value of the suspension was adjusted to two levels of 1.5 or 2.0 with citric acid at ambient temperature. Then the mixed solution was carried out at 75°C or 85°C for 2h or 3 h. The pH value was measured once per 30 minutes during pectin extraction process by a high temperature resistant pH electrode (METTLER TOLEDO, Switzerland) coupled with a pH meter (METTLER TOLEDO, Switzerland) which had the function of automatic temperature compensation. Each extraction process was repeated three times.

2.4 Pectin isolation

After the extraction was completed, the resulting mixture was cooled to room temperature with the circulating tap water. The cooling slurry was filtered through 400 mesh nylon cloth and then centrifuged at 8000rpm for 20 min. The obtained supernatants were dispersed into 3 volumes of 95% ethanol at room temperature for 3h. The precipitate of pectin was washed twice with 75% ethanol followed by 95% ethanol, and then oven-dried at 45°C for 24h and weighed.

The pectin yield was calculated using equation:

$$\text{yield (\%)} = 100 [\text{weight of dried pectin (g)}/\text{weight of dried sugar beet pulp powder (g)}]$$

2.5 Pectin characterization

All of the determinations were carried out three times on each sugar beet pectin sample.

2.5.1 Galacturonic acid and protein content

The galacturonic acid (GalA) content of extracted pectin was quantified colorimetrically by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). The protein content ($N \times 6.25$) was determined by the Kjeldahl procedure.

2.5.2 Neutral Sugars

The compositions of neutral sugars in different pectin samples were determined by high performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) according to the method of previous reported with minor modification (Haikel, Nicolas, Bernard, & Michel, 2004). A 20mg sample of sugar beet pectin was decomposed by an enzyme preparation of viscozyme L (Novozymes, Tianjin, China) at 45 °C for 24h and then hydrolysed by 0.2 M trifluoroacetic acid at 120 °C for 2h. The reaction mixture was cooled with ice water to stop the reaction. After filtered through a filter membrane of 0.22µm pore size, the hydrolysate (25µL) was injected into the Dionex ICS-5000 Bio-Lc system equipped with a CarboPac PA1 column (250×4mm) in combination with a guard column (50×4mm). All analyses were operated at a temperature of 30 °C and a flow rate of 1mL/min. The neutral sugars were eluted isocratically with 10 mM NaOH for 35 minutes. The column was washed with 500mM NaOH for 10 min and reequilibrated with 10mM NaOH for 10 min before the next injection. Total neutral sugars (NS) content of sugar beet pectin was calculated as the sum of individual neutral monosaccharides (rhamnose, arabinose, galactose, glucose and xylose) which were detected by HPAEC-PAD.

2.5.3 Degrees of methylation and acetylation

The degrees of methylation and acetylation of sugar beet pectin were determined

by the high performance liquid chromatography (HPLC) method according to the previous literature (Voragen, Schols, & Pilnik, 1986). A approximate 30 mg sample of sugar beet pectin was subjected to treatment with 1 mL of isopropanol-water mixture (1:1) containing 0.4M NaOH and held at room temperature for 2h. Methanol and acetic acid were released from the methoxy and acetyl groups of sugar beet pectin by saponification with sodium hydroxide mixture. The suspension was then filtered through a filter membrane of 0.45 μ m pore size. 25 μ L of the filtrate was injected into the Waters HPLC system equipped with an Aminex HPX-87H ion exchange column (300 $\times$ 7.8mm, BioRad, CA, USA). And the determination was operated at the temperature of 45 °C and a flow rate of 0.5ml/min with 5mM sulphuric acid as eluent. Degree of methylation (DM) and degree of acetylation (DA) were calculated as the percent molar ratio of methanol or acetic acid to the GalA (quantified by m-hydroxybiphenyl method).

2.5.4 Weight average molecular weight

The high performance size exclusion chromatography (HPSEC) method was used to determine the weight-average molecular weight (M_w) of the pectin samples. The samples (0.2% ,w/v) were dissolved in 100mM sodium nitrate (NaNO_3) solution at room temperature under magnetic stirring, and then filtered over a 0.45 μ m filters (Guo, Meng, Zhu, Zhang, & Yu, 2015b). Filtrates (100 μ L) were loaded onto the two Waters Ultrahydrogel columns (Ultrahydrogel linear and Ultrahydrogel 2000) and coupled on line with a Waters 2410 differential refractometer. Elution method was carried out with 100mM NaNO_3 solution containing 0.02% NaN_3 as the preservative at 35 °C and at a flow rate of 0.6 mL/min. The average molar mass of pectin was calculated by a calibration curve constructed with dextran standards.

2.6 Emulsifying property

2.6.1 Emulsion preparation

Oil-in-water (O/W) emulsions were prepared as follows: 1g pectin powders were added to a citrate buffer solution (pH 3.5, 50mM) containing 0.1% w/w sodium benzoate as a preservative at room temperature with gentle stirring. Then, 15g MCT and sufficient citrate buffer solution were added to achieve 100g total solution. The mixed solution was then pre-homogenized using a homogeniser of Silentcrusher (Heidolph, Germany) at a speed of 20000 rpm for 2min. Pre-emulsions were applied with three passes through the high-pressure homogenizer at 50MPa.

2.6.2 Determination of emulsions particle size

The particle size of emulsions was analyzed by the Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK). The refractive index of MCT and water were 1.45 and 1.33, respectively. The average droplet size of the emulsions was characterized by the $d_{4,3}$ (volume-weighted mean diameter). All experiments were performed in three times.

3. Results and discussion

3.1 The fluctuation of pH during the pectin extraction

Many studies have suggested that the extraction factors such as the pH, temperature, and time significantly affect the pectin yield and chemical characteristics (Levigne et al., 2002; Yapo, 2009b; Chan & Choo, 2013; Oliveira et al., 2016; Pereira et al., 2016). The pH, a not constant extraction parameter in the process of pectin extraction, is different from the other factors (time or temperature) which are constant under a certain extraction condition. Fig. 1 shows the changes of pH values with extraction time during the pectin extraction under different extraction conditions (initial pH 1.5 or 2.0, temperature 85°C or 75°C). The initial pH value (the first dot of every curve which had been labeled in the Fig. 1) of the mixture was measured at

room temperature and the other pH values were measured at extraction temperature (85°C or 75°C). Some important phenomena can be discovered from the Fig. 1 as follows: (1) At an equal initial pH (1.5 or 2.0), the pH values of citric acid extracting solution at 85°C were lower than 75°C during the early and middle stages of extraction (from beginning to approximate 2h) and then pH values at 85°C were higher than 75°C during the later stage of extraction (2-3h). (2) The pH value fluctuation of extraction solutions at 85°C was larger than that occurred at 75°C. (3) Under a certain extraction condition, the pH value was not constant in the process of pectin extraction and the final pH value was relatively higher than initial pH value.

The possible explanations for those experiment phenomena were suggested as follows: On the one hand, citric acid is a weak tri-protic organic acid whose ionization degree changed along with the rising of temperature. That was why, in this study, the pH value of extraction solution at 85°C was lower than the pH value of extraction solution at 75°C under the same initial pH value. On the other hand, during pectin extraction, the solubilization of pectin into the citric acid solution from the cell walls of sugar beet pulp and the degradation of extracted pectin could consume the hydrogen ions of the extraction solution. That was why the pH value of extraction solution increased as the extraction time went on. The last but not least, some studies reported that the relatively high extraction temperature could help to increase the yield and the degradation of pectin (Pagan, Ibarz, Llorca, Pagán, & Barbosa-Cánovas, 2001; Yapo et al., 2007; Chan & Choo, 2013; Oliveira et al., 2016; Pereira et al., 2016). The relatively high yield and degradation gave rise to greater consumption of hydrogen ions of the extraction liquid. Therefore, the variations of pH value at 85°C were larger than those at 75°C.

3.2 Pectin yield

The yields of beet pectin extracted under different extraction conditions were investigated. As can be seen in Table 2, the yield of pectin ranged from 8.7% to 21.6 % (w/w). And the highest yield was obtained when the extraction condition was set at 85 °C, pH 1.5, for 3h. The yield of pectin samples extracted at 85 °C were significantly higher than the yield of pectin samples extracted at 75°C. The possible reason for this case was that the higher temperature could help to destroy the cell walls of sugar beet pulp that could probably increase the contacting area (or/and reaction area) between hydrogen ions and pectin consisting in the cell walls of beet pulp. And the greater pH fluctuation of citric acid extracting solutions at 85 °C (Fig. 1) also demonstrated the relatively large consumption of hydrogen ions at higher extraction temperature. In conclusion, high temperature could significantly increase the reaction efficiency of hydrogen ions. Therefore, the pectin yield extracted at 85 °C was significantly higher than that extracted at 75 °C.

The bar lengths of the effect factors in the Pareto chart were used to compare their relative importance and were proportional to the absolute value of the corresponding estimated effects. The Pareto chart of the extraction yield (Fig. 2) showed that the significant single factors which were ranked by order of importance were the extraction temperature, the pH of the extraction, and the extraction time. The temperature was the most influential effect on the pectin yield. This result was not in agreement with previous literatures which considered that pH was the most important significant effect on the yield (Levigne et al., 2002; Yapo et al., 2007; Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Denman & Morris, 2015). A possible explanation for such a difference is that, in our experiment design, the decisive factor of pH fluctuation was temperature rather than the pH of the extraction solution or extraction time (Fig. 1). The larger variations of pH values may indicate the greater

pectin solubility from cell walls of sugar beet pulps, therefore the temperature became the most important factor on the pectin yield.

3.3 Galacturonic acid

The galacturonic acid (GalA) content of isolated pectin ranged from 55.4% to 70.2% (Table 2). And the highest GalA content was obtained at pH 1.5, 85°C for 3h which corresponded to the most drastic extraction condition. The GalA content of isolated pectin increased significantly with the decreasing pH, increasing temperature, and increasing extraction time. This trend was in accordance with the previous studies (Levigne et al., 2002; Robert et al., 2006; Yapo et al., 2007; Oliveira et al., 2016; Pereira et al., 2016).

All the individual factors significantly affect the GalA content of extracted pectin, with temperature being the most significant one (Fig. 2). This result was different from published papers (Yapo et al., 2007; Emaga et al., 2008; Denman & Morris, 2015). Those literatures argued that the pH was the most important significant effect factor on the GalA content of extracted pectin. The discrepancy could be attributed to the reason exhibited as follows: the solubilization of pectin from the cell walls and the degradation of extracted pectin could consume the hydrogen ions of the extraction solution, thus the greater pH change of the extraction solution may indicate the greater yield and degradation of extracted pectin. As Figure 1 shown, the pH value fluctuations of extraction solutions at 85°C were larger than 75°C, meanwhile the other factors did not have this obvious pH variation tendency. Therefore the temperature became the most important significant factor that can obviously affect the GalA content of extracted pectin.

3.4 Neutral sugars

The rhamnose content ranged from 2.4% to 4.5% (Table 2). The rhamnose

content of extracted pectin increased as the extraction pH decreased from 2.0 to 1.5, which is in agreement with previously published reports (Levigne et al., 2002; Denman & Morris, 2015). On the other hand, the extraction temperature had more significant effect on the rhamnose content of extracted pectin. The rhamnose content of pectin extracted at 85°C was higher than the rhamnose content of pectin extracted at 75°C when the other extraction parameters were at the same level. The galactose content varied from 6.6% to 10.4% (Table 2), and also had the similar changing trend to rhamnose (Table 2).

As shown in Table 2, the arabinose content of isolated pectins ranged from 0.8% to 10.1%. The arabinose content of pectin obtained at pH 1.5 was significantly lower than those obtained at pH 2.0. This observation was also demonstrated by Levigne et al. (2002) and Robert et al. (2006). Comparing to the rhamnose content and galactose content of extracted pectin, the arabinose content of extracted pectin was more sensitive to the pH of the extraction solution. A possible explanation for such a difference is that the arabinofuranosyl linkages were particularly acid labile (Thibault, Renard, Axelos, Roger, & Crépeau, 1993; Levigne et al., 2002; Yapo et al., 2007)

Rhamnose, arabinose, and galactose were the major components of pectin neutral sugars. Glucose (0.2-1%) and xylose (0.3-0.4%) were present in low amounts, and possibly came from sugar materials and hemicelluloses (Robert et al., 2006; Yapo et al., 2007; Emaga et al., 2008). The content of total neutral sugars (NS) was calculated as the sum of those individual neutral sugars such as rhamnose, arabinose, galactose, glucose and xylose (Yapo et al., 2007). The NS content ranged from 16.1% to 20.5% (Table. 2). The content of NS increased significantly with increasing pH value, decreasing extraction temperature, and reducing extraction time. This trend was in accordance with previous studies (Robert et al., 2006; Yapo et al., 2007; Garna et al.,

2007). The highest NS content was obtained at pH 2.0, 75°C, for 2h which corresponded to the mildest extraction condition (Table. 2).

As shown in Figure 2, temperature was the most influential effect on the rhamnose and galactose content, however for the arabinose and NS content the most influential effect was the pH. On the other hand, the significant effects (temperature, pH and time) had the same order of influence on rhamnose and galactose contents, but pH had more relative importance effect on rhamnose content according to the different bar lengths ratio of significant effects in each pareto chart for rhamnose and galactose. A possible explanation for those different trends of rhamnose, galactose, arabinose, and NS was that the different glycosidic bonds stability (GalA-GalA > Gal-Rha > Rha-GalA > neutral sugar-neutral sugar) of extracted pectin in the acid extraction solution (Thibault et al., 1993; Yapo et al., 2007). Those results also showed that the arabinose content is the indicative index for the NS content of extracted pectin, and relatively lower arabinose content represented the greater degradation of pectin neutral sugar regions.

3.5 DM and DA

The degree of methoxylation (DM) varied from 37.9 to 48 (Table 2). The highest DM was obtained under the condition of pH 2, 75 °C, 2h. The DM increased with the increasing pH, decreasing temperature and shortening the time of extraction. The reason for those observations could be the deesterification of polygalacturonic chain under the harsher extraction conditions (Mort, Feng, & Maness, 1993).

Table 2 also represents the degree of acetylation (DA) of obtained pectin samples under different extraction conditions. The DA ranged from 14.9 to 24.3 (Table 2). Lowering temperature or (and) increasing pH lead to an increase in the DA of extracted pectin. This result could be explained by the hydrolysis of acetic acid groups

from galacturonic acids under drastic extraction conditions (Garna et al., 2007). The highest DA was obtained under the condition of pH 2, 75°C, 2h which corresponded to the mildest extraction conditions.

The pareto chart for the DM (Figure. 3) shows the significant individual effects which were ranked by the order of importance were extraction time, extraction temperature, and extraction pH. The factors of time and temperature were more influential than pH on the DM, as described by Emaga et al (2008). But for DA, the pH was the most important significant factor in all of the significant effects (pH, temperature, time, pH-temperature interaction, pH-time interaction, and time-temperature interaction of extraction), as described by Levigne et al (2002). The difference between pareto charts of DM and DA revealed that DA was more sensitive to acid hydrolysis than DM.

3.6 Mw

The weight average molecular weight (Mw) of extracted pectin varied from 187 kDa to 377 kDa (Table 2). Similar data has been reported for sugar beet pectin (Levigne et al., 2002). Temperature had a more marked influence on molar mass than the other significant effects such as time and pH (Figure 3). The molar mass values of pectin samples from various extraction conditions at 75 °C were obviously higher than those values which were extracted at 85 °C. The pH had a relative slight effect on molar mass of extracted pectin. The highest pectin molar mass was obtained when the pectin extraction was operated under the condition of 2h, 75 °C, and pH 2. As shown in Table. 2, the values of pectin molar mass extracted at 75 °C were larger than those at 85 °C when extraction initial pH and extraction time were at the same level. In contrast, increasing pH when temperature and time kept constant only slightly increased the molar mass of pectin. And the effect of extraction time were similar to

the pH . Hence the data of Table. 2 could help create a better understanding of pareto charts for us.

3.7 Protein content

The protein content of extracted pectin varied from 5.3% to 8.5% (Table 2). These values of protein content were higher than those data of Li et al. (2015) reported under the similar extraction condition. The discrepancy was probably due to the different sugar beet pulp source and the different recovering and purification technology for the pectin extraction. Pectin samples extracted at pH 2.0 contained more protein than those at pH 1.5. The result was similar to the literature reported by the Yapo et al (2007).

3.8 Emulsifying property

The emulsifying property of extracted pectin samples were study by measuring the $d_{4,3}$ for the emulsions. The emulsions were prepared with 1% (w/w) pectin solutions. Figure. 4 shows the $d_{4,3}$ of the oil droplets in oil-in-water emulsions stabilized by different pectin samples. It was found that the $d_{4,3}$ of the emulsions decreased as the protein content increasing and the degradation of pectin molecule decreasing. This tendency was in accordance with the previous reports (Yapo et al., 2007; Funami et al., 2011; Li et al., 2015). The emulsifying ability of some extracted pectin samples such as E1, E7, and E8 was similar to the published literatures (Leroux et al., 2003; Funami et al., 2007; Chen et al., 2016). Based on the results mentioned above, it could be inferred that under some extraction conditions, citric acid extracted pectin from sugar beet pulp could act as an effective emulsifier of oil-in-water emulsions.

4. Conclusions

In this study, the pH value was not constant during pectin extraction and the pH

fluctuation of extraction solution at 85°C was larger than 75°C. During pectin extraction process, the pH was decreasing first and then increasing with the extension of extraction time. In the pectin extraction experiment design, the pH was the most significant factor on the Ara, NS, and DA contents of extracted pectin. But for yield, GalA, Rha, Gal, and Mw, temperature become the most important factor. And the extraction time could also become the most significant effect when the discussional composition was DM content of extracted pectin. Linking the most significant effect factor for a given pectin composition to the pH variation range under different extraction conditions, two possible explanation for those differences were that 1.) the different acid stability of those discussional pectin constituent, 2.) the different pH fluctuation range under those different extraction conditions in the process of sugar beet pectin extraction. Moreover, the emulsifying ability of the extracted sugar beet pectin samples were also investigated, the results showed that the extraction conditions could significantly affect the emulsifying activity of the extracted pectin samples, and the pectin samples extracted by citric acid under some given extraction conditions were effective emulsifiers for the oil-in-water emulsions system. Considering the different ionization properties between organic acid and mineral acid, the different initial temperature of extraction liquid adjusted by different acid extractant will be investigated further.

Acknowledgements

This work was supported by grants from the Science and Technology Planning Project of Guangdong Province (No.2013B051000015), the Science Foundation of State Key Laboratory of Pulp and Paper Engineering (No.C712035z), the Chinese National Science Foundation (No. 31601423), the Achievements Transformation Fund Project (No. 2016AB009).

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Table Captions:

Table 1. A two-level full factorial design was used for pectin extraction from sugar beet pulp, and the levels -1 and $+1$ correspond to 1.5 and 2 for pH, 2 and 3h for time (t), and 75 and 85 °C for temperature (T), respectively.

Table 2. Yield (% w/w), chemical composition (% w/w), degree of methoxylation (% mol), degree of acetylation (% mol) and weight-average molar mass (kg/mol) of extracted sugar beet pectin samples under different extraction conditions.

Table 1 A full factorial design was used for pectin extraction from sugar beet pulp, and the levels - 1 and + 1 correspond to 1.5 and 2.0 for pH, 2 and 3h for time (t), 75 and 85 °C for temperature (T), respectively.

Run	Variables		
	pH	t	T
E1	-1(1.5)	-1(2h)	-1(75 °C)
E2	-1(1.5)	+1(3h)	-1(75 °C)
E3	-1(1.5)	-1(2h)	+1(85 °C)
E4	-1(1.5)	+1(3h)	+1(85 °C)
E5	+1(2.0)	-1(2h)	-1(75 °C)
E6	+1(2.0)	+1(3h)	-1(75 °C)
E7	+1(2.0)	-1(2h)	+1(85 °C)
E8	+1(2.0)	+1(3h)	+1(85 °C)

654 Table 2 Yield (% w/w), chemical composition (% w/w), degree of methoxylation (% mol), degree of acetylation (% mol) and weight-average
655 molar mass (kg/mol) of extracted sugar beet pectin samples under different extraction conditions.

	E1	E2	E3	E4	E5	E6	E7	E8
yield	12.9±0.15	15.0±0.05	19.1±0.20	21.6±0.31	8.7±0.27	10.3±0.26	13.7±0.16	16.4±0.12
Gal A	59.2±0.10	61.7±0.40	64.6±0.80	70.2±0.46	55.4±0.36	61.1±0.76	62.9±0.83	67.3±0.37
Rha	3.1±0.12	3.7±0.3	4.4±0.06	4.5±0.2	2.4±0.06	2.8±0.15	3.3±0.12	3.8±0.06
Gal	7.9±0.31	8.8±0.21	9.9±0.12	10.4±0.35	6.6±0.21	7.3±0.12	8.7±0.15	9.6±0.15
Ara	5.6±0.06	4.8±0.15	2.0±0.21	0.8±0.17	10.1±0.10	8.2±0.06	6.5±0.12	4.5±0.10
Glu	0.9±0.10	0.7±0.17	0.3±0.06	0.2±0.06	1.0±0.10	0.8±0.17	0.5±0.12	0.3±0.06
Xyl	0.4±0.06	0.3±0.10	0.3±0.06	0.3±0.12	0.4±0.06	0.4±0.06	0.4±0.06	0.3±0.12
NS	17.9±0.26	18.3±0.30	16.9±0.31	16.1±0.25	20.5±0.21	19.4±0.25	19.3±0.10	18.5±0.17
DM	45.2±0.11	41.4±0.16	42.2±0.11	37.9±0.17	48.0±0.40	44.9±0.14	44.2±0.21	41.7±0.90
DA	22.5±0.12	19.2±0.11	18.8±0.24	14.9±0.06	24.3±0.14	22.8±0.08	23.0±0.15	20.8±0.46
protein	6.7±0.20	6.2±0.31	5.8±0.10	5.3±0.25	8.5±0.15	8.3±0.25	7.6±0.12	7.4±0.10
Mw	347±3.5	315±5.0	236±5.1	187±3.0	377±3.0	344±3.6	272±2.6	215±2.5

656

657 GalA: galacturonic acid, Rha: rhamnose, Ara: arabinose, Gal: galactose, Glu: glucose, Xyl: xylose, NS: total neutral sugars, DM: degree of
658 methoxylation, DA: degree of acetylation, Mw: weight-average molar mass. All data are expressed as mean values ± standard deviations of
659 triplicate analysis.

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Figure Captions:

Figure 1. The changing curves of pH values in the process of sugar beet pectin extraction under different extraction conditions.

Figure 2. Standard main effect pareto charts for pectin yield, GalA (galacturonic acid), Rha (rhamnose), Gal (galactose), Ara (arabinose) and NS (total neutral sugars) of pectin.

Figure 3. Standardized main effect pareto charts for DM (degree of methoxylation), DA (degree of acetylation) and Mw (Weight-average Molecular Weight) of pectin.

Figure 4. The $d_{4,3}$ (μm) of oil droplets in oil-in-water emulsions stabilized by different pectin samples.

Figure 1

Figure 2

Figure 3

Figure 4