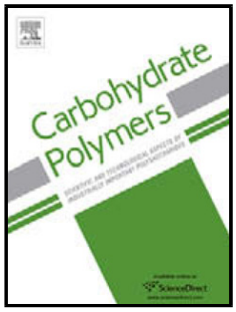

Title: Controlled methyl-esterification of pectin catalyzed by cation exchange resin

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PII: S0144-8617(15)01094-2
DOI: http://dx.doi.org/doi:10.1016/j.carbpol.2015.11.005
Reference: CARP 10523

To appear in:

Received date: 4-6-2015
Revised date: 2-11-2015
Accepted date: 3-11-2015

Please cite this article as: Peng, X., Yang, G., Fan, X., Bai, Y., Ren, X., and Zhou, Y.,Controlled methyl-esterification of pectin catalyzed by cation exchange resin, *Carbohydrate Polymers* (2015), http://dx.doi.org/10.1016/j.carbpol.2015.11.005

Highlights

- A new method is developed using cation exchange resin to methyl-esterify pectin.
- The method can product high DE of HG and RG-I pectin without destroying structures.
- Pectins with varying DE can be prepared by controlling the reaction conditions.

Controlled methyl-esterification of pectin catalyzed by cation exchange resin

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Abstract

This study developed a new method to methyl-esterify pectin using a cation exchange resin. Homogalacturonan (HG)-type pectin (WGPA-3-HG) and rhamnogalacturonan (RG)-I-type pectin (AHP-RG) obtained from the roots of panax ginseng and sunflower heads, respectively were used as models. Compared to commonly used methyl-esterification methods that use either methyl iodide or acidified methanol, the developed method can methyl-esterify both HG- and RG-I-type pectins without degrading their structures via β-elimination or acid hydrolysis. In addition, by modifying reaction conditions, including the mass ratio of resin to pectin, reaction time, and temperature, the degree of esterification can be controlled. Moreover, the resin and methanol can be recycled to conserve resources, lower costs, and reduce environmental pollution. This new methodology will be highly useful for industrial esterification of pectin.

Keywords: Pectin; Methyl-esterification; Cation exchange resin; Degree of esterification

1. Introduction

Pectins are complex polysaccharides present in all plant primary cell walls (Ridley, O'Neill, & Mohnen, 2001). The predominant structure of pectin is homogalacturonan (HG), which is mainly composed of α-(1→4)-**D**-Gal*p*A (De Vries, Den Uijl, Voragen, Rombouts, & Pilnik, 1983; Thibault, Renard, Axelos, Roger, & Crépeau, 1993). The second major structural element of pectin is rhamnogalacturonan I (RG-I), which consists of repeating disaccharide units [→4)-α-**D**-Gal*p*A-(1→2) -α-**L**-Rha*p*-(1→] in the backbone and neutral side chains composed of arabinan, galactan, or arabinogalactan (AG) (Yapo, 2011). The Gal*p*A residues in pectin can be methyl-esterified at their carboxyl groups, and the percentage of esterified Gal*p*A residues per total Gal*p*A residues is defined as the degree of esterification (DE), one of the most important properties of pectin (Jiang, Liu, Wu, Chang, & Chang, 2005).

Pectin has been widely used in the food industry as a gelling and stabilizing agent (Gamonpilas, Krongsin, Methacanon, & Goh, 2015), and the gelling mechanisms and properties are closely related to its DE (Garnier, Axelos, & Thibault, 1993; Ngouémazong et al., 2012; Ralet, Dronnet, Buchholt, & Thibault, 2001). Industrial demand for pectin with tunable abilities to gel or stabilize fruit and dairy products has increased the need for pectin with controllable DE, which also has a significant impact on the biological activities of pectin. For example, esterified cross-linking in pectin impacts its ability to induce apoptosis in prostate cancer cells (Jackson et al., 2007). The inhibitory potency of de-esterified RG-I-4 on galectin-3-mediated hemagglutination is decreased 50-fold when compared to normal RG-I-4 (Gao et al., 2013). In addition, structural analysis of pectin via β-elimination requires esterification of Gal*p*A residues (Deng, O'Neill, Hahn, & York, 2009; Deng, O'Neill

& York, 2006). Therefore, it is crucial to develop effective methods to control methyl-esterification.

Several methods have been reported to methyl-esterify carboxylic acid groups in pectin. The most commonly used are reactions of tetrabutyl ammonium pectinate with methyl iodide (Matricardi, Dentini, Crescenzi, & Ross-Murphy, 1995; Renard & Jarvis, 1999a, b) and the treatment of pectin with methanol acidified with sulfuric or hydrochloric acid (Rosenbohm, Lundt, Christensen, & Young, 2003; van Alebeek, Zabotina, Beldman, Schols, & Voragen, 2000; Willats et al., 2000). However, these approaches are also responsible for extensive depolymerization of pectin via β-elimination (Renard & Thibault, 1996) or acid hydrolysis of glycosidic linkages (Bertaud, Sundberg, & Holmbom, 2002; Rosenbohm et al., 2003; Willats et al., 2000). Furthermore, separation of the acid catalysts from the reaction mixture is very difficult and produces wastewater and equipment corrosion. Therefore, new methods for methyl-esterification of pectin need to be developed.

The objective of this study was to develop a new way to methyl-esterify both HG- and RG-I-type pectins using a cation exchange resin as a catalyst. The efficiency of methyl-esterification by this new method was compared to those using methyl iodide and hydrochloric acid-acidified methanol. The effects of different conditions were also assessed on the degree of esterification, including varying the mass ratio of resin to pectin and reaction time, as well as temperature.

2. Materials and methods

2.1. Materials

Strongly acidic cation exchange resin (AG 50W-X8) was purchased from Bio-Rad (Hercules, California, USA). The functional group of the resin is sulfonic

77 acid, and the mesh size is 100-200 with a mean particle size of 106-250 μm. Standard
78 polygalacturonic acid (DE=0%) and pectin (DE=92%) were purchased from
79 Sigma-Aldrich Co. (St. Louis, MO, USA). The roots of *Panax ginseng* and sunflower
80 heads were cultivated and collected from Changbai Mountain and Baicheng city in
81 Jilin province of China, respectively. All other chemicals were of analytical grade.

82 *2.2. Preparation of pectins*

83 Ginseng pectin WGPA and its fraction WGPA-3-HG were prepared and
84 characterized as described by Zhang et al. (2009). Briefly, water-soluble ginseng
85 polysaccharide (WGP) was extracted from the roots of *P. ginseng* using hot water and
86 precipitated with 80% ethanol. WGP was then applied to a DEAE-Cellulose column
87 (8.0×20 cm, Cl^-) and eluted with distilled water to give the neutral fraction (WGPN),
88 and the column was washed further with 0.5 M NaCl to give the acid fraction
89 (WGPA). WGPA was loaded onto a DEAE-Cellulose column (8.0×20 cm, Cl^-) and
90 eluted with a stepwise gradient of aqueous NaCl (0, 0.1, 0.2, 0.3 and 0.5 M) to give
91 five fractions: WGPA-N, WGPA-1, WGPA-2, WGPA-3 and WGPA-4. WGPA-3 was
92 applied to a semi-preparative Sepharose CL-6B column (3.0×90 cm) yielding two
93 fractions: WGPA-3-RG and WGPA-3-HG.

94 The heads of sunflower (*Helianthus annuus* L.) were extracted with 0.2% oxalic
95 acid (solid: liquid ratio 1: 16, w/v) at 100^{o}C for 1 h and filtered through four sheets of
96 gauze. The solid material was extracted again under the same conditions. The filtrates
97 were combined, centrifuged to remove water-insoluble materials, concentrated to
98 1500 mL and precipitated with 60% aqueous ethanol. After centrifugation, the
99 supernatant was precipitated with 80% aqueous ethanol. Following further
100 centrifugation and drying by solvent exchange (95% ethanol, acetone, and ether), the
101 polysaccharide fraction AHP-0.2-80% was obtained. AHP-0.2-80% was further

fractionated using a preparative Sepharose CL-6B column (3.0×90 cm) to yield two fractions: AHP-RG and AHP-HG.

2.3. Methyl-esterification of pectin

2.3.1. Methyl-esterification of pectin with methyl iodide

Pectin was methyl-esterified by treating with methyl iodide (MeI) and tetrabutyl ammonium fluoride (TBAF) in DMSO containing 8% water, as described by Deng et al. (2006). Briefly, a suspension of pectin (100 mg) in water (1.6 mL) and DMSO (20 mL) containing TBAF (200 mg) and MeI (100 μL) in a 50-mL round-bottom flask was stirred at room temperature for 18 h. The reaction mixture was poured into ice-cold water (60 mL) and centrifuged to remove iodine. The resulting supernatant was dialyzed (MWCO 3500) against deionized water for 48 h and then lyophilized.

2.3.2. Methyl-esterification of pectin with acidified methanol

Methyl-esterification of pectin by methanol acidified with hydrochloric acid (HCl-MeOH) was performed as previously described (Van Alebeek et al., 2000). Each pectin sample (100 mg) was added to anhydrous methanol (20 mL) containing 0.1 M HCl, and the suspension was stirred at room temperature (20^{o}C) for 3 days. The methyl-esterified pectin was filtered off and washed carefully with 80% aqueous ethanol until no more chloride was present in the washings. Finally, the product was washed with absolute ethanol and dried under reduced atmospheric pressure.

2.3.3. Methyl-esterification of pectin catalyzed by cation exchange resin in methanol

Methyl-esterification of pectin catalyzed by cation exchange resin was carried out as follows. Pectin (100 mg) and anhydrous methanol (200 mL) were placed in a 500-mL round-bottomed flask attached to a reflux condenser. The mixture was heated at reflux temperature (65^{o}C) in an oil bath and stirred with a magnetic stirring bar for 2 h until the swollen pectin formed a relatively homogeneous suspension. The cation

exchange resin was then added as the catalyst (mass ratio of resin to pectin, 0 to 3.0), and the suspension was stirred at 65°C or 20°C for 0 h to 24 h. Upon completion of the reaction, the mixture was filtered to remove methanol, and the resulting residue was dissolved in distilled water, filtered, and washed carefully with distilled water. The solution was freeze-dried to yield methyl-esterified pectin, and the remaining resin was regenerated by activation at 105°C.

2.4. Determination of degree of esterification (DE)

The DE of pectin was estimated by using FT-IR as previously described (Chatjigakis et al., 1998; Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015; Singthong, Cui, Ningsanond, & Goff, 2004). To quantify the DE of the products, a calibration curve was constructed based on pectin standards of known DE (20, 40, 50, 60 and 80%) that were prepared by mixing the appropriate quantities of commercial standards. The mixed pectin samples were dissolved in deionized water, and the pH was adjusted to 6.0 with KOH to guarantee total ionization of the carboxylic acid groups. The standard pectins and products were dried and desiccated in a vacuum jar prior to FT-IR analysis. FT-IR spectra were obtained using a Nicolet magna 750 FT-IR spectrophotometer equipped with a DTGS detector covering the frequency range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} with 128 co-added accumulated transients. Specific bands at 1740 and 1630 cm^{-1} corresponded to the absorption of the esterified carbonyl groups and carboxylic ions, respectively. The DE was proportional to the ratio of the area from the band at 1740 cm^{-1} over the sum of the areas from the bands at 1740 and 1630 cm^{-1}. The regression equation used for the calibration curve was $DE=138.15A_{1740}/(A_{1740}+A_{1630})-0.0705$ ($r^2=0.995$; where A_{1740} and A_{1630} are the areas from the bands at 1740 and 1630 cm^{-1}, respectively).

2.5. Sugar composition analysis

Sugar composition was analyzed using high-performance liquid chromatography (HPLC) as described previously (Yang, Zhao, Wang, Wang, & Mei, 2005). In brief, each pectin sample (2 mg) was first methanolyzed with anhydrous methanol (1.0 mL) containing 2 M HCl at 80°C for 16 h, and the products were hydrolyzed with 2 M trifluoroacetic acid (TFA, 0.5 mL) at 120°C for 1 h. The released monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) and analyzed on a DIKMA Inertsil ODS-3 column (4.6 mm×150 mm) connected to a Shimadzu HPLC system (LC-10ATvp pump and UV-VIS detector). The derivative (20 μL) was injected, eluted with 82.0% PBS (0.1 M, pH 7.0) and 18.0% acetonitrile (v/v) at a flow rate of 1.0 mL/min, and monitored by UV absorbance at 245 nm.

2.6. High performance gel permeation chromatography

The molecular weight of pectin was determined by high performance gel permeation chromatography (HPGPC) on a TSK-gel G-3000PW_{XL} column (7.8 mm×300 mm, TOSOH, Japan) coupled to a Shimadzu HPLC system as previously described (Zhang et al., 2009). The column was pre-calibrated using standard dextrans of known molecular weights of 1, 5, 12, 25, and 50 kDa. Each pectin sample (20 μL, 5 mg/mL) was injected, eluted with 0.2 M NaCl at a flow rate of 0.6 mL/min, and monitored using a refractive index RID-10A detector (Shimadzu, Tokyo, Japan).

2.7. ^{13}C NMR spectra

Each pectin sample (20 mg) was prepared by solvation in deuterated water (1.0 mL, 99.8%) and stirred overnight at room temperature. ^{13}C NMR spectra (57,000 transients) were acquired at 25°C using a Bruker AV600 NMR spectrometer operating at a ^{13}C frequency of 150 MHz. Chemical shifts (δ) were expressed in ppm relative to that of acetone (δ=29.77).

177 *2.8. Statistical analyses*

178 SPSS 11.0 programs were used in the statistical analysis. All results were
179 expressed as mean ± standard deviation (SD). Data obtained from the study were
180 analyzed statistically using ANOVA and dunnett-tests. Values of $P<0.05$ and $P<0.01$
181 were considered to be significant.

182

183 **3. Results and discussion**

184 *3.1. Methyl-esterification of pectin*

185 In this study, two types of pectin (WGPA-3-HG and AHP-RG) were used for
186 esterification. As shown in Table 1, WGPA-3-HG was an HG-type pectin with a DE
187 of 34.7%, composed of GalA (84.4%), Rha (4.6%), Gal (4.9%) and Ara (6.1%)
188 (Zhang et al., 2009), and its molecular weight was approximately 17 kDa (Fig. 1A).
189 AHP-RG, extracted from sunflower heads and fractionated by ethanol precipitation
190 and gel permeation chromatography (unpublished data), contained GalA (34.3%), Rha
191 (25.6%), Gal (33.5%), Ara (1.4%), Glc (4.0%), and Man (1.2%). The ratio of
192 Rha/GalA was 0.76, which fell in the RG-I range of 0.05 to 1.0 as defined by Schols
193 and Voragen (1996), suggesting that AHP-RG was an RG-I-type pectin. At the
194 beginning of the reaction, the DE was 27.2% and the molecular weight was
195 approximately 77 kDa (Fig. 1B and 2B). WGPA-3-HG and AHP-RG were used as
196 starting materials for methyl-esterification reactions catalyzed by cation exchange
197 resin in methanol, as well as by the two commonly used methods that employ methyl
198 iodide or hydrochloric acid-acidified methanol.

199

199 **Table 1** Yield and sugar composition of WGPA-3-HG, AHP-RG and their methyl-esterified products.

Sample	Yield (%)	DE (%)[a]	Sugar composition (%)					
			GalA	Rha	Gal	Ara	Glu	Man
WGPA-3-HG	100	34.7±0.9	84.4±0.6	4.6±1.4	4.9±0.3	6.1±0.4	—	—
HG-CH_3I[b]	73.2±1.0*	86.2±1.3**	68.6±0.5**	9.8±0.3*	7.3±0.4*	12.1±0.3**	2.2±0.2	—
HG-HCl[c]	71.9±1.2*	84.6±1.7**	90.6±0.5	—	4.3±0.3	2.1±0.2**	1.8±0.3	1.2±0.2
HG-resin[d]	85.5±0.5*	95.0±1.3**	84.2±0.6	3.9±0.2	4.3±0.3	6.3±0.3	1.3±0.2	—
AHP-RG	100	27.2±1.4	34.3±0.5	25.6±0.3	33.5±0.4	1.4±0.3	4.0±0.4	1.2±0.3
RG-CH_3I[b]	76.3±1.1*	85.2±1.5**	32.9±0.3	30.2±0.5	31.1±0.3	0.8±0.2*	3.6±0.3	1.4±0.2
RG-HCl[c]	79.8±1.2*	75.9±1.2**	52.9±0.6**	34.1±0.3	8.7±0.2**	0.4±0.2**	3.5±0.5	0.4±0.4*
RG-resin[d]	82.7±0.8*	84.6±1.3**	35.1±0.4	25.4±0.4	33.6±0.4	1.3±0.3	3.7±0.4	0.9±0.3

200 a, Data are expressed as mean ± SD of triplicate measurements. Compared with WGPA-3-HG or
201 AHP-RG, * means $p < 0.05$, ** means $p < 0.01$.
202 b, product of methyl-esterification using methyl iodide.
203 c, product of methyl-esterification using methanol acidified with HCl.
204 d, product of methyl-esterification catalyzed by cation exchange resin with a resin/pectin mass ratio of
205 1.0 at 65 °C for 24 h.
206
207 *3.1.1. Methyl-esterification of HG-type pectin*
208 The extent of esterification, estimated by the DE of products, was determined by
209 using Fourier transform infrared spectroscopy (FT-IR). Sugar composition and
210 molecular weight of the products obtained by the three methods are given in Table 1
211 and Fig. 1A. Methyl-esterification of WGPA-3-HG performed with methyl iodide
212 yielded a product (HG-CH_3I) with a DE of 84.6%. However, the GalA content of
213 HG-CH_3I decreased from 84.4% to 68.6%, and the molecular weight was markedly
214 decreased from 17 kDa to 8.8 kDa, suggesting that the polymer chain was partially
215 degraded by β-elimination due to the basic reaction conditions (Deng et al., 2006).
216 Methyl-esterification using methanol acidified with hydrochloric acid yielded a

product (HG-HCl) having a higher DE of 86.2%. However, its molecular weight was decreased slightly to 14 kDa, with the GalA content being increased to 90.6% and the Ara and Gal contents being decreased to 2.1% and 4.3%, respectively. These results indicated that the neutral sugars of the side chains were hydrolyzed under these acidic conditions, whereas the backbone remained unaffected. This finding is consistent with the fact that the glycosidic linkages between neutral sugar residues are more readily hydrolyzed than those between galacturonic acid residues (Garna, Mabon, Nott, Wathelet, & Paquot, 2006). Lastly, the methyl-esterification catalyzed by the cation exchange resin was highly specific, resulting in product (HG-resin) showing the highest DE (95.0%) and without marked changes in either molecular weight or sugar composition. This indicates that methyl-esterification catalyzed by cation exchange resin is a useful method for esterification of HG-type pectins.

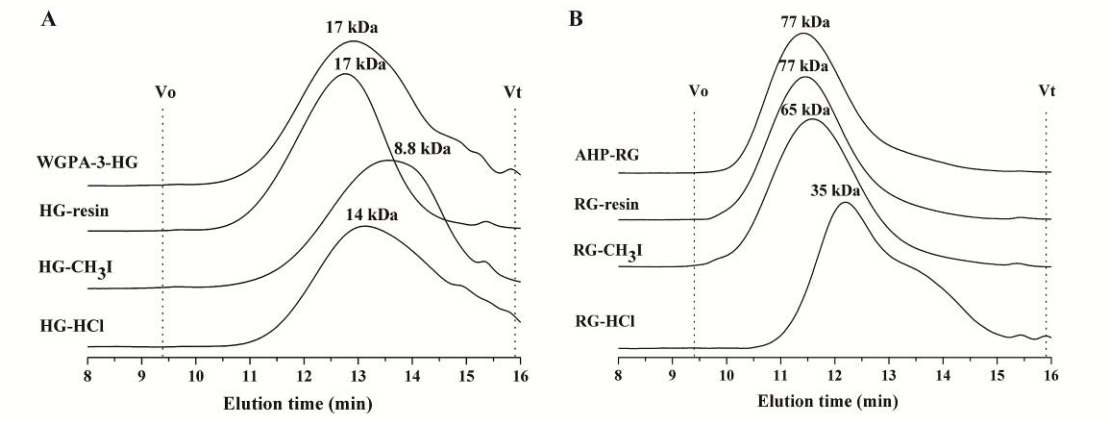


Fig. 1. HPGPC profiles of (A) WGPA-3-HG and its methyl-esterified products, and (B) AHP-RG and its methyl-esterified products.

The structures of the methyl-esterified product of WGPA-3-HG were further characterized by ^{13}C NMR (Fig. 2). Six prominent signals were observed and assigned to the non-esterified units of α-galacturonic acid (α-GalA): C-1, 98.5 ppm; C-2, 67.6 ppm; C-3, 68.3 ppm; C-4, 77.4 ppm; C-5, 70.8 ppm; and C-6, 174.9 ppm (Zhang et al.,

2009). Following methyl-esterification, the signal at 174.9 ppm was shifted to 170.2 ppm, which was attributed to methylated carboxyl groups, and the resonance at 98.5 ppm was shifted to 100.0 ppm, which was attributed to the C-1 of esterified α-GalA (Vriesmann & Petkowicz, 2009; Westereng, Michaelsen, Samuelsen, & Knutsen, 2008). The signal at 52.5 ppm, associated with methyl groups bonded to the carboxyls α-GalA, was significantly increased in intensity (Tamaki, Konishi, Fukuta, & Tako, 2008). These results further proved that WGPA-3-HG was highly esterified using our new method.

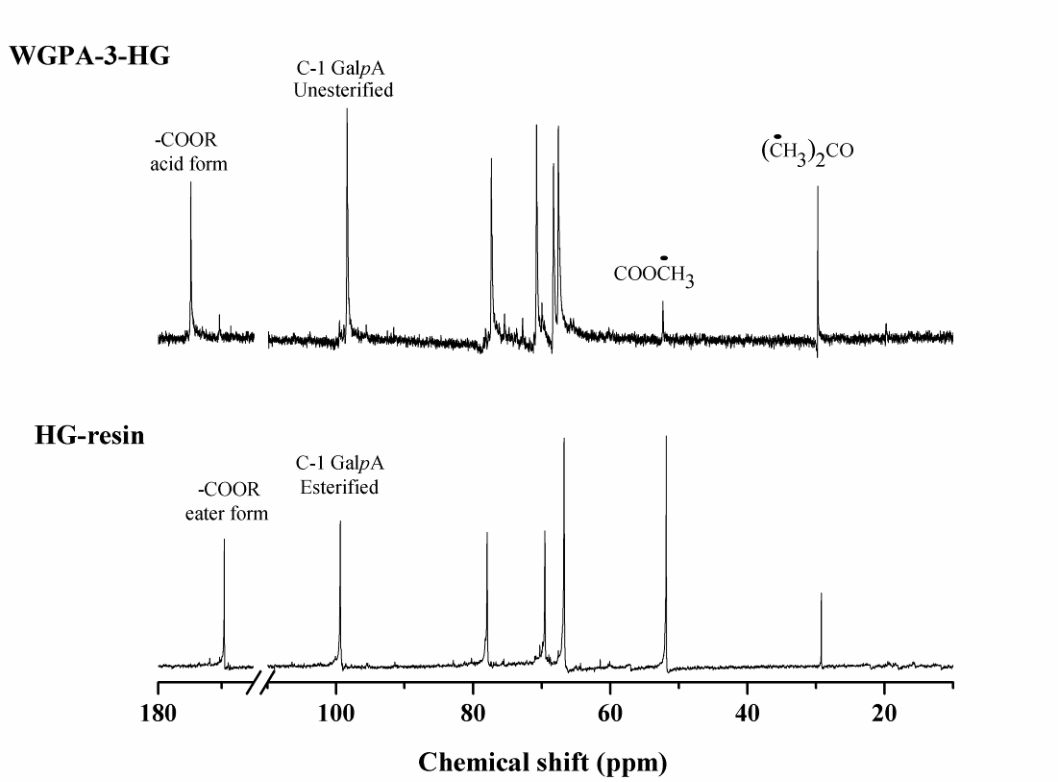


Fig. 2. The ^{13}C NMR spectra of WGPA-3-HG and its methyl-esterified product HG-resin.

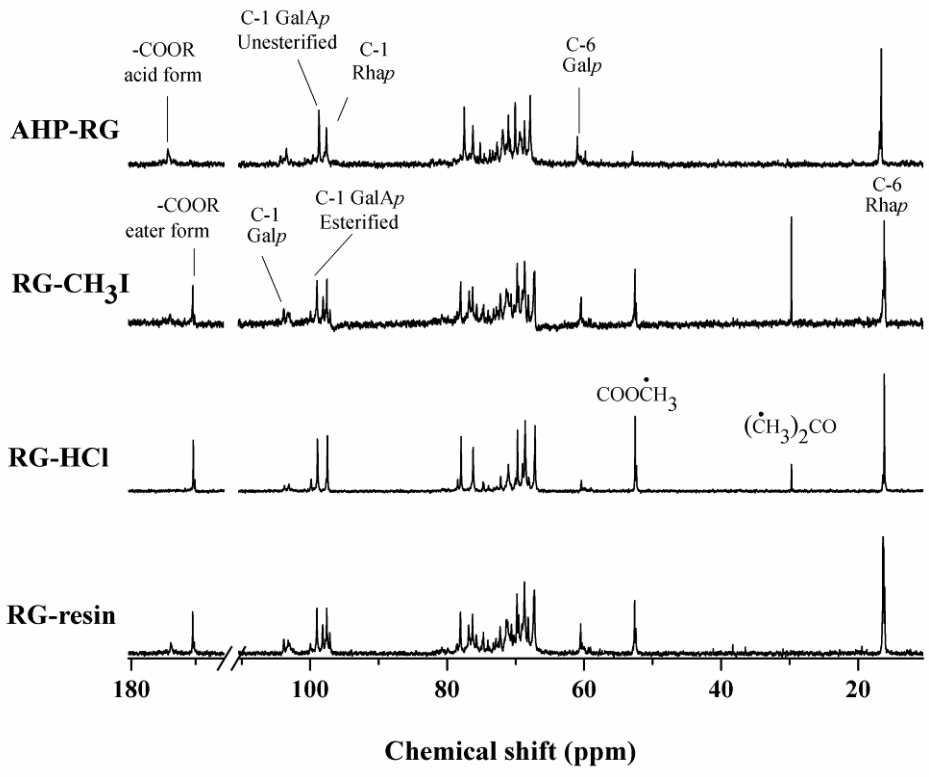
3.1.2. Methyl-esterification of RG-I-type pectin

The RG-I-type pectin AHP-RG was also methyl-esterified by the same three methods, with results shown in Table 1 and Fig. 1B. Methyl-esterification using the methyl iodide approach increased the DE to 75.9%, decreased the molecular weight from 77 kDa to 65 kDa and the GalA content to 32.9%, and increased the Rha content

252 to 30.2%. These results indicated that the partially esterified HG domains in AHP-RG
253 were somewhat degraded by β-elimination, whereas the RG-I domains were
254 minimally affected. The product (RG-HCl) that resulted from reaction with
255 HCl-acidified methanol showed that the DE increased to 85.2% and the molecular
256 weight decreased sharply to 35 kDa. Furthermore, the Gal and Ara content was
257 decreased to 8.7% and 0.4%, respectively, whereas the GalA and Rha content was
258 increased to 52.9% and 34.1%, respectively. These results revealed that the neutral
259 sugars in the side chains of RG-I domains were significantly hydrolyzed by acid
260 hydrolysis (Yapo, Lerouge, Thibault, & Ralet, 2007), whereas the HG domains
261 remained essentially unchanged. The methyl-esterification catalyzed by cation
262 exchange resin yielded the product RG-resin, which displayed a higher DE of 84.6%
263 and no changes in molecular weight or sugar composition. This indicates that the
264 method catalyzed by cation exchange resin could be used satisfactorily for
265 methyl-esterification of RG-I-type pectins as well.

266 The ^{13}C NMR spectrum of AHP-RG (Fig. 3) was more complex than that of
267 WGPA-3-HG, due to a higher content of neutral sugars. Thus, aside from the C-1 and
268 C-6 resonances of non-esterified α-GalA (98.2 and 173.9 ppm, respectively), those
269 from C-1 and C-6 of α-**L**-rhamnosyl units (97.1 and 16.1 ppm, respectively) and of
270 β-**D**-Gal units (103.9, 103.2 and 59.3 ppm, respectively) were observed in the
271 spectrum of AHP-RG (Westereng et al., 2008; Yu et al., 2010). In the spectra of all
272 three products, the C-1 signal at 98.2 ppm was shifted to 99.0 ppm, and the
273 corresponding high-frequency C-6 signal was shifted to 170.2 ppm due to
274 methyl-esterification of carboxyl groups in GalA residues. Moreover, the signal at
275 52.4 ppm, to which the methyl groups bound to the carboxyl groups of GalA were
276 assigned, was significantly increased in intensity. These results showed that AHP-RG

277 was methyl-esterified by all three methods. However, compared to AHP-RG, RG-HCl
278 produced a much simpler spectrum in which the C-1 and C-6 resonances of Gal were
279 decreased in intensity, suggesting that the neutral sugars in the side chains were
280 hydrolyzed. This finding is in agreement with the observed changes in molecular
281 weight and sugar composition. With the exception of signals from esterified GalA and
282 those from the methyl groups themselves, RG-CH_3I and RG-resin gave similar NMR
283 spectra to that of AHP-RG. These results suggest that the overall structure of AHP-RG
284 was not changed upon methyl-esterification using either methyl iodide or cation
285 exchange resin.



286

287 **Fig. 3.** The ^{13}C NMR spectra of AHP-RG and its methyl-esterified products.

288

289

290 Cation exchange resins are often used for esterification (Chen, Xu, Okuhara, &
291 Toshio, 1999; Harmer, & Sun, 2001; Heidekum, Harmer, & Hoelderich, 1999; Yadav

& Thathagar, 2002) and trans-esterification reactions (Dos Reis, Lachter, Nascimento, Rodrigues Jr, & Reid, 2005) in organic syntheses of small molecules, due to its non-corrosive properties and ease of separation. In the present study, cation exchange resin was used to catalyze the methyl-esterification of carboxylates in pectins, with its efficiency being compared to commonly used methods with methyl iodide and acidified methanol. Methyl-esterification using methyl iodide was sufficient for RG-I-type pectin, but not for HG-type pectin where the polymer backbone displayed significant degradation by β-elimination under basic conditions. On the other hand, methyl-esterification using acidified methanol was only optimal for HG-type pectin because the neutral sugars in the side chains of RG-I pectin were degraded by acid hydrolysis. Moreover, the acid was difficult to isolate, leading to wastewater and corrosive conditions for the equipment. However, these problems were avoided by using catalyzing the reaction using cation exchange resin, which readily modify both HG and RG-I pectins effectively without structural denaturation. The reaction scheme is illustrated in Fig. 4, showing that both the resin and methanol can be regenerated and recycled upon completion of the reaction, exemplifying the principle of "green chemistry".

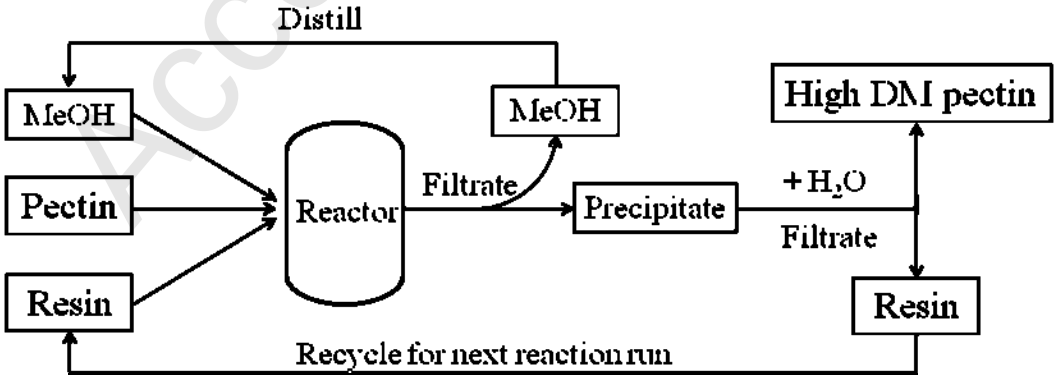


Fig. 4. Schematic diagram of the methyl-esterification of pectin catalyzed by cation exchange resin.

3.2. Effect of reaction conditions on methyl-esterification by cation exchange resin

Having demonstrated that methyl-esterification catalyzed by cation exchange resin functioned well with both HG- and RG-I-type pectins, we investigated how the reaction might be affected by varying reaction conditions, such as the mass ratio of resin to pectin, reaction time, and temperature. To further validate our method, WGPA isolated from the roots of *P. ginseng* was also methyl-esterified by cation exchange resin. WGPA was determined to be a mixture of HG- and RG-I-type pectins with a DE of 32.6%, containing GalA (53.4%), Rha (6.2%), Glu (10.8%), Gal (11.1%), Ara (16.3%) and GlcA (2.2%). Its molecular weight range was relatively broad, based on its presence in multiple eluent fractions from a Sepharose CL-6B column, similar to results described by Zhang et al. (2009).

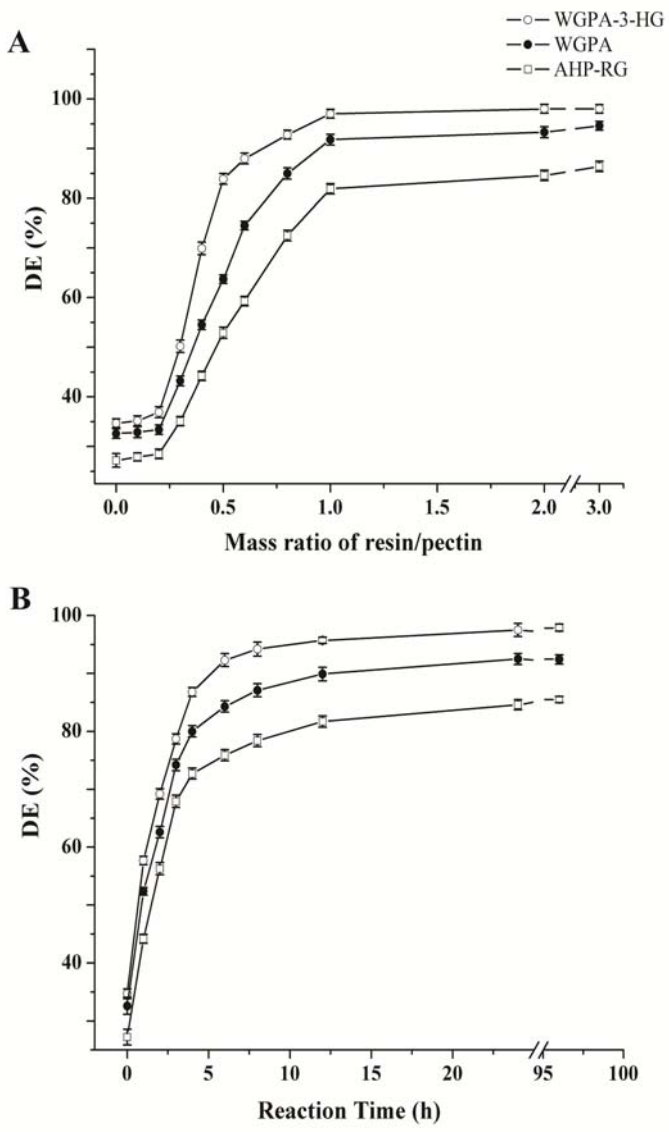
3.2.1. Effect of mass ratio of resin to pectin on the DE of products

The effect of the resin/pectin mass ratio on the DE of WGPA, WGPA-3-HG and AHP-RG was investigated using different mass ratios, with other reaction parameters being fixed at a reaction time of 24 h and temperature of 65^{o}C. As shown in Fig. 5A, there was an increasing trend in the DE of all three pectins. With WGPA, the DE changed little when the mass ratio fell below 0.2. As the mass ratio was increased from 0.3 to 1.0, the DE was significantly increased from 32.6% to 85.8%, but remained essentially unchanged upon further increases in mass ratio. Similar trends were observed with both WGPA-3-HG and AHP-RG. Therefore to obtain pectin with a relatively high DE, a mass ratio of resin to pectin greater 1.0 was required. On the other hand, it is possible to prepare a pectin with a specific DE by varying the resin/pectin mass ratio.

3.2.2. Effect of reaction time on the DE of products

The effect of reaction time on the DE of products was studied by running reactions at different times under otherwise constant conditions with a resin/pectin

338 mass ratios of 1.0 and reaction temperature of 65°C. The results in Fig. 5B showed
339 that the DE of the three pectins significantly increased as the reaction times were
340 increased from 1 to 12 h, with the highest DE being observed at or after 24 h.
341 Therefore, a reaction time of 24 h is required to obtain pectins with higher DE,
342 although the DE can be tailored to any desired value by controlling the reaction time
343 within the range of 1 to 12 h.



344

345 **Fig. 5.** Effect of (A) resin/pectin mass ratio and (B) reaction time on the DE of pectins. Reaction
346 conditions for (A): resin/pectin mass ratio from 0 to 3.0, 65°C for 24 h. Reaction conditions for (B):
347 reaction time from 0 to 96 h, resin/pectin mass ratios of 1.0 at 65°C. The values are means±SD from
348 three experiments.

3.2.3. Effect of reaction temperature on the DE of products

To study the effect of temperature on the DE of the products, the reaction was carried out at the boiling point of methanol (65°C) or at room temperature (20°C), while other reaction conditions were held constant with a resin/pectin mass ratio of 1.0 and reaction time of 24 h. Table 2 shows that the DE of pectins produced at 65 °C is approximately 13% higher than that obtained at 20°C. This result indicates, perhaps not unexpectedly, that a higher reaction temperature increases the DE of products.

Table 2 Effect of reaction temperature on the DE of pectins[a]

Sample	DE (%)		
	Original	20°C	65°C
WGPA	32.6±1.5	80.3±1.5**	92.5±1.1**
WGPA-3-HG	34.7±0.9	82.7±1.4**	95.0±1.1**
AHP-RG	27.2±1.4	71.2±1.3**	84.6±0.9**

[a] Data are expressed as mean ± SD of triplicate measurements. Compared with the original DE values, ** means $p < 0.01$.

Overall, it was found that all of the tested reaction conditions had significant effects on the extent of pectin methyl-esterification and that the DE of products could be tailored to any desired value by controlling reaction conditions (resin/pectin mass ratio, reaction time and temperature). In addition, the DE of WGPA-3-HG, AHP-RG and WGPA obtained under the same reaction conditions were different. HG-type pectin had the highest DE, while RG-I-type pectin had the lowest DE due to the

differences in their structures. Indeed, WGPA-3-HG is a linear chain polymer composed primarily of α-(1,4)-linked-**D**-galacturonic acid residues, and its carboxyl groups are exposed, making this molecule more susceptible to modification. AHP-RG consists of a backbone of alternating Rha and GalA residues with galactan side chains that typically inhibit esterification of carboxyl groups in adjacent regions. WGPA is a mixture of HG- and RG-I-type pectin, and its DE fell in between those of WGPA-3-HG and AHP-RG.

4. Conclusions

The simple catalysis with cation exchange resin was used to methyl-esterify pectin for the first time. Compared to the commonly employed methods using methyl iodide or acidified methanol, our resin-based method is most effective for methyl-esterification of HG- and RG-I-type pectins, because it can produces high DE pectin without marked degradation of their polymer chains. Moreover, it is possible to prepare these pectins with varying DE by controlling the resin/pectin mass ratio, reaction time, and reaction temperature. The resin and methanol can also be recycled to conserve resources, reduce costs, and minimize environmental pollution. These results will be highly useful for studying the structure-function relationships of pectins and may have future applications in the food industry.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31470798 and 31170770), the Doctoral Fund of the Ministry of Education of China (20120043130001), and the Chinese New Drug Creation and Manufacturing Program (2012ZX09502001-001).

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