Carbohydrate Polymers 114 (2014) 567–573

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Chemical and antihyperglycemic activity changes of ginseng pectin induced by heat processing

Lili Jiao[a], Xiaoyu Zhang[a], Mingzhu Wang[a], Bo Li[a], Zhen Liu[a], Shuying Liu[a,b,*]

[a] Jilin Ginseng Academy, Changchun University of Chinese Medicine, Jingyue Economic Development Zone, 1035, Boshuo Road, Changchun 130117, Jilin Province, China
[b] Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

ARTICLE INFO

Article history:
Received 4 May 2014
Received in revised form 25 July 2014
Accepted 12 August 2014
Available online 20 August 2014

Keywords:
Pectic polysaccharides
Panax ginseng C.A. Meyer
Processing temperature
Atiohyperglycemic effect
Antioxidant activity

ABSTRACT

Six pectic polysaccharides were obtained from white ginseng (GPW-1 and GPW-2), red ginseng (GPR-1 and GPR-2, steamed ginseng at 100 °C) and steamed ginseng (GPS-1 and GPS-2, steamed ginseng at 120 °C) by combination of water extraction, ion-exchange and gel permeation chromatographies. Based on the data from monosaccharide composition and ^{13}C NMR analysis, GPW-1, GPR-1 and GPS-1 were identified as type-I rhamnogalacturonan (RG-I)-rich pectins, GPW-2, GPR-2 and GPS-2 were homogalacturonan (HG)-rich pectins with different degrees of methyl-esterification. Remarkably, GalA increased with the increase of processing temperatures in these six fractions, which might be caused by the transformation of esterified GalA into un-esterified form during heat processing. In vivo animal experiments showed that GPs exhibited significant antiohyperglycemic and antioxidant activities in alloxan-induced diabetic mice, and the effects increased with the processing temperature, with the most potent activity in GPS.

1. Introduction

Diabetes is a global public health concern, affecting about 285 million adults (aged 20–79 years) in 2010, and is expected to increase to 439 million adults by 2030 (Shaw, Sicree, & Zimmet, 2010). Thus, the incidence of diabetes is increasing rapidly. This disease causes substantial morbidity, mortality and long-term complications and remains an important risk factor for cardiovascular disease (Yeh, Kaptchuk, Eisenberg, & Philips, 2003). Over 90% of diabetic populations suffer from type 2 diabetes (Skyler, 2004). The current available drugs for type 2 diabetes usually have many limitations, such as adverse side effects and high rates of secondary failure (Xie et al., 2005). Therefore, discovering and identifying novel drug for diabetes have been considerably conducted. An increasing number of herbs are used for diabetes glycamic control and prevention of diabetes complications. Among these herbs, *Panax ginseng* is one of the most commonly used. Its hypoglycemic effect has been supported by several studies in animals and humans (Reay, Kennedy, & Scholey, 2005; Sotaniemi, Haapakoski, & Rautio, 1995; Vuksan et al., 2008, 2000).

For storage and commercial production, fresh ginseng is often processed (drying) to white ginseng (WG) and red ginseng (RG). In Asia, the three preparations of this herb are WG, RG and steamed ginseng (SG). WG is a dried ginseng root, and RG and SG are ginseng roots which are steamed at about 100 °C and 120 °C, respectively, and then dried (Cho et al., 2008). RG and SG are reported to possess better pharmacological activities than WG, and the enhanced biological activities may be caused by the changes in the chemical constituents that occur during steam treatment (Kim et al., 2007; Kim, Murthy, Hahna, & Lee, 2008; Keum et al., 2000). Thus, the chemical constituents and bioactivity changes of ginsenosides and amino acid during heat processing have been widely studied (Cho et al., 2008; Lee et al., 2008). So far, reports on changes of other constituents, such as polysaccharide, between WG and SG are limited. *P. ginseng* polysaccharides have been reported to possess immunomodulation, antitumor, antiradiation, antioxidant and hypoglycemic activities (Luo & Fang, 2008; Park, Hwang, Song, & Jee, 2011; Suzuki & Hikino, 1989; Zhang et al., 2009). Hence, this study aimed to evaluate the changes in pectic polysaccharides from ginseng, including chemical components and the antihypoglycemic activity induced by heat processing.

* Corresponding author at: Jilin Ginseng Academy, Changchun University of Chinese Medicine, Jingyue Economic Development Zone, 1035, Boshuo Road, Changchun 130117, Jilin Province, China. Tel.: +86 43186045155; fax: +86 43186045258.
E-mail address: syliu@ciac.jl.cn (S. Liu).

http://dx.doi.org/10.1016/j.carbpol.2014.08.018
0144-8617/

2. Materials and methods

2.1. Chemicals

DEAE-cellulose, glibenclamide, monosaccharide standards and alloxan were purchased from Sigma Chemical Co. Triglyceride (TG), total cholesterol (TCH), liver glycogen and insulin levels, superoxide dismutase (SOD), maleic dialdehyde (MDA) and glutathione peroxidase (GSH-Px) kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other biochemicals and chemicals used in the experiment were of analytical grade.

2.2. Plant materials and SG preparation

P. ginseng roots aged 5 years were collected from Changbai Mountain, Jinlin Province, China in Augest 2012. Fresh ginseng roots were rinsed with water, and then SG, i.e., RG and SG, were prepared by steaming at 100 °C and 120 °C for 3 h by using an autoclave, respectively. The unsteamed ginseng and SG were dried at 50 °C until the moisture content was 14% to prepared WG, RG and SG.

2.3. Preparation of pectic polysaccharides from ginseng (GPs)

Air-dried *P. ginseng* roots were crushed and extracted with 95% ethanol under reflux extraction at 40 °C to remove pigments and small lipophilic molecules. The residue was further extracted thrice (4 h each) with distilled water at 100 °C. All aqueous extracts were combined, centrifuged under reduced pressure and precipitated by adding of 95% ethanol (4 volumes). The crude polysaccharide precipitate was collected by centrifugation, and dried under reduced pressure after washed with dehydrated alcohol and diethyl ether. The crude polysaccharides was dissolved in distilled water and centrifuged, and then the supernatant was applied to a column of DEAE-cellulose (Cl^-) column ($7.0 \times 30\ cm^2$). This column was initially eluted with distilled water (3000 mL), and an unbound fraction was recovered. The bound polysaccharides were eluted by 0.5 M NaCl solution (1000 mL), the eluent was collected and identified as the pectic polysaccharides of ginseng (GPs). Subsequently, Sephadex G-100 column ($2.5 \times 100\ cm^2$) were used to further purify the fractions obtained by anion-exchange step.

2.4. Chemical analysis of GPs

The sugar content of GPs was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The content of protein was determined by Bradford's method (Bradford, 1976). The content of uronic acids was measured by the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973).

2.5. Analysis of monosaccharide composition

The sample (2 mg) was hydrolyzed with 0.5 ml of 2 M trifluoroacetic acid (TFA) in an ampoule (2 ml). The ampoule was sealed under a nitrogen atmosphere and kept at 120 °C for 1 h, and the excess acid was completely removed by co-distillation with ethanol. After removing the acid, the products of hydrolysis were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) according to the method in the literature (Honda et al., 1989). The monosaccharide derivatives were analyzed by Aglient RRLC 1200 SL system (Agilent Technologies, Wilmington, USA), equipping with a DIKMA Inertsil ODS-3 column (4.6 i.d. ×150 mm, 5 μm, Dikma, Japan), detected by UV–vis DAD detector and connected to a Chemstation system. The PMP derivative (20 μl) was injected, eluted with 82.0% phosphate-buffered saline (PBS, 0.1 M, pH 7.0) and 18.0% acetonitrile (v/v) at a flow rate of 1.0 mL/min at room temperature. The wavelength for UV detection was 245 nm (Chambers & Clamp, 1971; Fu & O'neill, 1995; Strydom, 1994).

2.6. Homogeneity and molecular weight analysis of GPs

The homogeneity and the molecular weight distribution of samples were determined by high-performance lipid chromatography (HPLC), which was performed on a SHIMADZU HPLC system (Shimadzu, Japan) fitted with one TSK-G3000 PWXL columns (7.8 mm ID × 30.0 cm L) and a RID-10A detector. The column was calibrated by molecular mass markers (T-130, 80, 40, 20 and 10). The mobile phase was 0.7% Na_2SO_4, and the flow rate was 0.5 ml/min at 40 °C, with 1.6 mPa.

2.7. IR spectrum analysis

The FT-IR spectra were recorded with a Bruker (Germany) Vector 33 spectrometer in a range 4000–400 cm^{-1}. The samples were analyzed as KBr pellets.

2.8. NMR spectrometry

The samples were dissolved in D_2O (1 mL, 99.9%), and their ^{13}C-NMR spectra were recorded at 25 °C with a Bruker-600 FT-NMR spectrometer (600 MHz).

2.9. Animals

ICR male mice weighing 20.0 ± 2.0 g were obtained at 6–8 weeks of age. The mice were purchased from Changchun Institute of Biological Products Co., Ltd. The experimental animal production license number is SCXK (J2012-0001). They mice were randomly housed by tens into cages and received standard mouse chow and water. The room conditions were maintained at room temperature with 12/12-h light–dark cycle. Rodent laboratory mouse chow and water were provided ad libitum. All animals were maintained and used in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines issued by Experimental Animal Centre of Changchun University of Chinese Medicine. This study was approved by the university committee for animal experiments.

2.10. Experiment design

Mice were acclimatized for 7 days and fasted for 12 h prior to the experiments. Alloxan freshly prepared in 0.9% NaCl was immediately injected intraperitoneally with a single dose of 200 mg/kg body weight. After 72 h, fasting blood glucose levels were determined from tail vein blood samples using a glucose analyzer (ONETOUCH Select Simple, Johnson & Johnson, USA). Hyperglycemic mice with blood glucose levels higher than 16.8 mmol/L were used as diabetic mice and were selected for the following experiments (Fujita et al., 2005).

Fifty diabetic mice were randomly divided into five groups (10 mice in each group): model control group, positive control group and ginseng pectin treated groups. Ten normal mice served as normal control group and received distilled water orally. The mice in model control group were orally administrated with saline solution (0.2 mL). Mice in the positive group were orally administrated with 100 mg/kg of glibenclamide (0.2 mL). The WGPAs treated mice were orally administrated with 100 mg/kg of GPW, GPR and GPS, respectively. All groups were performed once daily for 4 weeks.

After the last drug administration, all mice were fasted overnight, and their bodyweight and blood glucose levels were

measured. The blood were then collected and centrifuged at 4000 × *g* at 4 °C for 10 min to separate the serum required. The livers were removed, washed and immediately homogenized in ice-cold physiological saline. The homogenate was centrifuged at 4000 rpm/min and 4 °C for 10 min, and the supernatant was collected for further analysis (Liu, Sun, Rao, Su, & Yang, 2013).

2.11. Biochemical analysis

The serum concentration of insulin, TG, TCH and liver glycogen levels were determined using commercial reagent kits according to the manufacturer's instructions.

The concentrations of SOD, MDA, CAT, GSH-Px, vitamin C and vitamin E in the supernatant of serum, livers and kidneys were assayed using commercial reagent kits obtained from the Institute of Biological Engineering of Nanjing Jianchen (Nanjing, China) according to their instructions.

2.12. Statistical analysis

Results were expressed as means ± SD and analyzed by one-way ANOVA followed by Tukey's test. This test was carried out by SPSS 13.0. A value of $P < 0.01$ was considered statistically highly significant, and a $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Chemical composition of the fractions separated by DEAE-cellulose charomatagory

The neutral sugar, uronic acid and protein contents of the GPW, GPR and GPS were determined first. The neutral sugar of GPS was higher (58.25%) than GPR (57.43%) and GPW (57.24%). The uronic acid contents evaluated in GPW, GPR and GPS were 17.26%, 27.97% and 35.47%, respectively. The content of protein was relatively lower in GPS (1.00%) than GPR (3.9%) and GPW (4.92%).

3.2. Fractionation, homogeneity and molecular weight of final fractions separated by Sepharose CL-6B chromatography

The three crude acid polysaccharides from WG, RG and SG obtained after the first run on DEAE-cellulose column were further fractionated using size-exclusion chromatography, and numbered according to their elution order from the Sepharose CL-6B column. When chromatographed on Sepharose CL-6B, all three polysaccharides revealed two main peaks, one rich in neutral sugars was eluted first and the other rich in uronic acid was eluted later. These observations are similar to previously fractionation for acid polysaccharide of white ginseng (Zhang et al., 2009). Therefore, six homogeneous polysaccharides, namely, GPW-1, GPW-2, GPR-1, GPR-2, GPS-1 and GPS-2, were obtained after Speharose CL-6B chromatography. The HPLC profiles (data not shown) demonstrated that the six polysaccharides each had a single and symmetrically sharp peak revealing that they were homogeneous polysaccharides. According to the retention time, their molecular weights were estimated to be 8.51×10^5 Da (GPW-1), 2.95×10^5 Da (GPW-2), 8.86×10^5 Da (GPR-1), 2.58×10^5 Da (GPR-2), 9.61×10^5 Da (GPS-1) and 3.39×10^5 Da (GPS-2). The results indicated that the molecular weights of the six polysaccharides increased slightly with the intensity of the steam treatment.

3.3. Monosaccharide composition of GPs

HPLC analysis showed that GPW, GPR and GPS were all composed of six monosaccharides Fru, Rha, GalA, Glc, Gal and Ara.

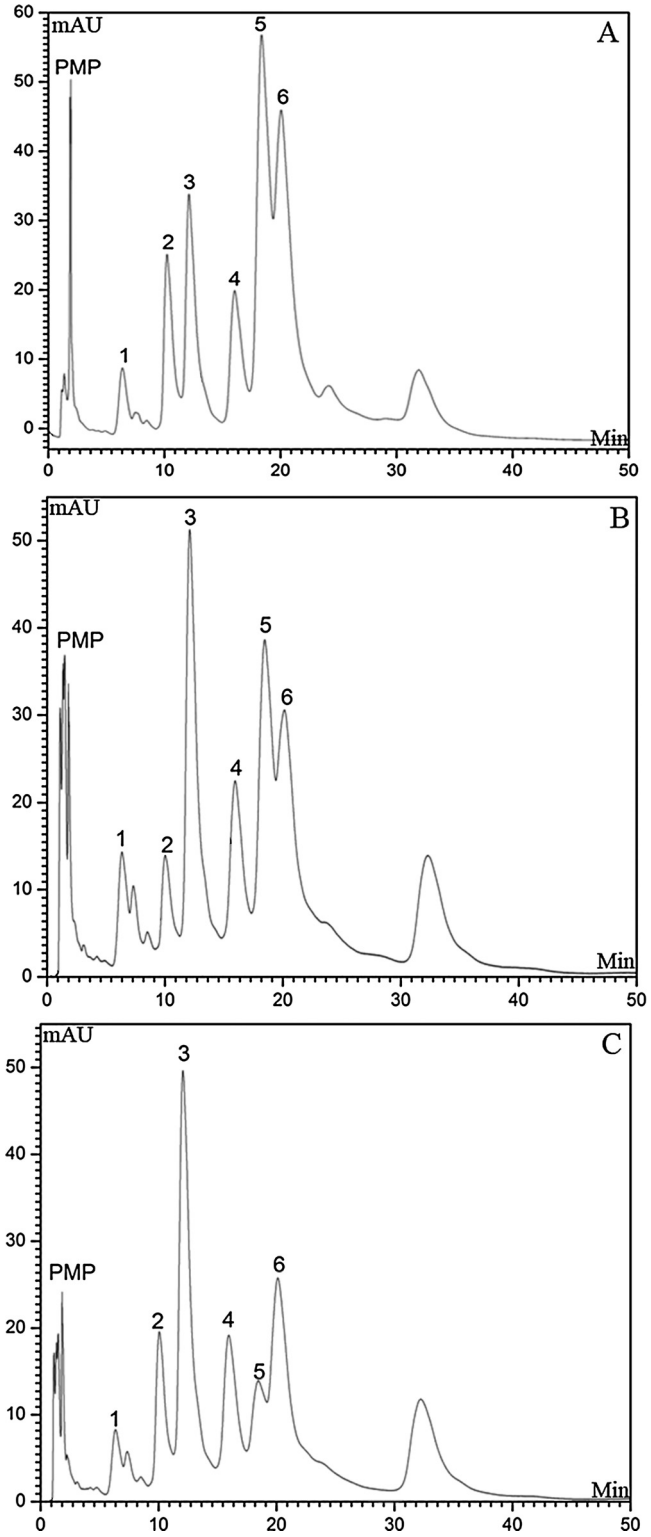

Fig. 1. HPLC analysis of monosaccharide composition of GPW, GPR and GPS. They were hydrolyzed, dried, PMP-labeled and analyzed by HPLC as described in the experimental methods.

Statistical analysis showed that processing temperature significantly affected the ratio of monosaccharide of ginseng acid polysaccharides, and the changes in the six sugars were shown in Fig. 1 and Table 1. The amount of GalA was high in GPS compared with that in GPW and GPR. In other words, GalA increased 1.62 fold after steaming at 100 °C, and 2.06 fold after steaming at 120 °C.

Table 1
Analysis of monosaccharide composition of GPs.

Group	Monosaccharide composition (%)					
	Fru	Rha	GalA	Glc	Gal	Ara
GPW	5.15	8.74	17.26	8.36	34.35	26.14
GPR	10.08	4.72	27.97	10.14	28.65	18.45
GPS	7.38	9.92	35.47	12.3	11.24	23.68
GPW-1	0.85	1.40	6.35	3.57	26.33	61.75
GPR-1	0.62	1.92	10.64	12.29	19.96	54.57
GPS-1	2.71	2.12	11.27	13.31	39.92	30.67
GPW-2	6.48	3.64	29.12	6.19	22.33	32.24
GPR-2	2.77	6.85	61.55	1.52	18.76	8.55
GPS-2	5.57	6.71	68.09	9.36	6.62	3.65

On the contrary, the total content of Gal and Ara was reduced by 22.13% after steaming at 100 °C, and abruptly reduced by 42.27% after steaming at 120 °C. Fru, Rha and Glc showed no significant change.

As shown in Table 1, sugar composition of the three fractions GPW-1, GPR-1 and GPS-1 was similar, and Ara, Gal and GalA were the major sugars in them. Rha in these three fractions was determined to be 1.40–2.12%, which was very low compared with the GalA content. According to the classification of pectins by Schols and Voragen (1996) that is based partly on the ratio of Rha/GalA, RG-I could be differentiated from HG by its ratio Rha/GalA that ranges from 0.05 to 1. The ratios of Rha/GalA in GPW-1, GPR-1 and GPS-1 were 0.22, 0.18 and 0.19, respectively. This indicated that they might consist mainly of RG-I domains whose side chains were composed of arabinans, galactan, type I and/or II arabinogalactan (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996). As shown in Table 1, Galacturonic acid was the prominent constituent in GPW-2 (29.12%), GPR-2 (61.55%) and GPS-2 (68.09%), suggesting a predominance of HG in the three fractions. Interestingly, the increase of GalA and decrease of the total amount of Gal and Ara with the processing temperature was observed in all the six fractions. According to previous reports (Tamaki, Konishi, Fukuta, & Tako, 2008; Zhang et al., 2009), GalA exits in two forms in ginseng pectin: methyl-esterified and un-esterified form, thus, the increase of GalA in GPR and GPS suggested the transformation of esterified GalA into un-esterified form during heat processing.

3.4. IR analysis of GPs

The IR spectra of GPW, GPR and GPS were quite similar only with some difference in the intensity of bands (Fig. 2). The FT-IR spectrum of GPW, GPR and GPS had very strong bands near 3240 cm^{-1} assigned to O—H stretching vibration. The bands in the region of 1762 cm^{-1} and 1611 cm^{-1} were ascribed to C=O stretching and asymmetric C=O stretching vibration of carboxyl group, respectively. The strong absorbance at 1436 cm^{-1} was assigned to C—O carboxylate stretching bands. Moreover, the bands observed

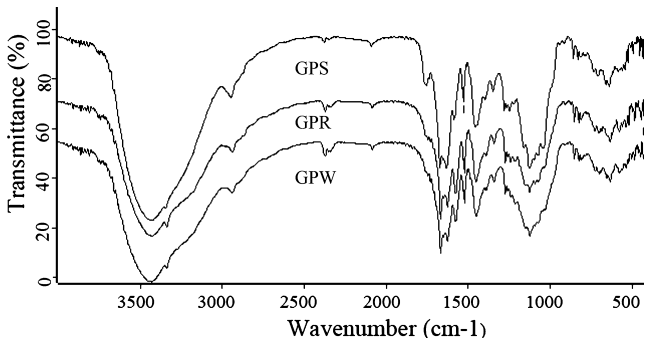

Fig. 2. IR spectrum of GPW, GPR and GPS.

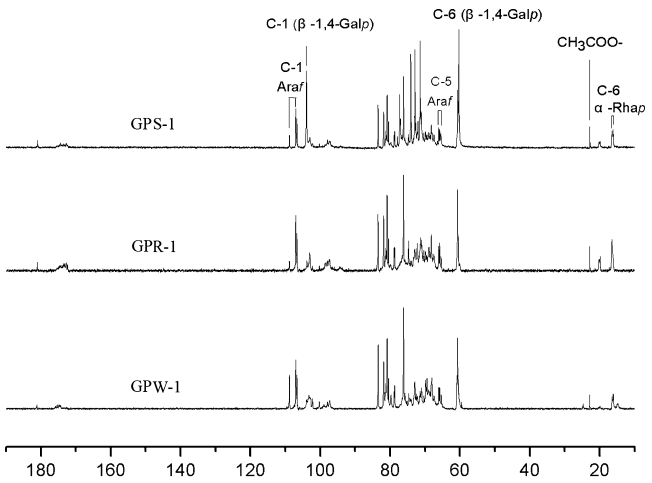

Fig. 3. ^{13}C NMR spectra of GPW-1, GPR-1 and GPS-1.

at 838 cm^{-1} indicated the existence of α-configuration in GPs (Synytsyaa, Copikova, Matejkab, & Machovic, 2003).

3.5. Structure features of purified GPs

The ^{13}C NMR spectra of GPW-1, GPR-1 and GPS-1 in D_2O were shown in Fig. 3. The anomeric resonances at 106.7, 107.1 and 108.9 ppm originated from α-1, 3-Ara*f*, α-1, 5-Ara*f* and non-reducing terminal Ara*f* units, respectively (Polle, Ovodova, Shashkov, & Ovodov, 2002), and the signals at 103.0–104.1 ppm were assigned to β-Gal*p*. The stronger signal appearing at 104.1 ppm in GPS-1 was assigned to C-1 of β-1, 4-Gal*p*, however, it could not be observed obviously both in GPW-1 and GPR-1. Moreover, GPW-1 and GPR-1 had more complex signals for C-1 of β-Gal*p* (102.6–103.9 ppm), indicating the existence of multiple linkage forms. Some weak signals (103.0–103.8 ppm) from C-1 in rhamnopyranosyl residues were observed in the three spectra, and the peaks of C-6 to α-Rha*p* appeared at 16.7–17.3 ppm. The signals at 22.8 ppm arised from O-acetyl function of β-1, 4-Gal*p*. These observations, when taken together, further demonstrated that GPW-1, GPR-1 and GPS-1 were RG-I-containing polysaccharides, and their side chain decorations, mainly linked to the O-4 of some of the α-L-Rha units, could be arabinans, galactans, type I and/or type II arabinogalactans.

GPW-2, GPR-2 and GPS-2 with high amount of GalA had complex ^{13}C NMR spectra, revealing the complexity of their structure, but we could still get some information about their structure from the characteristic signals of C-1 and C-6 (Fig. 4). In the anomeric region, the strongest resonance signal was attributed to α-GalA*p*. The signals appearing at 175.0 ppm and 170.1 ppm were indicative of the presence of methyl esterified and un-esterified carbonyl carbons in GalA. Moreover, the characteristic peaks of α-1, 4-GalA*p* could be clearly indentified in the spectra of GPR-2 and GPS-2, with the chemical shifts for carbon were C1 (98.7), C2 (67.7), C3 (68.3), C4 (77.32), C5 (71.2) and C6 (175.0) ppm. Comparing with GPR-2 and GPS-2, GPW-2 had more complex ^{13}C NMR spectrum, which might be related to its high esterification and more neutral sugars (Zhang et al., 2009). In addition, the peaks at 95.8, 103.9 and 106.7 were assigned to α-Rha*p*, α-GalA*p*, β-Gal*p* and α-Ara*p*. The signals at 22.8 and 16.3 ppm, which were attributed to O-acetyl groups in β-Gal*p* and C-6 in α-Rha*p*, could also be identified in these three ^{13}C NMR spectra. These results further proved that these three fractions were HG-rich pectins, and also contained small amount of other domains.

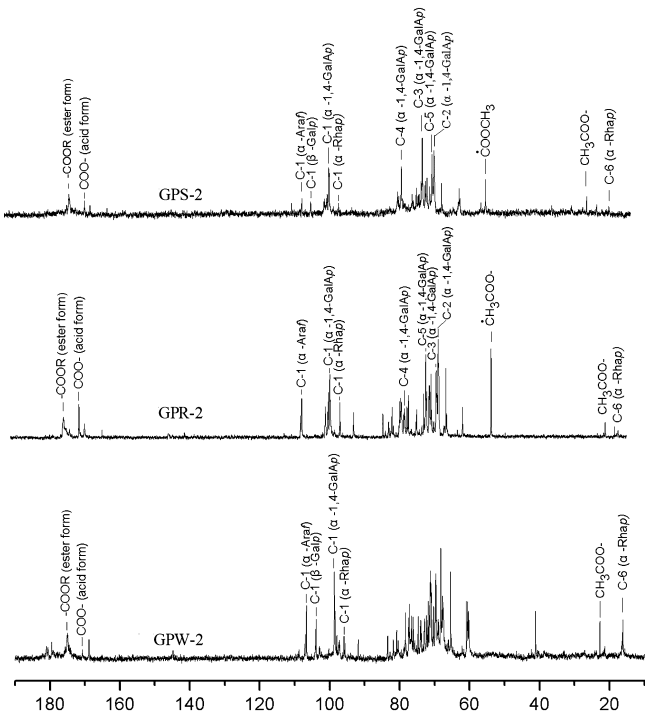

Fig. 4. ^{13}C NMR spectra of GPW-2, GPR-2 and GPS-2.

3.6. Effect of GPs on blood glucose level in alloxan-induced diabetic mice

To determine whether the processing temperature affected the hypoglycemic activities of pectic polysaccharides from ginseng, an assay was conducted on GPW, GPR and GPS. The level of fasting plasma glucose in untreated diabetic control mice was markedly higher than those of the untreated normal control mice. When diabetic mice were for 4 weeks, GPs at 50 mg/kg significantly decrease the level of blood glucose (Table 2). Compared with GPW, GPR and GPS showed significantly higher hypoglycemic activity (Table 2), and the trend was consistent at all dosages. GPs at 100 mg/kg showed better effect in decreasing glucose level than the other dosages. To compare the influence of different processing temperatures on the antihypoglycemic activities of ginseng pectins, an assay was performed with a single concentration (100 mg/kg) on GPW, GPR and GPS.

3.7. Effect of GPs on body weight in alloxan-induced diabetic mice

Weight loss and fatigue are the common symptoms of type 2 diabetes. We therefore detected the recovery of ginseng pectin on weight loss of diabetic mice, and the results were summarized in Table 2. The body weight of mice were significantly decreased after injected with alloxan compared with normal mice, and all these increases were improved obviously by administration with GPs (100 mg/kg) in different groups, and the effect in GPS group was most remarkable, followed by GPR and GPW. Previous reports revealed that ginseng could reduce HbA_{1c} level of type 2 diabetic individuals; however, those mice receiving ginseng also experienced a reduction in body weight. The present study found that pectic polysaccharides from ginseng decreased the glucose level, and the loss of body weight in mice was also recovered. Therefore, they are potential candidates for amelioration of type 2 diabetes.

3.8. Effect of GPs on serum insulin, glycogen, TG and TCH levels in alloxan-induced diabetic mice

Type 2 diabetes is a group of chronic metabolic diseases that result from insulin deficiency, insulin resistance, or both (Alberti & Zimmet, 1998; Kuzuya et al., 2002). It is characterized by high blood glucose level because of the inability of the body cells to use glucose properly (Ugochukwu & Babady, 2002). Type 2 diabetes is often accompanied by a variety of disorders in metabolic and regulatory processes, which lead to accumulation of lipids such as TC and TG in diabetic patients (Liu et al., 2013). The present study investigated the changes on the levels of serum insulin, TG, TCH and glycogen in mice, and the results were summarized in Table 3. In the diabetic control group, the insulin and glycogen levels were decreased markedly ($P < 0.05$), while TG and TC levels were increased compared to the untreated normal mice. Administration of GPs and glibenclamide effectively increased hepatic glycogen and insulin levels, and simultaneously decreased TG and TC levels of diabetic mice ($P < 0.05$ or $P < 0.01$). The hypoglycemic activities of GPs at 100 mg/kg were determined as GPW < GPR < GPS. This result indicated that the hypoglycemic activity of pectic polysaccharides of ginseng was related to the processing temperature, that is, the antihypoglycemic activity of ginseng pectins increased with the increase of processing temperature. The bioactivities of pectins can be affected by their contents in GalA and neutral sugars, the amounts and distribution of substituents (methoxyl and acetyl groups), as well as the molar mass and its distribution (Axelos & Thibault, 1991; Bonnin, Dolo, Le Goff, & Thibault, 2002). According to the aforementioned results, the molecular weights and the percentage of each sugar in GPs changed significantly during heat processing which may lead to structural changes of the pectic polysaccharides, and then affect their hypoglycemic activities.

3.9. Effect of GPs on MDA and non-enzymic antioxidants levels and antioxidant enzyme activities in serum and liver of alloxan-induced diabetic mice

Some biochemical pathways are activated during pathogenesis of type 2 diabetes, which can promote the production of

Table 2
Comparison of the body weight and blood glucose level in alloxan-induced diabetic mice among the GPs groups.

Group	Body weight (g)		Blood glucose (mmol/L)	
	Pre-experiment	Post-experiment	Pre-experiment	Post-experiment
Normal control	24.45 ± 1.25	31.67 ± 1.53[c]	5.0 ± 1.1[c]	5.6 ± 0.9[c]
Model control	24.68 ± 1.28	18.5 ± 1.3[a]	17.4 ± 4.8[a]	19.5 ± 3.6[a]
Positive control	24.28 ± 2.28	26.3 ± 0.9[a,c]	17.5 ± 4.9[a]	9.1 ± 1.4[a,c]
GPW	24.8 ± 1.3	25.48 ± 0.9[a,c]	17.7 ± 6.2[a]	12.2 ± 6.8[a,c]
GPR	24.27 ± 1.87	25.8 ± 1.07[a,c]	17.9 ± 4.4[a]	11.8 ± 4.0[a,c]
GPS	24.47 ± 1.67	26.07 ± 1.01[a,c]	18.3 ± 4.0[a]	10.5 ± 0.7[a,c]

Values are expressed as mean ± SD for six mice in each group. One-way ANOVA repeated measures with Duncan's multiple rang test was used to calculate statistical significance.
[a] $P < 0.01$, [b] $P < 0.05$, vs. control group.
[c] $P < 0.01$, [d] $P < 0.05$, vs. model control group.

Table 3
Comparison of the serum insulin, glycogen, TG and TCH content in alloxan-induced diabetic mice among the GPs groups.

Group	Insulin level (μIU/ml)	TG (mmol/L)	TCH (mmol/L)	Glycogen content (mg/g liver tissues)
Normal control	23.49 ± 4.60	1.16 ± 1.08	1.52 ± 0.77	7.61 ± 4.28
Model control	10.61 ± 2.98[a]	2.82 ± 0.31[a]	4.00 ± 1.43[a]	1.82 ± 0.31[a]
Positive control	19.52 ± 3.29[c]	1.44 ± 0.19[c]	1.60 ± 1.03[c]	5.22 ± 4.47[c]
GPW	12.72 ± 4.13[d]	2.22 ± 0.20[d]	3.51 ± 1.60[d]	2.88 ± 0.26[d]
GPR	15.25 ± 3.52[d]	1.91 ± 1.52[d]	1.90 ± 1.89[c]	2.91 ± 0.46[d]
GPS	18.04 ± 2.84[c]	1.73 ± 0.19[c]	1.80 ± 1.98[c]	4.58 ± 2.27[c]

Values were expressed as mean ± SD (n = 10).
[a] P < 0.01, [b] P < 0.05, vs. control group.
[c] P < 0.01, [d] P < 0.05, vs. model control group.

Table 4
Effect of GPs on MDA, VC and VE levels and activities of SOD, GSH-Px in alloxan-induced diabetic mice.

Group	Normal control	Model control	Positive control	GPW	GPR	GPS
Serum						
MDA (nmol/ml)	5.02 ± 1.17	8.47 ± 2.43[a]	5.44 ± 1.85[c]	6.82 ± 1.89[d]	6.21 ± 1.81[c]	5.81 ± 1.22[c]
SOD (U/ml)	109.19 ± 17.49	92.10 ± 3.62[a]	105.91 ± 9.92[c]	100.80 ± 6.17[d]	105.97 ± 10.66[c]	115.42 ± 10.06[c]
GSH-PX (U/ml)	888.42 ± 84.21	700.35 ± 120.7[a]	823.16 ± 86.31[c]	775.44 ± 62.45[d]	800.70 ± 42.81[c]	835.09 ± 171.23[c]
VC	14.02 ± 2.46	12.02 ± 0.88[b]	13.85 ± 0.6[d]	13.92 ± 2.70[d]	13.71 ± 0.59	13.89 ± 0.56[d]
VE	12.53 ± 3.13	9.27 ± 2.02[a]	10.03 ± 1.06[d]	11.29 ± 1.18[d]	11.23 ± 1.19[d]	11.80 ± 3.03[c]
Liver						
MDA(nmol/mg protein)	3.70 ± 0.92	4.59 ± 1.06[a]	3.70 ± 0.79[c]	4.01 ± 0.54[d]	3.91 ± 0.53[d]	3.85 ± 0.68[d]
SOD (U/mg protein)	276.62 ± 27.76	239.98 ± 16.35[a]	264.38 ± 21.06[c]	253.6 ± 14.17[d]	258.23 ± 4.57[c]	265.48 ± 12.88[c]
GSH-PX (U/mg protein)	1049.03 ± 32.71	673.8 ± 95.49[a]	986.67 ± 196.01[c]	783.89 ± 68.69[c]	800.38 ± 72.92[c]	920.92 ± 82.63[c]
VC	2.09 ± 0.31	1.15 ± 0.08[a]	1.77 ± 0.18[c]	1.75 ± 0.10[c]	1.75 ± 0.44[c]	1.77 ± 0.13[c]
VE	21.44 ± 2.03	16.53 ± 6.76[a]	19.65 ± 1.30[c]	19.06 ± 1.24[d]	19.24 ± 3.17[d]	20.82 ± 0.88[c]

Values were expressed as mean ± SD (n = 10).
[a] P < 0.01, [b] P < 0.05, vs. control group.
[c] P < 0.01, [d] P < 0.05, vs. model control group.

reactive oxygen species, and free radicals and reduce the antioxidant defense capacity (Ceriello & Motz, 2004; Evans, Goldfine, Maddux, & Grodsky, 2003; Gopaul et al., 2001; Vijayalingam, Parthiban, Shanmugasundaram, & Mohan, 1996). Free radicals can provoke lipid peroxidation and cause damage or destruction to a variety of tissues, and eventually lead to the development of diabetes and its complications. Thus, using antioxidants is regarded as a strategy to reduce the oxidative stress in type 2 diabetic patients. Many polysaccharides have recently shown to counteract hyperglycemia-induced alterations in vivo, and their hypoglycemic mechanisms are closely related to their antioxidant activities (Hegde et al., 2011; Huang et al., 2012; Zhao, Lan, Huang, Ouyang, & Zeng, 2011). Our results showed that the MDA level was significantly increased (P < 0.05 or P < 0.01, Table 4), whereas the concentrations of non-enzymatic antioxidants measured as VC and VE and the activities of antioxidant enzymes measured as SOD and GSH-Px were obviously decreased (P < 0.05 or P < 0.01) in both the serum and liver of diabetic mice when compared with normal control mice. After a 4-weeks administration, all GPs decreased the MDA level (P < 0.05 or P < 0.01), increased the non-enzymatic antioxidant concentrations and antioxidant enzyme activities of diabetic mice. GPS showed stronger antioxidant activity than GPR and GPW, suggesting that the increase of processing temperature had a positive effect on the antioxidant activity of ginseng pectin. This result was consistent with that of Cho's study; their findings showed that high-quality of ginseng can be obtained by steaming the ginseng root at higher temperature (120 °C), and its antioxidant activity remarkably increased (Cho et al., 2008).

4. Conclusion

In conclusion, all three pectic polysaccharides from WG, RG and SG were separated into two fractions which were identified as RG-I-rich and HG- rich pectins based on combination of monosaccharide composition and ^{13}C NMR analysis. The total amount of Gal and Ara in GPs decreased sharply with the processing temperature, however, the GalA content in GPS were markedly increased, compared with those in GPW and GPR. Furthermore, the molecular weights of the six pectic polysaccharides were slight increased. In addition, GPS showed stronger function in increasing the body weight, insulin and glycogen levels as well as reducing levels of blood glucose, TC, TG and MDA of alloxan-induced diabetic mice than GPR and GPW. Furthermore, GPS more effectively enhanced the non-enzymatic antioxidant levels and antioxidant enzyme activities in serum and liver of alloxan-induced diabetic mice than GPR and GPW. Given these results, heat processing could change the properties of ginseng pectins and change their physiological activities.

Acknowledgments

We are grateful for the financial support of this research from Scientific Research Foundation of Jilin Provincial Science & Technology Department of China (No. 20130206059YY), Science and Technology Bureau of Changchun City (No.13GH08) and program for Innovative Research Team (in Science and Technology) in University of Jilin Province (No.JTD201213).

References

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., & Voragen, A. G. J. (1996). An hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases progress in biotechnology* (pp. 47–55). Amsterdam: Elsevier Science.

Alberti, K., & Zimmet, P. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine, 15*, 539–553.

Axelos, M. A. V., & Thibault, J.-F. (1991). Influence of the substituents of the carboxyl and of the rhamnose content on the solution properties and flexibility of pectins. *International Journal of Biological Macromolecules, 13*, 77–82.

Bonnin, E., Dolo, E., Le Goff, A., & Thibault, J.-F. (2002). Characterisation of pectin subunits released by an optimised combination of enzymes. *Carbohydrate Research, 337*, 1687–1696.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.
Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. *Analytical Biochemistry*, *72*, 248–254.
Ceriello, A., & Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying IR, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*, 816–823.
Chambers, R. E., & Clamp, J. R. (1971). An assessment of methanolysis and other factors used in the analysis of carbohydrate-containing materials. *Biochemical Journal*, *125*, 1009–1018.
Cho, E. J., Piao, X. L., Jang, M. H., Baek, S. H., Kim, H. Y., Kang, K. S., et al. (2008). The effect of steaming on the free amino acid contents and antioxidant activity of *Panax ginseng*. *Food Chemistry*, *107*, 876–882.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Biochemistry*, *28*, 350–366.
Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. (2003). Are oxidative stress-activated signaling pathways mediators of IR and beta-cell dysfunction? *Diabetes*, *52*, 1–8.
Fu, D., & O'neill, R. A. (1995). Monosaccharide composition analysis of oligosaccharides and glycoproteins by HPLC. *Analytical Biochemistry*, *227*, 377–384.
Fujita, A., Sasaki, H., Ogawa, K., Okamoto, K., Matsuno, S., Matsumoto, E., et al. (2005). Increased gene expression of antioxidant enzymes in KKAy diabetic mice but not in STZ diabetic mice. *Diabetes Research and Clinical Practice*, *69*, 113–119.
Gopaul, N. K., Manraj, M. D., Hebe, A., Lee, K., Yan, S., Johnstone, A., et al. (2001). Oxidative stress could precede endothelial dysfunction and IR in Indian Mauritians with impaired glucose metabolism. *Diabetologia*, *44*, 706–712.
Hegde, S. V., Adhikari, P., Kotian, S., Pinto, V. J., D'Souza, S., & D'Souza, V. (2011). Effect of 3-month yoga on oxidative stress in type 2 diabetes with or without complications. *Diabetes Care*, *34*, 2208–2210.
Honda, S., Akao, E., Suzuki, S., Okuda, M., Kakehi, K., & Nakamura, J. (1989). High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl5-pyrazolone derivatives. *Analytical Biochemistry*, *198*, 351–357.
Huang, H. Y., Korivi, M., Chaing, Y. Y., Chien, T. Y., Chien, T. Y., & Tsai, Y. C. (2012). Pleurotus tuberregium polysaccharides attenuate hyperglycemia and oxidative stress in experimental diabetic rats. *Evidence-based Complementary and Alternative Medicine*, *2012*, 1–8.
Keum, Y. S., Park, K. K., Lee, J. M., Chun, K. S., Park, S. K., Kwon, H., et al. (2000). Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Letters*, *150*, 41–48.
Kim, K. T., Yoo, K. M., Lee, J. W., Eomd, S. H., Hwang, K., & Lee, C. Y. (2007). Protective effect of steamed American ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress. *Journal of Ethnopharmacology*, *111*, 443–450.
Kim, S. J., Murthy, H. N., Hahna, E. J., & Lee, H. L. (2008). Effect of processing methods on the concentrations of bioactive components of ginseng (*Panax ginseng* C.A. Meyer) adventitious roots. *LWT—Food Science and Technology*, *41*, 959–964.
Kuzuya, T., Nakagawa, S., Satoh, J., Kanazawa, Y., Kim, H. Y., Kang, K. S., et al. (2002). Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Research and Clinical Practice*, *55*, 65–85.
Lee, Y. J., Kim, H. Y., Kang, K. S., Lee, J. G., Yokozawa, T., & Park, J. H. (2008). The hemical and hydroxyl radical scavenging activity changes of ginsenoside-Rb1 by heat processing. *Bioorganic & Medicinal Chemistry Letters*, *18*, 4515–4520.
Liu, Y. T., Sun, J., Rao, S. Q., Su, Y. J., & Yang, Y. J. (2013). Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from Catathelasma ventricosum in streptozotoci-induced diabetic mice. *Food and Chemical Toxicology*, *57*, 39–45.
Luo, D., & Fang, B. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant activities. *Carbohydrate Polymers*, *72*, 376–381.
Park, E., Hwang, I., Song, J. Y., & Jee, Y. (2011). Acidic polysaccharide of Panax ginseng as a defense against small intestinal damage by whole-body gamma irradiation of mice. *Acta Histochemica*, *113*, 19–23.
Polle, A. Y., Ovodova, R. G., Shashkov, A. S., & Ovodov, Y. S. (2002). Some structural features of pectic polysaccharide from tansy *Tanacetum vulgare* L. *Carbohydrate Polymers*, *49*, 337–344.
Reay, J. L., Kennedy, D. O., & Scholey, A. B. (2005). Single doses of *Panax ginseng* (G115) reduce blood glucose levels and improve cognitive performance during sustained mental activity. *Journal of Psychopharmacology*, *19*, 357–365.
Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins: Structure elucidation using enzymes. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 3–19). Amsterdam: Elsevier Science BV.
Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, *87*, 4–14.
Skyler, J. S. (2004). Diabetes mellitus: Pathogenesis and treatment strategies. *Medicinal Chemistry*, *47*, 4113–4117.
Sotaniemi, E. A., Haapakoski, E., & Rautio, A. (1995). Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care*, *18*, 1373–1375.
Synytsyaa, A., Copikovaí, J., Matejkab, P., & Machovic, V. (2003). Fourier transform Raman and infrared spectroscopy of pectins. *Carbohydrate Polymers*, *54*, 97–106.
Strydom, D. J. (1994). Chromatographic separation of 1-phenyl-3-methy - Spyrazolone-derivatized neutral, acidic and basic aldoses. *Journal of Chromatography A*, *678*, 17–23.
Suzuki, Y., & Hikino, H. (1989). Mechanisms of hypoglycemic activity of Panaxans A and B, glycans of *Panax ginseng* roots: Effects on the key enzymes of glucose metabolism in the liver of mice. *Phototherapy Research*, *3*, 15–19.
Tamaki, Y., Konishi, T., Fukuta, M., & Tako, M. (2008). Isolation and structural characterisation of pectin from endocarp of Citrus depressa. *Food Chemistry*, *107*, 352–361.
Ugochukwu, N. H., & Babady, N. E. (2002). Antioxidant effects of Gongronema latifolium in hepatocytes of rat models of non-insulin dependent diabetes mellitus. *Fitoterapia*, *73*, 612–618.
Vuksan, V., Stavro, M. P., Sievenpiper, J. L., Zdravkovic, U. B., Leiter, L. A., Josse, B. G., et al. (2000). Similar postprandial glycemic reductions with escalation of dose and administration time of American ginseng in type 2 diabetes. *Diabetes Care*, *23*, 1221–1226.
Vijayalingam, S., Parthiban, A., Shanmugasundaram, K. R., & Mohan, V. (1996). Abnormal antioxidant status in impaired glucose tolerance and noninsulindependent diabetes mellitus. *Diabetic Medicine*, *13*, 715–719.
Vuksan, V., Sievenpiper, J. L., Koo, V. Y. Y., Francis, T., Beljan-Zdravkovic, U., & Xu, Z. (2008). Vidgen E: American ginseng reduces postprandial glycemia in nondiabetic and diabetic individuals. *Archives of Internal Medicine*, *160*, 1009–1013.
Xie, J. T., Wan, C. Z., Wang, A. B., Wu, J., Basila, D., & Yuan, C. S. (2005). Antihyperglycemic effects of total ginsenosides from leaves and stem of *Panax ginseng*. *Acta Pharmacologica Sinica*, *26*(9), 1104–1110.
Yeh, G. Y., Kaptchuk, T. J., Eisenberg, D. M., & Philips, R. S. (2003). Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care*, *26*, 1277–1294.
Zhao, L. Y., Lan, Q. J., Huang, Z. C., Ouyang, L. J., & Zeng, F. H. (2011). Antidiabetic effect of a newly identified component of *Opuntia dillenii* polysaccharides. *Phytomedicine*, *18*, 661–668.
Zhang, X., Yu, Li, Bi, H. T., Li, X. H., Ni, W. H., Han, H., et al. (2009). Total fractionation and characterization of the water-soluble polysaccharides isolated from *Panax ginseng* C A. Meyer. *Carbohydrate Polymers*, *77*, 544–550.