Structural characterization and anti-proliferative activities of partially degraded polysaccharides from peach gum

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\textbf{ABSTRACT}

LP100R, LP10R and LP5R were isolated from peach gum by ultrafiltration. They were identified as AG II arabinogalactans composed of mannose, rhamnose, glucuronic acid, galactose, xylose and arabinose, which had a \( \beta-(1\rightarrow6) \) -galactan backbone and were branched at O-3 and O-4. LP100R, LP10R and LP5R exist in a spherical conformation with the molecular weight of 8.50 \( \times \) 10\(^{5}\) g/mol, 4.77 \( \times \) 10\(^{5}\) g/mol and 2.40 \( \times \) 10\(^{5}\) g/mol, respectively. The binding affinities of LP fractions to galectin-3 (Gal-3) were 0.77 \( \mu \)M for LP100R, 2.88 \( \mu \)M for LP10R and 5.15 \( \mu \)M for LP5R, respectively. Meanwhile, an anti-proliferative assay revealed that LP100R possessed higher anti-proliferative activity against HepG2 cells (IC\(_{50}\) 4.5 ng/mL) and MCF-7 cells (IC\(_{50}\) 0.43 ng/mL) than did LP10R and LP5R, which were in accordance with their binding affinities to galectin-3. Therefore, LP fractions (especially LP100R) might exert the anti-tumor activity by directly inhibiting the Gal-3 mediated proliferation of cancer cells.

1. Introduction

Arabinogalactans have been widely found in plant tissues, such as fruits, leaves, and gum exudates (Martínez et al., 2015; Wang et al., 2015; Wang & Li et al., 2015; Yang et al., 2016). The primary structure of arabinogalactans is mainly assigned to two types, arabinogalactans type I (AG-I) having a backbone of \( \beta-(1\rightarrow4) \)-linked galactopyranosyl (Gaip) units, and arabinogalactans type II (AG-II) is composed of a main chain of \( \beta-(1\rightarrow3) \)-galactan with side chains of \( \beta-(1\rightarrow6) \)-linked galactooligosaccharides. A number of studies have reported that arabinogalactans display diverse bioactivities, such as immune-modulating, antitumor, and anti-diabetic activities (Bento et al., 2014; Dión et al., 2016; Wang et al., 2015; Wang & Zhang et al., 2015; Wang & Liu et al., 2015).

Peach gums are a type of exudate secreted from the branches and trunk of peach trees as a consequence of mechanical injury or microbial attacks (Wang et al., 2008). It was used as a traditional Chinese medicine for the treatment of strangury, hematuria, diarrhea and constipation (Wang et al., 2008) in ancient times. In recent years, additional functions of peach gum-derived polysaccharide have been investigated, such as improvement of spermatozoogenesis (Qian et al., 2017) and for the treatment of diabetes (Wang et al., 2017). However, the mechanism of action of peach gum-derived polysaccharide is still not clear. In addition, it remains undetermined whether peach gum polysaccharide has anti-proliferative activities. Peach gum is mainly composed of an acidic polysaccharide with a high molecular weight and a highly branched structure. It has been reported that peach gum polysaccharide is an acidic arabinogalactan with the core structure of \( \beta-(1\rightarrow6) \)-galactan (Simas et al., 2008). Although peach gum polysaccharide is an abundant and promising resource, its unclear of the fine structure has limited further structure-function relationship studies and also the development of peach gum polysaccharide based functional foods.

Thus, in the present study three isolated polysaccharides fractions from peach gum, designated as LP100R, LP10R and LP5R, were
prepared using free radical degradation. Their physicochemical properties, including composition of monosaccharide and functional groups, molecular weight (Mw) and conformation were investigated. Their precise structure was identified using methylation analysis and nuclear magnetic resonance (NMR) spectroscopy. The binding affinity of LP fractions to galactin-3 (Gal-3) and their anti-proliferation activities against HepG2 and MCF-7 cell lines in vitro were also evaluated, to explore its potential application as a functional component for the food industry.

2. Material and methods

2.1. Materials

Peach gum exudates from the trunk and branches of white peach (Prunus persica Batsch.) tree were collected at the Yuandong countryside peach farm (Jinhua, Zhejiang Province, China). Peach gum was ground into powder (80 mesh). All other chemicals were of analytical grade.

2.2. Preparation and isolation of partially degraded polysaccharides from peach gum

AEPG2.0 was peach gum extracted by 2.0 M NaOH according to the procedure of our previous work (Wei et al., 2018). Subsequently, we employed a Fenton-Cu²⁺ system to prepare partially degraded polysaccharides. A 0.5% (w/v) of AEPG2.0 was dissolved in distilled water containing 1 mM copper acetate at pH 6.0, and then reacted with 0.5% (w/v) H₂O₂ at 60°C for 30 min. Almost immediately, the peroxide in the reactive solution was terminated by 0.6% (w/v) NaHSO₃, then, the resulting solution was dialyzed (MWCO 3000Da), concentrated and freeze-dried to obtain a degraded polysaccharide (IP). Millipore Labscale TFF system was conducted to isolate LP as shown in Fig. 1A. Briefly, LP was fractionated by using 100 KDa, 10 KDa and 5 KDa ultrafiltration membranes (Millipore) separately, and was eluted with distilled water under a differential pressure of 20 psi. Samples remained in the chamber were collected and named LP100R, LP10R and LP5R, respectively.

2.3. Chemical composition

The monosaccharide composition of each LP fraction was analyzed by pre-column derivatization with 1-phenyl-3-methyl-5-pyrazole (PMP) according to the method of Zhang et al. (2013). IR spectra of LP fractions were recorded by using a Fourier-transform infrared spectrophotometer (Nexus IS10 FTIR, Thermo Nicolet, USA). LP fractions (2 mg) were ground with KBr powder and pressed into pellets for FT-IR measurements in the range of 4000–400 cm⁻¹.

2.4. Molecular weight and conformation

Size exclusion columns (a OHpak SB-G guard column, SB-806 HQ and SB-804 HQ column, 7.8 × 300 mm, Shodex, Japan), equipped with a multi-angle laser light scattering detector (DAWN HELOS II, Wyatt Technology, USA), a viscometer (ViscosStar III, Wyatt Technology, USA) and refractive index detector (SEC-MALLS-VISC-RH) were applied to measure molecular information of LP fractions at 25°C. The value of dn/dc (specific refractive index increment) was estimated at 0.138 ml/g. 0.2 M NaCl contained 0.02% NaNO₃ (pH 7.0) was used as mobile phase, at a flow rate of 0.5 ml/min. Meanwhile, eluent was filtered (0.22 μm filter membranes, Millipore) and degassed. Sample was dissolved directly in mobile phase (3 mg/mL) and filtered through 0.22 μm filter membranes (Millipore). The injection volume was 50 μL and the running time was 90 min. Data acquisition and calculations were performed by the ASTRA software, version 7.1.2 (Wyatt Technology).

2.5. Atomic force microscopy

Microstructures of LP fractions were acquired by atomic force microscopy (AFM) according to Wei et al. (2016). Briefly, LP fractions (1 mg/mL containing equal mass of sodium dodecyl sulfate (SDS) were depolymerized for 2h in water bath at 80°C, diluted to 1 g/mL and stirred for 24 h. A 5 μL of sample solution (filtered through a 0.22 μm filter) was dropped onto freshly cleaned mica substrate and dried in air prior to observation. Afterward, the topographies of LP fractions were obtained with an AFM (XE-70, Park Scientific Instruments, Suwon, Korea), operated in a tapping mode in air at room temperature (humidity: 50%-60%). The probe is a classical silicon cantilever (Si₃N₄) with a spring constant of 0.2 N/m and a resonance

Fig. 1. Fractionation and chemical compositions of low molecular weight polysaccharides from peach gum (Prunus persica Batsch.). (A) Scheme of preparation and fractionation. (B) FT-IR spectra of LP fractions. (C) Monosaccharide composition of LP fractions.
frequency of approximately 13 kHz. XEI data processing software (Version 1.8.0, Park Systems Corporation, Korea) was used for image manipulation.

2.6. Binding assay by surface plasmon resonance (SPR)

Binding affinities of LP fractions to human recombinant galectin-3 (Gal-3, expressed in HEK 293 cells, BioVision, CA, USA) were performed on a BiACore 3000 system (GE Healthcare, USA). Gal-3 was immobilized on an activated CMS sensor chip (GE Healthcare, USA), according to the standard amine coupling protocol by injecting 100 µg/ml Gal-3 at 5 µL/min, which was dissolved in 10 mM sodium acetate (pH 5.0), until an immobilized level of Gal-3 of over 10,000 resonance units (RU) was achieved. LP fractions were dissolved in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20, pH 7.4) and diluted to different concentrations. Five concentrations of each LP fractions were injected individually at a flow rate of 30 µL/min for 3 min, followed by HBS-EP buffer (for dissociation), 150 mM lactose (for regeneration) and HBS-EP buffer with a single cycle sensorgram. Data were analyzed by BiACore 3000 evaluation software 4.1.

2.7. Anti-proliferation activity assay

Anti-proliferation activities of LP fractions were evaluated in HepG2 (human hepatoma) and MCF-7 (human breast cancer) cell lines cultured in RPMI 1640 and DMEM medium, containing 10% fetal bovine serum at 37°C under 5% CO2, respectively. Briefly, 100 µl of HepG2 and MCF-7 cells were seeded in a 96-well plate for 24 h at the concentration of 5 x 104/mL. Then 100 µl of LP fractions with various concentrations were added to each well, respectively. Control cells were treated without LP fractions. After incubating for 48 h, 20 µl of methyl thiazolyl tetrazolium (MTT, 0.5 mg/mL) was added into each well for 4 h at 37°C. After discarding the medium, 200 µl dimethyl sulfoxide was added to each well to dissolve the resulting formazan. The absorbance of the cells was measured at 570 nm using an enzyme-labeling instrument (Bio-Rad, USA) and the IC50 values for each cell line treated with different samples were calculated.

2.8. Structural analysis

2.8.1. Methylation analysis

Methylation of LP100R was conducted as described by Ciauceu and Kerek (Ciauceu & Kerek, 1984) with some proper modifications, following the procedure described earlier (Wei et al., 2016). In brief, 5 mg of LP100R was prepared in 0.6 mL anhydrous NaOH-DMSO (dimethyl sulfoxide) solution under nitrogen, methylated three times with 1 mL of cold CH3I. The organic layer containing was washed with 3 mL water three times and dried. The resulting methylated products were depolymerized, hydrolyzed, reduced, acetylated and analyzed by a gas chromatography-mass spectrometer (GC-MS, Agilent 7890A/5975C). The degree of branching value (DB) was obtained from the following equation:

\[ DB = \frac{(NT + NB)}{(NT + NB + NL)} \]

Here, NT, NB and NL represent the number of terminal, branched and linear residues, respectively.

2.8.2. Nuclear magnetic resonance (NMR) spectroscopy

LP100R (30 mg) was dried for 48 h in a vacuum dryer with P2O5, and then dissolved in 0.5 mL D2O and lyophilized three times for replacing exchangeable protons: 1D and 2D NMR spectra (1H NMR, 13C NMR, 2H NMR-DEPT 135, 2H-1H COSY, HSQC, HMBC, TCCOSY and NOESY) of polysaccharide solution were recorded on Agilent DD2-600 MHz spectrometer (Agilent Technologies, Santa Clara, CA, USA) at 333.15 K with the operating frequency of 600 MHz. Chemical shifts were expressed in ppm relative to acetone at δ 30.2/2.22 (20°C/298K).

2.9. Statistical analysis

The data were expressed as the mean ± standard deviation (SD) with three replicates per sample. Statistical analysis involved use of the statistical analysis system software package (SPSS Statistics 17.0). The experimental data were evaluated by one-way analysis of variance (ANOVA) for a completely random design to determine the least significant difference at the level of P < 0.05.

3. Results and discussion

3.1. Preparation, fractionation and chemical composition

As shown in Fig. 1A, three fractions, LP100R (65.45%), LP10R (16.36%) and LPSR (18.18%) were obtained, respectively. Results showed that LP100R, LP10R and LPSR had a similar monosaccharide composition, which consisted of mannose, rhamnose, glucuronic acid, galactose, xylose and arabinose without significant differences among their proportions (Fig. 1C). The fractions obtained were very similar in monosaccharide composition to gum exudates that were previously reported (Qian, Cui, Wang, Wang, & Zhou, 2011). Galactose and arabinose were the prominent monosaccharide components in LP fractions, indicating that they were arabinoxylan-type polysaccharides.

The FT-IR spectra of LP100R, LP10R and LPSR were shown in Fig. 1B. The typical peaks for polysaccharides around 3388-3383 cm⁻¹, 2934 cm⁻¹ and 1419 cm⁻¹ were found. (Zhang, Zhang, Liu, Ding, & Ye, 2015). The broad bands, around 1617-1612 cm⁻¹, were due to part both intramolecularly absorbed water and the presence of a carbonyl group (Molai & Jahanbin, 2016). Wavenumbers around 1147-1159 cm⁻¹ corresponded to the vibrations of C–O–C glycosidic bonds (Chen et al., 2015). The bands, around 1076-1075 cm⁻¹, were assigned to β-galactose, while the peaks at 1037-1032 cm⁻¹ were characteristic of arabinans (Ksucuruova, Cepek, Sasaikova, Welsing, & Bhringerova, 2000), which indicate that an arabinoxylan-type polysaccharide was present in LP fractions. Moreover, the presence of the absorbance at 837-836 cm⁻¹ and 898 cm⁻¹ show that both α-linkages and β-linkages of aldopyranosides are present (Ksucruova et al., 2000). The bands at 780-778 cm⁻¹ and 710 cm⁻¹ are specific for the furan ring (Shan et al., 2015).

3.2. Heterogeneity, molecular weight and conformation of LP fractions

A single symmetrical peak was observed in corresponding chromatographic profiles of LP fractions (Fig. 2). This suggests that LP100R, LP10R and LPSR are homogeneous polysaccharide fractions. As shown in Table 1, the molecular weight of LP100R, LP10R and LPSR were 8.50 x 10⁶ g/mol, 4.77 x 10⁵ g/mol and 2.40 x 10⁵ g/mol, respectively. Polydispersity coefficients (Mw/Mn) of LP100R, LP10R and LPSR were 2.06, 2.17 and 1.98, respectively. This suggests that the LP fractions were polydisperse polysaccharides with a wide distribution of molecular weights.

Chain conformation further characterizes the spatial distribution of polysaccharide chain. Thus, Mark-Houwink-Sakurada equation (η = K[M]α) was applied to calculate chain conformations of LP100R, LP10R and LPSR in 0.2 M NaCl solution. As shown in Fig. 3A, the exponent (α) of Mark-Houwink-Sakurada equations for LP100R, LP10R and LPSR were 0.42, 0.33 and 0.26, indicating all of the three LP fractions exhibited a spherical conformation in 0.2 M NaCl solution.
Fig. 2. Chromatogram profiles of LP100R (A), LP10R (B) and LP5SR (C). LS, laser scattering signals; dIII, the refractive index signals. DP, differential pressure signals. Peaks after 40 min were solvent peaks.

Table 1
Molecular and conformation parameters of LP fractions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP100R</td>
</tr>
<tr>
<td>Mn (g/mol)</td>
<td>(4.33 ± 0.21) × 10⁶</td>
</tr>
<tr>
<td>Mp (g/mol)</td>
<td>5.29 ± 0.54 × 10⁶</td>
</tr>
<tr>
<td>Mw (g/mol)</td>
<td>8.60 ± 0.57 × 10⁶</td>
</tr>
<tr>
<td>Mw/Mn</td>
<td>1.23 ± 0.01</td>
</tr>
<tr>
<td>Rs (nm)</td>
<td>6.61 ± 0.05</td>
</tr>
<tr>
<td>[α] (mL/g)</td>
<td>12.72 ± 1.18</td>
</tr>
<tr>
<td>K (mL/g)</td>
<td>8.00 ± 0.15</td>
</tr>
<tr>
<td>n²</td>
<td>4.42 ± 0.03</td>
</tr>
</tbody>
</table>

(Burchard, 1999). The extension of LP fractions chains followed the order LP100R > LP10R > LP5SR.

Extensive studies have found that highly-branched polysaccharides in water exist as compact coils or spherical structures, such as in Acacia gum (ellipsoid-like conformation) (López-Torrez, Nigen, Williams, Doco, & Sanchez, 2015), BDII from Dendrobium devonianum (a globular shape) (Deng et al., 2019), p-LJP from Lycium barbarum L. (spherical molecules) (Liu et al., 2016), which might be partly due to self-organization and interaction among the side chains by hydrogen bonding and steric effects.

3.3 Atomic force microscopy

Atomic force microscope was used to directly detect the surface morphology of LP fractions in the natural circumstances (Fig. 3B-D). The chain morphology of LP10R and LP5SR were irregular spherical conformations with the height between 1–4 nm. Moreover, large amounts of compacted coils with high branch levels were found in Fig. 3D, which may be attributed to intra- and inter-molecular hydrogen bonds. Taken together, these AFM data verify the results of chain conformation analysis.

3.4. Binding affinity of LP fractions to galectin-3

Galectin-3 (Gal-3) is a specific β-galactoside binding lectin associated with immune reactions, cancer and many other serious diseases, and has become a molecular target in the development of anti-cancer therapeutics (Klyosov & Traber, 2012; Ledere, van Cutsem, & Michiels, 2013). Galactose-type inhibitors of Gal-3, such as modified citrus pectin and ginseng pectin, have been identified that show Gal-3 inhibitory effects by occupying the carbohydrate recognition domain (CRD) of Gal-3 with the galactan side chain of their RG-I domains (Gao et al., 2013). Due to a higher content of galactose in LP fractions, the SPR assay was adopted to determine the binding affinities of LP fractions to Gal-3, to further evaluate the Inhibitory potential of LP fractions on Gal-3.

Binding kinetics of LP fractions to Gal-3 are shown in Fig. 4B-D. Dissociation constant values (KD) were 0.77 μM for LP100R, 2.88 μM for LP10R and 5.15 μM for LP5SR, respectively (Fig. 4A). LP100R seems to display a higher affinity for Gal-3 than LP10R and LP5SR, which might be attributed to the greater relative extension of its chain conformation. According to some previous studies, Gal-3-binding affinity of LP100R was higher than potato galactan (272 KDa, 2.59 μM) and pumpkin pectic polysaccharide (PPC, 22.6 KDa, 1.26 μM), but lower than Ginseng pectin (RG-I, 60 KDa, 22.2 nM) and MGP (143 nM) (Gao et al., 2013; Zhao et al., 2017). These data suggest LP fractions from peach gum as new agents to inhibit Gal-3 binding to cellular receptors, that might better inhibit galectin-3-mediated adhesion and proliferation of colon cancer cells and block galectin-3-mediated effects in other diseases.

3.5. Anti-proliferation activities of LP fractions

The anti-proliferation activities of the LP fractions were tested on HepG2 and MCF-7 cell lines in vitro by MTT assay. The proliferation of HepG2 cells and MCF-7 cells were significantly inhibited by all of the three fractions in a dose-dependent manner (Fig. 4F,F). Moreover, the antiproliferation activities of LP fractions against MCF-7 cells (highly expressing Gal-3) were stronger than against HepG2 cells (with low Gal-3 expression). The IC₅₀ values for the anti-proliferative activities of LP100R against HepG2 cells and MCF-7 cells were 4.5 mg/mL and 0.43 mg/mL, respectively. Additionally, LP fractions had anti-proliferative activities of LP100R > LP10R > LP5SR against HepG2 cells and MCF-7 cells, which is consistent with the results of Gal-3-binding
affinity of LP fractions. These results suggest that the antiproliferation activities of LP fractions against MCF-7 cells and HepG2 cells might be due to the inhibition of Gal-3 mediated proliferation of cancer cells by LP fractions.

Compared to other plant polysaccharides, the antiproliferation activity of LP100R against HepG2 cells was lower than the polysaccharides from Viscum coloratum (Korn.) Nakai (Chai & Zhao, 2016), but higher than the polysaccharides from Zizyphus jujuba cv. Mussao.

Fig. 3. Chain morphology of LP fractions. (A) Mark-Houwink-Sakurada plot of LP fractions. AFM images of LP100R (B), LP10R (C) and LP5SR (D).

Fig. 4. SPR analysis of the binding affinities of LP fractions to galectin-3. The binding kinetic parameters (A), SPR sensorgrams of LP100R-Gal-3 binding (B), LP10R-Gal-3 binding (C) and LP5SR-Gal-3 binding (D). Anti-proliferation activities of the LP fractions against HepG2 (E) and MCF-7 (F) cell lines.
Table 2
Methylation analysis of LP100R by GC-MS.

<table>
<thead>
<tr>
<th>PMAA</th>
<th>Linkage pattern</th>
<th>Molar ratio (%)</th>
<th>it (%)</th>
<th>Ion fragmentation(m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3,5-Me₂-Ara</td>
<td>1-linked Ara'</td>
<td>27.8</td>
<td>13.8</td>
<td>71,93, 87,102,118,129,145,161,162</td>
</tr>
<tr>
<td>2.3,5-Me₂-Xyl</td>
<td>1-linked Xylp</td>
<td>1.6</td>
<td>14.8</td>
<td>83, 87,101,102,117,118,161</td>
</tr>
<tr>
<td>2.5-Me₂-Ara</td>
<td>1,3-linked Ara'</td>
<td>10.4</td>
<td>16.5</td>
<td>87,101,118,129,145,160,173,202,233</td>
</tr>
<tr>
<td>2.3-Me₂-Ara</td>
<td>1,3-linked Ara'</td>
<td>18.3</td>
<td>17.5</td>
<td>87,102,118,129,145,162,173,189</td>
</tr>
<tr>
<td>3-Me₂-Xyl</td>
<td>1,2,4-linked Xylp</td>
<td>2.3</td>
<td>20.2</td>
<td>87,100,114,129,134,145,157,173,189,190</td>
</tr>
<tr>
<td>2.4,6-Me₂-Gal</td>
<td>1,3-linked Galp</td>
<td>4.4</td>
<td>22.1</td>
<td>87, 101, 118, 129, 145, 161, 174, 202, 217, 234, 277</td>
</tr>
<tr>
<td>2.3,4,6-Me₂-Gal</td>
<td>1,6-linked Galp</td>
<td>1.3</td>
<td>23.3</td>
<td>87,99,102,118,129,145,160,189,233</td>
</tr>
<tr>
<td>2.6-Me₂-Gal</td>
<td>1,3,4,6-linked Galp</td>
<td>4.0</td>
<td>23.6</td>
<td>87, 100, 118, 129, 133, 160, 185, 205, 232, 265</td>
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<tr>
<td>4.6-Me₂-Gal</td>
<td>1,2,3,4-linked Galp</td>
<td>4.4</td>
<td>25.8</td>
<td>87, 101, 112, 118, 129, 143, 161, 186, 222, 252</td>
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<tr>
<td>2.4-Me₂-Gal</td>
<td>1,3,4,6-linked Galp</td>
<td>14.6</td>
<td>26.0</td>
<td>87, 97, 118, 129, 139, 166, 171, 187, 202, 217, 232, 259, 289, 333</td>
</tr>
<tr>
<td>2-Me₂-Gal</td>
<td>1,2,3,4,6-linked Galp</td>
<td>10.9</td>
<td>27.0</td>
<td>87, 97, 118, 129, 139, 166, 171, 187, 202, 217, 232, 259, 289, 333</td>
</tr>
</tbody>
</table>

* The molar ratios of residues were calculated by the integration of their peaks areas in total ion chromatogram.

(Wang & Shi et al., 2015; Wang & Zhang et al., 2015; Wang & Liu et al., 2015). The anti-proliferative activity of LP100R against MCF-7 cells was lower than that of Dicyophora indicausta polysaccharide (DP1) (Liao et al., 2015), but higher than that of an acidic polysaccharide from Angelica sinensis (Oliv.) Diels (Zhang et al., 2016). LP100R has potential antitumor activity because of its ability to inhibit cancer cells proliferation.

3.6 Structural characterization

Since the three fractions had similar chemical composition and structure (Fig. S1), and LP100R showed the best activities in the anti-proliferation assay and the SPR assay compared with LP10R and LPSR. Thus, LP100R was selected to perform further structural elucidation studies.

3.6.1 Methylation

Methylation performed with GC-MS analysis was further used to determine the linkage patterns of LP100R. LP100R was mainly composed of t-Linked Ara (27.6 mol%), 1,3-linked Ara (10.4 mol%) and 1,5-linked Ara (18.3 mol%), t-Linked Xylp (1.6 mol%) and 1,2,4-linked Xylp (2.3 mol%), 1,3,4-linked Galp (4.4 mol%), 1,6-linked Galp (1.3 mol%), 1,3,4-linked Galp (4.0 mol%), 1,3,4,6-linked Galp (4.4 mol%), 1,3,4,6-linked Galp (14.6 mol%) and 1,3,4,6-linked Galp (10.9 mol%) (Table 2). These results are similar to a previous reported composition (Siman et al., 2008). The high molar ratio of arabinose and galactose residues suggests that LP100R is an arabinogalactan. The large amount of 2,3-Gal-(1→3,4)-Gal-(1→3,4,6)-Gal-(1→ reveals that it is branched. Additionally, the relatively high amount of t-Linked Ara in LP100R combined with its molecular weight (85 kDa) suggests that this fraction is composed of highly branched structures. Moreover, the degree of branching value (DB) was 0.66, also indicating a highly branched structure.

3.6.2 NMR analysis

1D and 2D NMR spectra, including 1H NMR, 13C NMR, 15N-NMR DEPT 135, HSQC, HMBC, COSY, TOCSY and NOESY, were recorded (Figs. 5 and 6 and Fig. S2) to further identify the precise structure of LP100R. The major chemical shifts, based on the monosaccharide analysis, linkage analysis, and 1D and 2D NMR data are provided in Table 3. According to the HSQC spectrum (Fig. 6A), 16 cross peaks were present in the aromatic region, which indicate the existence of 16 glycosidic residues in LP100R, named residue A-P.

Two sharp peaks at 4.46 ppm and 4.45 ppm in 1H NMR spectrum (Fig. 5A) were assigned to β-o-Galp (Yuan et al., 2016), which correspond to the anomeric carbon at 103.16 ppm and 103.13 ppm in HSQC spectrum (Fig. 6A), respectively. COSY, TOCSY and HSQC spectra (Fig. 6A and Fig. S2) showed some downshift displacements at H-1/C-1 (δ 4.46/103.16 ppm), H-3/C-3 (δ 3.82/81.10 ppm) and H-6/C-6 (δ 4.01/68.84 ppm). Furthermore, HMBC spectrum (Fig. 6B) showed a correlation between H-1 (4.46 ppm) and C-6 (68.84 ppm). These data suggest that the glycosidic linkage of residue A is α-(3,6)-β-o-Galp-(1→, and that β-o-Galp-(1→ was the backbone, C5 and C4 were the branched points of the backbone. The correlation between H-1 of residue A and C-6 of residue B at 4.46/68.84 ppm, H-1 of residue B and C-6 of residue A at 4.45/68.84 ppm in HMBC spectrum (Fig. 6B), and H-1 of residue A and H-1 of residue B at 4.46/4.45 ppm in NOESY spectrum (Fig. S2), indicate the presence of the fragment of α-(3,6)-β-o-Galp-(1→3,4,6)-β-o-Galp-(1→, which forms the main chain of LP100R.

Five anomeric signals at 5.40/108.1, 5.25/109.24, 5.43/108.05, 5.25/109.86 and 5.09/107.09 ppm were revealed in HSQC spectrum (Fig. 6A), which are characteristic signals for α-L-Araf. HMBC spectrum (Fig. 6B) showed a correlation between H-1 (5.40 ppm) and C-3 (83.86 ppm), which suggested the presence of α-(2,3)-α-L-Araf-(1→ residue G1. In 15N-NMR DEPT 135 spectrum (Fig. 5G), anomeric signal at 109.24 ppm was observed and together with an inverted signal at 65.12 ppm from substituted C-5 of α-L-Araf units. Moreover, the cross-peak at 5.25 ppm and 3.98 ppm, confirmed the presence of α-(5)-α-L-Araf-(1→ (residue H) (Lee et al., 2015). Additionally, 5.43/108.05, 5.25/109.86 and 5.09/107.09 ppm was assigned to α-L-Araf-(1→, named residue K, residue I and residue J, respectively, according to the intensity of residues and a previous study (Yuan et al., 2016).

Combined analysis of HSQC, COSY and TOCSY spectra (Fig. 6 and Fig. S2), allowed some of the low intensity residues to be assigned. Anomeric signals at 4.50/102.81 ppm, 4.46/102.52 ppm were assigned to α-(6)-β-o-Galp-(1→ (residue E) and α-(3,6)-β-o-Galp-(1→ (residue C) (Lee et al., 2015). Anomeric signals at 5.09/101.50 ppm, 5.09/101.36 ppm were attributed to α-(2,3)-α-L-Araf-(1→ (residue F). The anomeric signals at 4.99/100.06 ppm were presented as a terminal α-L-Rhap-(1→ (residue N) (Yuan et al., 2016). Residue O was deduced to 4-O-Me-β-o-GlcAp (Nagel, Conrad, Leitenberger, Carle, & Neidhart, 2016). Residue P was assigned to a terminal β-o-Manp-(1→ (4.73/100.06 ppm) and anomeric signal at 4.45/101.99 ppm to α-(4,6)-β-o-Xylp-(1→ (residue L). Another anomeric signal at 8.44/100.69 ppm was attributed to terminal β-o-Xylp-(1→ (residue M) (Kang, Gao, & Shi, 2018).
correlation in HMBC between H-1 (5.43 ppm) of residue K and C-3 (83.86 ppm) of residue G, H-1 (5.40 ppm) of residue G and C-4 (73.96 ppm) of residue B, confirmed that the side chain α-l-Araf (1→3)-α-l-Araf (1→) is linked to the backbone at O-3. The NOESY spectra showed a cross peak between H-1 (5.25 ppm) of residue I and H-5 (3.98 ppm) of residue H, revealing the fragment of α-l-Araf (1→5)-α-l-Araf (1→). The correlation between H-1 (5.25 ppm) of residue H and C-3 of residue A/B in HMBC indicates another side chain attached to the backbone at O-3. Both of the signals, such as H-1 of residue N and H-1 of residue P, had a correlation with C-6 of residue D in HMBC spectrum. Therefore, α-L-Rhap (1→) and β-D-Manp (1→) are linked to →6)-β-D-Galp (1→ at C-6, while 4-O-Me-β-D-GlcAp is linked to →5)-α-L-Araf (1→, based on a correlation between H-1 of residue O and C-5 of residue H in HMBC spectrum.

HMBC spectrum (Fig. 6B) revealed the correlations between H-1 (4.46 ppm) of residue C and C-5 (65.12 ppm) of residue H, H-1 (4.50 ppm) of residue D and C-4 (65.12 ppm) of residue H. Correlations between H-1 (4.46 ppm) of residue C and H-5 (3.98 ppm) of residue H, H-1 (4.50 ppm) of residue D and H-5 (3.98 ppm) of residue H were observed in the NOESY spectrum (Fig. S2), and these suggested that the side chains of →6)-β-D-Galp (1→ and →3)-β-D-Galp (1→ were linked to →5)-α-L-Araf (1→ at O-5. The HMBC spectrum revealed a correlation between H-1 (5.69 ppm) of residue E/F and C-4 (73.96 ppm) of residue B, which indicates the attachment of residue E/F at O-4 of residue B. In addition, the terminals of residue E/F at C-2, C-3 and C-4 were substituted by residue J, as proven by signals in HMBC spectrum.

Residue M linked to residue L at O-4, which was proved by a correlation between H-1 (4.45 ppm) of residue M and C-4 (87.53 ppm) of residue L in the HMBC spectrum (Fig. 6B). Correlations between H-1 of residue L and C-5 of residue H in HMBC spectrum (Fig. 6B), and H-1 of residue L and H-5 of residue H reveal that the side chain β-D-Xylp (1→2,4)-β-D-Xylp (1→ is attached to →5)-α-L-Araf (1→ (residue H) at O-5. As described above, the putative structure of LP100R is characterized as Fig. 7. The structure of LP100R is an arabinogalactan with β-(1→6)-galactan as backbone and branches at O-3 and O-4 of Galp, and therefore is belonging to AG II arabinogalactan. Although, arabinogalactans with β-(1→6)-galactan backbone are rare in plants, some similar structures of arabinogalactans have been reported, such as from Lycium barbarum fruit (Martín, de Freitas, Sassaki, Evangelista, & Sierskowski, 2017), Litchi chinensis Sonn. pulp (Yang, Prasad, & Jiang, 2016), and Panax notoginseng flowers (Wang & Zhang et al., 2015).

4. Conclusion

Three polysaccharide fractions (LP100R, LP10R and LPSR) were prepared from peach gum by free radical degradation followed by separation using an ultrafiltration membrane. GPC-MALLS and AFM analysis indicated their molecular weight of $8.50 \times 10^4$ g/mol,
Fig. 6. Partial HSQC (A) and HMBC (B) spectra of LP100R.

Table 3

<table>
<thead>
<tr>
<th>Residue</th>
<th>Chemical shifts (δ, ppm)</th>
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<tr>
<td></td>
<td>H1/C1</td>
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<tr>
<td>(A)1,3,6-β-D-Galp</td>
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</tr>
<tr>
<td>(B)1,3,6-β-D-Galp</td>
<td>4.46/103.13</td>
</tr>
<tr>
<td>(C)1,3,6-β-D-Galp</td>
<td>4.46/102.52</td>
</tr>
<tr>
<td>(D)1,2,3-α-L-Rhap</td>
<td>5.09/101.50</td>
</tr>
<tr>
<td>(E)1,4-α-L-Rhap</td>
<td>5.09/101.36</td>
</tr>
<tr>
<td>(G)1,3-α-L-Araf</td>
<td>5.40/108.21</td>
</tr>
<tr>
<td>(H)1,5-α-L-Araf</td>
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<td>(I)1,2,4-β-D-Xylp</td>
<td>5.25/109.86</td>
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<tr>
<td>(J)1,2,4-β-D-Xylp</td>
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<td>(N)1,4-α-L-Rhap</td>
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<tr>
<td>(O)1,4,6,6-dimethyl-β-D-GlcAp</td>
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<tr>
<td>(P)1-β-D-Maup</td>
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4.77 × 10^7 g/mol and 2.40 × 10^7 g/mol, and all showed a spherical conformation in aqueous solution. NMR and methylation analysis suggested the polysaccharides were AG II arabinogalactan, with a backbone of β-(1-6)-galactan and mainly 1-3 and 1-5 linked arabinose branches. The degraded fraction showed binding affinity to Gal-3, with constant values of 0.77 μM, 2.88 μM and 5.15 μM for LP100R, LP10R and LP55R, respectively. Anti-proliferation assay also indicated LP fractions inhibited the growth of HepG2 cells and MCF-7 cells, and LP100R exhibited the lowest IC50. Thus, we suggest that the degraded peach gum fraction might be developed as a functional food component.

Acknowledgments

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Appendix A. Supplementary data

Supplemental material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.carbpol.2018.09.029.

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