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Artificial simulated saliva, gastric and intestinal digestion of polysaccharide from the seeds of *Plantago asiatica* L.

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ABSTRACT

The saliva, gastric and intestinal digestion of polysaccharide from *Plantago asiatica* L. seeds was investigated *in vitro*. It was found that salivary amylase had no effect on the polysaccharide; however, the polysaccharide was influenced in later gastrointestinal digestion. A steady decrease in molecular weight (M_w) of the polysaccharide from 1903.1 ± 93.0 to 4.7 ± 0.2 kDa was observed as digestion time increased. Meanwhile, the reducing ends were increased from 0.157 ± 0.009 to 0.622 ± 0.026 mM, indicating the decrease of M_w may due to the breakdown of glycosidic bonds. In addition, there was no monosaccharide released throughout the whole digestion period, suggesting that the gastrointestinal digestion did not result in a production of free monosaccharide. These results may provide some information on the digestion of polysaccharide from *P. asiatica* L. *in vitro*, and may contribute to the methods of studying the digestion of other carbohydrates.

1. Introduction

Increased interest in digestion of bioactive carbohydrates has occurred in recent years due to the beneficial effects on human health resulting from their consumption (Aherne, Daly, Jiwan, O'Sullivan, & O'Brien, 2010; Liang et al., 2011). Research has also focused on the changes in these polysaccharides after gastric and intestinal digestion in human (Thondre, Monro, Mishra, & Henry, 2010). However, it is not feasible to perform *in vivo* studies for every carbohydrate. Therefore, *in vitro* digestion models are likely to become increasingly important in assessment of carbohydrates, especially for determining the digestion of these substances (Oomen et al., 2002).

In vitro digestion models based on human physiology have been developed as simple, cheap, and reproducible tools to investigate the dissolution of pharmaceutical dosage forms (Blanquet et al., 2004), bio-accessibility of nutrients (Gervais et al., 2009; Tedeschi, Clement, Rouvet, & Valles-Pamies, 2009), and digestion of substances (Heaton, Marcus, Emmett, & Bolton, 1988; Muir & O'Dea, 1992). In the *in vitro* model, mobilization of the substances under gastric and intestinal pH conditions is simulated. The *in vitro* model could simulate transit through the human digestive tract by sequential exposure of substances to simulated mouth, gastric, and small intestinal conditions. In addition, the *in vitro* model can simulate more aspects of human physiology than *in vivo* studies, and a simple model is easy to perform and allows simultaneous determination of large numbers of samples (Oomen et al., 2002).

Plants of the *Plantago* family are used in folk medicine throughout the world (Samuelsen, 2000). Some have been studied widely, such as *Plantago afra* L., *Plantago psyllium* L., *Plantago ovata* Forsk. (isabgul), *Plantago indica* L. and *Plantago major* L. (Mahady, Fong, & Farnsworth, 1999). A petroleum extract of *Plantago asiatica* L. was found to have a pronounced antidepressant effect (Xu, Luo, & Tan, 2004). A gel extracted from *P. psyllium* seed husk promoted laxation as a lubricant in humans (Marlett, Kajs, & Fischer, 2000). Recently, the *Plantago* family has attracted much attention owing to its antispasmodic (Fleer & Verspohl, 2007), anticomplementary (Samuelsen et al., 1999), hyperglycemia-reducing (Hannan, Ali, Khaleque, & Akhter, 2006), antioxidant activity (Ye, Hu, & Dai, 2011), nitric oxide-inducing and tumor necrosis factor activities (Iringanine, Vray, & Ercruysse, 2005). Our research group has isolated a polysaccharide from the seeds of *P. asiatica* L., and it was found to consist of Rha, Ara, Xyl, Man, Glc and Gal, in a molar ratio of 0.05:1.00:1.90:0.05:0.06:0.10. Its uronic acid was GlcA. The polysaccharide was a highly branched heteroxylan which consisted of a β-1,4-linked Xyl*p* backbone with side chains attached to *O*-2 or *O*-3. The side chains consisted of β-terminal-linked Xyl*p*, α-terminal-linked Ara*f*, α-terminal-linked GlcA*p*, β-Xyl*p*-(1→3)-α-Ara*f* and α-Ara*f*-(1→3)-β-Xyl*p*, *etc.* (Yin et al., 2012). In addition, our recent studies also showed that the polysaccharide isolated from the seeds of *P. asiatica* L. could induce maturation of murine

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dendrite cells, have antioxidant activity *in vitro* and promote defaecation (Hang, Xie, Yin, Nie, & Xie, 2009; Tang et al., 2007; Wu, Tian, Xie, & Li, 2007; Yin, Nie, Zhou, Wan, & Xie, 2010). However, the saliva, gastric or intestinal digestion of the polysaccharide from the seeds of *P. asiatica* L. has not been investigated yet.

In this study, we developed an *in vitro* digestion model to provide information on the digestion of polysaccharide from the seeds of *P. asiatica* L. This information can contribute to the methods of studying the digestion of other carbohydrates. In addition, we investigated the possible mechanism by which the polysaccharide was digested.

2. Materials and methods

2.1. Materials

The seeds of *P. asiatica* L. were purchased from Ji'an County, Jiangxi Province, China and dried in the sun before use. The polysaccharide from *P. asiatica* L. seeds was prepared using our previous published method (Yin et al., 2010; Yin et al., 2012).

Monosaccharide standards, glucose (99.8%), galactose (99.9%), xylose (99.8%), mannose (99.8%), arabinose (99.8%) and rhamnose (99.9%) were purchased from TCI America (Portland, WA, USA). All other reagents, purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China) were of analytical grade.

2.2. Simulated saliva digestion

2.2.1. Saliva preparation

The fresh pooled saliva sample was collected from a healthy donor who did not have previous colonic disease and has not been treated with antibiotics for at least 3 months (Björck, Asp, Birkhed, & Lundquist, 1984; Sanz, Handschin, Nuessli, & Conde-Petit, 2007; Thorburn, Brand, & Truswell, 1987). The donor was refrained from eating or drinking prior to saliva collection.

There are several techniques for collecting human saliva. The most common method was spitting due to its simplicity. Specific protocol was carried out with the donor as follows (Navazesh, 1993; Saari, Halinen, Ganlöv, Sorsa, & Konttinen, 1997; Stokes & Davies, 2007):

(a) Several sips of water were consumed and/or swilled around the mouth for at least 30 s to obtain a 'neutral' mouth state;
(b) Saliva was expectorated at a set frequency for an initial 30-s period and discarded. The donor then expectorated into graduated test tube for a further 2 or 2.5 min. The preferred expectoration technique was to sit down with a slightly tilted head so that saliva was allowed to pool in the mouth. The saliva was then gently expectorated once after each 30-s period;
(c) Step (b) was repeated once;
(d) The saliva collected was immediately centrifuged at $1500 \times g$ for 10 min in order to pellet the cells and supernatant was stored at −20 °C.

After that, the amylase activity of the human saliva was determined according to the method of Van Ruth and Roozen (2000).

2.2.2. Digestion by human saliva

The saliva digestion was carried out according to the reported methods with some modifications (Asano, Hamaguchi, Fujii, & Iino, 2003; Yoon, Thompson, & Jenkins, 1983). Briefly, the polysaccharide was dissolved in distilled water at a concentration of 2 mg mL^{-1}. To test tube A was added 2 mL of saliva and 2 mL of the polysaccharide (2 mg mL^{-1}), to test tube B was added 2 mL of saliva and 2 mL of water, and to test tube C was added 2 mL of the polysaccharide (2 mg mL^{-1}) and 2 mL of water. All test tubes were put into a water bath (HH-SY21-N, Changfeng Equipment Corporation, Beijing, China) and maintained for 4 h at 37 °C. Then the test tubes were dipped into a boiling water bath for a few minutes to inactivate human salivary amylase (Yoon et al., 1983). Polysaccharide remaining after digestion was measured by high-performance gel permeation chromatography (HPGPC). Three independently replicated determinations were performed for each sample.

2.3. Simulated gastric and intestinal digestion in vitro

Digestion studies were performed using a flow-through dissolution system, RC806 (Tianfa Corporation, Tianjing, China) equipped with a temperature circulator-controller, which allowed us to maintain the temperature at 37 °C throughout the experiments. Stirring paddles (Tianfa Corporation, Tianjing, China) at the top of sample cells were also equipped to the flow-through dissolution system for simulating the gastric peristalsis. In addition, several pumps (HL-2D, Qingpu Corporation, Shanghai, China) were connected to the dissolution system in order to pump in the gastric and intestinal medium for digestion. Furthermore, an online pH meter (XB89-PH-2612, XG Corporation, Beijing, China) was also connected to the dissolution system for controlling the pH value in the digestion system. The pH value was monitored and continuously adjusted by adding either water or 1 M HCl into the stomach and either electrolytes or 1 M $NaHCO_3$ into the small intestine. Before each experiment, the system was washed with detergent, and rinsed with water (Blanquet et al., 2004).

For the gastric digestion test of the polysaccharide, three sample cells were loaded with 100 mL polysaccharide (2 mg mL^{-1}), and 150 mL of gastric medium was pumped through the cells at a constant flux of 0.4 mL min^{-1}. An additional cell was used as a reference cell, which contained no polysaccharide but water as the control through which the gastric medium was pumped.

For the intestinal digestion test, the polysaccharide, predigested by gastric medium was placed inside three sample cells. The small intestinal medium (200 mL) was pumped at a constant flux of 0.56 mL min^{-1}. In the control experiment, the reference cell was loaded with an equal amount of water and pumped with the same intestinal medium used for the sample cells.

For all the experiments, a fraction collector was used to withdraw samples at a programmed volume (*ca.* 3 mL) and specified different time intervals. The collected samples were then analyzed by HPGPC.

2.4. Digestion media used in the digestion experiments

All chemicals and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Amano Enzyme Inc., Japan. The simulated gastric and intestinal fluids were prepared according to the protocol used by a model gastrointestinal system (TNO Intestinal Model 1, TIM1 from TNO Zeist, The Netherlands), which simulates the physiological conditions of the human stomach and small intestine (Minekus, Marteau, Havenaar, & Huisintveld, 1995; Minekus et al., 1999; Salovaara, Alminger, Eklund-Jonsson, Andlid, & Sandberg, 2003).

2.4.1. Gastric medium preparation

The gastric medium was prepared using the method of Tedeschi et al. (2009) with some modifications. The gastric electrolyte solution (GES) consisted of 3.1 g L^{-1} NaCl, 1.1 g L^{-1} KCl, 0.15 g L^{-1} $CaCl_2 \cdot 2H_2O$, and 0.6 g L^{-1} $NaHCO_3$. The final pH was adjusted to 3 by addition of 0.1 N HCl. Then, 37.5 mg of gastric lipase (Lipase DF Amano 15, Amano Enzyme Inc., Japan) and 35.4 mg of pepsin from porcine gastric mucosa (Sigma) were added to a solution of 150 g of GES and 1.5 mL of CH_3COONa (1 M, pH 5). The solution was gently mixed for 10 min on a magnetic stirrer at room temperature and

simultaneously the pH adjusted to 3 by 0.1 N HCl. The solution was stored on ice before use. The gastric digestion of the polysaccharide and the control (water) were followed for 6 h. Samples were analyzed after 0.5, 1, 2, 4 and 6 h of digestion. Three independently replicated extractions were performed for each sample.

2.4.2. Preparation of small intestinal alkaline medium

The small intestinal medium was prepared according to the method of Tedeschi et al. (2009) with some modifications. The simulated small intestinal electrolyte solution (SIES) consisted of 5.4 g L^{-1} NaCl, 0.65 g L^{-1} KCl, 0.33 g L^{-1} $CaCl_2 \cdot 2H_2O$. The pH was adjusted to 7 using 0.1 N NaOH. To realistically simulate a duodenum residue solution, bile salt was added to a SIES containing the enzymes pancreatin and trypsin. A SIES with enzymes and bile salt was firstly prepared (SIENZY). The SIENZY solution contained 100 g of SIES, 100 g of pancreatin solution (7%, w/w, Sigma), 13 mg of trypsin (Sigma) and 200 g bile salt solution (4%, w/w). For the pancreatin solution, 14 g of pancreatin was dissolved in 200 g of water, and then the solution was mixed for 10 min on a magnetic stirrer and centrifuged for 10 min at 4800 × *g*. The supernatant (100 g) was used for the preparation of the SIENZY solution. The dilution was performed using SIES as dilution medium and the pH adjusted to 7.5 by 0.1 N NaOH. The intestinal digestion of the polysaccharide and the control (water) were also followed for 6 h. Samples were analyzed after 0.5, 1, 2, 4 and 6 h of digestion. Three independently replicated extractions were performed for each sample.

2.4.3. Control experiments

To find out the influence of the pH and salts in gastric and intestinal medium on the digestion of the polysaccharide, some control experiments were carried out as follows: (a) The polysaccharide was subjected to digestion at pH 1.5 and at 6.8 individually in the absence of enzyme. (b) The polysaccharide was dissolved in water along with the addition of the salts of gastric and intestinal medium (NaCl, KCl, $NaHCO_3$, $CaCl_2$, and CH_3COONa as described in Sections 2.4.1 and 2.4.2) individually or together.

2.5. Determination of molecular weight change

Molecular weight (M_w) was determined by HPGPC. Specifically, it was determined in a Waters high-performance liquid chromatography (HPLC) system (UK6 injector and 515 HPLC pump) equipped with a Waters Ultrahydrogel linear column (7.8 mm × 300 mm), and a Waters 410 refractive index (RI) detector, connected in series with a Millennium 32 workstation (Waters, Milford, MA, USA). A sample solution (20 μL) was injected in each run, with distilled water as the mobile phase at a flow rate of 0.5 mL min^{-1} (Chen, Xie, Nie, Li, & Wang, 2008). Dextran standards (T-2000, T-500, T-70, T-40 and T-10, Sigma–Aldrich, Shanghai, China) and glucose (M_w 180 Da, Sigma–Aldrich, Shanghai, China) were used to calibrate the column and establish a standard curve.

2.6. Determination of reducing sugar content (C_R)

The cleavage of glycosidic linkages was followed by measuring the increase of reducing sugars using dinitrosalicylic acid (DNS) method (Miller, 1959). Galacturonic acid with different concentrations was used to generate a calibration curve. A modified DNS reagent (1.5 mL) consisting of 1% DNS, 0.2% phenol, 0.5% sodium sulfite and 1% NaOH was added to 2 mL of sample. The mixture was then heated for 5 min at 100 °C. Then, 1 mL of a 40% solution of Rochelle salts was added to the mixture subsequently with the development of the color and prior to cooling. Lastly, the samples were detected by a double beam UV–vis spectrophotometer (TU-1901, PGENENAL, Beijing, China) at a wavelength of 540 nm.

2.7. Free monosaccharide determination after digestion by HPLC-ELSD

The free monosaccharide amounts after digestion of the polysaccharide were analyzed on an Agilent 1100 series HPLC (Agilent Technologies Palo, Alto, CA, USA) equipped with a Evaporative Light-scattering Detector (ELSD), using a Hypersil NH_2 column (4.6 mm × 250 mm, Dalian Elite Analytical Instrument Co. Ltd., China) by a published method with some modifications (Xu, Xia, Wang, & Wang, 2008). The flow rate was 0.8 mL min^{-1} and the mobile phase was acetonitrile–water (85:15). The injection volume of mixed monosaccharide standards and the digestion products was 10 μL. The temperature of column was set at room temperature. Identification of the free monosaccharides after digestion of the polysaccharide was carried out by comparing their retention times with those of standards under the same HPLC conditions. Quantitative determination was performed using the external standard method.

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD) of three determinations and were subjected to Student's *t*-test. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. M_w change of polysaccharide from P. asiatica L. after saliva digestion

For saliva from different people, the constituents were similar but the amount of every constituent was different. However, the values for every constituent amount are always found in a certain range (Boettcher & de la Lande, 1972; Kelsay, McCague, & Holden, 1972; Schneyer, 1956). The normal pH in the human saliva was reported to be approximately 7.2 (Ziebarth, Spiegelhalder, & Bartsch, 1997), and the enzyme in the human saliva was primarily salivary amylase (Ramasubbu, Paloth, Luo, Brayer, & Levine, 1996).

In this study, what we focus on is the effect of salivary amylase on the polysaccharide from *P. asiatica* L. during saliva digestion. The amylase activity of the human saliva used in this study was determined to be 102 ± 5 D units/ml, which agrees with values (20–250 D units/ml) reported by Spector (1956) and (18)-208 D units/ml range reported by Van Ruth and Roozen (2000), based on the same analysis.

The retention time of the polysaccharide from *P. asiatica* L. did not change before and after the saliva digestion (Fig. 1), indicating that the M_w of the polysaccharide was not changed by the saliva treatment. It also suggested that salivary amylase had no effect on the polysaccharide from *P. asiatica* L.

The salivary amylase was reported to have an effect on most soluble digestible starches (Ramasubbu et al., 1996; Zhang & Kashket, 1998), and it was the key enzyme for starch in the absence of pancreatic amylase for it may well represent a potential compensatory alternate pathway for the amylose, amylopectin and glycogen (Lebenthal, 1987). The salivary α-amylase, which hydrolyses α (1→4) linkage in starch and others, is secreted in the mouth from salivary glands. The action of amylase produces smaller carbohydrate segments that can be hydrolyzed further by enzymes at the brush border of the intestinal cells (Schneeman, 2002). However, there are many carbohydrates that were not be degraded or affected by salivary amylase, and the salivary amylase was found to liberate molecules containing the α-1:6-branch linkages of the

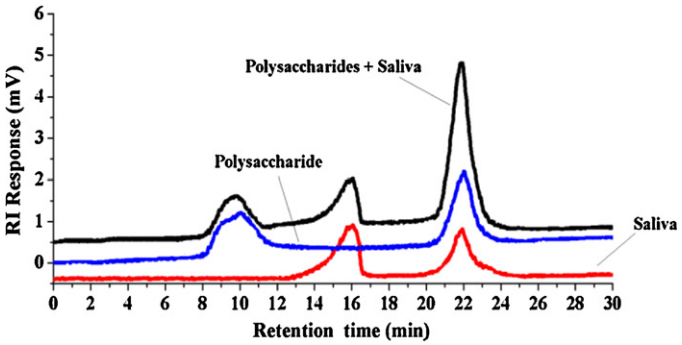

Fig. 1. HPGPC chromatogram of the polysaccharide from the seeds of *P. asiatica* L. before and after *in vitro* human saliva digestion with RI detection on Ultrahydrogel linear column (7.8 mm × 300 mm) with distilled water at 0.5 mL min^{-1}.

polysaccharide (α-limit dextrins) (Roberts & Whelan, 1960). Our results also showed that salivary amylase did not have any effect on the polysaccharide from *P. asiatica* L. Therefore, their results were similar to ours.

3.2. M_w change of polysaccharide from P. asiatica L. after artificial simulated gastric and intestinal digestion in vitro

As shown in Table 1, there was significant difference in M_w of the polysaccharide at different times during gastric and intestinal digestion. As the digestion time increased, a steady decrease in M_w was observed. Specifically, the M_w decreased from 1903.1 ± 93.0 to 8.9 ± 0.5 kDa during gastric digestion, and it continuously dropped to 4.7 ± 0.2 kDa after intestinal digestion.

Fig. 2a and b presents the average M_w of the polysaccharide from *P. asiatica* L. treated in simulated gastric digestion at different time points within 6 h and the blank gastric medium, respectively. As it can be seen from Fig. 2b, the peak for the substances in the gastric medium (peak G) appeared very late in the HPGPC chromatogram, indicating that the M_w of the substances in gastric medium did not contain polymers with very high M_w, which was consistent with the substances added for preparation of the gastric medium (in Section 2.4.1). In addition, the M_w of the polysaccharide was 1903.1 ± 93.0 kDa (Table 1) and it (peak A) appeared very early in HPGPC chromatogram (Fig. 2a). Thus, the substances in the gastric medium would not influence the determination of the M_w change of the polysaccharide during gastric digestion. Furthermore, as shown in Fig. 2a, polysaccharide treated by stimulated gastric digestion at different times (0–6 h) showed clear differences

Table 1
Average molecular weight and content of reducing sugars (C_R) of polysaccharide from the seeds of *P. asiatica* L. at different time points of gastric and small intestinal digestion.[a]

Samples	M_w (kDa)	C_R (mM)
Stomach		
0 h	1903.1 ± 93.0a[b]	0.157 ± 0.009a
0.5 h	39.0 ± 2.5b	0.490 ± 0.016b
1 h	19.5 ± 1.5 bc	0.532 ± 0.018 bc
2 h	14.6 ± 1.2bc	0.560 ± 0.012bc
4 h	10.3 ± 1.0bc	0.581 ± 0.019bc
6 h	8.9 ± 0.5bc	0.594 ± 0.011bc
Intestine		
0.5 h	7.2 ± 0.5c	0.604 ± 0.013c
2 h	6.4 ± 0.3c	0.610 ± 0.016c
4 h	6.3 ± 0.2c	0.618 ± 0.017c
6 h	4.7 ± 0.2c	0.622 ± 0.026c

[a] Data are presented as mean ± standard deviations of triplicate measurements.
[b] Mean values in the same column with different letters were significantly different (Tukey test, $p < 0.05$).

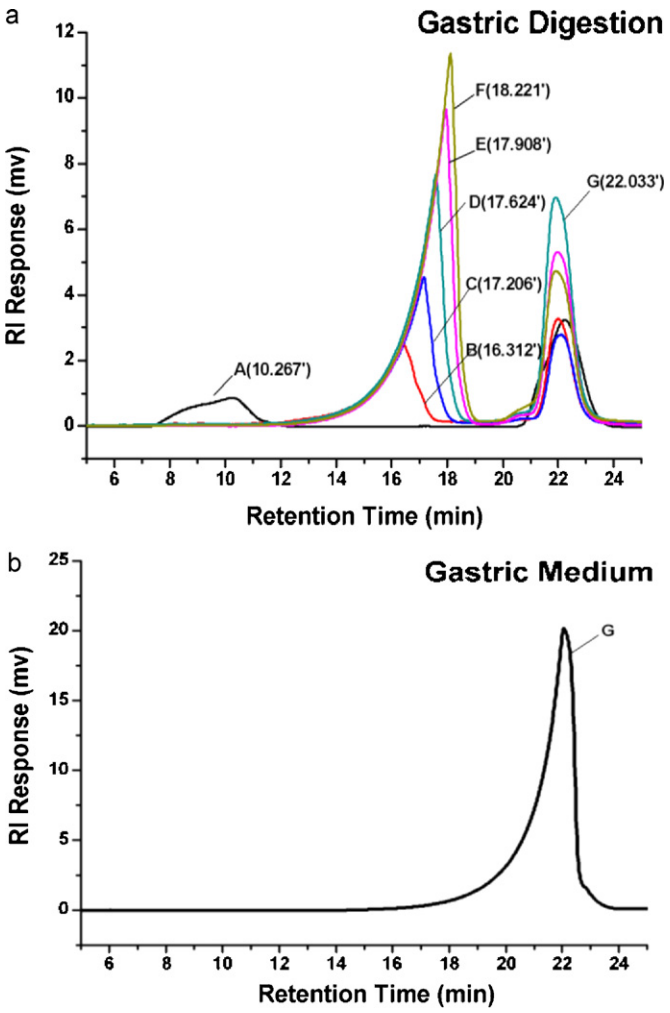

Fig. 2. HPGPC chromatogram of the polysaccharide from *P. asiatica* L. *in vitro* gastric digestion at different times within 6 h (a) and the chromatogram of gastric medium before digestion (b), with RI detection on Ultrahydrogel linear column (7.8 mm × 300 mm) with distilled water at 0.5 mL min^{-1}. Peaks (A–F) represent the polysaccharide at different digestion time points: (A) 0 h, (B) 0.5 h, (C) 1 h, (D) 2 h, (E) 4 h, (F) 6 h, while peak G represents the substances in gastric medium. The value in parentheses is the mean exposure time of three determinations.

in M_w distribution patterns. The elution volume of polysaccharide delayed with time (Fig. 2a), which means a reduction of polysaccharide M_w in stimulated gastric digestion. After polysaccharide was treated by gastric digestion at 0.5, 1, 2, 4 and 6 h, its average M_w was reduced from 1903.1 ± 93.0 (0 h) to 39.0 ± 2.5, 19.5 ± 1.5, 14.6 ± 1.2, 10.3 ± 1.0, and 8.9 ± 0.5 kDa, respectively (Table 1). It could be noted that the molecular weight of the starting polysaccharide declined quickly in the initial 0.5 h during the gastric digestion. Because the polysaccharide was very sensitive to the acidic pH (data shown later), the molecular weight loss was more easily generated when the gastric digestion started. Furthermore, the significant molecular weight loss of the polysaccharide was not only due to the breakdown of glycosidic bonds but also may result from disruption of agglomerates in the initial 0.5 h when the polysaccharide began to contact with the acidic gastric medium.

A reduction of polysaccharide M_w was also observed in stimulated intestinal digestion. Fig. 3a and b presented the average M_w of the polysaccharide treated by small intestinal digestion at different time points within 6 h and the blank intestinal medium, respectively. Also, the peak for the substances in the intestinal medium (peak E) appeared very late in the HPGPC chromatogram (Fig. 3b), indicating that the M_w of the substances in intestinal medium did

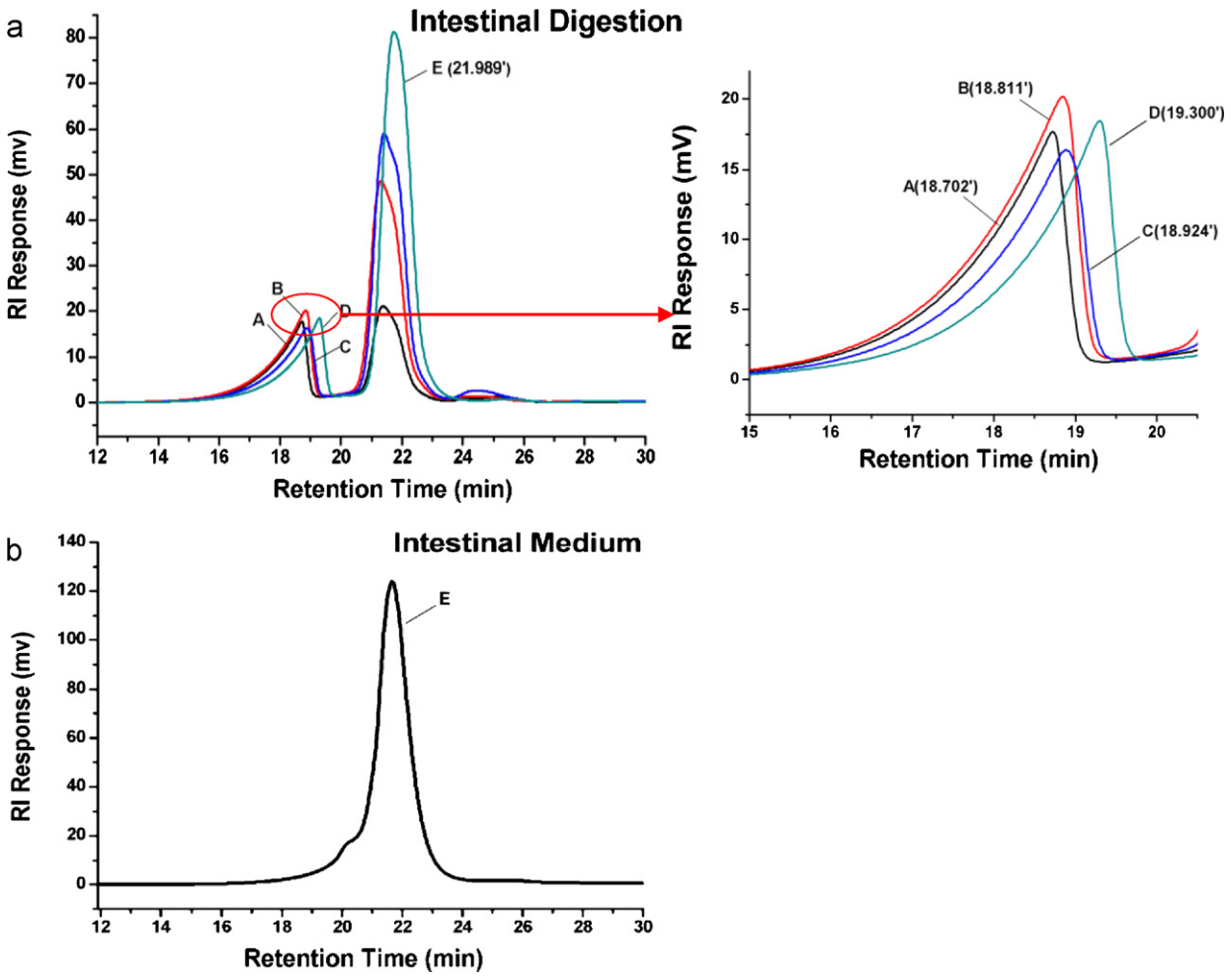

Fig. 3. HPGPC chromatogram of the polysaccharide from *P. asiatica* L. *in vitro* small intestinal digestion at different times within 6 h (a) and the chromatogram of small intestinal medium before digestion (b), with RI detection on Ultrahydrogel linear column (7.8 mm × 300 mm) with distilled water at 0.5 mL min^{-1}. Peaks (A–D) represent the polysaccharide at different digestion time points: (A) 0.5 h, (B) 2 h, (C) 4 h, (D) 6 h, while peak E represents the substances in intestinal medium. The value in parentheses is the mean exposure time of three determinations.

not contain polymers with very high M_w, which was consistent with the substances added for the preparation of the intestinal medium (in Section 2.4.2). In addition, the M_w of the polysaccharide after 6 h gastric digestion was 8.9 ± 0.5 kDa (Table 1) and it (peak F, Fig. 2a) appeared much earlier than the substances of intestinal medium (peak E, Fig. 3b) in HPGPC chromatogram. Thus, the substances in intestinal medium would also not influence the determination of the M_w of the polysaccharide during intestinal digestion. Furthermore, as shown in Fig. 3a, polysaccharide treated by stimulated gastric digestion at different times (0–6 h) resulted in clear differences in M_w distribution patterns. The elution volume of polysaccharide delayed with time (Fig. 3a), which means a reduction of polysaccharide M_w in stimulated intestinal digestion. And after polysaccharide was treated by intestinal digestion at 0.5, 2, 4 and 6 h, its average M_w was continuously reduced to 7.2 ± 0.5, 6.4 ± 0.3, 6.3 ± 0.2 and 4.7 ± 0.2 kDa, respectively (Table 1).

It was reported that pH and salt might caused polymer dissociation or breakdown (Fishman, Cooke, Hotchkiss, & Damert, 1993; Rate, McLaren, & Swift, 1993). To find out the influence of pH on the digestion of the polysaccharide, the polysaccharide was subjected to digestion at pH 1.5 and at 6.8 individually in the absence of enzyme. It was found that the M_w of the polysaccharide was obviously reduced after digestion at pH 1.5, and the extent of the decrease was more than digestion at pH 3.0 (Fig. 4a). In the contrast, the M_w of the polysaccharide was not changed under the digestion at pH 6.8 (Fig. 4b). These results mean that the pH is one of the factors that influence the M_w loss of the polysaccharide during digestion. The effect of salts on the digestion of polysaccharide was also evaluated. The M_w of the polysaccharide did not change when the salts in gastric and intestinal medium (NaCl, KCl, $NaHCO_3$, $CaCl_2$, and CH_3COONa) added individually or together. Therefore, it could be considered that the pH of the digestion medium would be a main reason that causes the M_w loss of the polysaccharide during digestion.

Some studies have also been carried out to investigate the gastric and intestinal digestion of other carbohydrates. Starch was reported to be degraded by gastric and intestinal media to oligosaccharides and could be digested by amylase or saccharidases in the small intestine to sugars that can be absorbed by the intestine (Heaton et al., 1988). In contrast, it was reported that pectin was not affected by gastrointestinal medium (Englyst & Cummings, 1985); oligosaccharides such as fructooligosaccharides (Losada & Olleros, 2002) and inulin (Cummings, 2001) cannot be digested by gastric acid and pancreatic enzymes.

3.3. Content of reducing sugars

It is known that polysaccharides tend to form aggregates in aqueous systems (Li, Wang, Cui, Huang, & Kakuda, 2006; Wang, Huang, Nakamura, Burchard, & Hallett, 2005). Sometimes it is

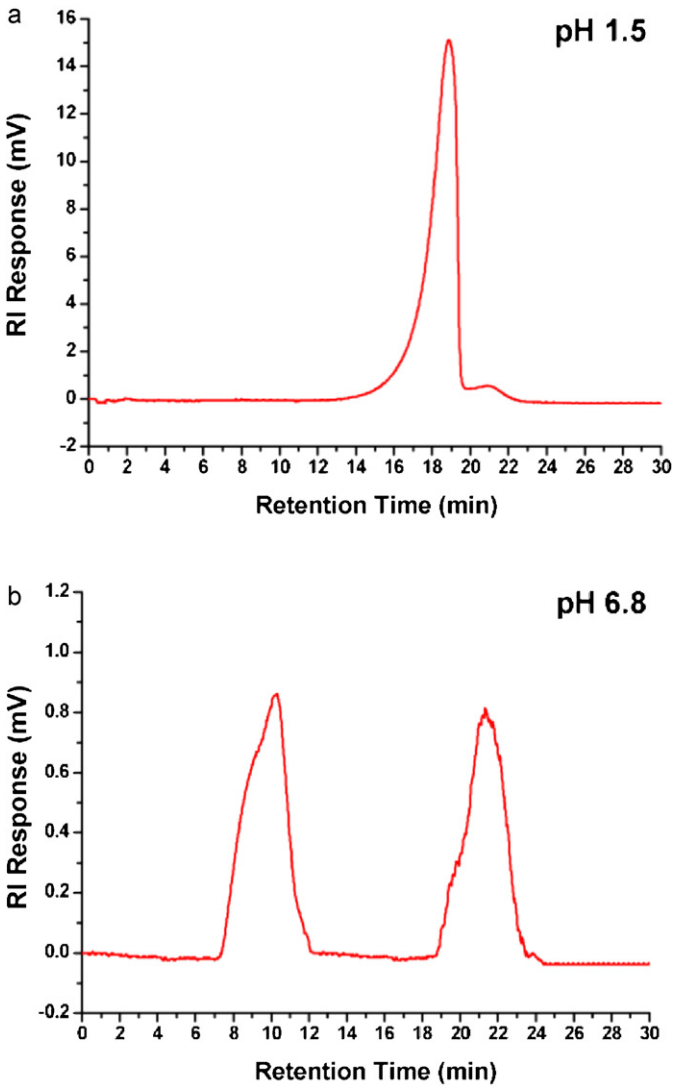

Fig. 4. HPGPC chromatogram of the polysaccharide from *P. asiatica* L. after *in vitro* digestion at pH 1.5 (a) and pH 6.8 (b) in the absence of enzyme, with RI detection on Ultrahydrogel linear column (7.8 mm × 300 mm) with distilled water at 0.5 mL min^{-1}.

difficult to determine whether the changes in M_w of polymers resulted from disruption of aggregates or due to covalent bond cleavage in the polymer chains. This problem can be resolved by measuring the amount of reducing sugars using 3,5-dinitrosalicylic acid (DNS) method (Chen et al., 2012). It was also reported that if the

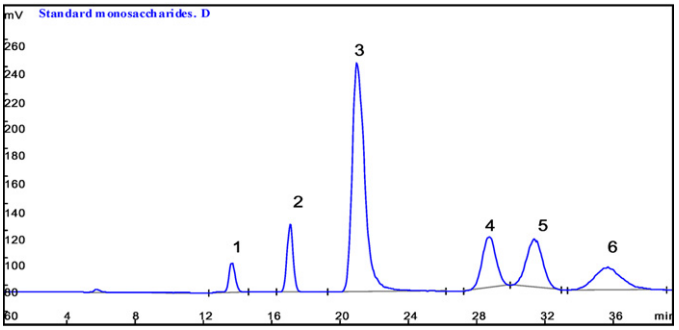

Fig. 5. HPLC-ELSD chromatogram of standard solution of monosaccharides. Peaks followed by the order: (1) rhamnose, (2) xylose, (3) arabinose, (4) mannose, (5) glucose, and (6) galactose.

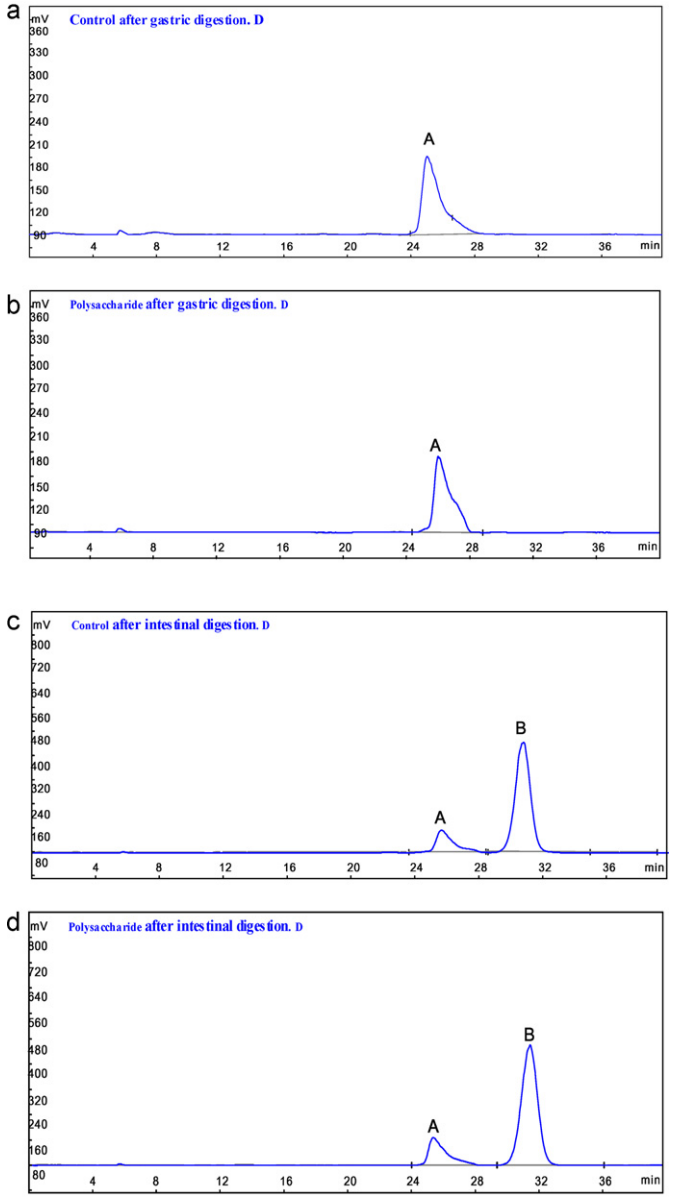

Fig. 6. HPLC-ELSD chromatograms for the released free monosaccharide determination of polysaccharide from *P. asiatica* L. after simulated gastric (b) and intestinal digestion (d), compared with chromatograms of control (water) after gastric (a) and intestinal digestion (c).

decrease of M_w was caused by the breakdown of glycosidic bonds, the number of reducing ends would increase (Chen et al., 2012; Harrington & Zimm, 1965). As shown in Table 1, the reducing sugars of the starting polysaccharide was 0.157 ± 0.009 mM. After the gastric and intestinal digestion, the reducing sugars were significantly increased to 0.622 ± 0.026 mM along with the time increasing, indicating the decrease of M_w may due to the breakdown of glycosidic bonds. In addition, the reducing ends of the polysaccharide were found to sharply increase from 0.157 ± 0.009 mM to 0.721 ± 0.036 mM after digestion at pH 1.5. The distinct rise of reducing sugars content may also due to breakdown of covalent bonds and formation of reducing ends. However, there was no change for the reducing ends of the polysaccharide during digestion at pH 6.8. This means that the breakdown of glycosidic bonds during digestion was mainly caused by the acidic pH.

3.4. Determination of free monosaccharide amounts after digestion

The polysaccharide from the seeds of *P. asiatica* L. consists of several monosaccharides including xylose, arabinose, rhamnose, galactose, glucose and mannose, which has been reported by our group previously (Yin, Nie, Fu, & Xie, 2008). Whether there was any monomeric monosaccharide released during digestion was analyzed in this study. A typical chromatogram presenting the separation of six standard monosaccharides was shown in Fig. 5, indicating the Hypersil NH_2 column satisfactorily separated the standard monosaccharides within 40 min.

Fig. 6 presents HPLC-ELSD chromatograms for the determination of free monosaccharides released after gastric and intestinal digestion of the polysaccharide, as compared with gastric and intestinal digestion of control (water). Specifically, Fig. 6a and b showed HPLC-ELSD chromatograms of water and polysaccharide after the gastric digestion. There was only a peak (peak A) in each of the two chromatograms which was the same with each other. In addition, there was also no difference between the chromatograms of water and polysaccharide after the intestinal digestion (Fig. 6c and d). The gastric and intestinal medium did not contain any carbohydrate, thus the peak A and B in Fig. 6a and c (water) was not the monosaccharide but other substances in digestion medium. Furthermore, there was no other peak which represented the free monosaccharide (according to the HPLC-ELSD chromatogram for standard monosaccharides in Fig. 5) in Fig. 6b and d for polysaccharide after digestion, compared with Fig. 6a and c of control (water) after digestion. Therefore, there was no free monosaccharide released after gastrointestinal digestion of the polysaccharide, suggesting that the gastrointestinal digestion did not result in a production of free monosaccharides.

4. Conclusions

Polysaccharide from the seeds of *P. asiatica* L. was investigated in artificial simulated saliva, gastric and intestinal digestion *in vitro*. It was found that salivary amylase did not have any effect on the polysaccharide. However, the M_w of the polysaccharide was decreased after gastric and intestinal digestion. Meanwhile, reducing ends were significantly increased, indicating the decrease of M_w may due to the breakdown of glycosidic bonds. In addition, there was no free monosaccharide released throughout the whole digestion time period, suggesting that the gastrointestinal digestion did not result in a production of free monosaccharide. These results may provide some insight into the digestion of polysaccharide from *P. asiatica* L. *in vitro*, and may contribute to the methods of studying the digestion of other carbohydrates.

Conflict of interest statements

We have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.10.072.

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