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Discrimination of polysaccharides from traditional Chinese medicines using saccharide mapping—Enzymatic digestion followed by chromatographic analysis

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ABSTRACT

Polysaccharides isolated from traditional Chinese medicines (TCMs) exhibit multiple pharmacological activities. However, quality control of polysaccharides is a challenge because of their complicate structure and macro-molecular mass. In this study, saccharide mapping based on specific enzymatic digestion of polysaccharides and chromatographic analysis was proposed to discriminate the polysaccharides from different TCMs. Endo-carbohydrase such as glucanase, arabinanase, xylanase, galactanase, cellulase, amylase and pectinase were used for enzymatic digestion of polysaccharides from 9 TCMs namely *Panax ginseng*, *P. notoginseng*, *P. quinquefolium*, *Cordyceps sinensis*, *C. militaris*, *Ganoderma lucidum*, *G. sinense*, *Astragalus membranaceus* and *Angelica sinensis*. By using high performance size exclusion chromatography (HPSEC) as well as derivatization with 1-Phenyl-3-methyl-5-pyrazolone (PMP) and HPLC analysis, the enzymatic hydrolysis properties of polysaccharides and their saccharide mapping were determined. The polysaccharides from 9 TCMs were firstly successfully distinguished based on their characteristic saccharide maps, which is helpful to improve the quality control of polysaccharides.

1. Introduction

Carbohydrate drugs have long been interested by pharmaceutical companies and biotech firms for their therapeutic effects [1]. As a class of carbohydrates with the degree of polymerization (DP) more than 10, polysaccharides are usually considered as main substrates for energy metabolism [2,3]. In fact, botanical polysaccharides such as water-soluble polysaccharides from traditional Chinese medicines (TCMs) have attracted a great deal of attention in last decades because of their broad spectrum of therapeutic properties and relatively low toxicity [4–7]. Indeed, immuno-modulation, anti-tumor, anti-oxidation, anti-virus, and anti-inflammatory bioactivities have been presented by many polysaccharides extracted from medicinal fungi and plants, such as *Cordyceps* sp. [8,9], *Ganoderma* sp. [10–15], *Panax* sp. [16–18], *Angelica sinensis* [19–21] and *Astragalus* [22,23]. However, quality control of polysaccharides is a challenge because of their complicate structure and macro-molecular mass. Since the activity of polysaccharides always shows strong relationship with their molecular mass [24,25], monosaccharide composition [26,27], configuration and position of glycosidic linkages [10,28], even the triple-helix conformation [29], the determination of these physical and chemical properties using chromatographic and electromigratic methods is the job for qualitative analysis of polysaccharides [30]. However, up to date, few reports focus on the discrimination of polysaccharides origin, which is crucial for quality control of polysaccharides from TCMs.

Generally, it is complex, difficult and time consuming to obtain structural information though it is unambiguous identification of polysaccharides. Currently, the fingerprints of high-performance thin-layer chromatography (HPTLC) [31] or gas chromatography (GC) [32] based on the constituent saccharides of polysaccharides, as well as chromatographic analysis of hydrolysates derived from total or partial acid hydrolysates, have been used for characterization of polysaccharides [33,34]. However, the selectivity of acid hydrolysis is poor, and the ratio of monosaccharide obtained in acid hydrolysate may be not in accordance with that in polysaccharides due to the degradation under acidic conditions [35,36]. Enzymatic digestion is specific, which has been used to find the novel characteristics of polysaccharides [37–40]. Actually, peptide mapping, the combination of specific enzymatic hydrolysis and characterization of hydrolysates, has been proved to be valuable for identification of protein [41,42]. Similarly, "saccharide mapping" based on carbohydrase hydrolysis followed by chromatographic analysis may be a powerful tool for characterization of polysaccharides.

In this study, polysaccharides from 9 traditional Chinese medicines, including *Panax ginseng*, *P. notoginseng*, *P. quinquefolium*, *Cordyceps sinensis*, *C. militaris*, *Ganoderma lucidum*, *G. sinense*, *Astra-*

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Table 1
Digestion conditions for enzymatic hydrolysis modified from operation manual of Megazyme and Sigma Co.

Enzyme	EC number	Buffer solution	pH	Temperature (°C)
Arabinanase	3.2.1.99	50 mM sodium acetate	4.0	40
Xylanase	3.2.1.8	25 mM sodium acetate	4.7	40
1,4-β-D-Galactanase	3.2.1.89	25 mM sodium acetate	4.0	40
Cellulase	3.2.1.4	25 mM sodium acetate	4.5	40
Pectinase	3.2.1.15	50 mM sodium acetate	5.5	40
α-Amylase	3.2.1.1	100 mM sodium acetate	7.0	40
Isoamylase	3.2.1.68	100 mM sodium acetate	4.0	40
β-Mannanase	3.2.1.78	50 mM sodium acetate	4.5	40
1,3-β-Glucanase	3.2.1.39	50 mM sodium acetate	6.0	40
Lichenase	3.2.1.73	25 mM sodium phosphate buffer	6.5	40

galus membranaceus and *Angelica sinensis* were firstly discriminated based on their carbohydrase enzymatic hydrolysis properties and chromatographic characteristics of enzymatic hydrolysates, i.e. saccharide mapping.

2. Experimental

2.1. Chemicals, reagents and materials

P. ginseng (PG), *P. notoginseng* (PN), *P. quinquefolium* (PQ) and *C. sinensis* (CS) were purchased from Zhong-qiao Corporation (Macau, China); *G. lucidum* (GL) and *G. sinense* (GS) were collected from Jinzhai, Anhui Province; *A. membranaceus* (AM) and *A. sinensis* (AS) were collected from Shanxi and Gansu provinces, respectively. The botanical origin of the materials mentioned above was identified by corresponding author. Mycelia of *C. militaris* (CM) were fermented in our lab (the fungus strain was from The Chinese Academy of Agricultural Sciences). All voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

D-galacturonic acid monohydrate (GalA), D-glucuronic acid (GlcA), D-arabinose (Ara), D-mannose (Man), D-galactose (Gal) and D-glucose (Glc) were purchased from Fluka (Buchs, France). Blue dextran 2000, L-rhamnose monohydrate (Rha), D-xylose (Xyl), maltose (Malt), pectinase (endopolygalacturonase, EC 3.2.1.15), cellulase (endo-1,4-β-D-glucanase, EC 3.2.1.4) and α-amylase (EC 3.2.1.1) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany). Ammonium acetate was obtained from Riedel-de Haën (Seelze, Germany). Deionized water was prepared by Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA). Sodium acetate, sodium phosphate monobasic and sodium phosphate dibasic from Riedel-de Haën were used in preparation of buffer solution for enzymatic digestion. Endo-arabinanase (EC 3.2.1.99), isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68), xylanase (EC 3.2.1.8), endo-1,4-β-D-galactanase (EC 3.2.1.89), β-1,3-D-glucanase (endo-1,3-β-D-glucanase, EC3.2.1.39), lichenase (EC 3.2.1.73) and β-mannanase (EC 3.2.1.78) were obtained from Megazyme (Wicklow, Ireland). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was a gift from Dr. Song Yue of Agilent Technologies Co., Ltd. (Shanghai, China), and recrystallization twice using methanol before use. Other reagents not mentioned here were of analytical-reagent grade from standard sources.

2.2. Preparation of polysaccharides from TCMs

The powders of medicinal material were immersed with 20-folds volume deionized water and refluxed in a Syncore parallel reactor (Büchi, Switzerland) for 1 h at the temperature of 100 °C with stir at 120 rpm. An aliquot of 5 mL extract solution was centrifuged at 4713 × *g* for 10 min (Allegra X-15R, Beckman Coulter, Fullerton, CA), and the supernatant was precipitated by addition of ethanol to final concentration of 75% (v/v), which stayed overnight (12 h) under 4 °C. After centrifugation (4713 × *g* for 10 min), the precipitate was washed with 4 mL of 95% ethanol twice and then ethanol was removed on water bath (60 °C). The dried residue was redissolved in 5 mL hot water (60 °C), then after centrifugation, the supernatant was collected and its polysaccharides were quantitatively determined using phenol-sulfuric acid assay [43] with glucose as reference standard. The polysaccharides solution was adjusted to about 0.15 mg/mL of polysaccharides calculated as glucose, and used for HPSEC analysis and further treatment.

2.3. Enzymatic digestion

Polysaccharide solution (1000 μL) was mixed with certain enzyme (the final concentration of arabinanase, xylanase, 1,4-β-D-galactanase, cellulase, pectinase, α-amylase and isoamylase were 1.4 U/mL, 4.5 U/mL, 9.4 U/mL, 10 U/mL, 95 U/mL, 12.5 U/mL and 10 U/mL, respectively) in 1.5 mL Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and digested overnight (≥12 h) under the optimum conditions (Table 1). Then the mixture was boiled at 85 °C for 30 min (Eppendorf Thermomixer Comfort, Eppendorf AG) to stop the enzyme digestion. The supernatant was applied for HPSEC analysis and derivatization, respectively, after centrifugation (5415 D, Eppendorf AG) at 15,700 × *g* for 30 min. Deionized water instead of polysaccharide solution, treated as mentioned above, was used as blank control.

2.4. Derivatization with PMP reagent

The sugar derivatization followed previous reports [44–46] with modifications. Briefly, the enzymatic hydrolysate (600 μL) was mixed with the same volume of NH_3 solution, and then 0.5 mol/L PMP methanolic solution (200 μL). The mixture was allowed to react on the Syncore parallel reactor (70 °C for 30 min) and then was cooled to room temperature with addition of water (2000 μL). The solution was vacuum evaporated to dryness under 50 °C, then repeatedly water was added (2000 μL) and dried twice to remove NH_3. The residue was dissolved in water and chloroform (1 mL each). After vigorous shaking and centrifugation at 15,700 × *g* for 5 min, organic phase was discarded to remove the excess reagents. The operation was performed triplicates, and finally the aqueous layer was filtered through a 0.45 μm syringe filter (Agilent Technologies) before liquid chromatography-diode array detector-mass spectrometry (LC–DAD–MS) analysis. A standard solution, containing 6 monosaccharides (Rha, Ara, Xyl, Man, Glc and Gal, ~1 mg/mL each), 2 uronic acids (GlcA and GalA, ~1 mg/mL each) as well as maltose (Malt, ~1 mg/mL), was also treated as mentioned above for reference.

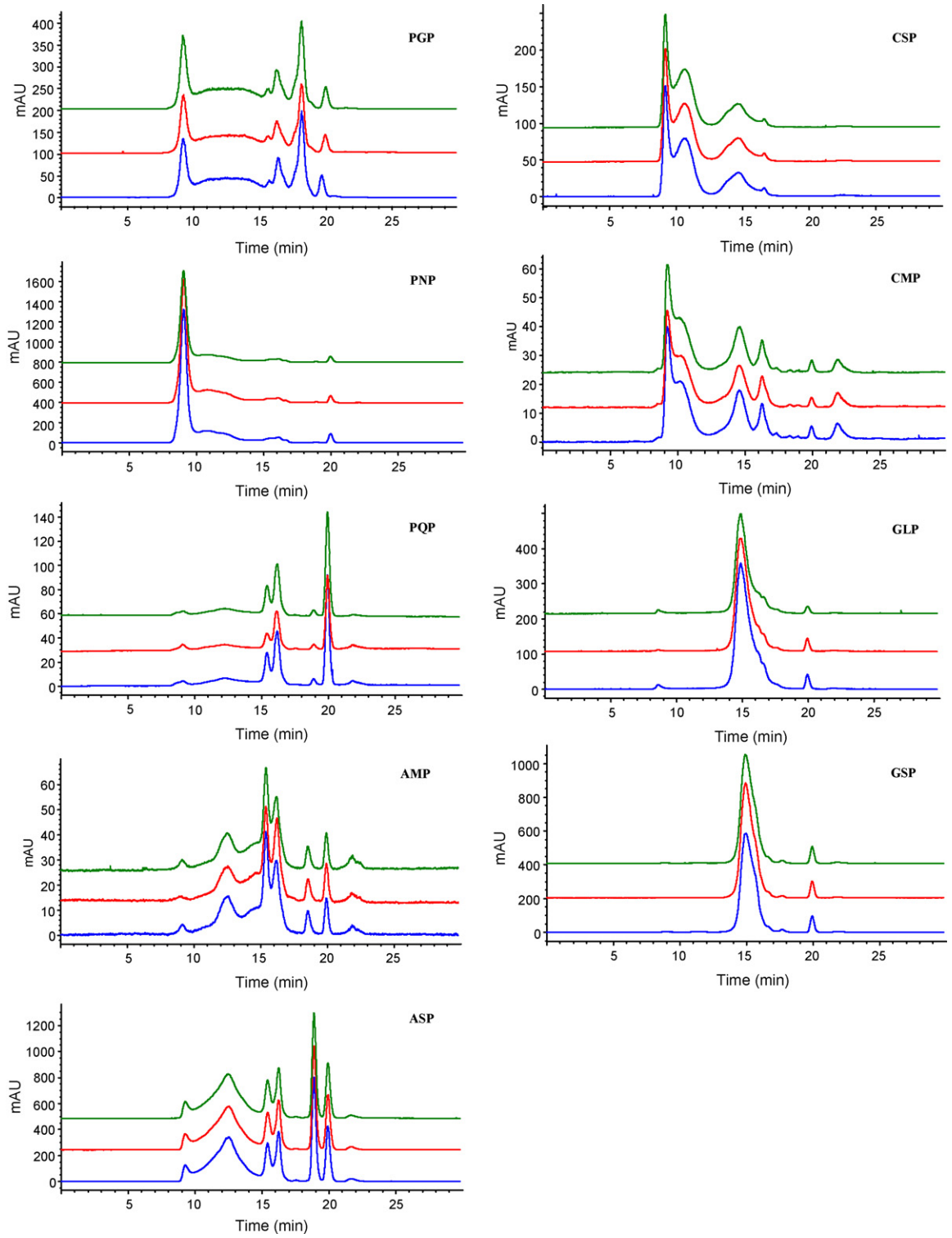

Fig. 1. HPSEC-ELSD profiles of three individually extracted polysaccharides from the investigated TCMs. PGP, PNP, PQP, CSP, CMP, GLP, GSP, AMP and ASP were polysaccharides from *P. ginseng*, *P. notoginseng*, *P. quinquefolium*, *C. sinensis*, *C. militaris*, *G. lucidum*, *G. sinense*, *A. membranaceus* and *A. sinensis*, respectively.

2.5. HPSEC–DAD–ELSD analysis

The analysis was performed on an Agilent 1100 series LC/DAD system (Agilent Technologies, Palo Alto, CA) coupled with evaporate light scattering detection (ELSD). The separation was achieved on a TSK G-4000PW$_{XL}$ column (300 mm × 7.8 mm i.d., 10 μm) operated at 30 °C. Isocratic elution with 20 mmol/L ammonium acetate aqueous solution was used at a flow-rate of 0.6 mL/min. Ultraviolet detection was set at 260 nm and 280 nm. The signal from Alltech ELSD 2000 (Alltech, Deerfield, IL, USA) was transmitted to the Chemstation for processing through an Agilent 35900E interface. The parameters of ELSD were set as follows: the drift tube temperature was 110 °C and nebulizer nitrogen gas flow-rate was at 3.0 L/min, impact off mode. An aliquot of 10 μL solution was injected for analysis.

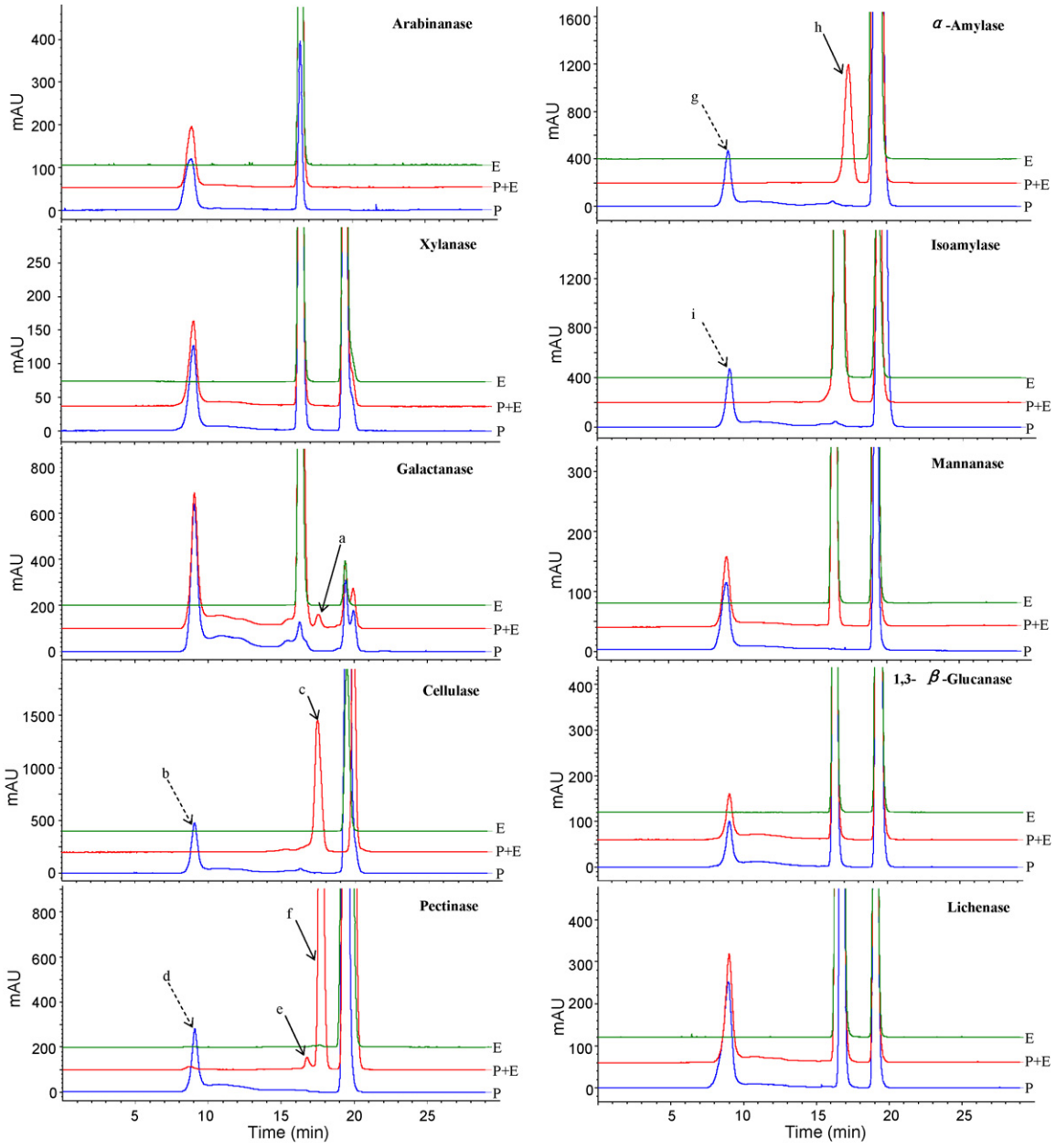

Fig. 2. HPSEC-ELSD profiles of *P. notoginseng* polysaccharides treated with (P + E) or without (P) selected enzymes (E). Peaks a-i were changed during enzymatic hydrolysis.

2.6. HPLC–DAD–MS^n characterization

The analysis of PMP derivates of saccharides derived from enzymatic hydrolysate of polysaccharides was performed on an Agilent 1200 series LC/MSD Trap system, equipped with a vacuum degasser, a binary pump, an autosampler and a diode array detector and an ion trap mass spectrometer with electrospray ionization interface connected to an Agilent LC/MSD Trap Software. Samples (10 μL) were injected onto a Zorbax Eclipse XDB-C_{18} column (150 mm × 4.6 mm i.d., 3.5 μm) operated at 25 °C. The separation was achieved using gradient elution with 20 mmol/L ammonium acetate aqueous solution (A) and acetonitrile (B) at a flow-rate of 1.0 mL/min: 0–1 min, 13–15% B; 1–40 min, 16% B. UV detection wavelength was set at 245 nm. MS spectra were acquired in positive ion mode. The full scan mass spectra were obtained from *m*/*z* 100 to 1500. ESI-MS conditions were as follows: dry gas (N_2) 8 L/min, dry temperature 350 °C, nebulizer pressure, 45 psi. ESI-MS/MS conditions: isolation width 4, fragment amplification 1.5, compound stability 50%. Identification of saccharides was achieved by comparison of their MS data with those of standard compounds.

3. Results and discussions

3.1. Extraction of polysaccharides from TCMs

Water-soluble polysaccharides are usually obtained by hot-water extraction and alcohol precipitation [4,9–11]. In order to improve the repeatability, a Syncore parallel reactor equipped with 24 vials was employed for parallel extraction of polysaccharide from TCMs. Few low molecular weight (MW) compounds especially monosaccharides incorporated in alcohol precipitate was

Table 2
Identification of polysaccharides from TCMs based on enzymatic digestion response.

Enzyme	Polysaccharides								
	PGP[a]	PNP	PQP	CSP	CMP	GLP	GSP	AMP	ASP
Arabinanase	+[b]	–	–	–	–	–	–	–	–
Xylanase	+	–	–	–	–	–	–	–	–
1,4-β-D-Galactanase	–	+	–	–	–	–	–	+	+
Cellulase	+	+	+	+	+	–	–	+	–
Pectinase	+	+	+	+	+	+	+	+	+
α-Amylase	+	+	+	+	+	–	–	+	–
Isoamylase	+	+	+	+	+	–	–	+	–

[a] PGP, PNP, PQP, CSP, CMP, GLP, GSP, AMP and ASP are polysaccharides from *P. ginseng*, *P. notoginseng*, *P. quinquefolium*, *C. sinensis*, *C. militaris*, *G. lucidum*, *G. sinense*, *A. membranaceus* and *A. sinensis*, respectively.
[b] +, positive response; –, negative response.

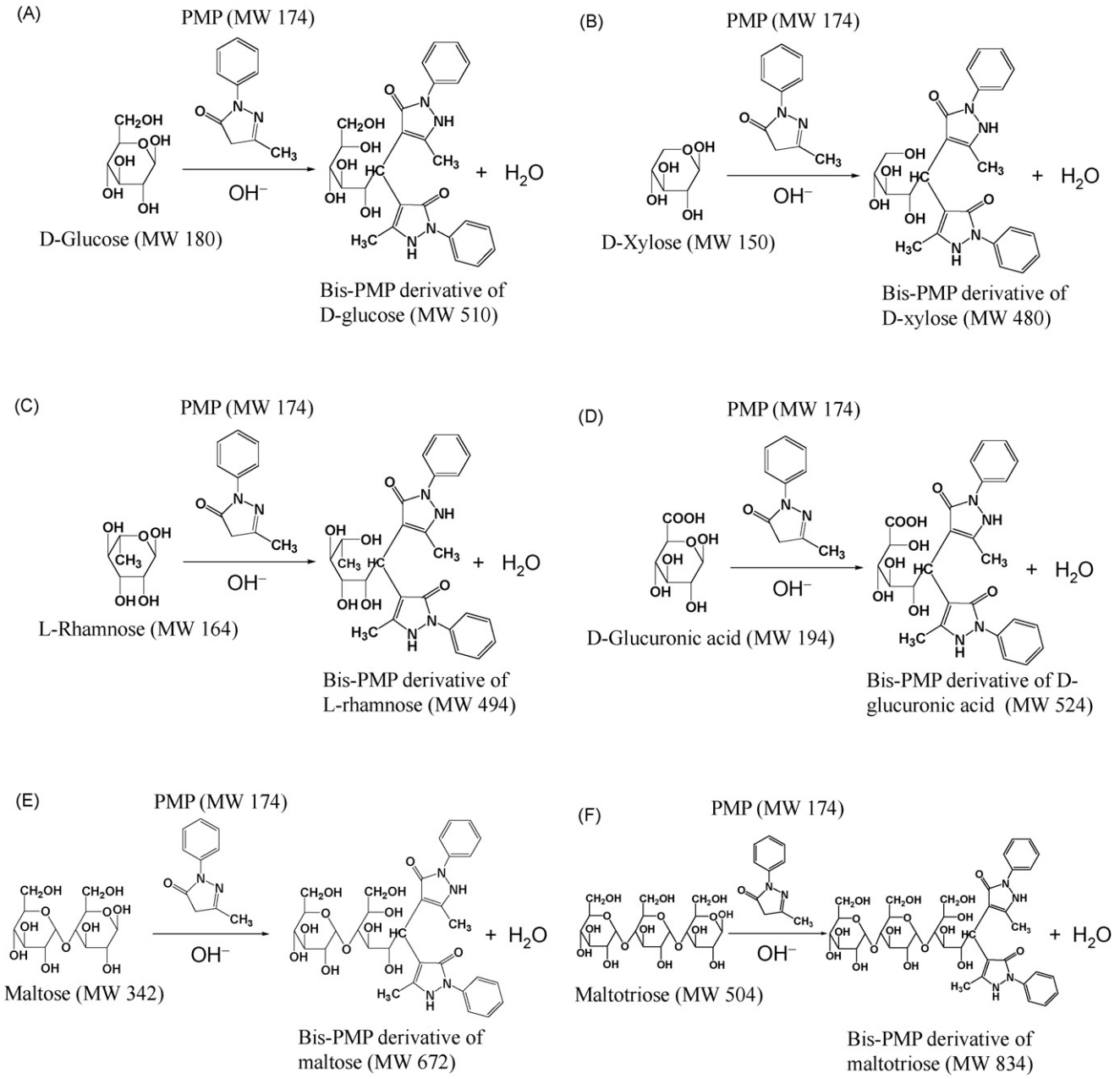

Fig. 3. Chemical reactions of standard (A–E) monosaccharides and (F) trisaccharide with PMP reagent.

removed by washing with 95% ethanol to avoid interfering the analysis of saccharides in enzymatic hydrolysate of polysaccharides. Three parallel extractions were performed, and the result indicated that extraction had a good repeatability and their HPSEC profiles detected by ELSD were very similar (Fig. 1). In addition, UV 260 nm and 280 nm were also selected for detection of conjugated nucleic acid or peptide. But only a few low peaks could be detected in the samples (data not shown).

3.2. Investigation on enzymatic hydrolysis of polysaccharides

Polysaccharides contain different monosaccharide (sugar) units may be linear or branched. Glucose, galactose, mannose, xylose, arabinose, rhamnose and glucuronic acid, galacturonic acid are usually found to be the composed units of backbone or branch of polysaccharides, and they joined together in a head-to-tail fashion by glycosidic linkages. Glucan with β-(1 → 3)-, β-(1 → 4)- and β-(1 → 6)-glycosidic linkages, xylan with β-(1 → 4)-backbone, arabinan with α-1,5-linked bonds, galactans or heteroglycan with β-(1 → 4)-linkages, and pectic polysaccharides with α-1,4-linked D-galacturonic acid residues

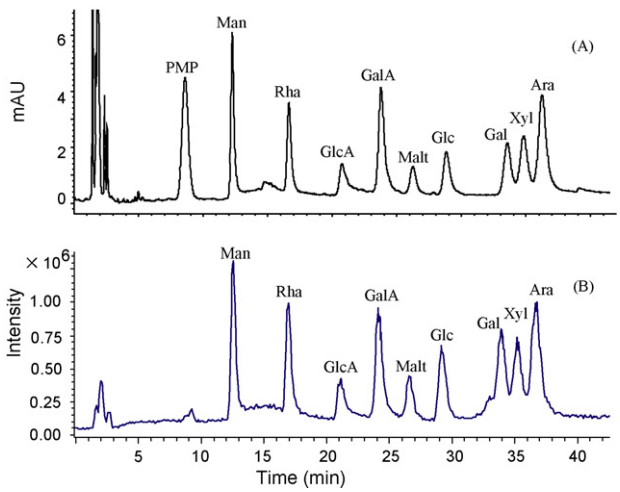

Fig. 4. HPLC chromatograms of PMP derivatized standard saccharides detected by (A) DAD (UV 245 nm) and (B) MS detection (TIC).

Table 3
Summary of the peaks detected in the full range scan (*m*/*z* 100–1500) of the hydrolyzed samples.

Enzyme	MS fragments (*m*/*z*)								
	PGP[a]	PNP	PQP	CSP	CMP	GLP	GSP	AMP	ASP
Arabinanase	Di[b](673.1) UN[c](613.0) UN(679.3) UN(613.1) Pen[d](481.1) UN(508.0)	–[f]	–	–	–	–	–	–	–
Xylanase	Di(673.1) UN(613.0) UN(679.4) UN(508.0)	–	–	–	–	–	–	–	–
1,4-β-D-Galactanase	–	UN(507.1) Di(673.1) UN(643.1) UN(537.0) GalA(525.1) Gal(511.1) UN(507.1)	–	–	–	–	–	Di(673.2) UN(643.1) UN(519.1) UN(679.5)	Di(673.2) GalA(525.1) Gal(511.1)
Cellulase	Man(511.1) Di(673.2) Tri[e](835.1) Malt(673.2) Glc(511.1) UN(679.4)	Man(511.1) Di(673.1) Tri(835.1) Malt(673.2) Glc(511.1) UN(679.4)	Man(511.1) Pen(481.1) UN(519.0) Malt(673.2) Glc(511.1) UN(679.4)	Man(511.1) Tri(835.2) Malt(673.2) Glc(511.1) UN(679.4)	Man(511.1) Pen(481.1) UN(451.2) Tri(835.1) Malt(673.2) Glc(511.1) UN(679.4)	–	–	Man(511.1) Rha(495.1) Di(673.1) UN(519.1) Malt(673.2) Glc(511.1) UN(679.4) Hex[g](511.1)	–
Pectinase	Man(511.1) Rha(495.1) GalA(525.1) Glc(511.1) UN(679.4) Gal(511.1) Ara(481.1)	Man(511.1) GalA(525.1) Glc(511.1) UN(679.4) Gal(511.1) Ara(481.1) UN(421.1)	Man(511.1) GalA(525.1) Glc(511.1) UN(679.4) Gal(511.1) Ara(481.1) UN(421.1)	Man(511.1) UN(451.1) Glc(511.1) Gal(511.1)	Man(511.1) Pen(481.1) GalA(525.1) Glc(511.1) UN(679.3) Gal(511.1) UN(421.1)	Man(511.1) Rha(495.1) GalA(525.1) Glc(511.1) UN(679.4) Gal(511.1) Ara(481.1) UN(421.1)	Man(511.1) Pen(481.1) GalA(525.1) Glc(511.1) UN(679.4) Gal(511.1)	Man(511.1) Pen(481.1) Glc(511.1) UN(679.4) Gal(511.1)	Man(511.1) Rha(495.1) GalA(525.1) Glc(511.1) Gal(511.1) Ara(481.1)
α-Amylase	Tri(835.1)	UN(679.4)	UN(679.4)	None[h]	None	–	–	UN(679.4)	–
Isoamylase	None	UN(679.4)	UN(679.4)	None	None	–	–	UN(679.4)	–

[a] The same as in Table 2.
[b] Disaccharides of hexose.
[c] Unknown.
[d] Pentose.
[e] Trisaccharides of hexose.
[f] Negative response to enzymatic digestion.
[g] Hexose.
[h] Positive response to enzymatic digestion but none fragments found.

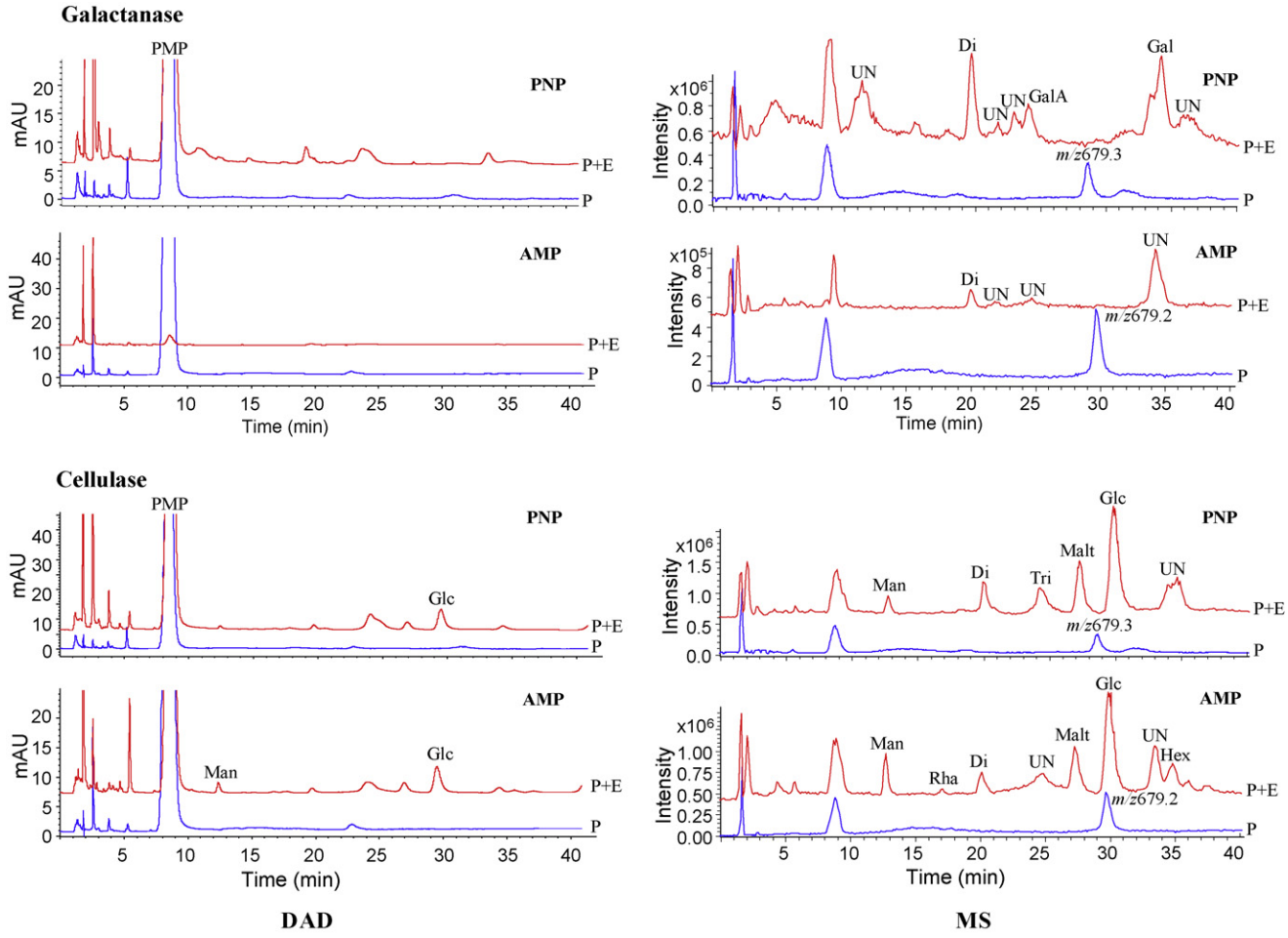

Fig. 5. HPLC chromatograms of PMP derivatized hydrolysate of polysaccharides from *P. notoginseng* (PNP) and *A. membranaceus* (AMP) treated with (P + E) or without (P) selected enzymes (E) detected by DAD (UV 245 nm) and MS detection (TIC). UN, unknown; Di, disaccharides of hexose; Tri, trisaccharides of hexose; Hex, hexose.

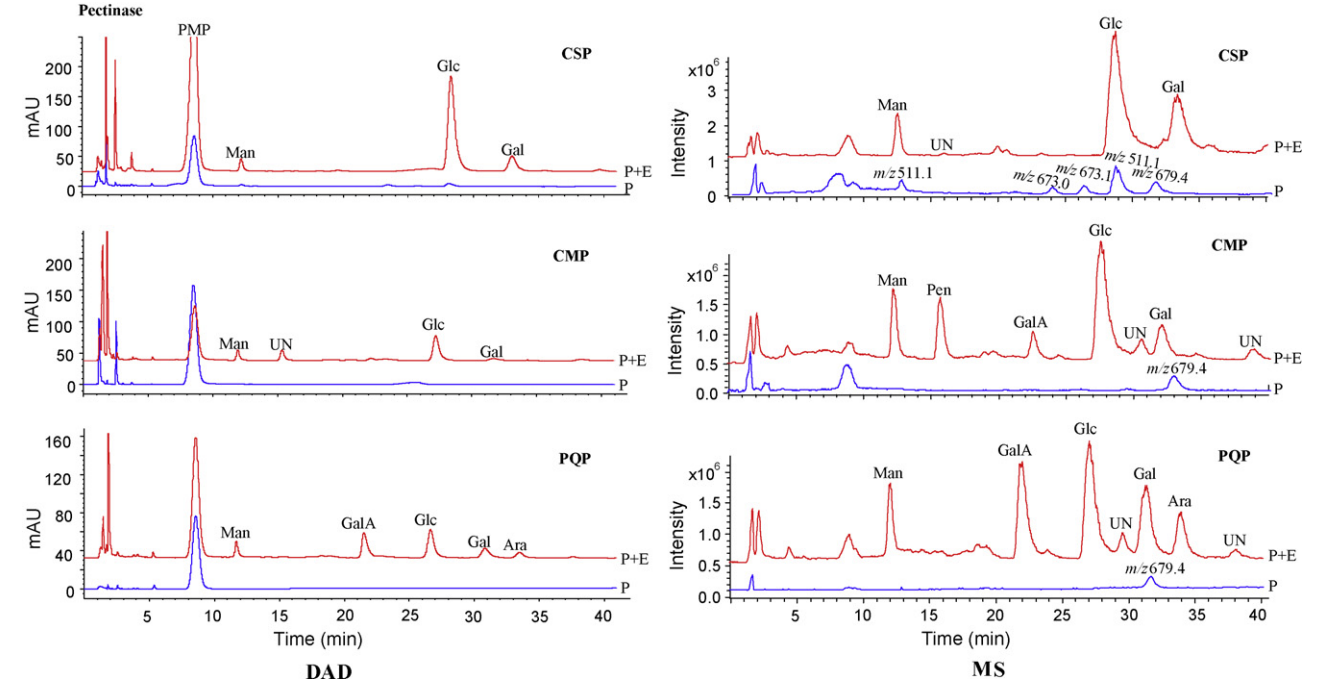

Fig. 6. HPLC chromatograms of PMP derivatized hydrolysate of polysaccharides from C. sinensis (CSP), *C. militaris* (CMP) and *P. quinquefolium* (PQP) treated with (P + E) or without (P) pectinase (E) detected by DAD (UV 245 nm) and MS detection (TIC). UN, unknown; Pen, pentose.

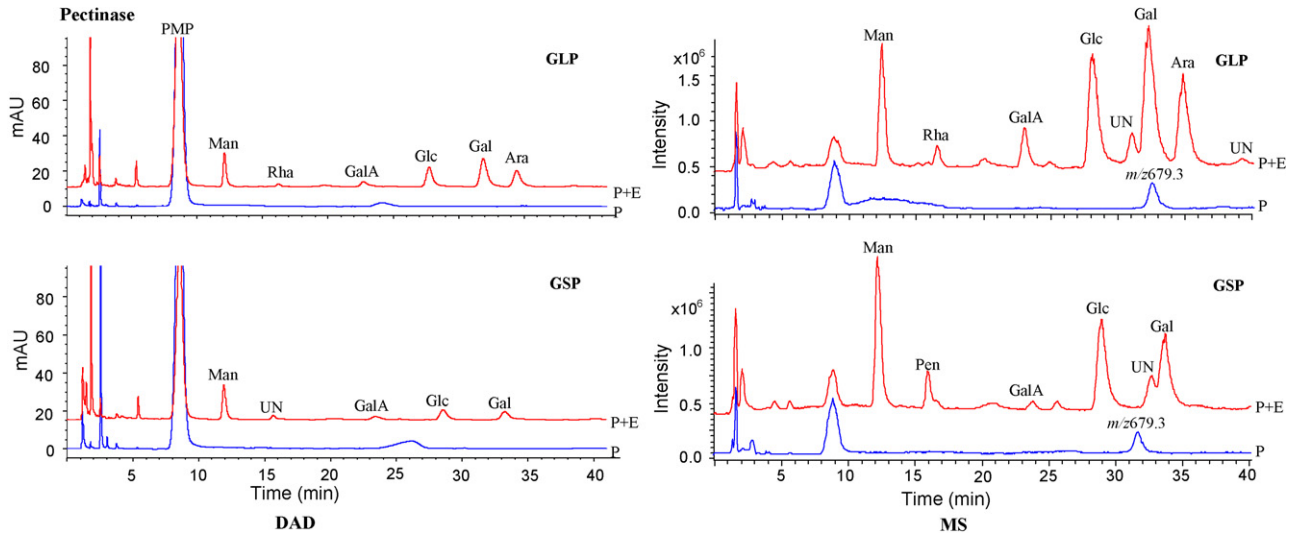

Fig. 7. HPLC chromatograms of PMP derivatized hydrolysate of polysaccharides from *G. sinense* (GSP) and *G. lucidum* (GLP) treated with (P + E) or without (P) pectinase (E) detected by DAD (UV 245 nm) and MS detection(TIC). UN, unknown; Pen, pentose.

widely existed in various glycans from plants and fungi [47–50]. In addition, polysaccharides can be endohydrolyzed to produce specific saccharide fragments which is helpful for identification. Therefore, endopolygalacturonase, endo-arabinanase, endo-1,4-β-D-galactanase, endo-1,4-β-D-glucanase, and endo-1,3-β-D-glucanase were selected for enzymatic hydrolysis of polysaccharides. Lichenase, β-mannanase, α-amylase, isoamylase, and xylanase were also used.

Fig. 2 showed HPSEC-ELSD profiles of polysaccharides before and after selected enzyme digestion. Meanwhile, the enzyme solution was also used as control. The results indicated that five enzymes, including arabinanse, xylanase, mananase, β-1,3-D-glucanase and lichenase had no significant effects on polysaccharides from PN. But galactanase (peak a found), cellulase (peak b → peak c), pectinase (peak d → peaks e and f), amylases (peak g → peak h), and isoamylase (peak i disappeared) can certainly hydrolyze the fraction of polysaccharides. The effects of selected enzymes on polysaccharides from different Chinese medicines were shown in Table 2. Based on this preliminary test, two polysaccharides from PG and AS could be discriminated out of nine investigated polysaccharides. The polysaccharides from 3 herbs of *Panax* genus can also be distinguished. PN and AM; PQ, CS and CM; as well as GL and GS respectively, were divided into three groups according to their enzymatic hydrolysis properties. The enzymatic hydrolysis was performed in triplicate and the results showed that the procedure had a good repeatability (data not shown). Herein, the responses of polysaccharides to different enzymatic digestion were firstly used for their rapid qualitative determination, which were based on their structure characters.

3.3. Identification of polysaccharides based on characterization of released saccharides

As shown in Table 2, some polysaccharides could not be discriminated just based on their enzymatic hydrolysis properties. Therefore, enzymatic hydrolysates of polysaccharides should be further investigated, which could provide their specific information. However, separation of underivatized carbohydrates often requires specific types of column [51,52] and simultaneous analysis of neutral, acid monosaccharides and oligosaccharides were difficult due to the poor selectivity. Especially, direct UV and MS detection of saccharides are usually unavailable because of their poor UV absorptivity and ionization. In order to improve the ionization sensitivity and detectability, pre-column derivatization was performed to trace released saccharides [52]. PMP (1-phenyl-3-methyl-5-pyrazolone) has been proved as a good derivatization reagent for the analysis of oligosaccharides [44,53–55] and monosaccharides [45,56,57]. Herein, PMP derivatization was used for the detection during chromatographic analysis of enzymatic hydrolysate of the investigated polysaccharides. Six monosaccharides, two uronic acids and maltose were employed as the reference for the derivatization, which could be sensitively detected at UV 245 nm and MS (Figs. 3 and 4). Using positive MS scan, pentoses (Xyl and Ara), hexoses (Man, Glc and Gal) and uronic acids (GlcA and GalA) showed, respectively, the peaks of m/z 481.1 ($[M+H]^+$), m/z 511.1 and m/z 525.1, except Rha gave the peak of m/z 495.1 and maltose had the peak of m/z 673.1 as previous report [53,58,59]. Besides, some peaks were tentatively identified as pentose (m/z 481), hexose (m/z 511), disaccharides (m/z 673) and trisaccharides (m/z 835) of hexose (Table 3) according to the references [53,58–60].

The response of polysaccharides from PN and AM, CS, CM and PQ, as well as GL and GS to enzymes were, respectively, similar (Table 2), so their enzymatic hydrolysates were further analyzed using PMP derivatization and HPLC–DAD–MS analysis. In order to confirm the saccharides in polysaccharides hydrolysate were derived from the enzymatic hydrolysis, the same sample without related enzyme treatment was also used for parallel analysis as control. The results showed that the peaks in polysaccharides had no interference to the analysis of their enzymatic hydrolysates (Fig. 5). The results show that the polysaccharides of PN and AM are easily discriminated according to the profiles of their endo-galactanase and cellulase hydrolysates (Fig. 5 and Table 3). But no obvious different peaks were found when the two polysaccharides treated with amylase (Table 3). Similarly, the polysaccharides from CS, CM and PQ could be distinguished based on their pectinase treated saccharide mapping (Fig. 6 and Table 3). Pectinase treated saccharide mapping (Fig. 7 and Table 3) also contributed to the difference of polysaccharides between GL and GS though they had similar compositional sugars. Therefore, the obtained saccharide maps provided structural fragments due to the specific enzymatic digestion rather than regular acidic hydrolyzed profiles.

4. Conclusion

A new "saccharide mapping" was proposed to discriminate crude polysaccharides from TCMs based on enzymatic (carbohydrase) digestion and subsequent chromatographic analysis of enzymatic hydrolysate. Polysaccharides extracted from 9 TCMs were successfully distinguished based on their characteristic profiles. The method provided a different approach on discrimination of polysaccharides from various origins and it is helpful to control the quality of polysaccharides from TCMs.

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