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Analysis of compositional monosaccharides in fungus polysaccharides by capillary zone electrophoresis

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ABSTRACT

A rapid analytical method of capillary zone electrophoresis (CZE) was established for the simultaneous separation and determination of 10 monosaccharides (aldoses and uronic acids). The monosaccharides were labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP), and subsequently separated using an uncoated capillary (50 μm i.d. × 58.5 cm) and detected by UV at 245 nm with pH 11.0, 175 mM borate buffer at voltage 20 kV and capillary temperature 25 °C by CZE. The 10 PMP-labeled monosaccharides were rapidly baseline separated within 20 min. The optimized CZE method was successfully applied to the simultaneous separation and identification of the monosaccharide composition in *Termitomyces albuminosus* polysaccharides (TAPs) and *Panus giganteus* polysaccharides (PGPs). The quantitative recovery of the component monosaccharides in the fungus polysaccharides was in the range of 92.0–101.0% and the CV value was lower than 3.5%. The results demonstrate that the proposed CZE method is precise and practical for the monosaccharide analysis of fungus polysaccharides.

1. Introduction

The nutritional properties and health benefits of edible or medicinal mushrooms have been recognized in China for over 2000 years (Dong, Cai, & Li, 2010; Mau, Chang, Huang, & Chen, 2004; Sadler, 2003). Interestingly, the active constituents that are involved in these effects of edible fungi mushrooms are considered to be polysaccharides, which possess numerous pharmacological effects on human physiology, such as satiety, gastric emptying, the regulation of blood glucose, insulin and cholesterol, as well as the balance of intestinal microflora (Adebayo-Tayo, Adegoke, Okoh, & Ajibesin, 2010; Lindequist, Niedermeyer, & Jülich, 2005; Mavundza et al., 2010). In recent years, rare edible fungi *Termitomyces albuminosus* and *Panus giganteus* used in popular medicines have been reported to contain water-soluble polysaccharides with a great variety of biological activities, including obvious antiviral and anticancer effects (Jong & Donovick, 1989; Lu et al., 2008; Wong et al., 2012). For these reasons, there has been great interest in reliable analytical methods for fungi polysaccharides, which can be used for routine quality control of commercial fungi mushrooms and their related polysaccharide products.

Monosaccharides are the most basic units of biologically important macromolecular polysaccharides. An understanding of the conjunct monosaccharides that frequently occur in natural polysaccharides can serve as the identification of basal chemical characteristic of commercial polysaccharides (Wang et al., 2012). However, the lack of chromophores or fluorophores in the structure of monosaccharides limits the modes of detection. High performance liquid chromatography (HPLC) with refractive index detection, and other related chromatographic methods do not often meet the demands of modern trace level analysis with regard to sensitivity and/or selectivity (Fu, Huang, Zhai, Li, & Liu, 2007; Wang & Fang, 2004). Therefore, the derivatisation of monosaccharides is indispensable to obtain highly sensitive detection (Honda, Akao, et al., 1989; Lv et al., 2009). The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP) is one of the popular labels that may react with reducing carbohydrates under mild conditions, requiring no acid catalysts and causing no desialylation and isomerization (Zhang, Xu, Zhang, Zhang, & Zhang, 2003). Recently, we have described a capillary zone electrophoresis (CZE) method for separation of 8 monosaccharides, where the introduction of PMP into the reducing monosaccharide molecules can be easily achieved by chelation of monosaccharides with a suitable ion of borate (Hoffstetter-Kuhn, Paulus, Gassmann, & Widmer, 1991; Honda, Iwase, Makino, & Fujiwara, 1989; Nguyen, Lerch, Zemann, & Bonn, 1997; Stefansson & Novotny, 1993). The CZE is shown to be a powerful separation technique which provides high-resolution, high efficiency, good selectivity, and short analysis time, and is becoming a standard tool for the analysis of many compounds (Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2003; El Rassi,

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1999; Morales, 2002; You et al., 2008). Although methods for the separation of the sugars in samples have been examined by numerous researchers, only 7–8 monosaccharides were usually baseline separated, which may significantly affect the analytical accuracy (Gao, Araiab, Lecka, & Emmerb, 2010; Rovio, Simolin, Koljonen, & Siren, 2008; Santos, Duarte, & Esteves, 2007). Therefore, potential CZE methods need to be further examined for the accurate identification of the 10 reductive monosaccharides possibly present in plant polysaccharides.

The aim of this study was therefore to develop a rapid and simple CZE analytical method for the simultaneous, efficient separation and determination of ten reducing monosaccharides (aldoses and uronic acids) possibly found in natural polysaccharides. This CZE method was successfully applied to the qualitative and quantitative analysis of the compositional monosaccharides of the polysaccharides derived from rare edible fungi *T. albuminosus* and *P. giganteus*.

2. Materials and methods

2.1. Materials and chemicals

The *T. albuminosus* and *P. giganteus* were purchased from local market of China, and identified according to the identification standard of Pharmacopeia of the People's Republic of China (2010 edition). Voucher specimens of the fungi mushroom materials were deposited at the School of Pharmacy, Fourth Military Medical University, China. The fungi mushrooms were thoroughly washed with water, air-dried and finely pulverized into a powder. D-Mannose, D-ribose, D-glucose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-xylose, D-galactose, L-arabinose and D-fucose were obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) and trifluoroacetic acid (TFA) were the products of Beijing Reagent Plant (Beijing, China). HPLC grade methanol was purchased from Honeywell (USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA). All of the other chemicals were of analytical-grade.

2.2. Extraction of the fungus polysaccharides

The fungi polysaccharides were isolated by hot-water extraction and ethanol precipitation as previously described (Tian, Zhao, Guo, & Yang, 2010). Briefly, the dried *T. albuminosus* and *P. giganteus* were defatted with 95% alcohol and then extracted three times with distilled water (w/v, 1:10) at 80 °C, 3 h each time. The combined extracts were pooled and concentrated under a reduced pressure and then centrifuged at 3000 × *g* for 15 min. The supernatant was collected and 95% alcohol was added slowly by stirring to precipitate the polysaccharide and adjusted to 75% alcohol, and then kept at 4 °C for 24 h. The polysaccharide pellets were obtained by centrifugation at 4000 × *g* for 15 min and repeatedly washed sequentially with ethanol, acetone and ether, respectively. The refined polysaccharide pellets were completely dissolved in appropriate volume of distilled water and intensively dialyzed for 3 days against distilled water (cut-off Mw 8000 Da), and then the retentate portion was deproteinized by a freeze-thaw process (FD-1, Henan Yuhua Instrument Co., China) repeated 10 times followed by filtration. Finally, the filtrate was lyophilized to yield brownish water-soluble polysaccharides.

2.3. Hydrolysis of the polysaccharides

Hydrolysis of the polysaccharides was performed according to our previous procedure (Lv et al., 2009). 18.6 mg of the analyte of *T. albuminosus* polysaccharides (TAPs) or 21.3 mg of *P. giganteus* polysaccharides (PGPs) was placed in 2 mL of 2 M TFA in an ampoule (10 mL). The ampoule was sealed under a nitrogen atmosphere and then kept in an oil bath at 110 °C for 8 h to hydrolyze the polysaccharide into component monosaccharides. The resultant mixture was cooled to room temperature, followed by a centrifugation at 1000 × *g* for 5 min. The supernatant was collected and dried to remove the excess TFA under a stream of nitrogen gas, and then 1.0 mL of distilled water was added for the further experiments.

2.4. PMP derivatization procedures for reducing monosaccharides

The PMP derivatization procedure was carried out as described previously (Yang, Zhao, Wang, Wang, & Mei, 2005). Briefly, 20 μL of standard monosaccharides (aqueous) or the monosaccharide hydrolysate of the polysaccharides was spiked with 400 μL of 0.3 M aqueous NaOH and 400 μL 0.5 M PMP-methanol solution. Galacturonic acid was added as an internal standard to each sample before the derivatization. After the solution was shaken for 10 s, the mixture was allowed to react for 30 min at 70 °C, and then cooled to room temperature and neutralized with 400 μL of 0.3 M HCl. The resulting solution was extracted with chloroform, and the organic phase was discarded, and the extraction process was repeated for 3 times. Finally, the aqueous layer was filtered through a 0.22 μm membrane for CZE analysis. The reagent solution was freshly prepared before derivatization.

2.5. Preparation of buffer and standard solution

The stock solution of 400 mM boric acid was prepared in water. The running buffers were prepared by diluting the stock solution to the appropriate concentrations and were adjusted to the desired pH values with sodium hydroxide (NaOH). The stock solution of standard monosaccharides (10 mM) were prepared and diluted with ultrapure water. Both of the buffer and sample solution were filtered through a 0.45 μm membrane filter and degassed by ultrasonic apparatus for 2 min before use. All the solutions prepared were stored in the dark at 4 °C until use.

2.6. Apparatus and electrophoretic procedures

Separation and analysis were performed on a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector and an automatic injector. Data acquisition and processing were carried out with Beckman System Gold software. All fused-silica capillary tubes have an internal diameter of 50 μm with effective length of 48.5 cm in total length of 58.5 cm. The capillary (from Yongnian Optical Fiber Factory, Hebei) was conditioned prior to the first use by sequentially rinsing with 1 M NaOH, 0.1 M HCl, and ultrapure water for 20 min. At the start of each sequence, the capillary was washed with water for 5 min, 0.1 M HCl for 5 min, 0.1 M NaOH for 10 min and water for 5 min. Between analyses, the capillary was rinsed with 0.1 M NaOH for 3 min followed by water for 2 min and then equilibrated with running buffer at a pressure of 20 psi for 3 min. Samples were introduced into the capillary by pressure injection mode for 5 s at 0.5 psi. At the end of the working day, the capillary was washed with water for 5 min and the capillary end dipped in a vial containing water. Absorbance was detected with a UV detector set at 245 nm.

3. Results and discussion

3.1. Optimization of CZE separation of PMP-monosaccharides

3.1.1. Effects of buffer pH on separation

It is well known that the CZE separation of carbohydrates with vicinal hydroxyl groups is best performed in alkaline borate buffer, where the analytes can be transformed into negatively charged

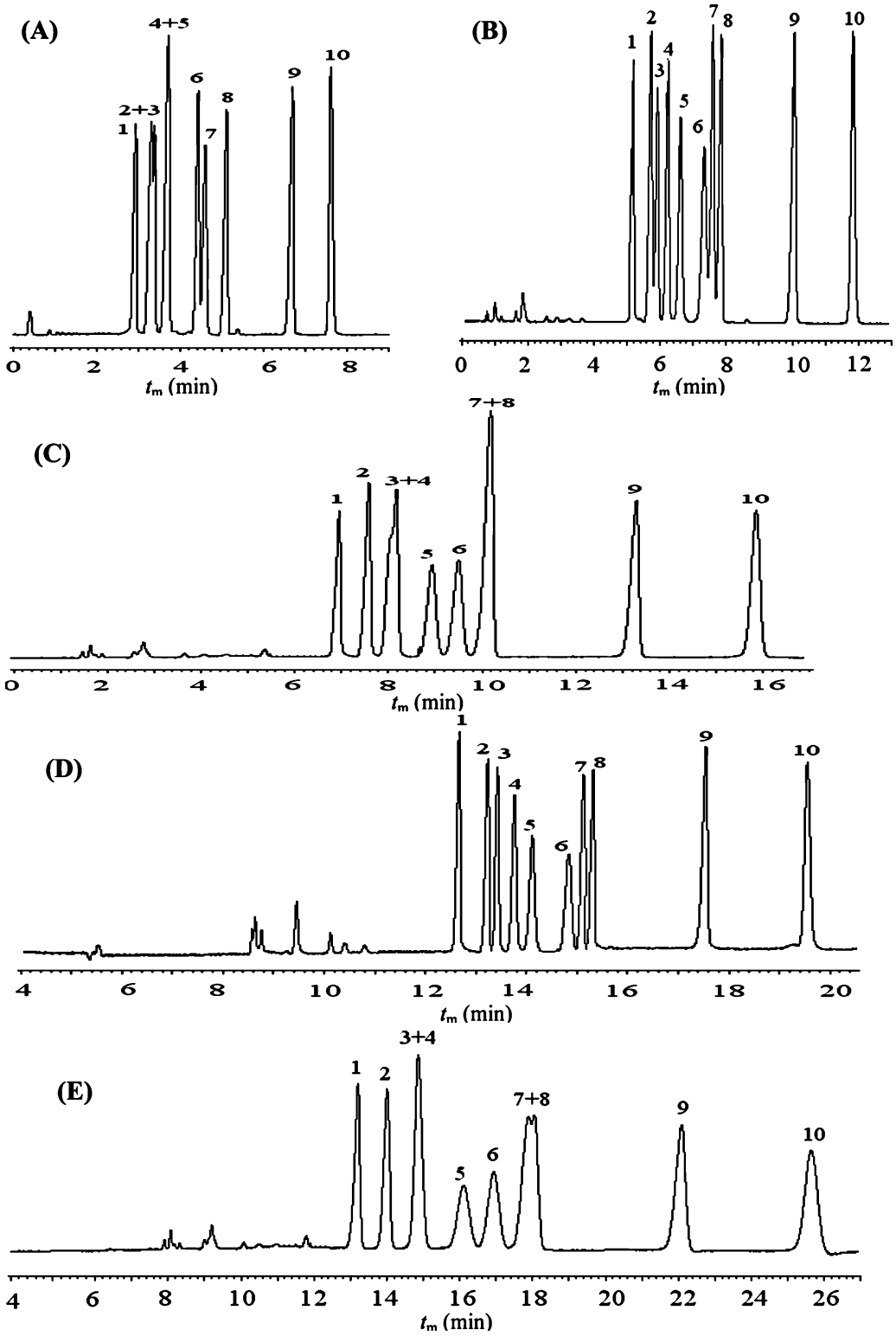

Fig. 1. Effects of pH values on the separation of ten PMP-labeled standard monosaccharides by CZE. The pH of borate buffer: (A) pH 9.5, (B) pH 10.0, (C) pH 10.5, (D) pH 11.0 and (E) pH 11.5. Analytical conditions: fused-capillary 58.5 cm (48.5 cm to the detector) × 50 μm i.d., 175 mM borate buffer, applied voltage 20 kV, capillary temperature 25 °C, and UV detection at 245 nm. Peaks: 1, xylose; 2, arabinose; 3, glucose; 4, ribose; 5, rhamnose; 6, fucose; 7, galactose; 8, mannose; 9, glucuronic acid; 10, galacturonic acid.

complexes with borate (Hoffstetter-Kuhn et al., 1991; Landers, Oda, & Schuchard, 1992). In CZE, the acidity of the buffer can also affect the migration time (t_m) and separation of analytes by changing the zeta potential (ζ) and the overall charge of the analytes (Yang, Zhao, Ruan, & Yang, 2008). In this regard, the effects of different pH values (9.5, 10.0, 10.5, 11.0, 11.5) of alkaline borate buffers on the separation of the tested PMP-labeled monosaccharides were investigated under a constant condition of 175 mM borate buffer, applied voltage 15 kV and capillary temperature 25 °C. As shown in Fig. 1, t_m of PMP-labeled derivatives of the 10 reductive monosaccharides

was prolonged and the resolution was increased with increasing pH values since each PMP-monosaccharide probably migrated in negatively charged form by complexation with borate and electroosmotic flow (EOF) was decreased, resulting in the gradual increase in t_m (Lin & Lin, 2004; Lin, Lin, Liao, & Liu, 2004). However, at pH 9.5, 10.0 and 10.5, all the tested monosaccharides could not reach baseline separation (Fig. 1A–C). Interestingly, baseline separation of the tested 10 PMP-monosaccharides was achieved within 20 min when the pH was increased to 11.0 (Fig. 1D), and the peaks for the PMP-monosaccharides were identified in the order of xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid and galacturonic acid. However, a further increase in the pH of up to 11.5 (Fig. 1E) led to a decrease of the resolution. At the same time, the baseline drift or a deteriorated peak shape was also noted and t_m significantly prolonged (27 min). As a result, pH 11.0 was chosen as the optimum acidity of the alkaline borate buffer.

3.1.2. Influence of the borate background electrolyte

The separation of the analytes which have complexation with background electrolyte generally requires a careful optimization on background electrolyte composition. In the present study, borate was not only used as a background electrolyte but also exhibited complexation with the tested monosaccharides, which influenced EOF, electrophoretic mobility, and the symmetry of the peaks in CZE (Hoffstetter-Kuhn et al., 1991; Honda, Suzuki, & Taga, 2003; Yang et al., 2008). Hence, the effects of borate concentration on separation behavior of a large number of various kinds of the PMP derivatives of the aldoses and uronic acids mentioned above were examined at the concentrations of 125, 150, 175 and 200 mM under constant conditions of pH 11.0, applied voltage 20 kV and capillary temperature 20 °C. It was found that the current was less than 95 μA with the voltage at 20 kV when the borate concentration was as high as 200 mM, indicating that high concentration of borate did not lead to Joule heating. As illustrated in Fig. 2, with the increase in concentrations of borate buffer, an enhanced separation of the tested monosaccharides tagged with PMP was obtained, and the t_m was also prolonged since the ionic strength of the running buffer increased with the increase in borate concentrations, resulting in the decrease of mobility of the solute in the capillary (Frazier, Inns, Dossi, Ames, & Nursten, 2000). As depicted in Fig. 2A–D, the PMP derivatives of arabinose and glucose (peak 2, 3) or ribose and rhamnose (peak 4, 5) could not separated at 125 mM borate buffer and only gave a single peak. When the borate concentration increased to 150 mM, the PMP derivatives of fucose and galactose (peak 6, 7) were not separated, and arabinose and glucose (peak 2, 3) or ribose and rhamnose (peak 4, 5) could not achieve a fully baseline separation, and the best separation was obtained at a borate concentration of 175 mM. However, the excessive borate concentration (200 mM) led to long t_m and irregular peak shapes. As a result, 175 mM borate buffer was chosen as the preliminary optimized buffer concentration for further investigation.

3.1.3. Selection of separation temperature and voltage

In our study, the temperature of capillary column was found to significantly affect the t_m possibly due to its effects on the stability of the monosaccharide complexes with borate, the viscosity of buffer, and the dissociation of silicon hydroxyls in CZE (Yang et al., 2008). The results indicated that the mobility of all the analytes expectedly increased as the temperature increased from 10 °C to 30 °C, and the t_m was shortened, with the best separation achieved at 25 °C. When temperature was higher than 25 °C, the baseline gradually worsened due to higher current. Therefore, the capillary temperature was maintained at 25 °C. Furthermore, effects of working voltage on separation of the PMP-labeled monosaccharides over a range of 5–25 kV were also studied under a constant condition of pH 11.0, 175 mM borate buffer, and capillary temperature 25 °C. The result showed that the t_m was shortened with an increase of the voltage. It was found that too low a voltage (5 kV) gave a tail peak with a long t_m. While the applied voltage was higher than 20 kV, the high currents reduced the separation efficiency. Considering the resolution and analysis time, the optimal voltage was set at 20 kV to accomplish a good compromise.

Table 1
Regression analysis and limit of detection (LOD) of PMP-labeled monosaccharides by the proposed CZE method.

Monosaccharides	t_m (min)[a]	Equation ($y = ax + b$)[b]	R^2	LOD (μM)[c]
Xylose	12.307	$y = 3.7539x + 0.0109$	0.9901	1.4
Arabinose	13.093	$y = 3.8409x + 0.0241$	0.9908	1.3
Glucose	13.196	$y = 2.8712x + 0.0317$	0.9900	1.5
Ribose	13.382	$y = 3.0998x + 0.0245$	0.9905	0.9
Rhamnose	14.066	$y = 2.8134x + 0.0120$	0.9922	1.2
Fucose	14.407	$y = 2.8967x + 0.0599$	0.9906	1.1
Galactose	15.063	$y = 3.6489x + 0.0060$	0.9912	1.7
Mannose	15.195	$y = 2.9005x + 0.0123$	0.9931	1.6
Glucuronic acid	17.202	$y = 4.0869x + 0.0221$	0.9909	1.8

[a] Migration time.
[b] The y and x are peak area ratio of the monosaccharides to internal standard (galacturonic acid) and concentration of the monosaccharides, respectively.
[c] The detection limits correspond to concentrations giving a signal-to-noise ratio of 3.

3.2. Validation of the CZE analysis

The rapid CZE method was validated in terms of linearity, limit of detection (LOD) and precision. Under the optimized conditions of 175 mM of borate buffer at pH 11.0, running voltage 20 kV and capillary temperature 25 °C, the linear calibration regression equations were established by the analysis of five points ranging from 5.0 to 400 μM of standard sugars (xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid and galacturonic acid). Each point of the calibration plot was repeated three times in an independent solution. The linear regression equations for all the PMP-labeled monosaccharides were listed in Table 1. As a consequence, the CZE assay had excellent linearity between Y (peak area ratio of the analytes with internal standard) and X (concentration of the standards) from 5.0 μM to 400 μM with the correlation coefficients (r) in the range of 0.9900–0.9931. Furthermore, LOD of each tested monosaccharide was obtained by injecting 10 μL of gradational dilutions of a mixed monosaccharide standard solution, and peak height with a signal-to-noise ratio (S/N) of 3 was used to evaluate the LOD. As shown in Table 1, the LOD of the monosaccharides was in the range from 0.9 to 1.8 μM, suggesting that the sensitivity of the method was satisfactory.

Moreover, the method precision was also determined by measuring the repeatability as reflected by coefficient of variation (CV, %). CV% was obtained by analyzing both intra-day variability and inter-day variability of the t_m and corrected peak areas (A/t) for each monosaccharide, which was calculated as the relative standard deviation by making five repetitive injections of a standard mixture solution (10 μM for each monosaccharide) under the same optimum conditions. As summarized in Table 2, the CV values in intra-day were less than 3.3% for the t_m, and 2.7% for the peak areas, and the inter-day CV values were less than 3.9% for the t_m and 3.8% for the peak areas, indicating that the precision of the method was satisfactory.

3.3. Analysis for monosaccharide composition of fungus polysaccharides

The purpose of the present method was to develop a rapid and accurate CZE analytical method for the quantification of the

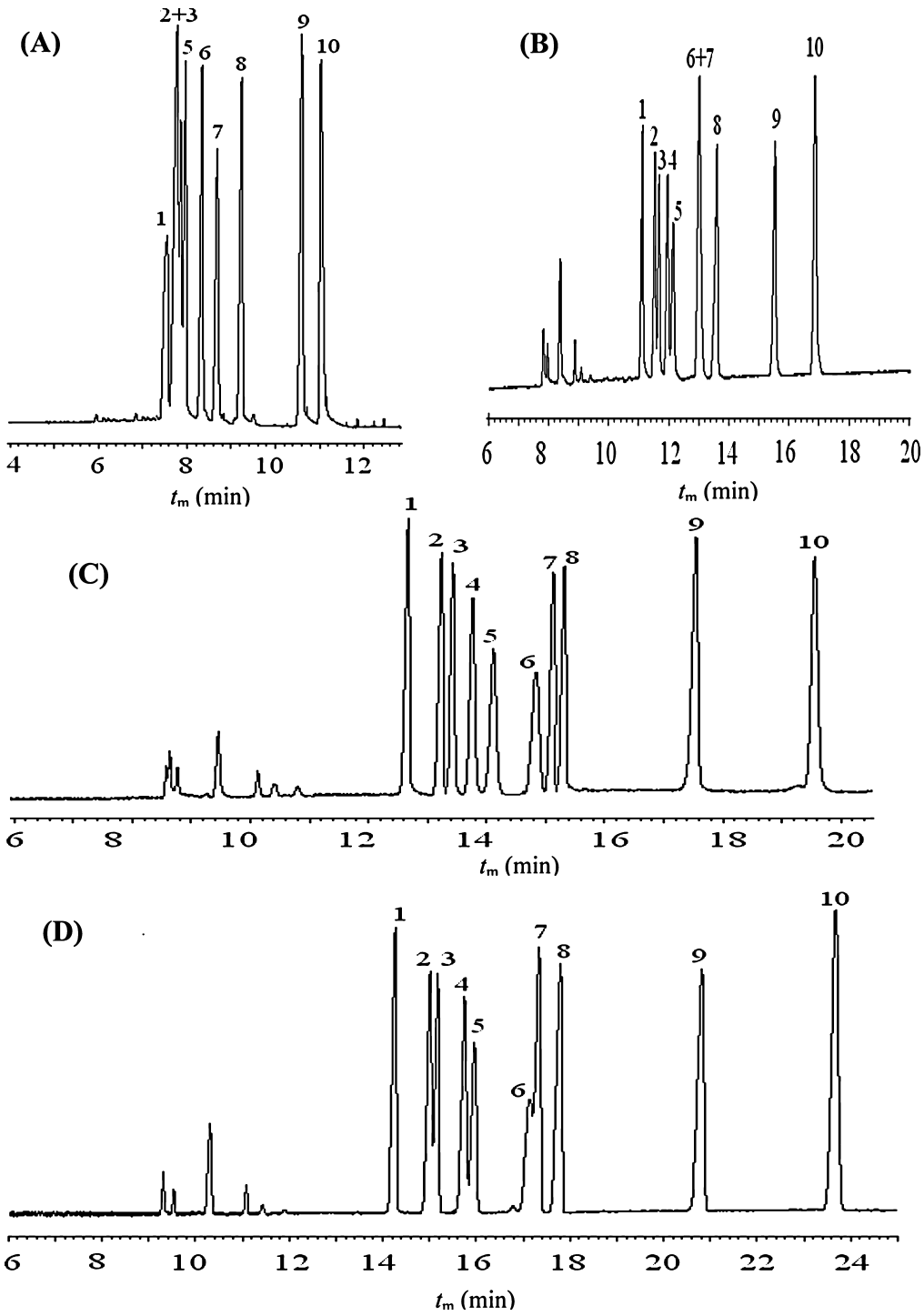

Fig. 2. Effects of borate concentrations at pH 11.0 on the separation of ten PMP-labeled standard monosaccharides by CZE. Borate concentration: (A) 125 mM, (B) 150 mM, (C) 175 mM and (D) 200 mM. CZE conditions and peak assignments were same as described in Fig. 1.

component monosaccharides in fungus polysaccharides. To demonstrate the applicability of the proposed CZE method, the two fungus polysaccharides isolated from *P. giganteus* and *T. albuminosus* were hydrolyzed with TFA, and the hydrolysate was neutralized and derivatized with PMP as described in the experimental section. The released monosaccharides tagged with PMP were analyzed by the optimized CZE method using galacturonic acid as internal standard. The typical electropherograms of the compositional monosaccharides released from TAPs and PGPs were shown in Fig. 3A and B, respectively, and the detected contents were listed in Table 3. As can be seen, ten PMP-labeled monosaccharides could be baseline separated, and the component monosaccharides were identified by comparing their t_m with those of the monosaccharide standards spiked in the samples (Fig. 3C). TAPs and PGPs were shown to be the typical heteropolysaccharides with significantly differentiable properties in monosaccharide composition. TAPs were composed of xylose, arabinose, glucose, galactose, mannose and glucuronic acid in the molar contents of 9.2, 12.8, 277.3, 21.2, 355.7 and 9.2 μM (Table 3), and their corresponding mole percentages were 1.4%, 1.9%, 41.7%, 3.2%,

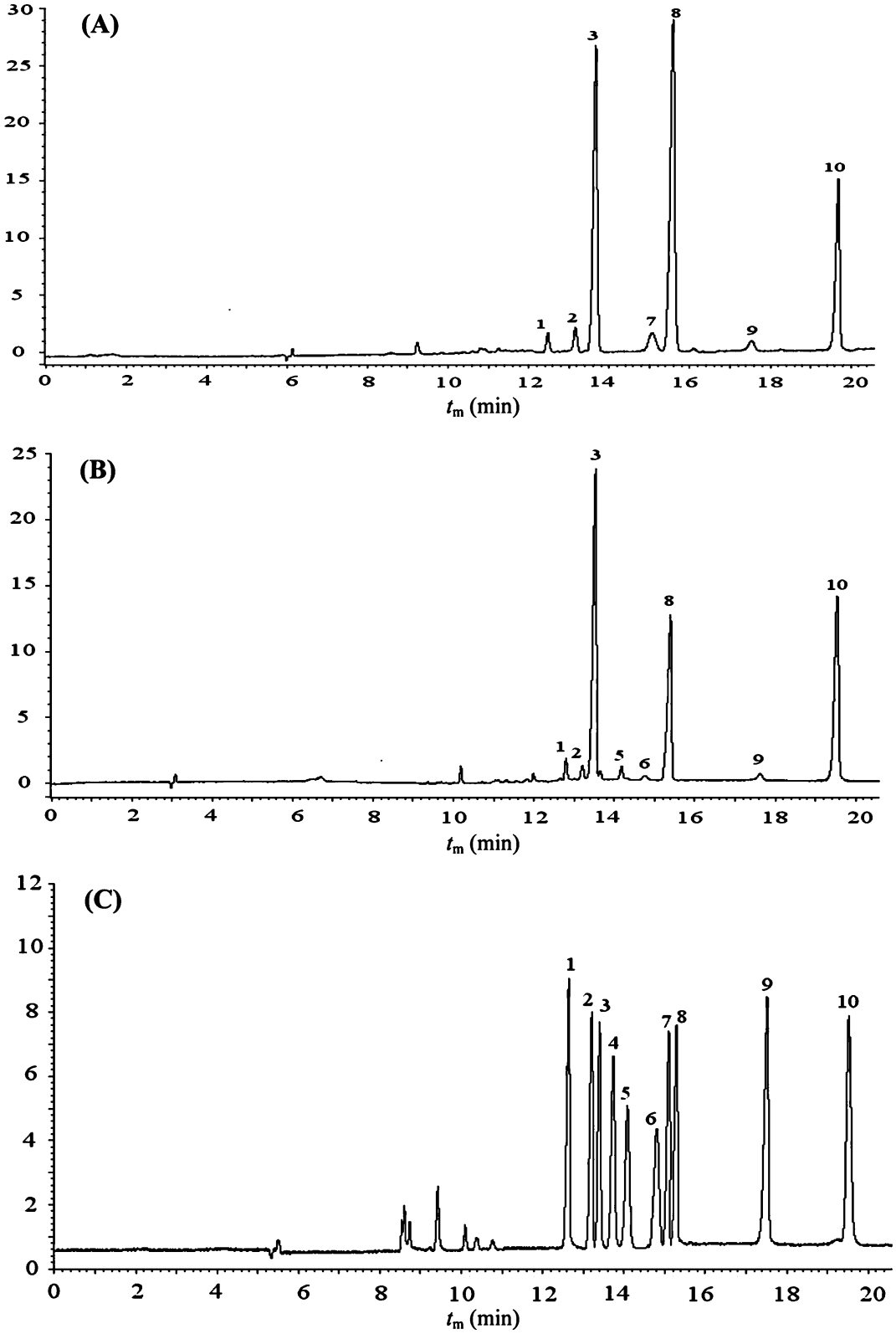

Fig. 3. Typical electropherograms of the compositional monosaccharides of *Termitomyces albuminosus* polysaccharides (TAPs, A), *Panus giganteus* polysaccharides (PGPs, B), and ten PMP-labeled standard monosaccharides (C). The polysaccharides were hydrolyzed with TFA at 110 °C for 8 h and then the hydrolyte was labeled with PMP. The CZE analysis was carried out as described in Section 2.

50.5% and 1.4% (mol.%), respectively (Fig. 3A). PGPs consisted of xylose, arabinose, glucose, rhamnose, fucose, mannose and glucuronic acid in the molar contents of 13.4, 30.0, 471.7, 10.3, 7.3, 23.7 and 18.7 μM (Table 3), and their corresponding mole percentages were 2.3%, 5.2%, 82.0%, 1.8%, 1.3%, 4.1% and 3.2%, respectively (Fig. 3B). It was clear that the predominant monosaccharides in TAPs were glucose and mannose up to 92.2% (mol.%) of all the quantitative monosaccharides, whereas 82.0% glucose

Table 2
Repeatabilities of the migration time (t_m) and corrected peak area (A/t) of the tested monosaccharides by the developed CZE method.

Monosaccharides	Intra-day precision (%, $n=5$)		Inter-day precision (%, $n=5$)	
	t_m	A/t	t_m	A/t
Xylose	1.0	1.4	2.4	1.9
Arabinose	1.7	1.9	1.9	2.4
Glucose	2.3	2.0	2.0	3.1
Ribose	3.1	2.7	2.3	3.8
Rhamnose	3.2	1.1	1.7	2.4
Fucose	2.1	2.2	2.0	3.2
Galactose	2.7	1.1	2.8	3.0
Mannose	1.9	2.1	2.9	2.7
Glucuronic acid	3.3	1.7	3.9	3.7

Table 3
Recovery and precision (CV%) of the monosaccharide analysis in fungus polysaccharides by spiking method ($n=3$).

Samples	Components	Content (μM)	Spiked (μM)	Found (μM)	Recovery (%)	CV (%)
TAPs	Xylose	9.2	10.0	18.9	97.0	2.9
	Arabinose	12.8	10.0	22.9	101.0	3.5
	Glucose	277.3	100.0	377	99.7	3.2
	Galactose	21.2	20.0	40.9	98.5	2.8
	Mannose	355.7	100.0	454.9	99.2	3.0
	Glucuronic acid	9.2	10.0	18.7	95.0	3.4
PGPs	Xylose	13.4	10.0	22.8	94.0	2.2
	Arabinose	30.0	20.0	48.8	94.0	2.7
	Glucose	471.7	100.0	570	98.3	3.4
	Rhamnose	10.3	10.0	19.6	93.0	3.1
	Fucose	7.3	5.0	11.9	92.0	2.8
	Mannose	23.7	20.0	43.3	98.0	1.8
	Glucuronic acid	18.7	20.0	38.3	98.0	3.1

was the main monosaccharide composition in PGPs. Moreover, recovery experiments were further performed in order to investigate the accuracy of the method by the addition of known amounts of each standard monosaccharide solution into the hydrolysate of fungus polysaccharides, and the spiked sample subjected to the entire CZE analytical sequence. Each sample was spiked close to the concentration occurring naturally, and recoveries were calculated based on the difference between the total amount determined in the spiked samples and the amount observed in the non-spiked samples. All analyses were carried out in triplicate. The results showed that the recoveries of all the detected monosaccharides ranged from 92.0% to 101.0%, and the CV values fell within 1.8–3.5%, respectively (Table 3). Taken together, it was confirmed that the developed CZE method was precise and applicable for the compositional monosaccharide analysis of the fungus polysaccharides derived from *P. giganteus* and *T. albuminosus*.

It is widely recognized that the separation and analysis of a large number of carbohydrates are a challenge for research. Gas chromatography (GC) has frequently been applied to carbohydrate determination, but this method has some drawbacks such as, a complicated derivative procedure, iso-peak occurrence, low resolution of aldose and its uronic acid (Yang et al., 2005). A HPLC method for separation of 10 monosaccharides labeled with PMP has been developed (Lv et al., 2009). The present study demonstrates an alternative approach to rapid, high performance separation of 10 monosaccharides (aldoses and uronic acids) via direct addition of borate to CZE buffer in less than 20 min. The new CZE approach offers a sharp reduction in analysis time, compared to current practice (He et al., 2003; Nuchtavorn & Suntornsuk, 2012; Wang et al., 2012), with no significant compromise in chromatographic resolution. This proposed CZE method could also analyze monosaccharide composition of fungus polysaccharides. It is widely recognized that fungus polysaccharides are generally heteropolysaccharides and have been shown to possess wide biological activities (Djordjevic et al., 2009; Feng, Li, Wu, He, & Ma, 2010; VanCott et al., 1996). The present results suggest that the described CZE method may be able to discriminate and predict fungus polysaccharides from *P. giganteus* and *T. albuminosus* by using monosaccharide profile data. The ability to discriminate fungal polysaccharides may be useful to help determine the origins of these potentially bioactive compounds.

4. Conclusion

The present study is specifically concerned with the simultaneous separation of 10 reducing monosaccharides as aldoses and uronic acids found in natural polysaccharides using CZE with pre-column PMP derivatization and UV detection at 245 nm. Hence, a fast and sensitive CZE method has been successfully optimized, and the newly established method showed good linearity, precision and an adequately low limit of detection. The method has been successfully applied to the qualitative and quantitative determination of monosaccharide components of polysaccharides from two commercial fungus species (*P. giganteus* and *T. albuminosus*). This proposed CZE method offered shorter analysis time and allowed the detection of higher numbers of monosaccharide constituents in natural polysaccharide matrices, and provides an accurate and economic alternative for monosaccharide analysis with a number of potential applications.

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