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Analysis of compositional carbohydrates in polysaccharides and foods by capillary zone electrophoresis

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ABSTRACT

A simple, sensitive and specific analytical method has been established for high efficient separation and simultaneously high sensitive determination of thirteen reducing carbohydrates, including aldohexose and aldopentoses as well as maltose and lactose. Reducing carbohydrates were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP), separated by capillary zone electrophoresis (CZE) with use of methanol modifier in 175 mM borate buffer (pH 11.0), and detected by UV at 245 nm. The optimized CZE method was found to be well suited to examine the compositional reducing monosaccharides of the isolated polysaccharides from *Lycopus lucidus* and Jujube, and free mono- and disaccharides in beer and milk. Quantitative recoveries of the compositional carbohydrates in the samples were in the range of 93.2–104.0%, and RSD values ranged from 2.9% to 4.9%. The developed CZE method proves to be precise and practical for quality control of reducing carbohydrates, and will provide more highly efficient separation in food analysis in the future.

1. Introduction

Carbohydrates are the major source of energy in the human diet with a consumption ranging from 40 to 80% of total energy requirements. In addition to the provision of energy, it is becoming clear that carbohydrates play a essential role in the process of life, and are found to have a wide range of effects on human physiology including effects on satiety and gastric emptying, control of blood glucose, insulin metabolism, and serum cholesterol and influencing colonic microflora and gastrointestinal processes such as laxation and fermentation (Muir et al., 2009). Carbohydrates as the major classes of importance to human nutrition are a diverse and complex family of compounds, including monosaccharides, oligosaccharides and the polysaccharides (starch and nonstarch polysaccharides) (Molnár-Perl, 2000; Muir et al., 2009). Clearly, a greater understanding of the physiological effects of carbohydrates will only be possible with the separation, identification, and quantification of the different classes of carbohydrates present in foods.

Monosaccharides are the most basic units of biologically important carbohydrates, which mainly include the reducing hexoses (glucose, glucosamine, galactose, mannose, etc.) and pentoses (ribose, arabinose, etc.). In this regards, a understanding of free or conjunct monosaccharides frequently occur in foodstaffs or natural oligo- or polysaccharide preparations can serve as incentive for the greater consumption of functional mono-, oligo- and polysaccharides with beneficial effects on health (Lachman, Havrland, Fernández, & Dudjak, 2004; Parr & Bolwell, 2000; Wang et al., 2002). At the same time, lactose and maltose as common reducing disaccharide sweeteners are widely applied to many foods (Paulus & Klockow, 1996). Therefore, the monitoring of reducing monosaccharides and oligosaccharides which frequently occur in food samples is very important for in the fields of nutrition, biology and food science.

The separation of complex mono- and oligosaccharide mixtures is a challenging analytical task for numerous reasons. At first, the analytes show differences only in the configuration of the hydroxyl groups and possess therefore very similar separation properties (Frügel, Carle, & Schieber, 2005; Yang, Zhao, Wang, Wang, & Mei, 2005). Furthermore, they generally lack UV-absorbing or fluorescent functions as well as readily ionizable charged groups. For these reasons, many different analytical methods (Currie & Perry, 2006; Rumpel & Dignac, 2006; Sanz, Martínez-Castro, & Moreno-Arribas, 2008; Tetsuo, Zhang, Matsumoto, & Matsumoto, 1999; Yang & Ding, 2000), especially those based on high-performance liquid chromatographic (HPLC) techniques using various different detectors, have been assayed (Blanco, Muro, & Gutierrez, 2004; Lopes & Gaspar, 2008; Nogueira, Silva, Ferreira, & Trugo, 2005; Panagiotopoulos, Sempere, Lafont, & Kerherve, 2001). Although HPLC coupled with refractive index detector (RID) has been accepted as one of the major techniques for the direct analysis

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of carbohydrates, it has many disadvantages, such as lack of sensitivity and incompatibility with gradient elution (Yang & Ding, 2000). To overcome the high detection limit of carbohydrates, most of the methods described in the literatures rely on some sort of derivatization technique since the introduction of a chromophoric moiety can substantially improve the sensitivity, and a suitable tagging of reducing saccharides usually results in enhanced resolution and less matrix disturbances (Honda, Isawe, Makino, & Fujiwara, 1989). The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP) with strong UV absorbance at 245 nm is one of the popular labels for the HPLC method that can react with reducing carbohydrates under mild conditions, requiring no acids catalyst and causing no desialylation and isomerization (Fu & O'Neill, 1995; Kakehi, Ueda, Suzuki, & Honda, 1993; Yang, Zhao, & Lv, 2007).

As an alternative to HPLC, capillary zone electrophoresis (CZE) is shown to be a powerful separation technique which provides high-resolution results and is becoming a standard tool for the analysis of many compounds (Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2003; Morales, 2002; You et al., 2008). Recently, we have also described a CZE method for separation of 8 monosaccharides, where the introduction of a chromophic PMP into the reducing monosaccharide molecule can be easily achieved by chelation of the monosaccharides with a suitable ion of borate (Yang, Zhao, Zhou, et al., 2007; Yang, Zhao, Yang, & Ruan, 2008). Although methods for the separation of the sugars in samples have been examined by a great quantity of researchers, only 7–8 monosaccharides were usually baseline separated, which significantly affected the analytical accuracy (Gao, Araiab, Lecka, & Emmerb, 2010; Ijiri et al., 2010; Rovio, Simolin, Koljonen, & Siren, 2008; Santos, Duarte, & Esteves, 2007; Yang, Zhao, Zhou, et al., 2007). Therefore, the potential CZE method needs to be further challenged for the accurate identification of complex mono- and oligosaccharide ingredients.

The aim in this work was to present an optimized, powerful and robust CZE analytical technique capable of simultaneously separating thirteen saccharides, including a set of stereoisomers of aldopentose and aldohexose as well as reducing lactose and maltose which frequently occur in natural plants or food industry. Furthermore, the developed CZE method has been successfully applied to the analysis of the compositional monosaccharides released from the polysaccharides from herbal *Lycopus lucidus* Turcz. and Jujube as well as the analysis of free monosaccharides and disaccharides in food such as beer and milk.

2. Materials and methods

2.1. Materials and chemicals

The aerial parts (leaves and stems) of *L. lucidus* Turcz., and Chinese jujube (*Zizyphus jujuba*) were harvested from the Northern countryside region of Shaanxi province, China. The samples were thoroughly washed with water, air-dried and finely pulverized into a powder. The milk and beer samples were purchased at local markets. D-Mannose, D-ribose, D-glucose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, glucosamine, D-xylose, D-galactose, L-arabinose, D-fucose, maltose, and lactose 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), and all of the other chemicals were of analytical grade.

2.2. Extraction of the plant polysaccharides

The plant polysaccharide extracts were isolated by hot-water extraction and ethanol precipitation as previously described (Tian, Zhao, Guo, & Yang, 2010). In brief, the dried powder of *L. lucidus* and Chinese jujube (150 g) were defatted with 95% alcohol and then extracted with distilled water (w/v, 1:10) at 80 °C for three times, 1 h each time. After extraction, the combined extracts were pooled, concentrated to 30% of the original volume under a reduced pressure and then centrifuged at 3000 rpm for 15 min. The supernatant was collected and 3 volume of 95% alcohol was added slowly by stirring to precipitate the polysaccharide, and then kept at 4 °C overnight and finally the polysaccharide pellets were obtained by centrifugation at 4000 rpm for 15 min and repeatedly washed sequentially with possibly less amounts of 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 M_w 8000 Da), and then the retentate portion was deproteinized by a freeze–thaw process (FD-1, Henan Yuhua Instrument Co., China) for repeating 10 times followed by filtration. Finally, the filtrate was lyophilized to yield brownish water-soluble polysaccharides.

2.3. Hydrolysis of the polysaccharides

22.4 mg of the *L. lucidus* polysaccharide or 19.8 mg of Jujube polysaccharide sample 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. The resulting reaction solution was cooled to room temperature, and was further centrifuged at 1000 rpm for 5 min. The supernatant was collected and dried to remove the excess TFA under a stream of nitrogen gas. The sample solution was added with 1.0 mL distilled water and then ready for the further experiments.

2.4. 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. The stock solution of standard monosaccharides and disaccharides (10 mM) were prepared and diluted with ultrapure water. The sample solutions were filtered through a 0.22 μm syringe filter and were degassed using an ultrasonic bath for 2 min prior to use. All the solutions prepared were stored in the dark at 4 °C until being used.

2.5. PMP derivatization procedures for reducing monosaccharides and disaccharides

The PMP derivatization procedure was carried out as our described previously (Lv et al., 2009; Zhang, Xu, Zhang, Zhang, & Zhang, 2003). Briefly, 20 μL aqueous of standard monosaccharides or disaccharides or the hydrolyzed samples of the polysaccharides was spiked with 400 μL of 0.3 M aqueous NaOH and 400 μL 0.5 M PMP–methanol solution. It was showed no glucosamine existed in all the tested samples, and thus glucosamine as an internal standard was added to each sample before the derivatization. After the solution was shaken for 10 second, the mixture was allowed to react for 30 min at 70 °C, 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 after shaking, 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, and for the beer and milk analysis, the

samples were degassed before being derivatized and diluted by 1:20 with water and passed through a 0.22 μm membrane filter prior to injection.

2.6. Apparatus and electrophoretic procedures

Separation and analysis were carried out with a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA). Detection was done with direct UV monitoring. Data acquisition and processing were carried out with Beckman System Gold software. All capillaries (fused silica) had an internal diameter of 50 μm and were 58.5 cm in total length (effective length 48.5 cm). The new capillaries (from Yongnian Optical Fiber Factory, Hebei), were conditioned by rinsing with 1 M sodium hydroxide for 20 min, 0.1 M hydrochloric acid (20 min), and deionized water (30 min). At the start of each working day, 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 capillaries were 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 by pressure injection mode at 0.5 psi for 5 s into the capillary. At the end of the working day, the capillary was washed with water for 5 min and dipping the capillary end in a vial containing water. Absorbance was detected with a photodiode array detector set at 245 nm.

3. Results and discussion

3.1. CZE separation profile of the PMP-labeled reducing carbohydrates

For quantification of reducing saccharides by selective, sensitive, and robust CZE methods, pre-column derivatization is among the most popular approaches. In the present case, reductive 1-phenyl-3-methyl-5-pyrazolone (PMP) as the strong UV-active tag was chosen and an excess of PMP label was used to guarantee optimal conversion into the labeled saccharide derivatives, which works in a simplified aqueous methanol system, and affords stable PMP derivative as our previously described (Yang et al., 2005). In our study, the CZE separation of the standard PMP-carbohydrate derivatives was performed in alkaline borate buffers, where analytes can be transformed into negatively charged complexes with borate (Grőßl, Harrison, Kamla, & Kenndler, 2005; Wang & Fang, 2004; Zhao et al., 2007), and detection was carried out at 245 nm and identified by spiking samples with standards.

To guarantee the ionization of the analytes, we made a detailed study of the pH of the running borate buffer over a range between pH 9 and 11.2 under a constant condition of 175 mM borate buffer, applied voltage 15 kV and capillary temperature 25 °C, and the effects are shown in Fig. 1. It was found that with the buffer pH increased, the migration time (t_m) of the tested nine aldohexose and two aldopentoses as well as maltose and lactose tagged with PMP were prolonged and the resolution was obviously increased initially, and when the pH was higher than 11.0, the resolution decreased and a further deteriorated peak shape was observed (Fig. 1F). As illustrated in Fig. 1A–D, at the pH 9.0, 9.5, 10.0 and 10.5, all the tested thirteen reducing monosaccharides and disaccharides can not reach baseline separation. As the pH increased to 11.0, the resolutions of all the analytes were obviously improved, and it can be identified thirteen significant peaks in spite of ribose and glucose, fucose, mannose and galactose can not still reach completely baseline separation. It seems to show that the order of the peaks remained changeless, and the elution of the PMP-labeled saccharides was in a order of glucosamine, maltose, lactose, xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid and galacturonic acid (Fig. 1E). As a result, pH 11.0 was chosen as the preliminary optimized pH value for further investigated.

Borate concentration is another important parameter in our separation procedure, and therefore, it must also be optimized. Herein, the borate was not only used as a background electrolyte but also exerted complexation with the tested carbohydrates, which can influence both electro-osmotic flow and electrophoretic mobility, and may also affect the symmetry of the peaks in CZE (Zhao, Yang, Sun, Jiang, & Zhang, 2006). As expected, different borate concentrations (125, 150, 175, 200 and 250 mM) at pH 11.0 were shown to have a greater impact on the resolution and t_m of the reducing carbohydrates. As shown in Fig. 2A–E, the best resolution versus the lowest t_m was obtained at 175 mM borate buffer at pH 11.0. It was also noted that when the excessive concentrations of borate high than 200 mM, it was troublesome to obtain a stable baseline, and the electric field in the capillary may become distorted because of Joule heating (Cross & Cao, 1998), leading to an even worse separation and irregular peak shapes, and the reproducibility of the separation was poor (Frazier, Inns, Dossi, Ames, & Nursten, 2000). As a result, 175 mM borate buffer at pH 11.0 was chose to accomplish a good compromise.

In this study, it was difficult to achieve a fully simultaneous CZE baseline separation for a large number of the PMP-labeled nine aldohexose and two aldopentoses as well as maltose and lactose only via optimizing borate buffer concentrations and pH values as described above. Therefore the separation condition needed to be further optimized to achieve a robust CZE approach for improving the accuracy for sugar composition analysis. It is well known that the addition of an organic modifier can either improve or deteriorate the CZE separation (Egeberg & Bergli, 2002; Koike, Kitagawa, & Otsuka, 2007). Interestingly, a steady improvement in the resolution of the tested reducing carbohydrates was observed with increased proportions of methanol in the increasing range of 0.5–4% (v/v), where the mobility of the PMP–carbohydrate derivatives increased concomitantly (Fig. 3A–E). The best separation was achieved at 4% methanol under the condition of 175 mM borate buffer at pH 11.0, and thirteen PMP-labeled carbohydrate derivatives were baseline separated as respect. Fig. 3E shows a simultaneous separation of the tested thirteen standard carbohydrates as PMP derivatives, and the peaks were identified in the order of glucosamine, maltose, lactose, xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid, and galacturonic acid. Taking all effects into account, pH 11.0, 175 mM borate buffer containing 4% methanol at 25 °C and 15 kV are generally recommended as the optimum condition.

3.2. Validation of the CZE analysis

The CZE method was validated by determining linearity, sensitivity and repeatability. The linearity was verified by the analysis of six points in the range of 3.0–137.0 μM of standard sugars (maltose, lactose, xylose, arabinose, glucose, ribose, rhamnose, galactose, mannose, fucose, glucuronic acid and galacturonic acid), each point of the calibration plot was repeated three times in an independent solution, and the linear regression parameters of the calibration curves are shown 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 3 μM to 137 μM with the correlation coefficients (r) in the range of 0.9902–0.9985. Furthermore, limit of detection (LOD) of each tested analytes at the signal-noise ratio (S/N) of 3 were determined to be ranged from 1.3 to 1.9 μmol by successive dilutions of PMP-labeled

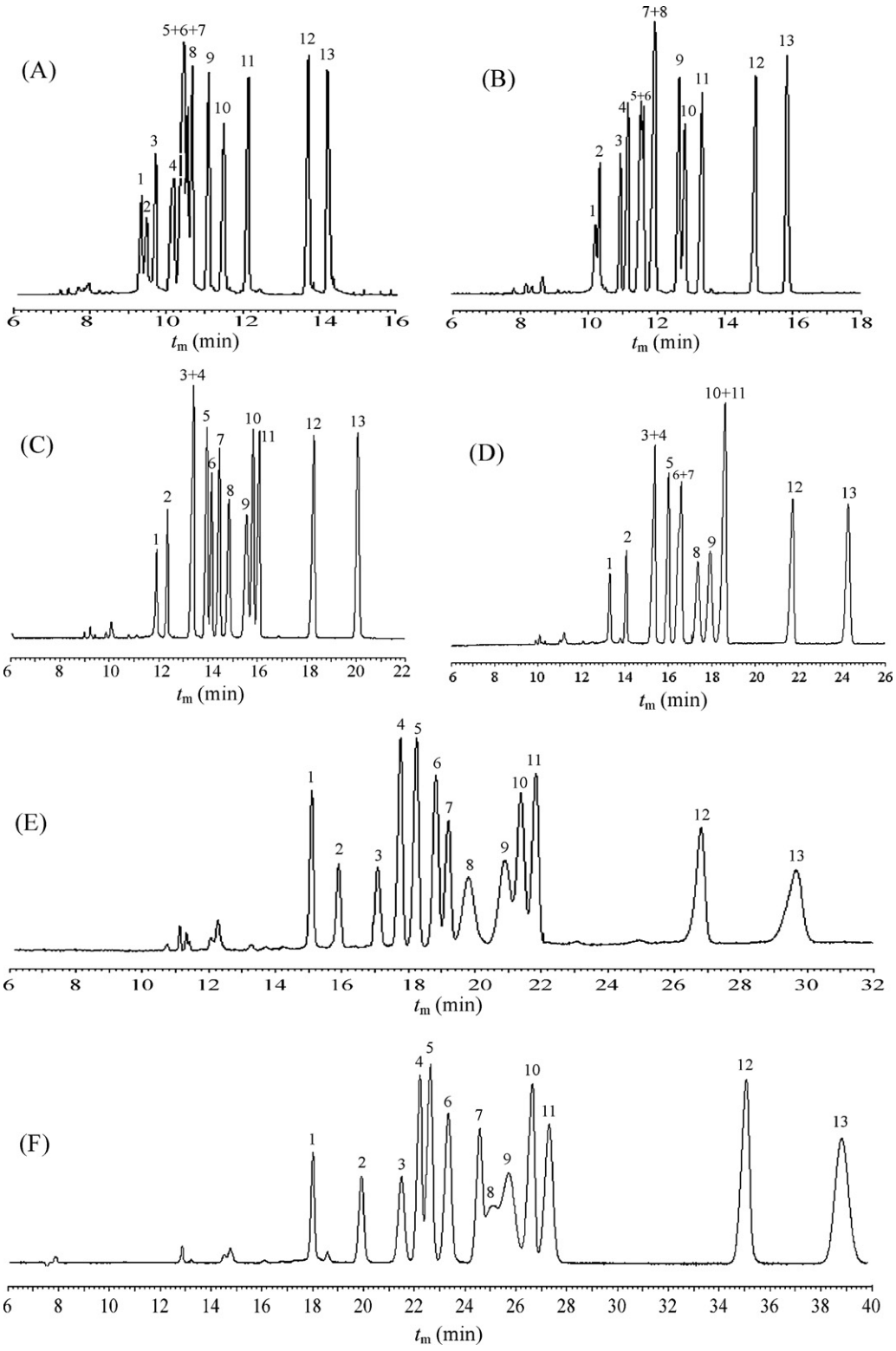

Fig. 1. Effects of pH values on the separation of thirteen PMP-labeled standard reducing carbohydrates by CZE. Analytical conditions: fused-capillary 58.5 cm (48.5 cm to the detector) × 50 μm i.d., 175 mM borate buffer, applied voltage 15 kV, capillary temperature 25 °C, UV detection at 245 nm. The pH of borate buffer: (A) pH 9.0, (B) pH 9.5, (C) pH 10.0, (D) pH 10.5, (E) pH 11.0, and (F) pH 11.2. Peaks: 1. glucosamine, 2. maltose, 3. lactose, 4. xylose, 5. arabinose, 6. glucose, 7. ribose, 8. rhamnose, 9. fucose, 10. galactose, 11. mannose, 12. glucuronic acid, and 13. galacturonic acid.

carbohydrate mixture (Table 1), indicating that the sensitivity of the method was satisfactory.

As shown in Table 2, the method repeatability was also determined by estimating the method precision expressed as coefficient of variation (CV%). CV% was obtained by the analysis of both intra-day variability and inter-day variability of t_m and corrected peak areas (A/t), which was estimated by making five repetitive injections of a standard mixture solution (10 μM for each analyte) under the same CZE conditions. The results showed that the CV values in intra-day were less than 3.7% for t_m and 4.1% for A/t, and the

Table 1
Migration time (t_m), regression analysis and limit of detection (LOD) of the proposed CZE method.

Carbohydrates	t_m (min)[a]	Equation, $Y = aX + b$[b]		Correlation coefficient	LOD (μM)[c]
		a	*b*		
Maltose	18.856	118.64	−0.0175	0.9948	1.5
Lactose	22.387	121.31	−0.0959	0.9985	1.5
Xylose	23.207	177.65	−0.0285	0.9902	1.5
Arabinose	24.617	227.01	−0.0639	0.9908	1.4
Glucose	26.104	181.29	−0.0952	0.9915	1.3
Ribose	27.790	211.42	−0.0531	0.9936	1.3
Rhamnose	29.725	173.73	−0.0801	0.9965	1.6
Fucose	31.304	190.50	−0.0202	0.9943	1.6
Galactose	33.108	244.06	−0.1542	0.9918	1.3
Mannose	34.687	251.29	−0.0885	0.9941	1.3
Glucuronic acid	49.007	218.89	−0.1304	0.9913	1.9
Galacturonic acid	63.899	270.28	−0.2463	0.9974	1.9

[a] t_m: migration time.
[b] The *Y* and *X* are peak area ratio of the analytes to internal standard (glucosamine) and concentration of the analytes (3–137 μM), respectively.
[c] LOD correspond to concentrations giving a signal-to-noise ratio of 3.

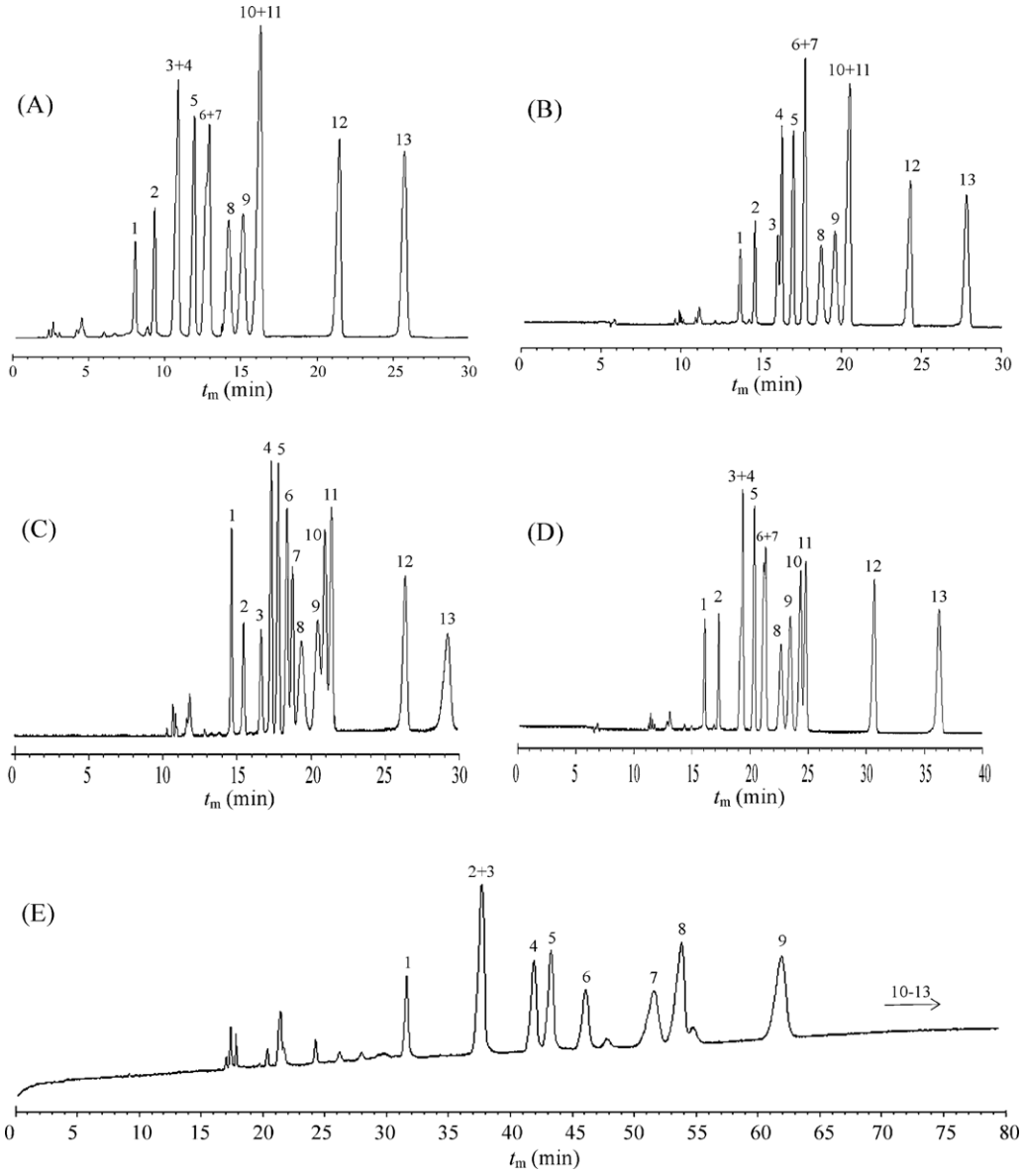

Fig. 2. Effects of borate concentrations at pH 11.0 on the separation of thirteen PMP–carbohydrates. Borate concentration: (A) 125 mM, (B) 150 mM, (C) 175 mM, (D) 200 mM, and (E) 250 mM. CZE conditions and peak assignments as described in Fig. 1.

Table 2
Precision of the migration time (t_m) and corrected peak area (A) of the tested carbohydrates by the developed CZE method.

Carbohydrates	Intra-day precision (CV%, $n = 5$)		Inter-day precision (CV%, $n = 5$)	
	t_m	A	t_m	A
Maltose	1.0	1.0	1.5	1.3
Lactose	1.0	1.2	1.3	2.4
Xylose	2.0	1.8	1.8	3.2
Arabinose	1.9	1.4	1.7	2.8
Glucose	3.3	4.1	2.8	4.4
Ribose	3.5	3.7	3.0	4.2
Rhamnose	2.2	1.4	2.0	2.3
Fuccose	2.0	2.7	2.1	3.2
Galactose	2.6	1.5	2.4	3.6
Mannose	2.4	2.9	3.1	5.1
Glucuronic acid	3.7	2.8	4.0	4.6
Galacturonic acid	3.1	2.3	3.5	5.4

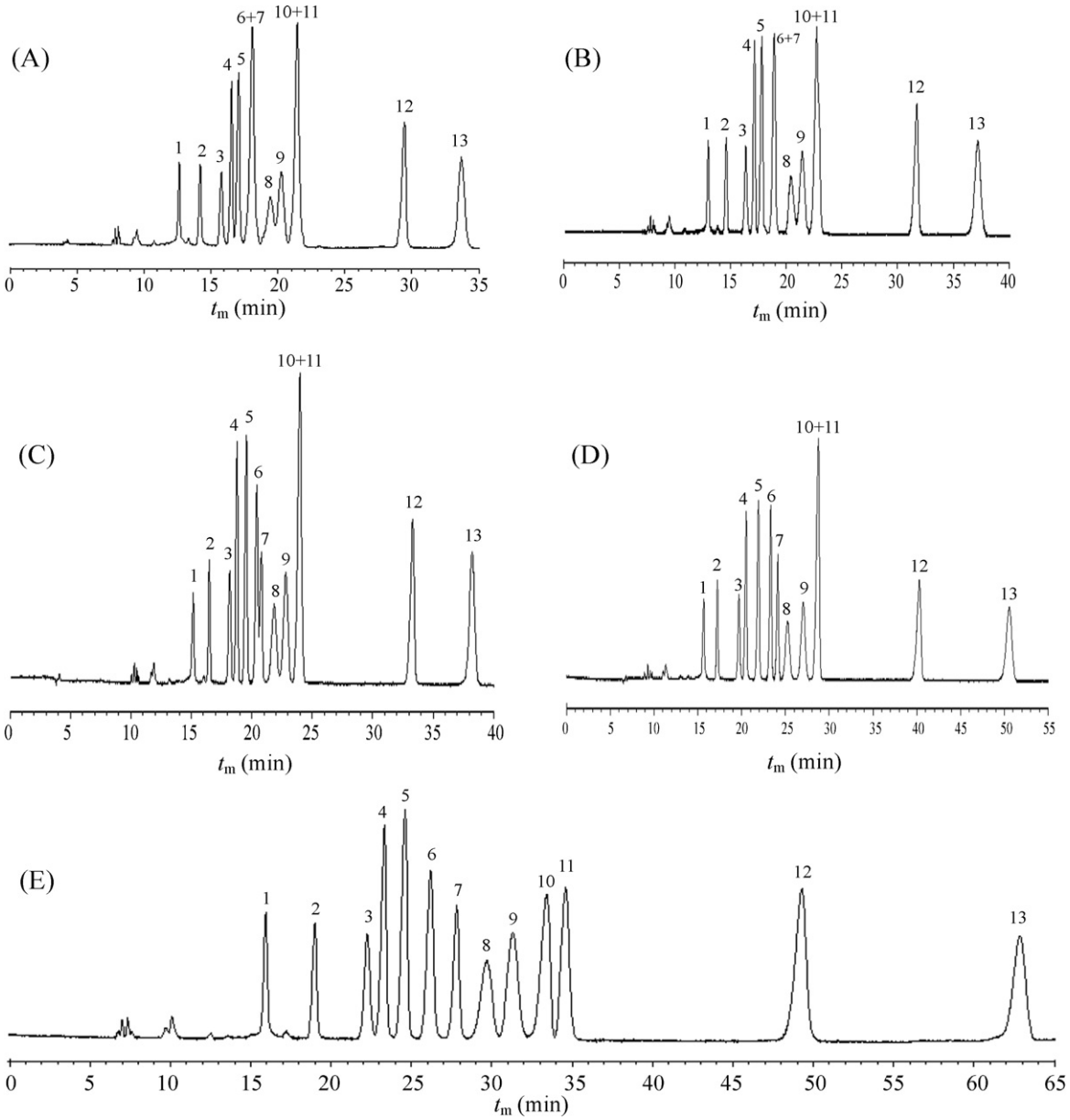

Fig. 3. Influence of methanol modifier on the separation of thirteen PMP-labeled standard carbohydrates. Methanol concentrations in borate buffer: (A) 0.5%, (B) 1%, (C) 2%, (D) 3%, and (E) 4%. CZE conditions and peak assignment as mentioned in Fig. 1.

inter-day CV values were less than 5.4% for all variables, respectively. The validated results indicated that this method was accurate and reliable.

3.3. Application to real polysaccharide samples derived from L. lucidus and Jujube

The present study was designed to develop a repeatable and accurate CZE analytical method for the quantification of most component reducing monosaccharides and disaccharides. To evaluate the applicability of the proposed CZE method, the two isolated polysaccharides from *L. lucidus* (LPs) and Jujube (JPs) were hydrolyzed with TFA, dried and PMP-labeled as described in Section 2 and finally the released monosaccharide as PMP derivatives were analyzed by the described CZE method under the optimized conditions using glucosamine as internal standard. The typical electropherograms of the hydrolyzed monosaccharides from LPs and JPs are shown in Fig. 4B and C, respectively. Peaks were identified by comparing their t_m with those of the standards spiked in the samples (Fig. 4A). As can be seen in Fig. 4B and Table 3, LPs were composed of xylose, arabinose, glucose, ribose, rhamnose, galactose, mannose, glucuronic acid, and galacturonic acid in the molar contents of 31.5, 27.5, 22.5, 17.5, 6.0, 71.5, 13.5, 5.5 and 25.0 μM, respectively, and their corresponding mole percentages were 14.3%, 12.5%, 10.2%, 7.9%, 2.7%, 32.4%, 6.1%, 2.5% and 11.3%, respectively. JPs consisted of arabinose, glucose, ribose, rhamnose, and galactose in the molar contents of 8.4, 103.2, 44.1, 5.7, and 19.8 μM, and their corresponding mole percentages were 4.6%, 57.1%, 24.3%, 3.1% and 10.9%, respectively (Fig. 4C and Table 3). The results showed that LPs and JPs were the typically acidic and neutral heteropolysaccharide with significantly differentiable properties in monosaccharide composition, respectively.

3.4. Analysis of reducing mono- and oligosaccharides in beer and milk

Fig. 4D shows a typical electropherogram of the compositional reducing monosaccharides and disaccharides tagged with PMP derived from beer sample. Maltose and glucose were found in beer in the molar content of 16.3 and 6.6 μM, respectively, indicating that the predominantly composition reducing carbohydrates in beer was maltose. As can be seen in Fig. 4E, milk included maltose, lactose and glucose in the molar content of 56.6, 21.2 and 6.1 μM.

Furthermore, recovery experiments were performed in order to investigate the accuracy of the CZE method. Known amounts of each standard monosaccharides or disaccharides with the samples were added to the hydrolyzed polysaccharide samples or food samples, and the resulting spiked sample was subjected to the entire analytical sequence. Each solute was spiked at a close concentration with the sample 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 show that the recoveries of all the nine monosaccharides in the polysaccharide samples ranged between 93.2 and 104.0% and the RSD values fell within 3.0–4.9% (Table 3). In the analysis of beer and milk, the recovery was between 94.0% and 102.5% (Table 3), and the RSD values fell within 2.9–3.9%. These results also showed that the proposed CZE method was not only suitable for the determination of the component reducing monosaccharides in polysaccharides, but also practical for the analysis of the reducing mono- and oligosaccharides in beer and milk.

The major aim of this study was to develop an analytical technique, based on CZE with PMP-tagged UV detection, which could be used to quantify the major reducing carbohydrates frequently occur in foods. Although there is some published information in this area (Honda, Suzuki, & Taga, 2003; Sjőberg, Adorjan, Rosenau, & Kosma, 2004), no previous studies have attempted to quantify all of these carbohydrates. To our knowledge, this is the first report showing a CZE separation method for the thirteen aldohexose, aldopentoses, maltose and lactose. It is well known that the separation and identification of a large number of carbohydrates is a complex and challenging area of research. One of the major problems is the risk of coelution of the different sugars. Here, a combined optimization techniques of borate buffer with methanol modifier provided a good separation of most reducing monosaccharides and disaccharides of interest in foods. The developed CZE method has been tested with quality control samples of polysaccharides, beer and milk samples and has been found to provide accurate and precise results.

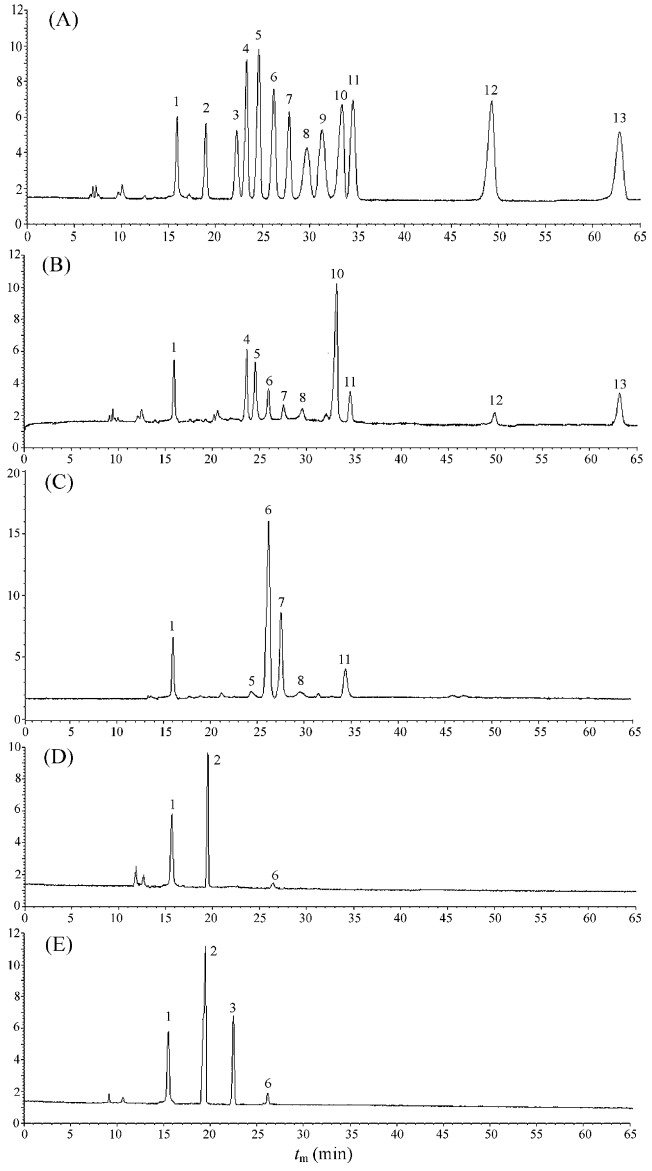

Fig. 4. Typical electropherograms of thirteen standard reducing carbohydrates (A), and the compositional mono- and di-saccharides of *L. lucidus* polysaccharides (LPs, B), Jujube polysaccharides (JPs, C), beer (D), and milk (E) under the optimum conditions of pH 11, 175 mM borate buffer containing 4% methanol at applied voltage 15 kV and capillary temperature 25 °C and detection wavelength at 245 nm. The polysaccharide samples were hydrolyzed with TFA and then were labeled with PMP, and food samples were diluted 1:20 with distilled water, and then was labeled with PMP as described in Section 2. CZE conditions and peak assignment as described in Fig. 1.

Table 3
CZE determination of the component carbohydrates in the sample of polysaccharide and food and their recovery analysis ($n = 3$).

Samples	Components	Content (μM)	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
LPs	Xylose	31.5	20.0	52.0	102.5	4.9
	Arabinose	27.5	20.0	47.1	98.0	4.0
	Glucose	22.5	20.0	43.2	103.5	4.1
	Ribose	17.5	20.0	36.9	97.0	3.6
	Rhamnose	6.0	5.0	11.2	104.0	3.0
	Galactose	71.5	50.0	119.3	95.6	4.1
	Mannose	13.5	20.0	33.8	101.5	3.6
	Glucuronic acid	5.5	5.0	10.2	94.0	4.4
	Galacturonic acid	25.0	20.0	44.1	95.5	4.6
JPs	Arabinose	8.4	5.0	13.2	96.0	3.7
	Glucose	103.2	50.0	149.8	93.2	4.7
	Ribose	44.1	50.0	94.9	101.6	3.7
	Rhamnose	5.7	5.0	10.5	96.0	4.0
	Galactose	19.8	20.0	40.2	102.0	4.2
Beer	Maltose	16.3	20.0	36.8	102.5	3.9
	Glucose	6.6	5.0	11.3	94.0	3.0
Milk	Maltose	56.6	50.0	105.9	98.6	3.6
	Lactose	21.2	20.0	40.7	97.5	2.9
	Glucose	6.1	5.0	11.2	102.0	3.2

4. Conclusions

Reducing carbohydrates is considered to be one of the most abundant ingredients in nature. To investigate their natural distribution and biological roles, a robust analytical system must be used to isolate, identify, and quantify them. Herein, we report the development of such a system that can chromatographically separate and specifically quantify nine aldohexoses, and two aldopentoses, and two disaccharides tagged with PMP. Furthermore, the CZE method with indirect UV detection was optimized and validated for quantification of reducing carbohydrates in different types of polysaccharides, beer and milk. The proposed CZE method provides a repeatable, accurate and economic alternative for the separation of the natural mono- and disaccharides. The proposed method is particularly suitable for determining the component monosaccharides in natural polysaccharide and can also be applied to routine analysis of monosaccharides or disaccharides in the other real-life samples, such as other plant polysaccharides, fruits, vegetables, juices, wines, and grain. Besides, the analytical cost is lower and the CZE analysis can be carried out in common laboratory. This finding is of significance in the discovery of new functional foods, and expanding our understanding of food composition, and will assist in the refinement of design of dietary strategies to improve health outcomes.

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References

Blanco, D., Muro, D., & Gutierrez, M. D. (2004). A comparison on pulsed amperometric detection and spectrophotometric detection of carbohydrates in cidar brandy by liquid chromatography. *Analytica Chimica Acta*, *517*, 65–70.

Cortacero-Ramírez, S., Segura-Carretero, A., Cruces-Blanco, C., & Fernández-Gutiérrez, A. (2003). Analysis of beer components by capillary electrophoretic methods review. *Trends in Analytical Chemistry*, *22*, 440–455.

Cross, R. F., & Cao, J. (1998). Salt effects in capillary zone electrophoresis. II Mechanisms of electrophoretic mobility modification due to Joule heating at high buffer concentrations. *Journal of Chromatography A*, *809*, 159–171.

Currie, H. A., & Perry, C. C. (2006). Resolution of complex monosaccharide mixtures from plant cell wall isolates by high pH anion exchange chromatography. *Journal of Chromatography A*, *1128*, 90–96.

Egeberg, P. K., & Bergli, S. O. (2002). Fingerprinting of natural organic matter by capillary zone electrophoresis using organic modifiers and pattern recognition analysis. *Journal of Chromatography A*, *950*, 221–231.

Frazier, R. A., Inns, E. L., Dossi, N., Ames, J. M., & Nursten, H. E. (2000). Development of a capillary electrophoresis method for the simultaneous analysis of artificial sweeteners, preservatives and colours in soft drinks. *Journal of Chromatography A*, *876*, 213–220.

Frügel, R., Carle, R., & Schieber, A. (2005). Quality and authenticity control of fruit purées, fruit preparations and jams—A review. *Trends in Food Science & Technology*, *16*, 433–441.

Fu, D., & O'Neill, R. A. (1995). Monosaccharide composition analysis of oligosaccharides and glycoproteins by high-performance liquid chromatography. *Analytical Biochemistry*, *227*, 377–384.

Gao, Q., Araiab, M., Lecka, C., & Emmerb, A. (2010). Characterization of exopolysaccharides in marine colloids by capillary electrophoresis with indirect UV detection. *Analytica Chimica Acta*, *662*, 193–199.

Grőßl, M., Harrison, S., Kamla, I., & Kenndler, E. (2005). Characterisation of natural polysaccharides (plant gums) used as binding media for artistic and historic works by capillary zone electrophoresis. *Journal of Chromatography A*, *1077*, 80–89.

Honda, S., Isawe, S., Makino, A., & Fujiwara, S. (1989). Simultaneous determination of reducing carbohydrates by capillary zone electrophoresis as the borate complexes of N-2-pyridyl-glycamines. *Analytical Biochemistry*, *176*, 72–77.

Honda, S., Suzuki, S., & Taga, A. (2003). Analysis of carbohydrates as 1-phenyl-3-methyl-5-pyrazolone derivatives by capillary/microchip electrophoresis and capillary electrochromatography. *Journal of Pharmaceutical and Biomedical Analysis*, *30*, 1689–1714.

Ijiri, S., Todoroki, K., Yoshidaa, H., Yoshitakec, T., Nohtaa, H., & Yamaguchi, M. (2010). Sensitive determination of rhodamine 110-labeled monosaccharides in glycoprotein by capillary electrophoresis with laser-induced fluorescence detection. *Journal of Chromatography A*, *1217*, 3161–3166.

Kakehi, K., Ueda, M., Suzuki, S., & Honda, S. (1993). Determination of hyaluronic acid by high-performance liquid chromatography of the oligosaccharides derived therefrom as 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone derivatives. *Journal of Chromatography A*, *630*, 141–146.

Koike, R., Kitagawa, F., & Otsuka, K. (2007). Simultaneous determination of amphoteric surfactants in detergents by capillary electrophoresis with indirect UV detection. *Journal of Chromatography A*, *1139*, 136–142.

Lachman, J., Havrland, B., Fernández, E. C., & Dudjak, J. (2004). Saccharides of yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] tubers and rhizomes and factors affecting their content. *Plant, Soil and Environment*, *50*, 383–390.

Lopes, J. F., & Gaspar, E. M. S. M. (2008). Simultaneous chromatographic separation of enantiomers, anomers andstructural isomers of some biologically relevant monosaccharides. *Journal of Chromatography A*, *1188*, 34–42.

Lv, Y., Yang, X. B., Zhao, Y., Ruan, Y., Yang, Y., & Wang, Z. Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection. *Food Chemistry*, *112*, 742–746.

Molnár-Perl, I. (2000). Role of chromatography in the analysis of sugars, carboxylic acids and aminoacids in food. *Journal of Chromatography A*, *891*, 1–32.

Morales, F. J. (2002). Application of capillary zone electrophoresis to the study of food and food-model melanoidins. *Food Chemistry*, *76*, 363–369.

Muir, J. G., Rose, R., Rosella, Q., Liels, K., Barrett, J. S., Shepherd, S. J., et al. (2009). Measurement of short-chain carbohydrates in common Australian vegetables and gruits by high-performance liquid chromatography (HPLC). *Journal of the Science of Food and Agriculture*, *57*, 554–565.

Nogueira, L. C., Silva, F., Ferreira, I. M. P. L. V. O., & Trugo, L. C. (2005). Separation and quantification of beer carbohydrates by high-performance liquid chromatography with evaporative light scattering detection. *Journal of Chromatography A*, *1065*, 207–210.

Panagiotopoulos, C., Sempere, R., Lafont, R., & Kerherve, P. (2001). Subambient temperature effects on the separation of monosaccharides by high-performance anion-exchange chromatography with pulse amperometric detection. Application to marine chemistry. *Journal of Chromatography A*, *920*, 13–22.

Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential of possible nutritional enhancement of the diet by modifying the phenol content or profile. *Journal of the Science of Food and Agriculture*, *80*, 985–1012.

Paulus, A., & Klockow, A. (1996). Determination of carbohydrates by capillary electrophoresis. *Journal of Chromatography A*, *720*, 353–376.

Rovio, S., Simolin, H., Koljonen, K., & Siren, H. (2008). Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis. *Journal of Chromatography A*, *1185*, 139–144.

Rumpel, C., & Dignac, M. F. (2006). Gas chromatographic analysis of monosaccharides in a forest soil profile: Analysis by gas chromatography after trifluoroacetic acid hydrolysis and reduction–acetylation. *Soil Biology & Biochemistry*, *38*, 1478–1481.

Santos, S. M., Duarte, A. C., & Esteves, V. I. (2007). Development and application of a capillary electrophoresis based method for the assessment of monosaccharide in soil using acid hydrolysis. *Talanta*, *72*, 165–171.

Sanz, M. L., Martínez-Castro, I., & Moreno-Arribas, M. V. (2008). Identification of the origin of commercial enological tannins by the analysis of monosaccharides and polyalcohols. *Food Chemistry*, *111*, 778–783.

Sjöberg, J., Adorjan, I., Rosenau, T., & Kosma, P. (2004). An optimized CZE method for analysis of mono- and oligomeric aldose mixtures. *Journal of Pharmaceutical and Biomedical Analysis*, *339*, 2037–2043.

Tetsuo, M., Zhang, C., Matsumoto, H., & Matsumoto, I. (1999). Gas chromatographic–mass spectrometric analysis of urinary sugar and sugar alcohols during pregnancy. *Journal of Chromatography B*, *731*, 111–120.

Tian, L. M., Zhao, Y., Guo, C., & Yang, X. B. (2010). A comparative study on the antioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuynia cordata*. *Carbohydrate Polymers*, *83*, 537–544.

Wang, Q. J., & Fang, Y. Z. (2004). Analysis of sugars in traditional Chinese drugs. *Journal of Chromatography B*, *812*, 309–324.

Wang, Y., Zhao, H., Sheng, X., Gambino, P., Costello, B., & Bojanowski, K. (2002). Protective effect of Fructus Lycii polysaccharides against time and hyperthermia-induced damage in cultured seminiferous epithelium. *Journal of Ethnopharmacology*, *82*, 169–175.

Yang, W., & Ding, M. Y. (2000). Analysis of carbohydrates in drinks by high-performance liquid chromatography with a dynamically modified amino column and evaporative light scattering detection. *Journal of Chromatography A*, *904*, 113–117.

Yang, X. B., Zhao, Y., & Lv, Y. (2007). Chemical composition and antioxidant activity of an acidic polysaccharide extracted from *Cucurbita moschata*. *Journal of Agricultural and Food Chemistry*, *55*, 4684–4690.

Yang, X. B., Zhao, Y., Wang, Q. W., Wang, H. F., & Mei, Q. B. (2005). Analysis of monosaccharide components in *Angelica* polysaccharides by high performance liquid chromatography. *Analytical Sciences*, *21*, 1177–1180.

Yang, X. B., Zhao, Y., Yang, Y., & Ruan, Y. (2008). Isolation and characterization of immunostimulatory polysaccharide from an herb tea, *Gynostemma pentaphyllum* Makino. *Journal of Agricultural and Food Chemistry*, *56*, 6905–6909.

Yang, X. B., Zhao, Y., Zhou, Y. J., Lv, Y., Mao, J. L., & Zhao, P. (2007). Component and antioxidant properties of polysaccharide fractions isolated from *Angelica sinensis* (OLIV.) DIELS. *Biological & Pharmaceutical Bulletin*, *30*, 1884–1890.

You, J. M., Sheng, X., Ding, C. X., Sun, Z. W., Suo, Y. R., Wang, H. L., et al. (2008). Detection of carbohydrates using new labeling reagent 1-(2-naphthyl)-3-methyl-5-pyrazolone by capillary zone electrophoresis with absorbance (UV). *Analytica Chimica Acta*, *609*, 66–75.

Zhang, L., Xu, J., Zhang, L., Zhang, W., & Zhang, Y. (2003). Determination of 1-phenyl-3-methyl-5-pyrazolone-labeled carbohydrates by liquid chromatography and micellar electrokinetic chromatography. *Journal of Chromatography B*, *793*, 159–165.

Zhao, Y., Yang, X. B., Sun, X. L., Jiang, R., & Zhang, S. Y. (2006). Enantiomeric separation of synthetic 2,3-dihydroxy-3-phenylpropionate compounds by β-cyclodextrin-modified capillary electrophoresis. *Journal of Chromatography A*, *1108*, 258–262.

Zhao, Y., Yang, X. B., Wang, Q. F., Sun, X. L., Jiang, R., & Zhang, S. Y. (2007). Determination of enantiomeric excess of aromatic 1,2-diols with HP-β-cyclodextrin as chiral selector by CE. *Chromatographia*, *66*, 601–605.