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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