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Rapid determination and quantitation of compositional carbohydrates to identify honey by capillary zone electrophoresis

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ABSTRACT
A simple, sensitive, and specific capillary electrophoretic method based on 1-phenyl-3-methyl-5-pyrazolon (PMP) derivatization has been developed for simultaneous separation and determination of 11 aldoses (maltose, xylose, arabinose, ribose, glucose, rhamnose, fucose, galactose, mannose, glucuronic acid, and galacturonic acid). The separation of PMP-labeled maltose and monosaccharides was carried out under the selected optimum conditions with pH 11.0, 200 mM borate buffer containing 4% methanol at voltage 20 kV, and capillary temperature 25°C, and the 11 PMP aldoses could be perfectly separated from each other within 41 min. Our experiment results demonstrated that the molar ratios of carbohydrates in 10 kinds of honey were greatly different, and the carbohydrates’ constitutions varied greatly between commodity honey and natural honey. This proposed zone electrophoresis method provides an accurate and economic alternative for monosaccharide and maltose analysis to identify honeys, and can also be applied to routine detection of reducing carbohydrates in real-life samples.

1. Introduction
Honey is the sweet substance produced in honeycomb by honeybees from nectars of blossoms or other active secretions of plants (Megherbi, Herbreteau, Faure, & Salvador, 2009). It is not only a remarkably complex natural nutritious tonic which contains at least 181 substances but also a type of supersaturated solution which contains small amounts of proteins, minerals, inorganic salts as well as a variety of enzymes which have the capability of nourishing, moistening, and detoxifying, such as catalase, invertase, and diastase (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Baroni et al., 2009; Daglia, Ferrari, Collina, & Curti, 2013; Noori & Al-Waili, 2004). However, carbohydrate is the main substance (95–99%) containing fructose, glucose, maltose, and other complex carbohydrates. Because of different floral source and external factors, the composition of carbohydrates in honey is different (Arráez-Román, Gómez-Caravaca, Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2006; Blasa et al., 2006; LaGrange & Sanders, 1988; Shin & Ustunol, 2005).

Honey has been widely used in food and pharmaceutical with its high nutritional and medicinal values (Hamdy, Ismail, Al-Ahwal, & Gomaa, 2009; Mendes et al., 1998). Nevertheless, more and more honeys are intentionally adulterated with cheap sugar in order to increase its economic benefits (Megherbi et al., 2009; Paradkar & Irudayaraj, 2001). Even though many methods have been successfully applied to evaluate the quality of honey, such as stable carbon isotope ratio analysis (Cotte et al., 2007; Eiffein & Raekze, 2008; Padovan, Jong, Rodrigues, & Marchini, 2003), high-performance liquid chromatography (HPLC) (Cavazza, Corradini, Musci, & Salvador, 2013; Ouchemoukh, Schweitzer, Bey, Djoudad-Kadjji, & Louaillec, 2010), gas chromatography coupled to a flame-ionization detector (Cotte, 2010), etc., there is an urgent need to develop more accurate and economic methods that could be used in routine quality control of honey.

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2. Materials and methods

2.1. Materials and chemicals

The proposed assay was applied to 10 honey samples, including multifloral honey, *Acacia* sp. honey, and so on. Among them, four honey samples, such as multifloral honey (Multifloral), *Acacia* sp. honey (*Acacia* sp.), *Ziziphus jujuba* flower honey (*Ziziphus jujuba*), and *Fagopyrum esculentum* honey (*Fagopyrum esculentum*) were from the apiary, which were not processed. Rongshiwang Mountain *Chrysanthemum morifolium* honey (*Chrysanthemum morifo-

nium*), Rongshiwang Mountain Wuxing *Eriobotrya japonica* honey (*Eriobotrya japonica*), Rongshiwang Mountain Salum *Sophora japonica* honey (*Sophora japonica*) were collected from Shaanxi Old Beekeepers Biological Technology Co., China. *Astragalus spp.* honey (*Astragalus spp.*) was from Shanghai Guanshengyuan Bee Products Co., China. Bai Hua *Acacia* sp. honey (Bai Hua *Acacia* sp.) was from Beijing Baihua Honey Co., China, and the *Sophora japonica* honey (Organic *Sophora japonica*) came from Shanghai Guanshengyuan Bee Products Co., China. *Fagopyrum esculentum* honey and *Eriobotrya japonica* honey (Multi-2003 floral) were all obtained from Sigma (St. Louis, U.S.A.). 1-Phenyl-3-methyl-5-pyrazolone (PMP)-tagged UV detection method to detect compositional carbohydrates in honey.

The purpose of the present study was to develop a rapid and simple CZE analytical method for the simultaneous, efficient separation and determination of 11 reducing carbohydrates possibly found in honey. Considering the complex carbohydrate composition of honey, the developed CZE method was effectively applied to quantitative analysis of the carbohydrate composition in the honey samples. In addition, this method was successfully used in quantitative analysis of the monosaccharides which were extracted and degraded of polysaccharides in honey sample. Furthermore, the developed CZE method proves to be precise and practical for quality control of reducing carbohydrates in honey.

were the products of Beijing Reagent Plant (Beijing, China). HPLC-grade methanol was purchased from Honeywell (U.S.A.). Doubly deionized water was obtained with a Milli-Q water purification system (Millipore, Bedford, MA), and all of the other chemicals were of analytical grade.

2.2. Extraction of the honey polysaccharides

The honey polysaccharides were isolated by hot-water extraction and ethanol precipitation with slight modification. Briefly, 50 g honey was extracted with 400 mL distilled water at 80°C for twice, 2 h each time. The mixture was concentrated to 150 mL under a diminished pressure and centrifuged at 3000 g for 15 min. After the supernatant was collected, the residue was redissolved by adding 500 mL of 95% ethanol slowly by stirring and kept at 4°C overnight. Finally, the polysaccharide pellets were obtained by centrifugation at 3000 g for 10 min and repeatedly washed sequentially with ethanal, acetone, and ether, respectively. The refined polysaccharide pellets were completely dissolved in appropriate volume of distilled water and intensively dialyzed for 2 days (water change regularly) against distilled water (cutoff Mw 8000 Da), and then the remaining portion was concentrated, deproteinated by a freeze–thaw process (FD-1, Henan Yuhua Instrument Co., China) repeated 10 times followed by filtration. Finally, the filtrate was lyophilized to obtain the crude water-soluble honey polysaccharides.

2.3. Hydrolysis of the honey polysaccharides

The honey polysaccharides were hydrolyzed extensively by TFA as our previously described by Lv et al. (2009). In brief, a suitable amount of honey polysaccharide (Multifloral/21.4 mg, *Acacia* sp./20.4 mg, *Ziziphus jujuba*/18.6 mg, *Fagopyrum esculentum*/22.9 mg, *Chrysanthemum morifolium*/19.7 mg, *Eriobotrya japonica*/16.1 mg, *Salix Sophora japonica*/19.3 mg, *Astragalus spp./17.6 mg, Bai Hua *Acacia* sp./20.1 mg, Organic *Sophora japonica*/17.7 mg) were hydrolyzed with 2 mL of 2 M TFA in ampoules (10 mL), respectively. The ampoules were sealed under a nitrogen atmosphere. After hydrolysis at 110°C for 8 h with an oil bath, the mixture was cooled to room temperature and centrifuged at 1000 g for 5 min. The supernatant was collected and dried with nitrogen gas to remove the excess TFA and redissolved in 1.0 mL distilled water, which was ready for derivatization.

2.4. Derivatization procedure

The PMP derivatization of monosaccharides and maltose was carried out as described previously with proper modification (Honda et al., 1989; Honda, Suzuki, & Taga, 2003). Briefly, 10 kinds of honey samples (0.3 g for each) were hydrolyzed with 10 mL distilled water in ampoules. 20 μL hydrolyzed solution of monosaccharides (aqueous) or the diluted samples of honey was dissolved in 400 μL of 0.3 M aqueous NaOH and 400 μL of 0.5 M PMP–methanol solution. After shaking for 10 s, the solution was allowed to stand for 30 min at 70°C in a water bath, and then cooled to room temperature and neutralized with 400 μL 0.3 M HCl. The
resulting solution was extracted with 1.0 mL chloroform, and after vigorous shaking and centrifuging, the organic phase was carefully discarded, and the extraction process was repeated for three 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 the samples were dried by nitrogen and diluted with 0.5 ml of distilled water.

2.5. Preparation of buffer and standard solution

As Hu, Wang, Yang, and Zhao (2014) reported that the concentration of 200 mM of working buffer solution was obtained by adding 500 mL of deionized water to the 250 mL of stock buffer solution (400 mM), and neutralized to pH 11.0 with saturated sodium hydroxide. The stock standard monosaccharide solutions (10 mM) were prepared by dissolving each standard monosaccharide in ultrapure water. The sample solutions were filtered through a 0.22-µm syringe filter and 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.

2.6. Apparatus and electrophoretic procedures

The PMP-labeled monosaccharides and maltose were analyzed by a Beckman P/ACE MDQ capillary electrophoresis instrument equipped with a UV detector, an automatic injector, and an uncoated fused silica capillary (Beckman-Coulter, Fullerton, CA, U.S.A.). Data acquisition and processing were carried out with Beckman System Gold software. Fused silica capillaries had an internal diameter of 50 µm and were 58.5 cm in total length (effective length 48.5 cm). At the start of each working day, the capillary was preconditioned by flushing with the following reagents for 5 min: water, 0.1 M NaOH, water, 0.1 M HCl, water, and then running buffer. Between injections, the capillary was rinsed sequentially with water, 0.1 M NaOH and water, both for 5 min, and then equilibrated with running buffer at a pressure of 20 psi for 3 min. After all analysis of the day, the capillary was rinsed with water for 5 min and stored overnight with water inside. Samples were introduced by pressure injection at 0.5 psi for 5 s into the capillary. The UV absorbance was measured at 245 nm.

3. Results and discussion

3.1. Establishment of standard analysis method

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 could work in a simplified aqueous methanol system, and could afford stable PMP derivative as previously described. Separation of the PMP-labeled derivatives of standard aldoses was performed by CZE, 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 on some important parameters, including the pH of running borate buffer, the borate concentrations, and the proportions of methanol. The best resolution versus the lowest migration time was obtained at the optimized conditions of pH 11.0, 175 mM borate buffer containing 4% methanol at 25°C and 15 kV, at which value the PMP-carbohydrate derivatives were negatively charged. An optimum chromatographic profile of the standard PMP-labeled carbohydrates is shown in Figure 1. The result indicated that 11 kinds of reducing sugars with PMP label, including maltose and 10 monosaccharides (xylose, arabinose, ribose, glucose, rhamnose, fucose, galactose, mannose, gluconic acid, and galacturonic acid as internal standard), were simultaneously separated under the optimized conditions.

In this study, we have tried to analyze the reducing carbohydrate composition of honey samples and honey polysaccharides using the established CZE method. For the 10 kinds of diluted honeys, they were directly derivatized with PMP and subsequently analyzed by the developed CZE method. For the 10 kinds of isolated honey polysaccharides, they were hydrolyzed with TFA, and then the acid

![Figure 1](image)

**Figure 1.** Typical electropherograms of standard PMP-labeled reducing carbohydrates (maltose and 10 monosaccharides). Analytical conditions: fused capillary 58.5 cm (48.5 cm to the detector) ×50 µm, i.e. 200 mM borate buffer, pH = 11.0, applied voltage 15 kV, capillary temperature 25°C, UV detection at 245 nm; Peaks: 1. maltose, 2. xylose, 3. arabinose, 4. glucose, 5. ribose, 6. rhamnose, 7. fucose, 8. galactose, 9. mannose, 10. glucuronic acid, 11. galacturonic acid (internal standard).

**Figura 1.** Electroferograma típico de carbohidratos reductores (maltosa y 10 monosacáridos) estándar, etiquetados PMP. Condiciones analíticas: Amortiguador de borato de capilar fundido 58.5 cm (48.5 cm para el detector) ×50 µm i.d., 200 mM, pH = 11.0, voltaje aplicado 15 kV, temperatura capilar 25 ºC, detección de UV a 245 nm; Picos: 1. maltosa, 2. xilosa, 3. arabinosa, 4. glucosa, 5. ribosa, 6. raminosa, 7. fucosa, 8. galactosa, 9. manosa, 10. Ácido glucurónico, 11. Ácido galacturónico (estándar interno).
hydrolysates were neutralized and derivatized with PMP. Finally, the released monosaccharide as PMP derivatives was analyzed by the described CZE method. As shown in Figure 1, all of the peaks were identified within 41 min by comparing their \( t_m \) values with those of the standards spiked in the samples. Nevertheless, the results showed that all the samples did not contain galacturonic acid (GalUA), and thus, GalUA is used as internal standard for its good separation from other standards. As can be seen, the sequence of peaks is maltose, xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid, and galacturonic acid. Therefore, the final optimized experimental conditions in this work were 200 mM borate buffer at pH 11.0, running voltage 15 kV, and capillary temperature 25°C.

3.2. Rationality and linearity

Method precision expressed as coefficient of variation (CV, %) was obtained by measuring the repeatability for both intraday variability and inter-day variability of retention time \((t_r)\) and corrected peak areas \((A/t)\), which was estimated by making six repetitive injections of a standard mixture solution \((10 \mu g/mL\) for each analyte) under the same CZE condition. The results showed that the CV values in intraday were less than 1.8% for \( t_r \) and 2.7% for \( A/t \), and the inter-day CV values were less than 5% for all variables. The validated results indicated that this method was rapid, accurate, and reliable.

Under the optimized conditions, five series of concentrations in the range of 1.2–140.8 \( \mu M \) of the standard sugars (maltose, xylose, arabinose, glucose, ribose, rhamnose, fucose, galactose, mannose, glucuronic acid, and galacturonic acid) were tested to determine the linearity by CZE, each point of the calibration plot was repeated three times in an independent solution, and the regression equation and correlation coefficient were listed in Table 1. As a consequence, a good linearity with the correlation coefficients \((r)\) in the range of 0.9908–0.9967 was obtained between Y (peak area ratio of the analytes with internal standard) and X (concentration of the standards).

3.3. Aldoses analysis of honeys and its isolated polysaccharides

In this study, the developed CZE analytical method was further applied to the quantification of reducing carbohydrate composition of natural honey and the component monosaccharides in the honey polysaccharides. The analytical results were shown in Tables 2 and 3, respectively. As depicted in Table 2, reducing glucose and maltose were dominant components in all tested honeys, and all the 10 different kinds of honeys contained maltose, xylose, glucose, and galactose with different mole ratios (2.8:1.2:8.8:1 for Multifloral, 0.9:1.4:5.2:1 for Acacia sp., 2.6:1.7:5.6:1 for Ziziphus jujuba, 3.7:2.3:11.9:1 for Fagopyrum esculentum, 1.9:1.8:9.4:1 for Chrysanthemum morifolium, 2.7:1.0:9.4:1 for Erionotus japonica, 4.1:2.1:7.6:1 for Salum Sophora japonica, 4.5:0.68:2.1 for Astragalus spp., 2.7:0.5:13.9:1 for Bai Hua Acacia sp., 1.9:0.4:9.7:1 for Organic Sophora japonica). Besides, multifloral honey and Ziziphus jujuba flower honey contained glucuronic acid, and only Astragalus spp. honey did not contain arabinose. In addition, only multifloral honey contained free mannose and only Acacia sp. honey contained free fucose.

As can be seen in Table 3, the compositional monosaccharides released from all the purified 10 kinds of honey polysaccharides consisted of xylose and glucose, and their corresponding mole ratios in different samples were 1:8.1; 1:2.7; 1.0.7; 1:1.1; 1:1.4; 1:2.4; 1:1.2; 1:4.2; 1:3.9; 1:3.9, respectively. It was clear that all the honey polysaccharides did not contain arabinose except for Ziziphus jujuba flower honey and Fagopyrum esculentum honey, while only two kinds of polysaccharides from honeys covered rhamnose. Glucose was the main part of the monosaccharide composition in honey polysaccharides.

The results of CZE assay indicated that all the tested natural honeys contained xylose, glucose, and galactose, while all the commercial honey did not contain rhamnose, mannose, fucose, and galactose, and the main free aldose in honeys was glucose and maltose, suggesting that the proportion of compositional reducing disaccharide and monosaccharides altered with different nectars. It was found that all the honey polysaccharides consisted of xylose and glucose. Except for Rongshiwang Mountain Wuxing Eriobotrya japonica honey, all the commercial honey polysaccharides did not contain galactose. Multifloral honey and Ziziphus jujuba flower honey and their polysaccharides contained rhamnose and glucuronic acid. Besides, Acacia sp. honey and its polysaccharide were composed of fucose, whereas commercial Acacia sp. honey and their polysaccharides did not contain it.

3.4. Recovery experiments

In order to verify the accuracy and stability of the detection method, the recovery experiments were carried out for the main compositional aldoses. The standard solutions of glucose and maltose were accurately added into sample diluents, and the analysis was performed with the method described in 3.1. Ultimately, the analytical data in recovery experiments were given in Table 4. In this study, the average recoveries were calculated based on the difference between the amount determined in the spiked samples and the amount observed in the non-spiked samples. All the analysis was carried out in triplicate. The results showed that the recoveries of glucose in all the honey samples ranged from 96.2% to 103.8%, and the relative standard deviation (RSD) values were not more than 3.9%. The recovery of maltose was between 94.5% and 102.5%, and the RSD values were not more than 4.1%. These results showed that the developed method was accurate and practical for the component analysis of monosaccharides and oligosaccharides in honey samples.

### Table 1. Experimental results of linearity relationship and precision of analysis of reducing monosaccharides and disaccharide (maltose) reductors.

| Carbohydrates | Equation \((y = ax + b)\) | Correlation coefficient \((r^2)\) |
|---------------|---------------------------|-----------------------------|
| Maltose       | \(y = 3.2006x + 0.0408\)  | 0.9908                      |
| Xylose        | \(y = 3.3976x + 0.0007\)  | 0.9931                      |
| Arabinose     | \(y = 3.3812x + 0.0296\)  | 0.9908                      |
| Glucose       | \(y = 2.3671x + 0.0471\)  | 0.9915                      |
| Ribose        | \(y = 3.0132x + 0.0331\)  | 0.9933                      |
| Rhamnose      | \(y = 2.7369x + 0.0401\)  | 0.9915                      |
| Fucose        | \(y = 2.8251x + 0.0199\)  | 0.9940                      |
| Galactose     | \(y = 3.6392x + 0.0356\)  | 0.9912                      |
| Mannose       | \(y = 3.7105x + 0.0343\)  | 0.9967                      |
| Glucuronic acid | \(y = 4.4539x + 0.0390\) | 0.9922                      |
Table 2. Determination of free reducing monosaccharides and maltose in the samples of the tested honeys.

| Samples        | Maltose | Xylose | Arabinose | Glucose | Ribose | Rhamnose | Fucose | Galactose | Mannose | Glucuronic acid |
|----------------|---------|--------|-----------|---------|--------|----------|--------|-----------|---------|----------------|
| Multifloral    | 21.4    | 8.8    | 5.8       | 67.0    | 9.2    | 1.9      | ND     | 7.6       | 1.6     | 1.7            |
| Acacia sp.     | 10.4    | 15.6   | 5.6       | 57.5    | 8.3    | ND       | 2.2    | 11.0      | ND      | ND            |
| Ziziphus jujuba| 23.4    | 15.5   | 5.2       | 50.5    | ND     | 2.1      | ND     | 9.0       | ND      | 1.50          |
| Fagopyrum esculentum | 19.8 | 12.4   | 4.1       | 63.2    | 7.9    | ND       | 5.3    | ND        | ND      | ND            |
| Chrysanthemum morifolium | 11.2 | 10.4 | 3.2       | 54.8    | 4.9    | ND       | ND     | 5.8       | ND      | ND            |
| Eriobotrya japonica | 19.7 | 7.3    | 5.2       | 68.9    | 6.8    | ND       | ND     | 7.3       | ND      | ND            |
| Salum Sophora japonica | 29.8 | 15.2  | 4.4       | 54.9    | ND     | ND       | ND     | 7.2       | ND      | ND            |
| Astragalus spp. | 31.2    | 4.1    | ND        | 56.3    | ND     | ND       | ND     | 6.9       | ND      | ND            |
| Bai Hua Acacia sp. | 16.4 | 3.1    | 3.6       | 83.3    | ND     | ND       | ND     | 6.0       | ND      | ND            |
| Organic Sophora japonica | 17.6 | 3.3    | 5.7       | 88.7    | ND     | ND       | ND     | 9.1       | ND      | ND            |

ND: not detected.

Table 3. Determination of the component monosaccharides of the isolated polysaccharides derived from 10 kinds of honeys.

| Samples        | Maltose | Xylose | Arabinose | Glucose | Ribose | Rhamnose | Fucose | Galactose | Mannose | Glucuronic acid |
|----------------|---------|--------|-----------|---------|--------|----------|--------|-----------|---------|----------------|
| Multifloral    | ND      | 2.12   | ND        | 17.1    | 9.29   | 1.99     | ND     | 1.6       | 2.1     | 2.0            |
| Acacia sp.     | ND      | 5.67   | ND        | 15.5    | ND     | 2.2      | ND     | 1.8       | ND      | ND            |
| Ziziphus jujuba| ND      | 15.65  | 1.5       | 11.1    | ND     | 2.12     | ND     | 3.0       | ND      | 3.3            |
| Fagopyrum esculentum | ND | 12.54  | 2.1       | 13.8    | ND     | ND       | ND     | 2.4       | ND      | ND            |
| Chrysanthemum morifolium | ND | 10.64  | ND        | 14.4    | ND     | ND       | ND     | ND        | ND      | ND            |
| Eriobotrya japonica | ND | 7.63   | ND        | 18.5    | ND     | ND       | ND     | 1.6       | ND      | ND            |
| Salum Sophora japonica | ND | 15.72  | ND        | 19.1    | ND     | ND       | ND     | ND        | ND      | ND            |
| Astragalus spp. | ND      | 4.01   | ND        | 16.8    | ND     | ND       | ND     | ND        | ND      | ND            |
| Bai Hua Acacia sp. | ND      | 3.91   | ND        | 15.3    | ND     | ND       | ND     | ND        | ND      | ND            |
| Organic Sophora japonica | ND | 3.23   | ND        | 12.6    | ND     | ND       | ND     | ND        | ND      | ND            |

ND: not detected.

Table 4. Results of the recovery test of glucose and maltose (n = 3).

| Samples        | Components | Content (µM) | Spiked (µM) | Found (µM) | Recovery (%) | RSD (%) |
|----------------|------------|--------------|-------------|------------|--------------|---------|
| Multifloral    | Glucose    | 67.0         | 50.0        | 116.0      | 98.0         | 3.2     |
| Acacia sp.     | Maltose    | 10.4         | 20.0        | 29.3       | 94.5         | 2.7     |
| Ziziphus jujuba| Maltose    | 23.4         | 20.0        | 43.9       | 102.5        | 3.5     |
| Fagopyrum esculentum | ND | 19.8 | 20.0 | 39.1 | 96.5 | 3.2 |
| Chrysanthemum morifolium | ND | 11.2 | 20.0 | 30.8 | 98.0 | 2.9 |
| Eriobotrya japonica | ND | 19.7 | 20.0 | 38.9 | 96.0 | 2.2 |
| Salum Sophora japonica | ND | 29.8 | 20.0 | 49.5 | 98.5 | 3.1 |
| Astragalus spp. | ND        | 31.2         | 20.0        | 50.1       | 94.5         | 2.9     |
| Bai Hua Acacia sp. | ND      | 16.4         | 20.0        | 35.0       | 97.0         | 3.2     |
| Organic Sophora japonica | ND | 17.6 | 20.0 | 36.7 | 95.5 | 4.1 |

It is widely recognized that the separation and identification of a large number of carbohydrates are a complex and challenging area for research. One of the major problems is the risk of coelution of the different sugars. Another reason is that honey as a natural sweetener mainly contains carbohydrates, in which fructose is the most followed by glucose and other complicated sugars (Cavia et al., 2002; Kaškonienė, Venskutonis, & Ėckšteitytė, 2010). However, fructose as a ketose can not be derivatized for the absence of aldehyde group. Although there is some published information in this area, (Honda et al., 2003; Sjöberg, Adorjan, Rosenau, & Kosma, 2004), no previous studies have attempted to quantify all of these carbohydrates. Therefore, an efficient method has been developed to control the quality of honey by determining the components of reducing sugars, and the different ratio of each component was established in this study. To our knowledge, this is the first report showing a new CZE method for separating 11 kinds of reducing monosaccharides and oligosaccharides from honeys within 41 min, which provides a rapid and effective method to control quality of honeys and detect the adulteration of honeys.
4. Conclusions
An efficient simultaneous CZE separation of 10 monosaccharides and disaccharide (maltose) as common reducing carbohydrate components found in honeys was detailedly examined and optimized. The newly established CZE method has been successfully applied to the qualitative and quantitative determination of reducing monosaccharides and disaccharide components in four natural and six commercial honeys with high separation efficiency, precision, and simple analysis process. This study has also allowed the conclusive identification of 10 monosaccharides released from isolated polysaccharides in the 10 kinds of honeys. The different proportion of reducing carbohydrate components between natural and commercial honeys were found by the CZE analysis. This proposed CZE method provides an accurate and economic alternative for monosaccharide and oligosaccharides analysis to identify honeys, and can also be applied to routine detection of monosaccharide and oligosaccharides in real-life samples.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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