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Rapid Screening of Natural Liquid Sweeteners by Capillary Electrophoresis

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Abstract

Capillary electrophoresis (CE) is an instrumental method of chemical analysis that has been developed for the rapid screening of liquid sweeteners (corn syrups, honeys, maple syrups and nectars) from different geographic regions. CE can separate organic compounds in each sweetener sample based on their charge-to-size ratios in a background electrolyte (BGE) solution. Ultraviolet (UV) light absorption can detect the separated compounds for quantitation. Electrophoretic mobility values were determined for all the CE-UV peaks to identify whether flavonoids (e.g., quercetin) and phenolic compounds (e.g., rutin) were present in the sweetener. A novel approach was also developed to perform CE-UV analysis by spiking honeys in the background electrolyte. Due to an increase of the viscosity, both the electroosmotic flow (as indicated by a neutral marker) and the electrophoretic mobility of polydopamine-coated magnetic nanoparticles were decreased by approximately 50%. The electrical conductance was also decreased by approximately 30% due to a higher BGE solution viscosity. These results have demonstrated that CE-UV is a promising technique for the rapid screening of natural liquid sweeteners to detect adulteration by corn syrup.

Keywords: *sweetener adulteration, electrical conductivity, electrophoretic mobility, solution viscosity, polydopamine-coated magnetic nanoparticles*

1. Introduction

Rapid screening of liquid sweeteners is of outmost importance as the consumption of processed foods with added sweeteners has been on the rise in recent years. Growing demand for tasty and healthy food has driven the development of low-calorie sweeteners, sweet taste modulators, and bitter masking compounds originated from natural sources [An 2024]. Natural sweeteners such as steviol glycosides are found to produce significant improvement of glucose metabolism in random controlled trials with adults [Bai 2024]. Stevia rebaudiana is a perennial shrub that is grown around the world for its production of non-caloric sweetener [Patel 2024] as an alternative for natural sweeteners against diabetes [Silver de Andrade 2024]. As a replacement for sugar, xylitol, erythritol, and D-allulose seem promising as alternative sweeteners due to favorable metabolic outcomes. They replicate some benefits of sugars (e.g., sweetness and gastrointestinal hormone release) while circumventing any detrimental effects on human health [Teysseire 2024]. Other natural sweeteners (crude trilobatin, crude rubusoside, and glycyrrhizinic acid) exhibit promising inhibitory effects against α-glucosidase and α-amylase in an efficacious approach to manage diabetes [Jiang 2024]. While non-nutritive sweeteners are widely being used in different food products with the assumption that they would lower calorie intake and help to manage weight and blood sugar levels better, studies using animal models have reported that chronic exposure to sweeteners leads to increased food consumption, weight gain and insulin resistance [Mathur 2024]. These sweeteners do not change plasma glucose levels but modify the weight of metabolic organs such as the liver and contribute to weight gain (in female mice) [Balcón-Pacheco 2024]. In view of the scientific evidence, reducing sugar concentration and sweetness in foods should be the priority strategy for public policies to focus on [García-Cordero 2024].

Since the Middle Ages, honey has played a significant role in sweetening a wide range of family dishes [Salama 2024]. Honey as a natural sweetener provides a seamless substitution for sugar in a variety of

recipes; its source of energy is about 64 calories per tablespoon [Kumar 2024]. Nowadays, honey has wide-spread use in the food and beverage industry as a sweetening or flavoring ingredient. Flavors of honey vary based on the nectar source, and various types or grades of honey are available [Badders 2024]. Honey can be a good source of phenolic compounds and antimicrobial agents [Kebede 2024; Mohammed 2024] with various health benefits [Escuredo 2012; Tirfie 2024]. It is an extremely composite natural product that faces great qualitative variance due to fluctuations in geographical climate and yearly weather conditions [Petrovic 2024], hence making it an easy target for product adulteration [McComb 2012]. When honeys from two vegetation zones in Nigeria were analyzed for their pollen contents from flowers, a total of twenty-eight pollen types were identified belonging to eighteen families [Ige 2013]. The numerous pollen types contained in the honeys confirmed their botanical origin [Canli 2024]. A recent test, however, revealed that 76 percent of honeys bought at groceries in ten states of U.S.A. had all the pollen removed. Filtering out pollen in honey further makes adulteration easy [Schneider 2011]. With the global honey market valued at over $9 billion [Sokhai 2024; Huang 2024], honey adulteration and counterfeiting are becoming ever more frequent. Most fraud is committed by blending honey with one or more syrups and new adulteration techniques have been developed to bypass current testing methodologies. Consuming adulterated and contaminated honey threatens safety, food security, and environmental sustainability [Morariu 2024]. Natural sweeteners do not change plasma glucose levels but modify the weight of metabolic organs such as the liver. Corn syrup is a viscous sweet liquid produced by hydrolysis of corn starch using enzymatic reactions [Cereda 2024]. It consists largely of glucose that is added to soften the texture and prohibit the crystallization [Gabarra 1998]. Light corn syrup, decolorized, is used as an ingredient in many food products including candies because it does not crystallize when heated [Papageorgio 1995]. Dark corn syrup is used when a more distinctive flavor is desired at the table [Britannica Online Encyclopedia 2011]. High-fructose corn syrup (HFCS) is widely used in the manufacture of soft drinks because it is considerably cheaper than sucrose. A study has found that countries using HFCS in their food supply have a significantly higher prevalence of type 2 diabetes than countries that do not use the sweetener [Kietowicz 2012; Piñalvo 2024]. It is proven by clinical tests that the human body has very limited ability to handle fructose [Úbeda 2024]. Intravenous fructose very quickly produces liver dysfunction and even death whereas glucose does not [Editors of Encyclopaedia Britannica 2022]. Although fructose from honey is well suited in human metabolism and has enormous beneficial effects [Erejuwa 2012], it was found that rapeseed honey [Na 2024] led to an increase of blood fructose concentration due to inhibition of the fructose metabolism by unidentified substances present [Münstedt 2011]. Excess calories from free sugars are implicated in the epidemics of obesity and type 2 diabetes [Ahmed 2023].

The purity of honey can be monitored by different analytical tests to detect whether cheap sugar syrups have been added, and numerous research investigations for honey adulteration are reported in the literature. Ruiz-Matute et al. (2010) developed a GC-MS technique that detects fructose, fructose dianhydrides, high fructose inulin syrups (HFIS), inulobiose, inulotriose, ketoses and sucrose towards the development of new markers for the detection of adulterants not naturally present in honey. Chen et al. (2011) rapidly determined honey adulterated with HFCS via fiber optic diffuse reflectance near infrared spectroscopy with chemometric techniques. Li et al. (2012) used Raman spectroscopy with partial least squares-linear discriminant analysis (PLS-LDA) to detect such adulterants as HFCS and maltose syrup in honey. Rios-Corripio et al. (2012) employed attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and multivariate analysis (principal component analysis and partial least squares methods) to determine honey adulteration by sugars and syrups. Near-infrared spectroscopy along with chemometrics is becoming a powerful non-destructive analytical technique that can authenticate honey, determine its geographical/botanical origin, and detect adulteration [Biswas 2024]. Nikolova et al. (2012) determined the presence of a glucose adulterant in honey by measurement of β-carotene content, color coordinates, electrical conductivity, individual carbohydrate contents, potassium content, refractive index and water content (using differential thermal calorimetry, high performance liquid chromatography, polarimetry, refractometry, etc.). The glass transition temperature and optical rotation of honey provided assessments of glucose adulteration exceeding 20%. After AOAC advised in 2010 that the isotope ratio of 13C/12C should be measured in order to detect the addition of HFCS to honey, Simsek et al. (2012) determined percent adulteration in honey using a mass spectrometer coupled to an elemental analyzer. Their method exploited the isotope ratio differences between dicots (C3 plants, from which honey is naturally derived) and monocots (C4 plants such as

corn and sugar cane). Further qualitative data was collected, including diastase activity, electrical conductivity, hydroxymethyl-furfural content and moisture content of honey. Alfalfa, sage and sourwood honeys, which vary in their oligosaccharide contents, were compared with sucrose, HFCS and inulin in their ability to support activity, growth and viability of lactic acid bacteria and bifidobacteria typically used in yoghurt manufacturing [Popa et al. 2011]. The end products of fermentation (lactic and acetic acids) were not influenced by the oligosaccharide content or floral source of the honeys. Mass spectrometry, due to its great sensitivity and specificity, proved to be a very effective method for characterizing food components including amino acids, carbohydrates, nutrients, oils, polyphenols, and vitamins [Nwachukwu 2024]. All these analytical methods provide a direction for the development of more robust techniques to determine honey adulteration. An enhancement to the well-established two-tier testing strategy utilizes field deployable FTIR spectroscopy for rapid and facile first-tier screening, followed by high resolution mass spectrometry for confirmatory analysis [Makni 2024]. This approach not only addresses fraud but also other key attributes such as quality and purity. The best tools to detect food fraud are FTIR, polymerase chain reaction, and gas chromatography-mass spectroscopy combining chemometrics [Vinothkanna 2024]. The latest trends in honey contaminant analysis, challenges, and opportunities for green chemistry development have recently been reviewed [Sixto 2024]. Conventional approaches for detecting honey adulteration are often associated with extensive time requirements and restricted sensitivity. A novel approach to address the adulteration issue is employing convolutional neural networks for the classification of honey samples based on the use of thermal imaging technique that can reveal differences in temperature in honey samples caused by variations in sugar composition, moisture levels, and other substances used for adulteration [Boulbarj 2024].

Capillary electrophoresis (CE) is an analytical technique that can rapidly separate different types of food compounds as per their charge to size ratios [Domínguez-Rodríguez 2024]. The CE technique was first reviewed [Zeece 1992] as a new analytical tool to detect compounds in foods. It was successfully applied to the determination of inorganic ions in beverages [Sádecká 1999] and feeds [Blatný 1999], analysis of natural food pigments [Watanabe 2000], and determination of organic acids in foods [Klampfl 2000]. Song et al. (1999) developed a CE method with field-amplified sample stacking injection for the determination of formoterol in a dry syrup pharmaceutical preparation. Delgado et al. (1994) proposed CE as an alternative to HPLC for determination of honey flavonoids. Suárez-Luque et al. (2005) reported a CE method for the determination of cations (Ni^{2+} and Li^{+}) in honey without any treatment. Arráez-Román et al. (2006) identify phenolic compounds in rosemary honey by CE–electrospray ionization-mass spectrometry. Rizelio et al. (2012a) developed a fast CE method for the determination of carbohydrates and cations (K^{+}, Na^{+}, Ca^{2+}, Mg^{2+} and Mn^{2+}) in honeys. They next validated the method for the classification of honey by geographical origin using principal components analysis [Rizelio 2012b]. The technique evolved into a highly mature and versatile separation technique [Voeten 2018]. Modern CE has been used for the analysis of both large and small molecules in many applications where it performed better than, or is complementary to, liquid chromatography [Torano 2019].

The development of a rapid screening tool for natural liquid sweeteners was the prime objective of this work. Initial efforts were focused on the CE-UV characterization of corn/table syrups, honeys, maple syrups and nectars that were available at local stores from different countries. Electrophoretic mobility (μ_{ep}) values were calculated for all the CE-UV peaks to determine whether flavonoids and phenolic compounds were present in these natural sweeteners. Subsequently CE-UV analysis was performed by adding honeys to the background electrolyte inside the capillary. The electrical conductance was monitored to gauge the overall content of charged compounds, the electroosmotic flow was measured using a neutral marker to assess the total amount of acidic sugars, and the electrophoretic mobility of polydopamine-coated magnetic nanoparticles was calculated to determine any increase in viscosity.

2. Experimental

2.1. Materials

Honeys, corn/table syrups, maple syrups and nectars of different brands, as listed in Table 1, were obtained from local stores in Ottawa (Ontario, Canada). Fructose, glucose and sucrose were obtained

from Sigma-Aldrich (Oakville, ON, Canada) as well as acetic acid (CH_3COOH), dopamine (DA) hydrochloride, disodium hydrogen phosphate (Na_2HPO_4), gallic acid (GA), mesityl oxide (MO), rutin (RUT) and sodium dodecyl sulfate (SDS). All chemicals were used as received without any further purification.

Table 1. *Honeys, syrups and nectars.*

Country of Origin	Notation	Ingredients
Brazil honey	H1	100% pure honey
Canada honey 1	H2	100% pure honey
Canada honey 2	H3	100% pure honey
China honey	H4	100% pure honey
Taiwan honey	H5	100% pure honey
Canada maple syrup 1	M1	100% pure maple syrup
Canada maple syrup 2	M2	100% pure maple syrup
Canada maple syrup 3	M3	100% pure maple syrup
Canada maple syrup 4	M4	100% pure maple syrup
Canada corn syrup 1	C1	Glucose, glucose-fructose, water, salt, vanillin.
Canada corn syrup 2	C2	Glucose, glucose-fructose, refiner's syrup, salt
Canada table syrup 1	T1	Artificial flavor, caramel color, sodium benzoate, sodium carboxy-methylcellulose, sodium hexametaphosphate, sorbic acid, sugar
Canada table syrup 2	T2	Corn syrup, glucose-fructose, water, cellulose gum, caramel color, salt, sodium benzoate, sorbic acid, artificial and natural flavors, sodium hexametaphosphate
Mexico agave nectar	AN	Syrup produced from distillation of Agave plant sap. No animal by-products.

2.2. Polydopamine-Coated Magnetic Nanoparticles

Coating of magnetic nanoparticles (MNPs) with polydopamine (PDA) was adopted from a method previously reported [Iqbal et al. 2012], with some modifications. 2.63 g of MNPs were added into 500 mL of 10 mM Na_2HPO_4 (pH 7.5±0.2) buffer in a 500-mL three-necked round bottom flask equipped with a mechanical stirrer. The mixture was stirred for 2 hours until the nanoparticles were well-dispersed. Then 1.0 g of DA was added and the reaction mixture was stirred for another 3 hours at room temperature. The product was collected with a magnet and washed five times with 3% (v/v) CH_3COOH containing 0.1% (w/v) SDS, and then with DDW. The black MNPs@PDA particles were dried in air for 48 hours.

2.3. Capillary Electrophoresis

CE-UV analysis was performed on a laboratory-built system including a Spellman CZE1000R high voltage power supply (Hauppauge, New York, USA). Fused-silica capillary (51 µm i.d., 356 µm o.d. and 16 µm polyimide coating) was obtained from Polymicro Technologies (Phoenix, AZ, USA). The background electrolyte (BGE) was composed of 20 mM Na_2HPO_4 in deionized distilled water (DDW) to attain pH 8.5±0.2. The capillary was preconditioned by flushing with methanol, 1.0 M HCl, 1.0 M NaOH, DDW and BGE for 15 min each. After extended use of the capillary, it was reconditioned quickly by flushing the capillary with MeOH, 1.0 M HCl, 1.0 M NaOH, DDW and BGE for 3 min each. The capillary was finally equilibrated with the BGE at an applied voltage of 20 kV for 10 min. Each honey, syrup or nectar sample was dissolved in the BGE before electrokinetic injection at 17 kV for 6 s typically to start the CE-UV analysis. A Bischoff Lambda 1010 (Leonberg, Germany) UV detector was employed, at a wavelength of 190 or 200 nm, to detect the migration of food compounds. The detector output signal was acquired through a SRI model 203 Peak Simple chromatography data system (Torrance, CA, USA). The mean migration time determined in triplicates was used to calculate the electrophoretic mobility of each food compound. The capillary inlet and outlet BGEs were changed after every set of CE analyses to maintain purity and level in the vials.

3. Results and Discussion

A new CE-UV method was first developed for the characterization of honeys from different geographic regions of the world. The BGE was optimized by evaluating the tendency of analyte peaks to undergo migration and dispersion. An optimal composition was defined as 20 mM Na_2HPO_4 at pH 8.5. This CE-

UV method showed satisfactory results in terms of rapid analysis time (under 30 min), good separation efficiency, and adequate resolution. **Figure 1a** shows the electropherograms of five honeys from Brazil, Canada, China and Taiwan. Essentially, these honeys showed larger sugar peaks plus a cluster of unresolved negatively-charged peaks (which are believed to be polyphenols and flavonoids) after the migration time of 3.9 min for mesityl oxide. Apparent similarities can be seen between the honeys from China and Taiwan, which in turn are very different than those from Brazil and Canada.

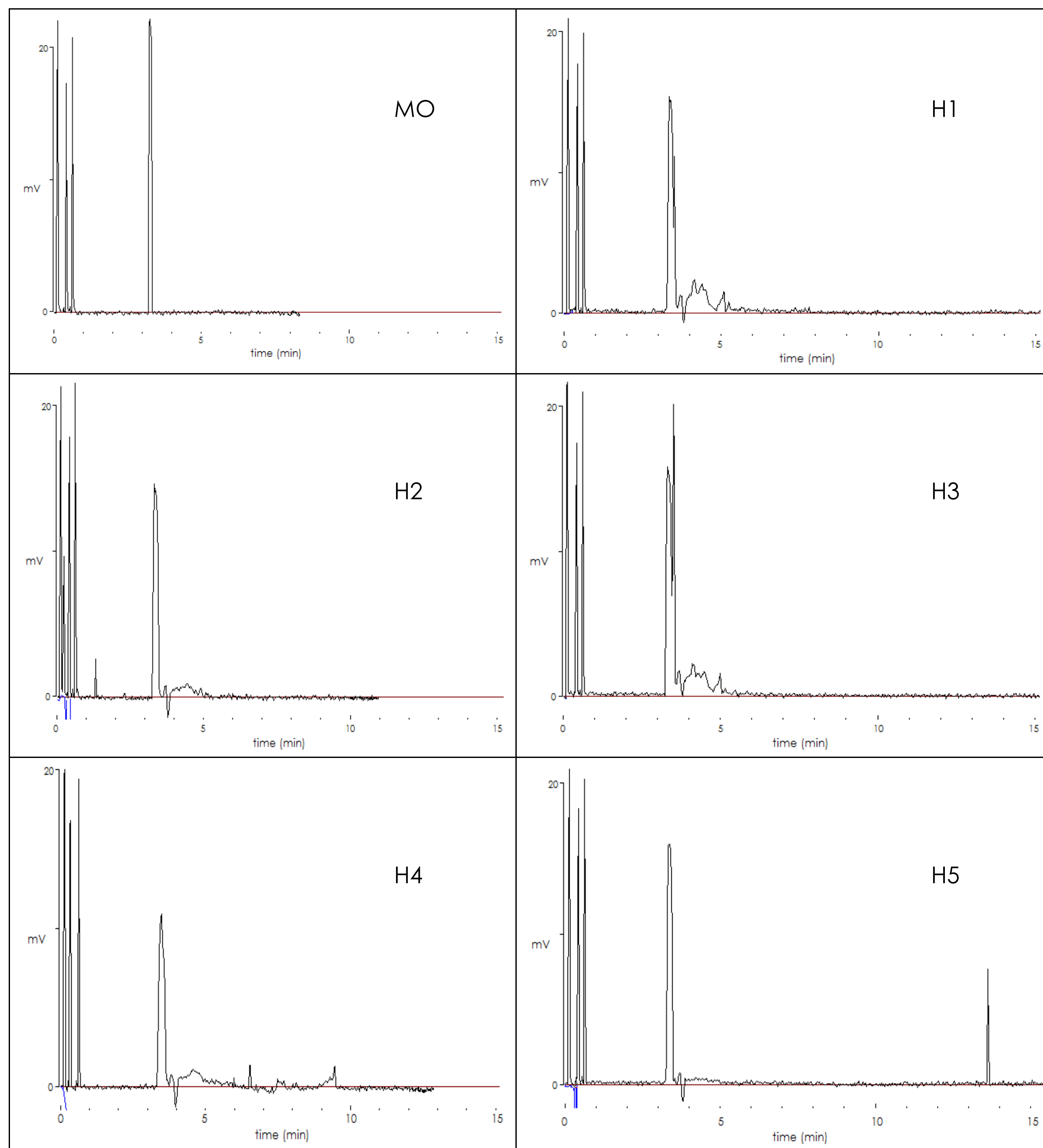
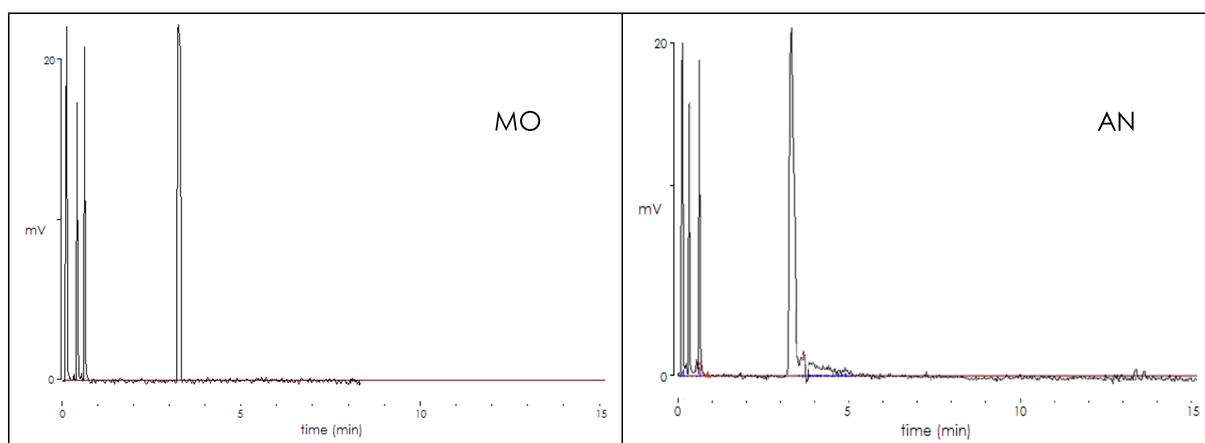


Figure 1a. *CE-UV electropherograms of the neutral marker mesityl oxide (MO) and the five honey samples (H1-5) analyzed at 190 nm. In order from left to right and from top to bottom: MO, H1 (Brazil), H2 (Canada1), H3 (Canada2), H4 (China), and H5 (Taiwan).*

The maple syrup samples (**Figure 1b**) displayed much more predictable results than the honeys. Two very negative, and hence very late, peaks were observed in all four maple samples (M1-4): one peak at the 15-16 minute mark and the other peak around 18-21 minutes. Furthermore, a slightly negative marker could be detected after 7-8 minutes of CE-UV analysis in samples M1, M2 and M4. The early peak aside, the maple samples differentiated themselves from the honeys both by the range and the consistency of their component markers.

Figure 1b. *CE-UV electropherograms of the maple syrup samples analyzed in BGE at 190 nm. In order from left to right and from top to bottom: mesityl oxide (MO) (for comparison), maple syrup1 (M1), maple syrup2 (M2), maple syrup3 (M3) and maple syrup4 (M4).*

The remaining liquid sweeteners exhibited moderate peaks in comparison to the honey and maple syrup samples (**Figure 1c**). Agave nectar was the only sample in the experiment lacking constituent non-carbohydrate components. The table syrup samples T1 and T2 produced several early component peaks (5-8 min), following shortly after the total sugar peak. Similarly corn syrup samples C1 and C2 displayed several neutral or only slightly negative peaks around the 5-min mark, though C2 produced a distinct peak at 14.5 min in defiance of the precedent set by the other artificial liquid sweeteners.



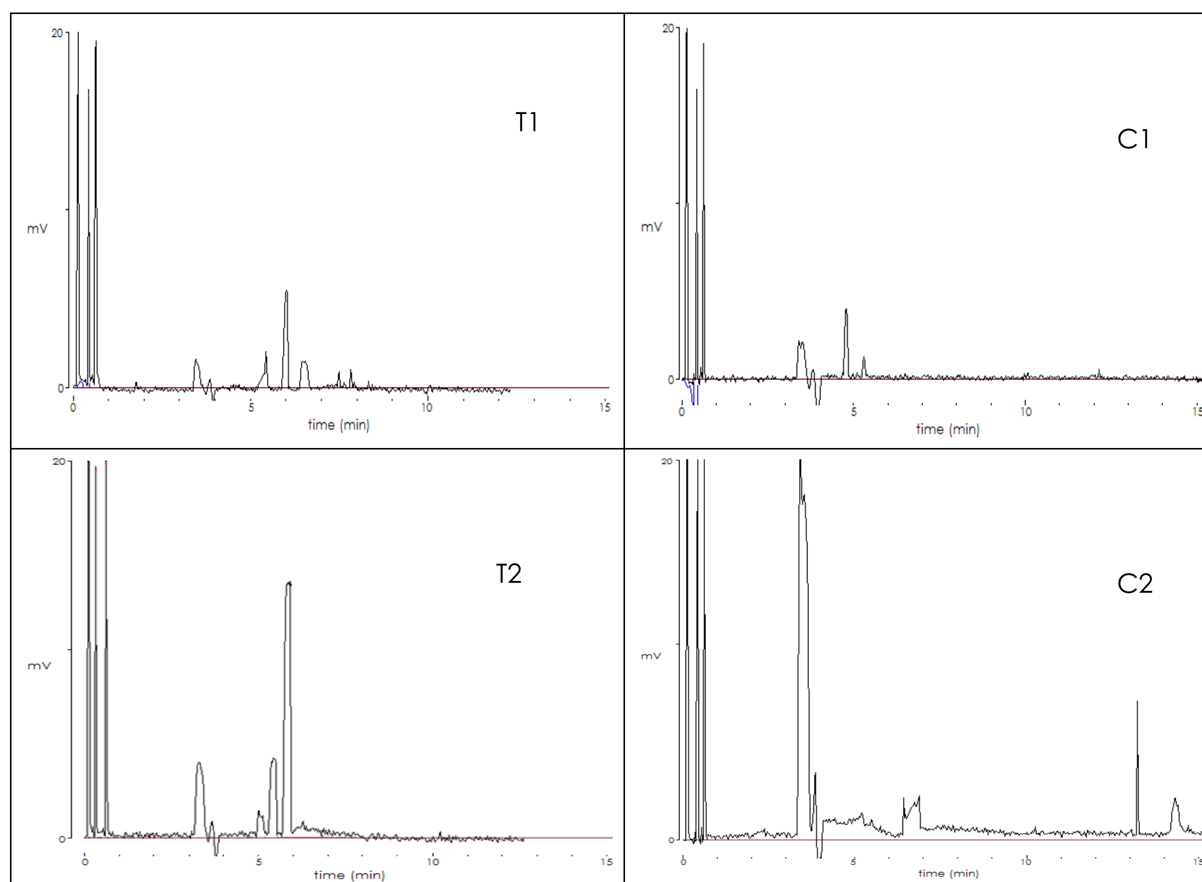
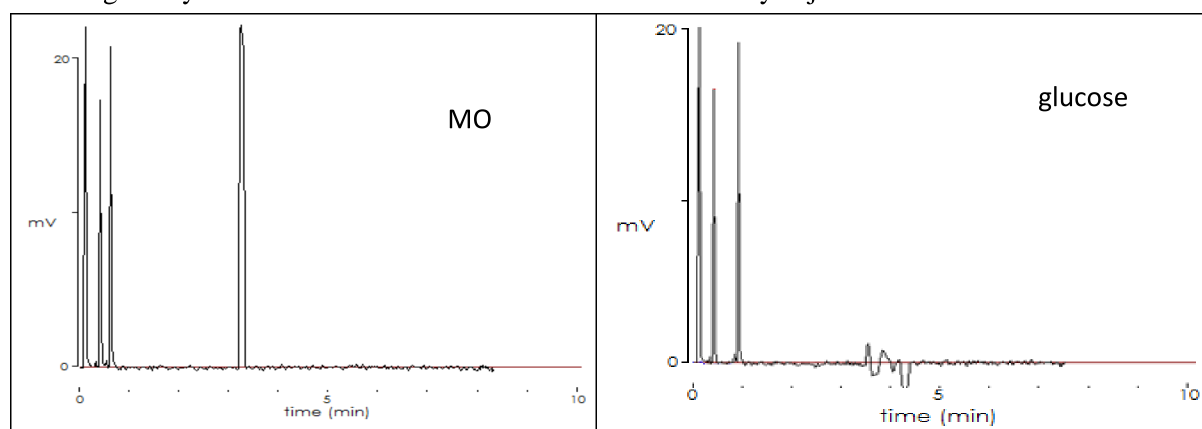


Figure 1c. *CE-UV electropherograms of the agave nectar, corn syrup and table syrup samples analyzed in BGE at 190nm. In order from left to right and from top to bottom: mesityl oxide (MO) (for comparison), agave nectar (AN), table syrup1 (T1), corn syrup1 (C1), table syrup2 (T2) and corn syrup2 (C2).*

Adulterants in honey are normally considered to be glucose, fructose, sucrose, and corn syrup [http://elibrary.asabe.org/abstract.asp?aid=6092&t=2&redir=&redirType=]. Honey gets its sweetness from the monosaccharides (fructose and glucose). Fructose may be considered as a marker sugar in honey since CE supports its analysis with good sensitivity at a UV detection wavelength of 200 nm, but not glucose and sucrose which require 190 nm for their UV detection. However, **Fig. 2** shows mesityl oxide (serving as a neutral marker to determine the electroosmotic flow) and three sugar standards. Obviously CE cannot separate basically neutral compounds such as these three sugars because their migration times (3.30 min, 3.28 min and 3.25 min) were too close. It is commonly known that honey has no defined proportion of sugars (due to different types of flora used to produce it by the bees). Fructose is also found in fruits, flowers, berries and root vegetables. Therefore the real challenge is how to distinguish between adulterant fructose and honey fructose by CE-UV. If not distinguishable, fructose can always be added into corn syrup to imitate honey. Moreover, both fructose and glucose increase in most honeys upon storage over one year [Cavia 2002]. Hence sugars may not be the best marker for detecting honey adulteration as their concentrations can be easily adjusted.



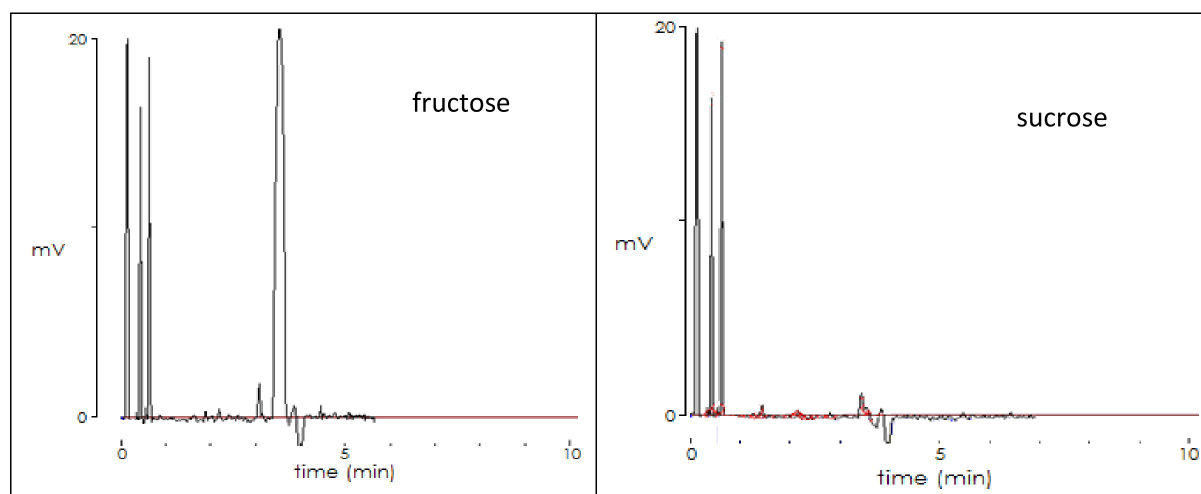
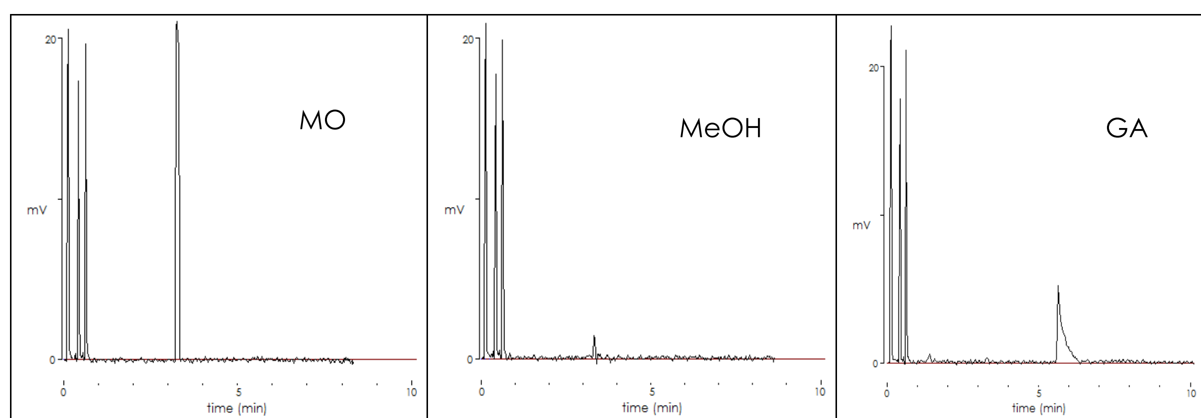


Figure 2. *CE-UV electropherograms of the neutral marker mesityl oxide (MO) and the three sugar standards.*

Fortunately, in stark contrast to honeys, the electropherogram for corn syrup exhibit two distinct negatively-charged peaks at ~4.7 min and ~5.2 min (after the neutral sugar peaks at 3.9 min). Table syrup has three peaks at ~5.4 min, ~5.9 min and ~6.4 min, and Aunt Jemima syrup also has three peaks at ~4.9 min, ~5.4 min and ~5.8 min. It would be great if these negatively-charged peaks can be identified. They may be present in trace amounts but have high molar absorptivity of UV light. However, Bee Hive corn syrup did not exhibit any negatively-charged peaks even though the label showed refiners' syrup in addition to glucose and glucose-fructose. Interestingly, Agave nectar had no negatively-charged peaks but only the total sugar peak. There are two types of HFCS available in the market, namely HFCS-42 and HFCS-55. HFCS-42 contains 42% fructose and 58% glucose, whereas HFCS-55 contains 55% fructose and 45% glucose [Lowndes 2012]. Honey contains a similar proportion of sugars as in HFCS-55. However, the probability of honey being adulterated by HFCS-55 is debatable due to its higher price than regular corn syrup.

The cluster of small negatively-charged peaks (between 4 min and 6 min) was investigated by CE-UV analysis of several major polyphenols and flavonoids. Gallic acid had previously been found in lavender Portuguese honeys by high performance liquid chromatography (HPLC) as a phenolic acid marker of their botanical origin [Andrade 1997]. Rutin, quercetin and other flavonoids are secondary metabolites that are synthesized in higher plants in order to protect them against UV radiation, diseases and predators [Kreft 2003]. The average polyphenol content was determined by Perna et al. (2012) to be ~12 mg gallic acid equivalent (GAE) per 100 g honey and ~8 mg quercetin equivalent per 100 g honey, for total phenolic and flavonoid contents respectively. This seemed like a simple way to sort honeys out from syrups. **Figure 3** shows the CE-UV electropherograms of seven phenolic compounds (gallic acid, rutin, caffeic acid, kaempferol, 5-methoxyflavone, naringenin and quercetin dihydrate), chosen as standards by which to compare and analyze the liquid sweetener component peaks. All seven compounds displayed marker peaks around 5-7 minutes, indicative of low to moderately negative electrophoretic mobility (μ_{ep}) values. Methanol is a neutral compound and was used in this experiment to dissolve and analyze naringenin, quercetin dihydrate and 5-methoxyflavone. It was demonstrated that 5-methoxyflavone produces a cumulative neutral marker peak together with MO.



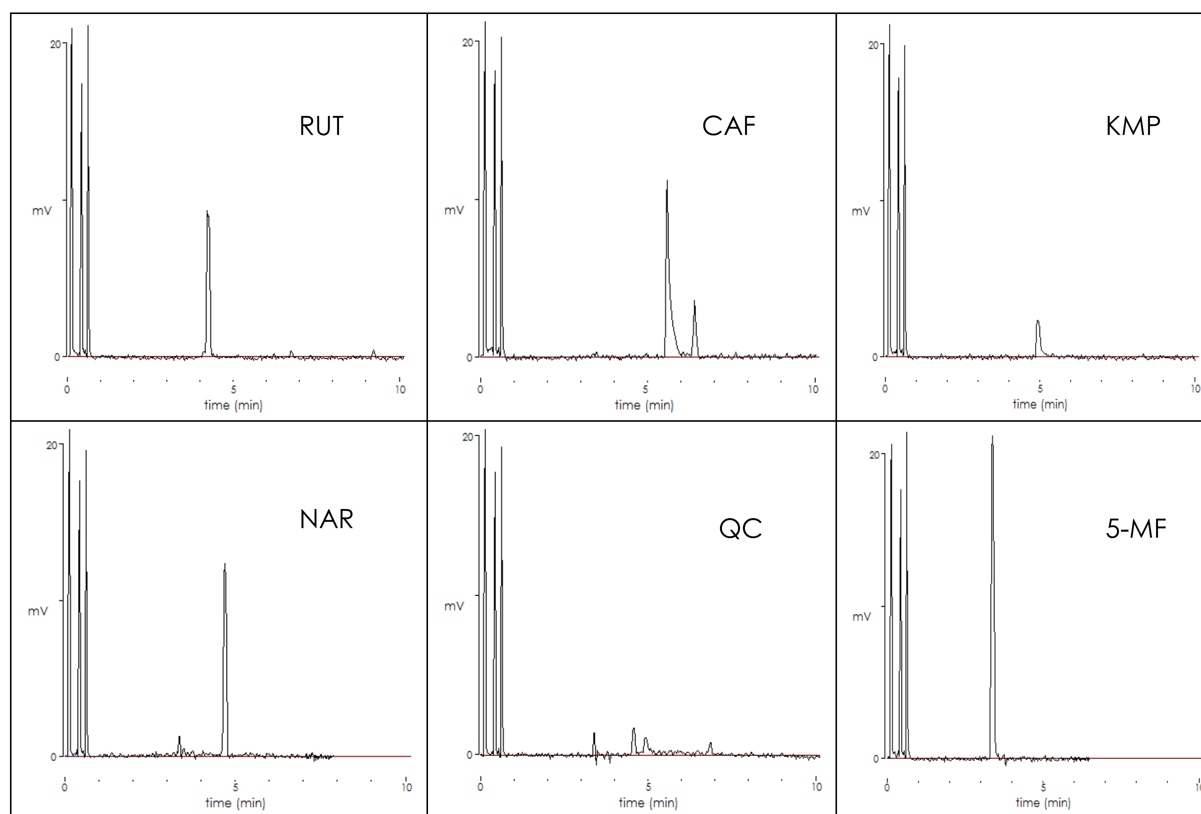


Figure 3. *CE-UV electropherograms of phenolic standards analyzed in BGE at 190 nm. In order from left to right and from top to bottom: mesityl oxide (MO), methanol (MeOH), gallic acid (GA), rutin (RUT), caffeic acid (CAF), kaempferol (KMP), naringenin with 5% MeOH (NAR), quercetin dihydrate with 5% MeOH (QC) and 5-methoxyflavone with 5% MeOH (5-MF).*

As summarized in **Table 2**, the calculated μ_{ep} values help determine whether any of these phenolic standards are found in the liquid sweeteners. Apparently the characteristic peaks for honey compounds have electrophoretic mobility values ($m^2V^{-1}s^{-1}$) in the range from $-1.37x10^{-8}$ to $-1.52x10^{-8}$, corn syrup compounds from $-2.02x10^{-8}$ to $-3.05x10^{-8}$, and maple syrups from $-4.36x10^{-8}$ to $-5.06x10^{-8}$. Spiking honeys with rutin, for instance, confirmed its presence in Brazil honey and Canada honey 2; the peak at 4.09-4.16 min rose above the cluster of small negatively-charged peaks. Phenolic compounds in maple syrups can be as many as 41 (http://pubs.acs.org/doi/abs/10.1021/bk-1992-0506.ch015). UV detection at 190 nm was fairly selective, thereby reducing the number of CE peaks to just the several observed.

Table 2. *Electrophoretic mobility values of the non-carbohydrate constituents in the fourteen liquid sweetener samples (H = honey, C = corn syrup, T = table syrup and M = maple syrup) analyzed by CE-UV in 20 mM Na_2HPO_4 background electrolyte (pH 8.50±0.2). Seven clusters are represented as follows: ✓-#1, ✓-#2, ✓-#3, ✓-#4, ✓-#5, ✓-#6 and ✓-#7.*

Sample: Electrophoretic mobility ($m^2V^{-1}s^{-1}$):	H1	H2	H3	H4	H5	C1	C2	T1	T2	M1	M2	M3	M4
$-1.90 x10^{-8}$				✓									
$-1.95 x10^{-8}$	✓		✓										
$-2.12 x10^{-8}$		✓											
$-2.16 x10^{-8}$ (rutin)													
$-2.42 x10^{-8}$ (naringenin)													
$-2.47 x10^{-8}$										✓			
$-2.49 x10^{-8}$			✓										
$-2.53 x10^{-8}$	✓												
$-2.69 x10^{-8}$ (kaempferol)		✓											
$-2.78 x10^{-8}$							✓						

-3.06 x10^{-8}												✓	
-3.09 x10^{-8}						✓							
-3.15 x10^{-8}			✓										
-3.25 x10^{-8}	✓												
-3.35 x10^{-8}									✓				
-3.36 x10^{-8} (caffeic acid)													
-3.44 x10^{-8}												✓	
-3.64 x10^{-8}						✓							
-3.72 x10^{-8} (gallic acid)													
-3.75 x10^{-8}									✓				
-3.76 x10^{-8}								✓					
-3.84 x10^{-8}							✓						
-3.96 x10^{-8}							✓						
-4.07 x10^{-8}										✓			
-4.12 x10^{-8}				✓									
-4.13 x10^{-8}									✓				
-4.22 x10^{-8}								✓					
-4.24 x10^{-8} (quercetin)													
-4.29 x10^{-8}											✓		
-4.39 x10^{-8}									✓				
-4.41 x10^{-8}													✓
-4.53 x10^{-8}								✓					
-4.78 x10^{-8}				✓									
-5.05 x10^{-8}								✓					
-5.15 x10^{-8}								✓					
-5.35 x10^{-8}					✓								
-5.47 x10^{-8}										✓			
-5.69 x10^{-8}													✓
-5.74 x10^{-8}							✓						
-5.77 x10^{-8}										✓			
-6.02 x10^{-8}													✓
-6.05 x10^{-8}											✓	✓	
-6.34 x10^{-8}												✓	
-6.36 x10^{-8}											✓		

The clustering of component electrophoretic mobility values in **Figure 4** shows a wide array of distinct CE-UV peaks. Seven clusters were deemed appropriate via scree plot analysis and in knowledge of the diverse non-carbohydrate composition of the honeys and maple syrups. The color of each cluster corresponds to a subset of electrophoretic mobility values as depicted in Table 2. While its use is limited, one-sample t-test analysis can indicate a few results of note. Firstly, the true mean of Cluster 1 (-3.79 x10^{-8}) is not shown to differ from the μ_{ep} value of gallic acid (-3.72 x10^{-8}), t (4) = -1.32, p = 0.26. The true mean of Cluster 2 (-3.22 x10^{-8}) also cannot be shown to differ from the μ_{ep} value of caffeic acid (-3.36 x10^{-8}), t (5) = 2.22, p = 0.08. Alternatively, there is at least 95% confidence interval that the μ_{ep} value of rutin (-2.16 x10^{-8}) and the true mean of Cluster 4 (-1.98 x10^{-8}) as well as the μ_{ep} value of naringenin (-2.42 x10^{-8}) and the true mean of Cluster 7 (-2.59 x10^{-8}) both differ, t (3) = 3.74, p = 0.03 and t (4) = -2.83, p = 0.05, respectively. However, a coincidence between the μ_{ep} value of kaempferol (-2.69 x10^{-8}) and the true mean of Cluster 7 cannot be ruled out (t (4) = 1.61, p = 0.18) nor can the μ_{ep} value of quercetin (-4.24 x10^{-8}) be shown to differ from the true mean of Cluster 6 (-4.27 x10^{-8}). Clusters 3 and 5 are far removed from the electrophoretic mobility values of the phenolic standards analyzed in this work.

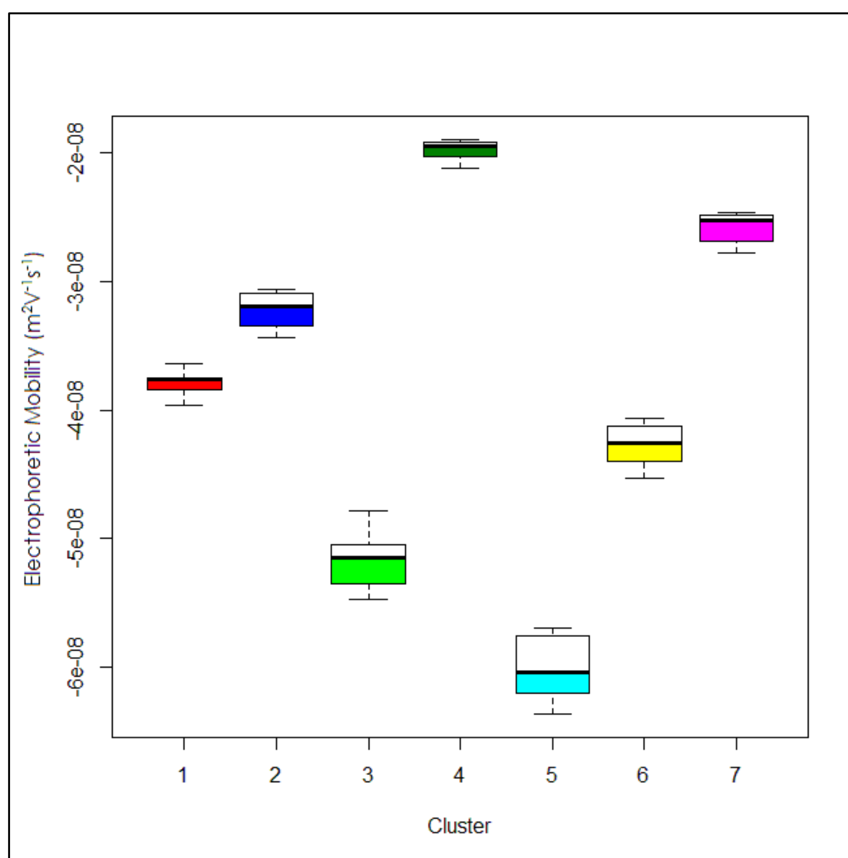
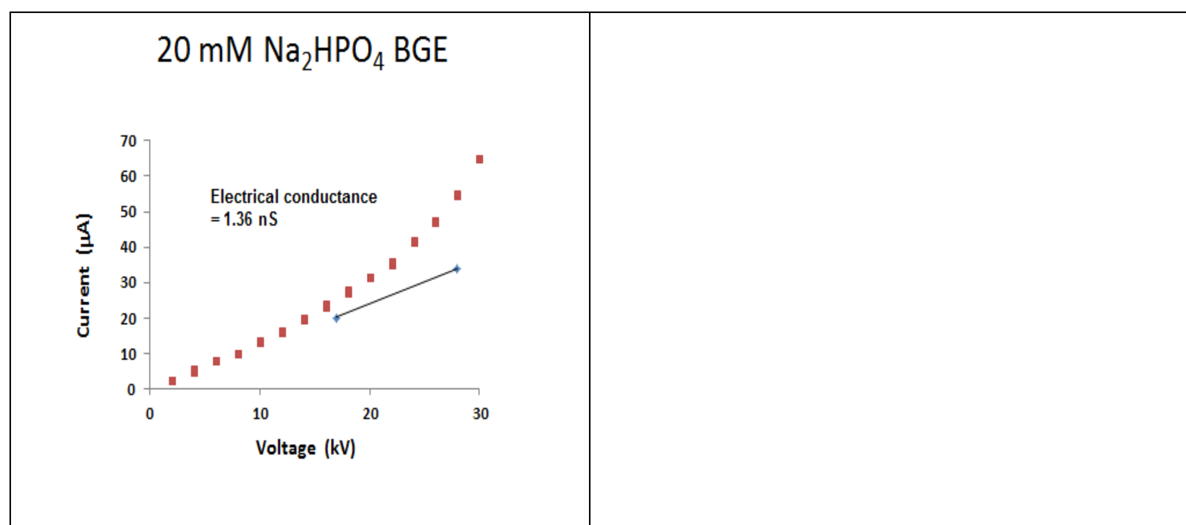


Figure 4. *Clustering of experimental electrophoretic mobility values of liquid sweetener sample component peaks.*

4.1. Novel CE-UV analysis

Conductivity measurements are used routinely in many food applications as a fast, inexpensive and reliable way of measuring the ionic content in a solution [Mitchell 1989]. Hence CE-UV analysis was performed by adding honeys to the background electrolyte inside the capillary and the electrical conductance was monitored to gauge the overall content of charged compounds. **Figure 5** shows the current versus voltage plots for the BGE before and after spiking with two different honeys (5 g in 20 mL). An electrical conductance (G) was determined from the slope of each straight trend line under 12 kV. In comparison with the original BGE, spiking with honey (Canada and China) decreased the G value significantly by over 30%.



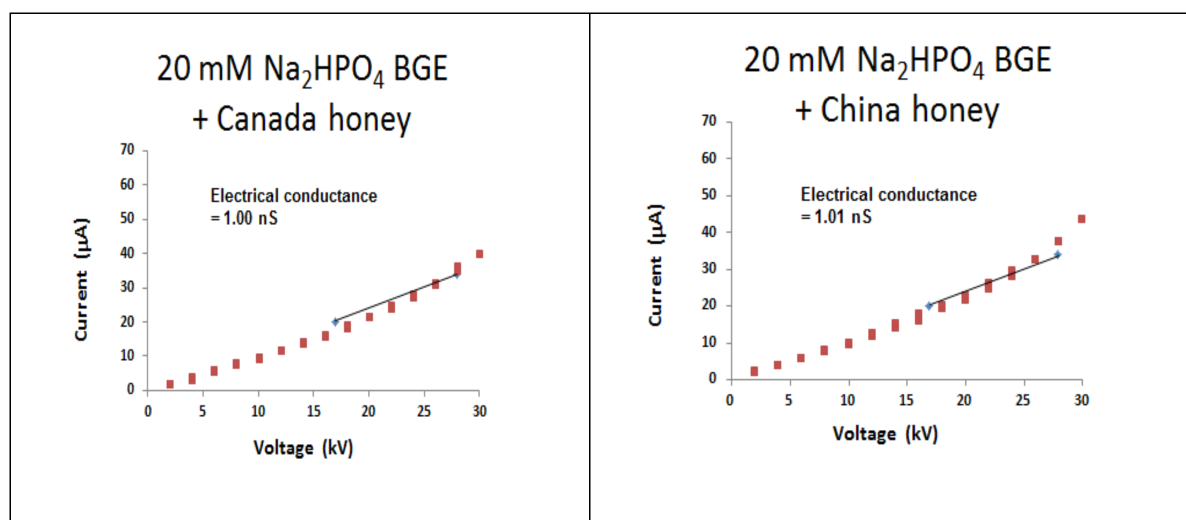


Figure 5. *Current versus voltage plots for BGE before and after spiking with five different honeys. Reference line is included for visual comparison with experimental data points.*

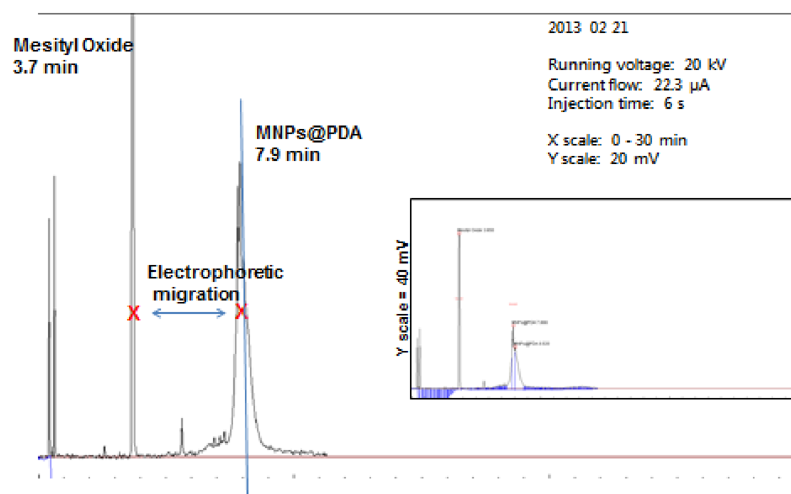


Figure 6. Electropherogram of mesityl oxide and polydopamine-coated magnetic nanoparticles using 20 mM Na_2HPO_4 (pH 8.5±0.2) as background electrolyte.

CE is an instrumental method that can separate analytes on the basis of their difference in charge and size, inside a fused silica capillary filled with BGE, under a high electric field. The capillary allows for a high loading capacity of nanoparticles in aqueous suspension for electrophoretic analysis. **Figure 6** shows a typical CE-UV analysis of MO and MNPs@PDA using 20 mM Na_2HPO_4 (pH 8.5±0.2) as BGE. The electroosmotic flow (EOF) and electrophoretic mobility (μ_{ep}) were determined from their mean migration times in triplicate analysis. After honeys were spiked in the BGE, both the EOF as indicated by a neutral marker and the $\mu_{ep\ p}$ value of polydopamine-coated magnetic nanoparticles were decreased by approximately 50% due to an increase of the viscosity, as summarized **Table 4**. The physical properties of honey vary with temperature (depending on water content), the type of flora used to produce it and the proportion of specific sugars it contains. At room temperature, honey is a super cooled liquid in which the glucose will precipitate into solid granules. This forms a semisolid of precipitated glucose crystals in a solution of fructose and other ingredients [http://en.wikipedia.org/wiki/Honey#cite_note-Prescott_1999-3]. Honeys that are supersaturated with a very high percentage of glucose will crystallize almost immediately after harvesting, while honeys with a low percentage of glucose do not crystallize. The above CE-UV results were obtained after the BGE spiked with each honey was filtered through a 0.22-μm PVDF membrane. If filtration was omitted, numerous noise peaks would appear randomly in the electropherogram as illustrated in **Figure 7**. Gas bubbles (probably due to fermentation) and nucleation crystallites are two main reasons for those noise peaks. Induction times for onset of nucleation and nucleation rates had previously been determined for amorphous sugar matrices made with sucrose and corn syrup held at temperatures between 60 and 110°C [Levenson 2005]. Both the classical nucleation rate equation and the Williams–Landel–Ferry

(WLF) equation fit the data. Within the measurement variability, the type of corn syrup used did not cause a significant difference in either the induction times or nucleation rates. The kinetic barrier to nucleation was determined to be −76.7 to −127.2 kJ/mole, which was 4–6 times higher than the activation energy for translational diffusivity of sucrose. These results suggested that rotational diffusivity of sucrose and the interaction between corn syrup and sucrose also must play an important role in nucleation.

Table 4. *Electrophoretic mobility results of polydopamine-coated magnetic nanoparticles in 20 mM Na_2HPO_4 background electrolyte (pH 8.5±0.2) before and after spiking with different honeys, syrups and nectars.*

Honey spiked into 20 mM Na_2HPO_4 BGE (5 g / 20 mL)	Mesityl Oxide Migration Time (min)	Electroosmotic Flow ($m^2V^{-1}s^{-1}$)	MNPs@PDA Migration Time (min)	MNPs@PDA Electrophoretic Mobility ($m^2V^{-1}s^{-1}$)
Nil	4.2 ± 0.1	$4.8x10^{-8}$	8.2 ± 0.2	$-2.3x10^{-8}$
Nil	3.7 ± 0.1	$5.6x10^{-8}$	7.9 ± 0.2	$-3.0x10^{-8}$
Canada (filtered)	6.9 ± 0.1	$2.9x10^{-8}$	14.5 ± 0.2	$-1.5x10^{-8}$

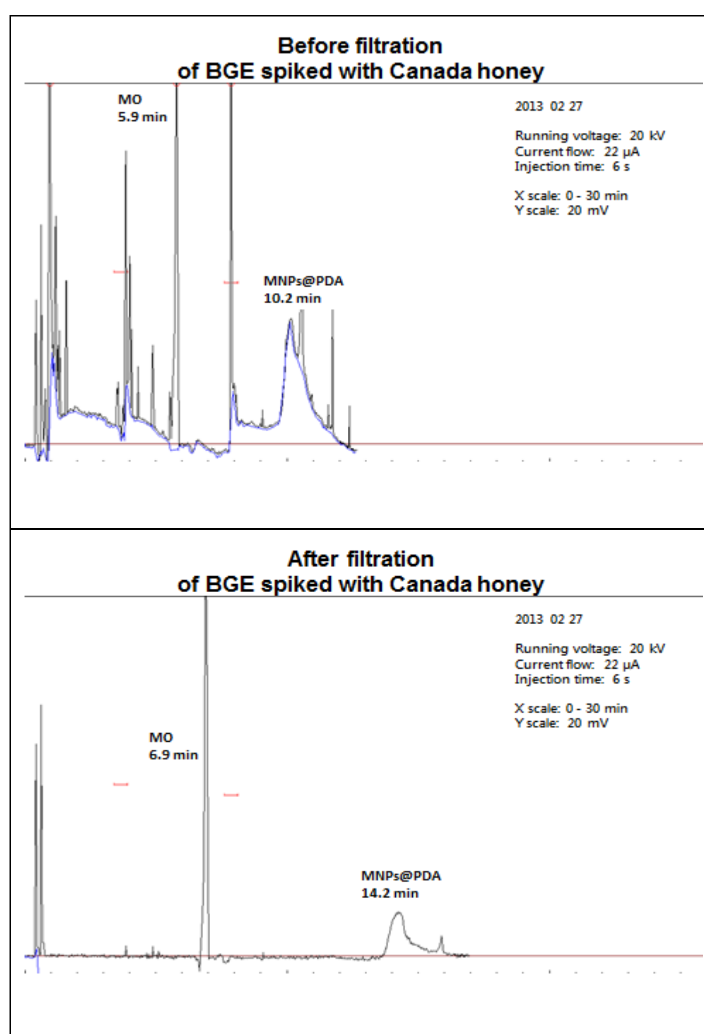


Figure 7. *Electropherograms of mesityl oxide and polydopamine-coated magnetic nanoparticles using 20 mM Na_2HPO_4 (pH 8.5±0.2) spiked with Canada honey as background electrolyte, before and after filtration through 0.22 mm PVDF membrane.*

CONCLUSION

CE-UV was evaluated in this work as a simple analytical technique that can rapidly screen honey, corn/table syrups, maple syrups and nectar to distinguish them from one another. Adulterated honey is not good for our health as the overall nutrients in honey are reduced and the adulterant fructose is just a sweet poison. Our effort was particularly focused on sugars, phenolic acids and other negatively-

charged compounds (flavonoids and polyphenols) which can easily be characterized by their electrophoretic mobility values. CE-UV analysis can be performed in a novel way by spiking honeys in the background electrolyte. The electrical conductance, the electroosmotic flow and the electrophoretic mobility of polydopamine-coated magnetic nanoparticles all change their values to demonstrate that CE-UV is a promising technique for the rapid screening of natural liquid sweeteners. Our future work will include the development of a more sensitive detector, based on laser-induced fluorescence, for the quantification of trace adulterants after their CE separation.

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