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Development of a fast capillary electrophoresis method for determination of carbohydrates in honey samples

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ABSTRACT

In this study, the determination of fructose, glucose and sucrose by capillary electrophoresis (CE) was investigated. The tendency of the analyte to undergo electromigration dispersion and the buffer capacity were evaluated using the Peakmaster® software and considered in the optimization of the background electrolyte, which was composed of 20 mmol L^{-1} sorbic acid, 0.2 mmol L^{-1} CTAB and 40 mmol L^{-1} NaOH at pH 12.2. Under optimal CE conditions, the separation of the substances investigated was achieved in less than 2 min. The detection limits for the three analytes were in the range of 0.022 and 0.029 g L^{-1} and precision measurements within 0.62–4.69% were achieved. The proposed methodology was applied in the quantitative analysis by direct injection of in honey samples to determine the main sugars presents. The samples were previously dissolved in deionized water and filtered with no other sample treatment. The mean values for fructose, glucose and sucrose were in the ranges of 33.65–45.46 g 100 g^{-1}, 24.63–35.06 g 100 g^{-1} and <0.22–1.32 g 100 g^{-1}, respectively. The good analytical performance of the method makes it suitable for implementation in food laboratories for the routine analysis of honey samples.

1. Introduction

Honey is a natural food produced by honey bees from the nectar of a variety of plants. It is an aqueous supersaturated sugar solution that also contains a complex mixture of other minor substances, such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals [1,2]. The composition and the quality of honey varies mainly according to the botanical and geographical origin, but is also influenced by the environmental, processing and storage conditions [2].

Sugars are the main components of honey, representing around 95 g/100 g dry matter [2,3]. The reducing sugars fructose and glucose are the major constituents, and disaccharides, trisaccharides and other oligosaccharides are present in honey in small concentrations [3,4]. It has been verified that adulterated honey obtained from the deliberate addition of sucrose syrups or from bees feeding on sugars and syrups is available on the market. In this context, the determination of fructose, glucose and sucrose is a common approach to describing the quality of honey [2,5].

Sugar composition can be determined by different methods. The most commonly used analytical technique is high performance liquid chromatography (HPLC) with refractometric detection [6,7]. Other methods employed are HPLC with pulsed amperometric detection [8], ion exchange chromatography with pulsed amperometric detection [9], gas chromatography with flame ionization detection [3,10,11], gas chromatography–mass spectrometry [12], Fourier transformed infrared spectroscopy [13,14], dispersive Raman spectroscopy [15] and enzymatic tests [16,17]. As an alternative technique, capillary electrophoresis (CE) has been occasionally selected for the determination of sugars in honey [5] and also in other food products including juice, yogurt, apricots, wine, rice and beverages [18–23].

Capillary electrophoresis is a powerful separation technique that can provide high resolution efficiency and is becoming a standard tool for the analysis of many compounds [18]. There are many advantages associated with CE compared with other analytical techniques, for instance, the ultra-small sample volume, low consumption of solvents, low time of analysis, high-resolution separation, and minimal sample preparation [5,19]. The aim of our study was to design a rapid method for the determination of the sugars fructose, glucose and sucrose in honey samples, using a CE methodology. The method was validated and then applied in the determination of these sugars in honey samples of different geographical origin. No reports of a method faster than that presented in this paper using CE could be found in the literature.

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2. Experimental

2.1. Materials

Sorbic acid and cetyl trimethylammonium bromide (CTAB) were purchased from Sigma Aldrich (Santa Ana, CA, USA). Sodium hydroxide (NaOH), D-(+)-glucose monohydrate, D-fructose, and sucrose were obtained from Merck (Rio de Janeiro, RJ, Brazil). All reagents were of analytical grade, and the water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA).

Stock solutions of fructose, glucose and sucrose were prepared daily, stored at 4 °C, and diluted with ultra pure water to give the concentrations required for the CE experiments. In the indirect analysis of the three carbohydrates an optimal background electrolyte (BGE) was used, composed of 20 mmol L^{-1} sorbic acid, 0.2 mmol L^{-1} CTAB and 40 mmol L^{-1} NaOH at pH 12.2.

2.2. Instrumentation

CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 254 nm (indirect detection, with a reference at 360 nm for peak inversion), a temperature control device maintained at 25 °C and an acquisition and data treatment software program supplied by the manufacturer (HP ChemStation, rev. A.06.01). Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 60.0 cm total length, 8.5 cm effective length, 50 μm inner diameter and 375 μm outer diameter were used. At the beginning of each day, the capillary was conditioned by flushing with 1 mol L^{-1} NaOH (10 min) followed by a 10 min flush with deionized water and an electrolyte solution (15 min). In between runs, the capillary was reconditioned with the BGE solution (2 min flush). At the end of each working day, the capillary was rinsed with 1 mol L^{-1} NaOH (5 min) and water (10 min) and then dried in air (2 min). Standard solutions and samples were introduced at the extremity of the capillary nearest the detector and injected hydrodynamically (at 50 mbar for 3 s; 1 mbar = 100 Pa) with negative pressure. The applied separation voltage was 25 kV with negative polarity at the injection end.

2.3. Samples

The proposed method was applied to seven multifloral honey samples, obtained from local producers, and their collection was organized through a state government research center called Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI). The honey samples were harvested in November and December 2010, from different locations across the state of Santa Catarina and stored at ambient temperature.

Honey samples were accurately weighed (2.5 g), dissolved in deionized water in a 50 mL volumetric flask and the volume was properly completed. The honey sample solution was filtered through 0.45 μm membrane filters (Millipore, Bedford, MA, USA) and dissolved in the proportion of 1:10 (v/v) in deionized water. This solution was directly injected into the CE equipment with no other sample treatment previously applied.

2.4. Method validation

In order to validate the proposed analytical method the following validation parameters were evaluated: linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

Analyte standard solutions at six different concentration levels were prepared and injected in triplicate, in three independent runs. The peak areas of fructose, glucose and sucrose were plotted against concentration to construct the calibration curves. In order to examine the linearity, the least squares procedure was employed to obtain the coefficient of determination of the curves.

The LOD and LOQ of the honey samples were determined considering the concentrations at which the peak responses were 3 and 10 times the average noise level, respectively.

The system suitability in terms of precision was tested by means of twenty consecutive injections of the standard mixture, through the percentage relative standard deviation (%RSD). Both intra-day and inter-day precisions were determined. Six separate solutions were prepared at each concentration level. Electropherograms were obtained on the same day to assess the intra-day precision ($n = 6$) and over a period of 3 days (6 injections/day) to assess the inter-day precision ($n = 18$). The results were expressed as the %RSD.

The accuracy, expressed as the percentage recovery, was determined for one honey sample using the standard addition method, at three levels for each carbohydrate, and each measurement was carried out in triplicate. The results obtained for the spiked honey sample were compared to those with no addition of standards, and the percentage recovery was calculated.

3. Results and discussion

3.1. Method development

To analyze carbohydrates by CE the BGE solution needs to provide a highly alkaline medium, with a pH value above 12, in order to ionize the hydroxyl groups ($pK_a \approx 12$) present in the sugars [18,23]. Moreover, since carbohydrates lack any strong chromophore in the UV region available in the equipment (190–600 nm), indirect detection of UV absorption by adding a UV absorbing chromophore with a high molar absorptivity into the background electrolyte is preferred.

Considering these aspects, sorbate was selected as the co-ion because it is a strong chromophore with a mobility similar to that of fructose, glucose and sucrose, which is of interest in terms of reducing the electromigration dispersion (EMD). The EMD phenomenon for strong electrolytes can be described by models that are principally based on the difference between the effective mobility of the analyte and that of its co-ion in the BGE [24]. The concentration of sorbate selected was 20 mmol L^{-1}, based on the fact that higher concentrations led to reduced electroosmotic flow and increased run time while lower concentrations promoted a desirable shorter run time at the price of inferior resolution.

Sodium hydroxide was selected as the counter-ion in order to provide a pH value above 12. The ideal concentration of sodium hydroxide was chosen with the assistance of Peakmaster® software, developed by Gaš and co-authors [24–26]. This software also allows the EMD values, the formation of system zones, the buffer capacity and the BGE conductivity to be verified. Fig. 1, which was constructed using Peakmaster® software, shows the effective mobility curves, EMD, buffer capacity and conductivity for a BGE with a constant sorbic acid content of 20 mmol L^{-1} and variable sodium hydroxide content of 25–75 mmol L^{-1}, which generated different pH values.

The satisfactory separation condition was determined with a BGE comprised of 20 mmol L^{-1} sorbate and 40 mmol L^{-1} NaOH, at pH 12.2. At this pH value, the buffer capacity is sufficient, and the EMD value is low enough to ensure a symmetric peak. Moreover, higher pH values did not improve the critical pair resolution (fructose/glucose) and increased the BGE conductivity, which results in higher current values. To support a co-electroosmotic separation, the addition of 0.2 mmol L^{-1} of the cationic surfactant CTAB was sufficient to revert the electroosmotic flow [27].

Once the co-ion, counter-ion and flow reversal surfactant had been selected, other CE parameters, including tension and

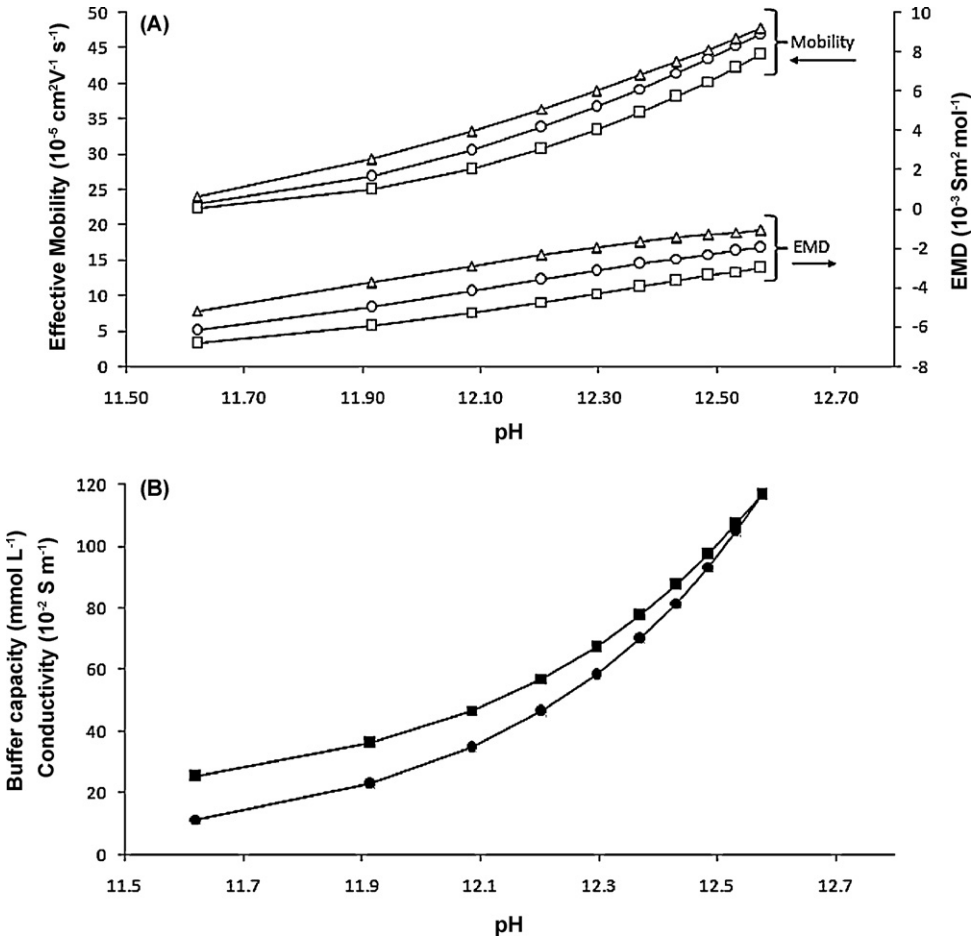

Fig. 1. Optimization of the BGE pH and composition using Peakmaster® software. Conditions: constant concentration of 20 mmol L^{-1} sorbic acid and NaOH varying from 25 to 70 mmol L^{-1}, which generates the pH values show in the figure (axis *x*). (A) Effective mobility and EMD *versus* pH curves for all analytes. Arrows indicate the *y* axis for each parameter. (B) BGE buffer capacity and conductivity *versus* pH curves. Legends: (△) fructose; (○) glucose; (□) sucrose; (■) conductivity; (●) buffer capacity.

capillary length, were also optimized using Peakmaster® software. An experimental electropherogram of the fructose, glucose and sucrose standard mixture under the optimized conditions is shown in Fig. 2, where it can be observed that the separation of components of the substance investigated was achieved in less than 2 min.

3.2. Method validation

3.2.1. Linearity

After the optimization of analytical conditions, the linearity was studied. The analytical curves were constructed from five standard solutions in the concentration range of 0.180–3.603 g L^{-1} for fructose and sucrose and 0.171–1.172 g L^{-1} for sucrose, with a triplicate injection at each concentration level. A linear relationship between the peak area and concentration of the analyte was obtained with a satisfactory coefficient of determination ($R^2 > 0.999$) and intercepts close to the origin. The linear range, slope, intercept, and coefficients of determination for all analytes are summarized in Table 1.

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ)

Signal to noise ratios (S/N) of 3 and 10 were considered to estimate the LOD and LOQ, respectively. LOD and LOQ values for the honey samples were, respectively, 0.026 and 0.088 g L^{-1} for fructose, 0.029 and 0.097 g L^{-1} for glucose and 0.022 and 0.074 g L^{-1} for sucrose (Table 1).

3.2.3. Precision

The precision of the injection system was examined by performing twenty consecutive injections of the same solutions using 1.801 g L^{-1} of fructose, 1.801 g L^{-1} of glucose and 0.685 g L^{-1} of sucrose. All determinations were carried out on the same day and under the same experimental conditions. The electropherograms were evaluated considering the migration time and peak area values. The RSD values for the migration time and peak area were

Table 1
Method validation in terms of linearity, limit of detection (LOD) and limit of quantification (LOQ).

Analyte	Linear range[a]	Regression[b]	R^2	LOD[a]	LOQ[a]
Fructose	0.180–3.603	$y = 80.3969x - 4.9679$	0.9993	0.026	0.088
Glucose	0.180–3.603	$y = 77.1357x - 5.1156$	0.9995	0.026	0.097
Sucrose	0.171–1.172	$y = 52.5345x - 1.4397$	0.9997	0.022	0.074

[a] Values expressed in g L^{-1}.
[b] The *x* value is the concentration of analytes (g L^{-1}), the *y* value is the peak area (mAU).

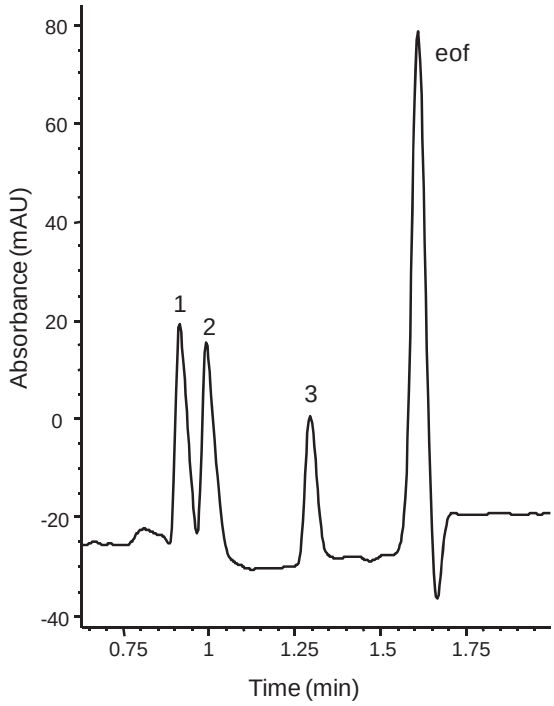

Fig. 2. Electropherogram of standard solution containing fructose (1), glucose (2) and sucrose (3). Separation conditions: 20 mmol L^{-1} sorbic acid, 0.2 mmol L^{-1} CTAB and 40 mmol L^{-1} NaOH, at pH 12.2; injection at −50 mbar for 3 s; applied voltage, +25 kV; capillary 60 cm (L_{tot}) × 8.5 cm (L_{det}) × 50 μm (i.d.); 25 °C; indirect detection (sorbic acid) at 254 nm. eof – electroosmotic flow.

all below 5%, as shown in Table 2, which indicates the acceptable instrumental precision of the method.

The repeatability (intra-day precision) was established by performing six consecutive injections of 1.801 g L^{-1} of fructose, 1.801 g L^{-1} of glucose and 0.685 g L^{-1} of sucrose. The RSD values obtained for the migration time and the peak area values were between 0.62 and 3.87% RSD. Intermediate precision (inter-day precision) was established through 6 injections of a standard solution, on 3 different days. The results ranged from 1.06 to 3.58% RSD (Table 2). The RSD values obtained indicate an acceptable level of inter-day and intra-day precision.

3.2.4. Accuracy

The method accuracy was investigated by analyzing three different final concentrations of each sugar added to the honey samples: 0.901, 1.801 and 2.702 g L^{-1} for fructose and glucose and 0.342, 0.685 and 1.027 g L^{-1} for sucrose. The recovery ranged from 99.53 to 109.02% for fructose, 98.98 to 107.14% for glucose and 96.20 to 108.15% for sucrose, demonstrating the good reliability of the method for the analysis of these sugars in honey samples (Table 3).

Table 2
Precision validation parameters.

Parameter	Value (RSD %)		
	Fructose	Glucose	Sucrose
Instrumental precision (n = 20); peak area	4.66	2.86	4.69
Instrumental precision (n = 20); migration time	0.66	0.70	1.29
Intra-day precision (n = 6); peak area	3.87	2.43	2.02
Intra-day precision (n = 6); migration time	0.69	0.62	0.93
Inter-day precision (n = 18); peak area	1.06	2.76	3.58
Inter-day precision (n = 18); migration time	2.06	2.93	3.50

Table 3
Results of recovery test.

Analyte	Concentration added (g L^{-1})	Concentration found (g L^{-1})	Recovery (%)	RSD (%)
Fructose	0.901	0.899	99.53	1.62
	1.801	1.845	102.45	1.88
	2.702	2.946	109.02	2.23
Glucose	0.901	0.892	98.98	1.52
	1.801	1.692	93.83	2.09
	2.702	2.895	107.14	1.83
Sucrose	0.342	0.341	99.85	1.48
	0.685	0.659	96.20	1.78
	1.027	1.111	108.15	2.38

3.3. Analysis of honey samples

The proposed method, after being optimized and evaluated in terms of the parameters described above, was successfully applied to determine the main carbohydrates in honey samples (n = 7), which were prepared as indicated above. All analyses were carried out in triplicate. The sample concentrations of fructose, glucose and sucrose are given in Table 4. A typical electropherogram for sample F is shown in Fig. 3.

The quantitative analysis of the honey samples revealed that fructose is the sugar present in highest concentrations (33.65–45.46 g 100 g^{-1}) followed by glucose (22.34–35.39 g 100 g^{-1}). The proportion of these sugars in honey is largely dependent on the source of the nectar, and the fructose:glucose ratio may have an impact on the honey flavor since fructose is much sweeter than glucose [2]. Furthermore, honey with a high fructose:glucose ratio remains fluid for longer periods, because fructose has higher solubility than glucose. In fact, it was observed that sample F, with

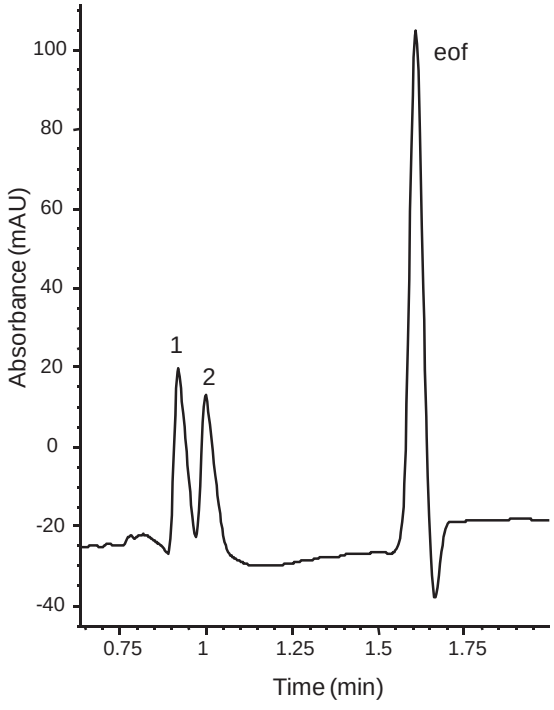

Fig. 3. Electropherogram of honey sample F. Peak identification: (1) fructose, (2) glucose, eof – electroosmotic flow. Separation conditions: see Fig. 2.

Table 4
Amounts of fructose, glucose and sucrose (g/100 g) in honey samples analyzed.

Sample	Fructose	Glucose	Fructose + glucose	Fructose/glucose	Sucrose
A	38.93 ± 0.61	34.98 ± 0.29	73.91	1.11	<LOD
B	45.46 ± 0.69	22.34 ± 0.48	67.80	2.03	<LOQ
C	38.56 ± 0.83	26.90 ± 0.33	65.47	1.43	<LOQ
D	33.21 ± 0.44	26.51 ± 0.03	59.72	1.25	1.32 ± 0.01
E	40.22 ± 1.00	32.47 ± 0.67	72.69	1.24	0.89 ± 0.01
F	33.65 ± 0.69	35.39 ± 0.30	69.04	0.95	<LOQ
G	39.33 ± 0.74	32.34 ± 1.36	71.68	1.22	0.91 ± 0.01

Data are mean ± SD of triplicate measurements.

the lowest fructose:glucose ratio, showed a high level of crystallization, while all other samples were completely fluid.

The determination of sucrose content is useful in detecting the adulteration of honey by the addition of syrups [2]. However, a high sucrose concentration in honey can also reflect the early harvesting of the honey, because the sucrose has not been fully transformed into glucose and fructose by the action of invertase [4]. The mean percentage considering sucrose of all of the honey samples was below the proposed maximum allowable limit of 6 g/100 g [28].

4. Conclusions

In this study the fructose, glucose and sucrose contents of honey samples were determined by CE. The results showed that this method is of high efficiency, involving a short analysis time, low-cost separation with minimum consumption of reagents and simple sample preparation. Under the optimum conditions, the three carbohydrate compounds were completely separated within 2 min and the method provides good linearity, reproducibility and detection limits.

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