



Capillary electrophoresis method for the simultaneous determination of carbohydrates and proline in honey samples



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ABSTRACT

A capillary electrophoresis method with indirect UV detection for the simultaneous determination of three carbohydrates (fructose, glucose and sucrose) and the amino acid proline in honey samples was developed. This method included the use of a background electrolyte consisting of 10 mM sodium benzoate and 1.5 mM cetyltrimethylammonium bromide, pH 12.4. Under optimal capillary electrophoresis conditions, the separation of the investigated substances was achieved in less than 5 min and single dilution of each sample was employed. The detection limits for fructose, glucose and sucrose were 0.58 g L^{-1} , 0.67 g L^{-1} and 0.12 g L^{-1} respectively, and 0.72 mg L^{-1} for proline. Precision measurements calculated in terms of %RSD in the range of 0.92 to 5.43%, were obtained. The proposed method was applied to honey samples from Argentina and Sweden and enables the determination of the three carbohydrates and the amino acid proline. The results show that the proposed method is simple, requires short analysis times, low consumption of reagents and sample, minimum waste and that there is no need to perform any sample pre-treatment. This method is a good alternative to carry out the quality control of honey samples. Finally, it is a promising methodology for achieving green chemistry goals.

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1. Introduction

The composition of honey not only varies according to the botanical origin, but is also influenced by the environmental, processing and storage conditions [1]. Botanical origin depends on the geographical region where the bee collects the pollen [2]. In order to evaluate the quality of honey related to maturity, the content of reducing sugars (fructose and glucose), sucrose and proline must be determined. The sugar content of honey, besides contributing to nutritional and energetic value affects the physical characteristics of the product. For example, the crystallization phenomenon in honey is due to the lower solubility of glucose [3]. Moreover, due to the presence of glucose oxidase, glucose is turned into gluconic acid and this affects the acidity of the product. The authenticity of the honey may be associated with the fructose/glucose ratio, and a value less than one, may indicate adulteration or yeast proliferation [4]. Thus, the determination of fructose, glucose and sucrose is used to describe the quality and authenticity of honey, considering that the deliberate addition of sucrose or fructose syrups provides adulterated honey [5]. Proline is the predominant free amino acid from which the total amino acid content can be approximated [6]. The proline content of honey is measured as a criterion for estimating the quality, and in

some cases also as a criterion for estimating the maturity of honey as well as an indicator for detecting sugar adulteration [5].

Codex Alimentarius [7] establishes a minimum of 60% (w/w) for monosaccharides and a maximum of 5% (w/w) for sucrose. Furthermore, genuine honeys must contain a minimum of 180 mg proline/kg honey, but this value may vary depending on the type of honey [8]. Código Alimentario Argentino [9] establishes a minimum of 65% (w/w) for monosaccharides and a maximum of 8% (w/w) for sucrose, but does not provide values for proline.

The determination of carbohydrates and proline in honey samples by official methods required large amounts of reagents and long analysis time. Fehling Causse Bonnans method is employed to quantifying reducing sugar [10]. Besides, the determination of proline is carried out spectrophotometrically at 520 nm after generating the complex formed with ninhydrin, by the addition of 2-propanol [11].

On the other hand, the determinations of these analytes are carried out using different methods, such as high resolution liquid chromatography with pulse amperometric detection [12] or with fluorimetric detection [13]. Gas chromatography [14], Fourier transform infrared spectroscopy [15] and liquid chromatography with UV detection and mass spectrometry [16] are also used. However, these methods require long analysis time, sample pretreatment, large amounts of reagents and causes the consequent generation of large amounts of waste.

As an alternative technique, capillary electrophoresis (CE) has been used for the determination of carbohydrates in honey and also in

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other food products. CE is a powerful separation technique, with many advantages compared to 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. Several works employ CE with indirect UV detection for carbohydrates analysis employing many suitable co-ions added to the background electrolyte. Soga et al. develop a CE method for the simultaneous monosaccharide composition analysis of glycoproteins. The determination was carried out in less than 30 min, with indirect UV detection using 2,6-pyridinedicarboxylic (PDC) acid as a background electrolyte [17]. An easy and reproducible CE method for the determination of 28 carbohydrates in food samples was also described by Soga et al. The electrolyte solution was prepared containing PDC and cetyltrimethylammonium hydroxide (CTAH). The separation required an analysis time of 25 min [18]. Indirect UV detection at 254 nm using sorbate as background electrolyte was employed by Zemmann et al. for monitoring carbohydrates in soft drinks. The quantitative analysis could be accomplished within an overall analysis time of 2 min [19].

Some authors employ CE for the simultaneous determination of carbohydrates and amino acids. Soga et al. quantify these analytes in soy sauce, nutrient tonic and pineapple [20]. On the other hand, CE and mid-UV detection was described by Sarazin et al. [21]. Moreover, a parallel and serial dual electrode detector for CE was described for the simultaneous determination of a mixture of carbohydrates and amino acids [22].

Several authors have proposed a CE method for the determination of carbohydrates in honey samples. Fructose, glucose and sucrose were completely separated within 2 min by a CE method proposed by Rizelio et al. [23]. The method provides good linearity, reproducibility and detection limits. Biluca et al. [24] proposed a method for the determination of 5-hydroxymethyl furfural (5-HMF) and carbohydrates (fructose, glucose and sucrose) in different honey samples. Although the method showed good results and the separation was carried out in less than 2 min, the determination of 5-HMF and carbohydrates was performed separately. Tezcan et al. quantify organic acids and saccharide composition, as well as total phenolic contents in some Turkish honey samples. The separation of the carbohydrates was performed in about 6 min [25]. To the best of our knowledge, we could not find any article for the simultaneous determination of fructose, glucose, sucrose and proline in honey samples. For this reason the aim of this work was to develop an analytical method for the simultaneous determination of these analytes in honey samples from Argentina and Sweden using capillary electrophoresis methodology.

2. Material and methods

2.1. Chemicals and reagents

Reagents of analytical grade and ultra pure water ($18 \text{ M}\Omega \text{ cm}^{-1}$, Barnstead, Dubuque, USA) were used. D-(+)-glucose and D-fructose were purchased from Merck (Buenos Aires, Argentina) and sucrose and cetyltrimethylammonium bromide (CTAB) from Mallinckrodt (St. Louis, USA). Sodium benzoate and sodium hydroxide were obtained from Anedra (Buenos Aires, Argentina) and proline from Fluka (Buchs, Switzerland) standard stock solutions of each monosaccharide (50 mmol L^{-1}) and proline (0.5 mg L^{-1}) were prepared in water. The working solutions containing the mixture of the four analytes were obtained by dilution of the standard stock solutions with water and were prepared freshly every day. All the solutions were stored at 4°C .

2.2. Honey samples

Six commercial honey samples (three from Bahía Blanca, Buenos Aires, Argentina and three from Stockholm, Sweden) were purchased from local markets. To perform the determination, 200 mg of honey were weighed and diluted with water to a final volume of 10.0 mL.

2.3. Instrumentation

A Beckman Coulter capillary electrophoresis instrument MDQ equipped with a diode array detector operating at 224 nm was used. The capillaries were also from Beckman System. Control and data processing were carried out with 32 Karat software.

2.4. Capillary electrophoresis analysis

The separation was carried out in a fused-silica capillary (58 cm effective length, 68 cm total length, $50 \mu\text{m}$ i.d.) with a negative power supply of 25 kV at 25°C . Sample injections were performed in hydrodynamic mode for 3 s at 0.5 psi. The background electrolyte (BGE) was 10 mmol L^{-1} sodium benzoate with 1.5 mmol L^{-1} CTAB at pH 12.4. New capillaries were equilibrated by flushing 1 mol L^{-1} hydrochloric acid (15 min), 1 mol L^{-1} sodium hydroxide (15 min), 0.1 mol L^{-1} sodium hydroxide (5 min), water (5 min) and buffer solution (15 min). The capillary was conditioned daily by flushing it with 0.1 mol L^{-1} sodium hydroxide (5 min), water (3 min) and buffer solution (5 min). Between runs the capillary was reconditioned with the BGE solution (2 min).

3. Results and discussion

3.1. Optimization of the capillary electrophoresis conditions

The separation was carried out by capillary zone electrophoresis with indirect UV detection since carbohydrates and proline lack any strong chromophore in the UV region. For this reason, a UV absorbing chromophore with a high molar absorptivity was added into the background electrolyte.

There are several criteria that have to be considered when choosing a substance for indirect UV detection. High molar absorptivity and a mobility closely matched to the mobility of the analytes are two such demands. The use of 2,6-pyridinedicarboxylic acid (PDC) at 350 nm, and sodium benzoate at 224 nm has earlier been described and was thus evaluated allowing the separation of the four analytes with satisfying resolution [24]. However, the baseline for PDC was highly unstable. On the other hand, by using sodium benzoate, efficient resolution and stable baseline were obtained. Furthermore, this chromophore is more suitable for analysis of compounds with low mobility [26].

The influence of the concentration of sodium benzoate was studied in the range 10 to 35 mM. It was concluded that the sodium benzoate concentration did not influence the resolution, the lowest evaluated concentration, 10 mM, was hence employed.

Furthermore, the effect of pH in the separation of the four analytes was studied. The studied range was 11.6 to 12.6, considering the pKa values of the carbohydrates, 12.03 for fructose, 12.20 for glucose, 12.51 for sucrose and 10.64 for proline [27]. Between pH 11.6 and 11.9 the separation of fructose and glucose was not observed. The best resolution was achieved when the pH was increased up to a value of 12.4. Besides, the current and the baseline were unstable at pH higher than 12.4. Accordingly, this pH was selected for all subsequent analysis.

Both carbohydrates and proline are anionic at alkaline conditions and would therefore migrate towards the positive electrode, that is, against the electroosmotic flow (EOF) in a normal CE system. Here, CTAB, a cationic surfactant, was used to reverse the direction of EOF by a positively charged dynamic coating of the inner wall surface of the silica capillary. By this means, the anionic analytes migrate in the same direction as the EOF. CTAB at a concentration of 1.5 mM provided the best capillary electrophoresis performance with stable baseline as has earlier been shown for a similar BGE solution [28].

Furthermore, the applied voltage was optimized and the studied range varied from 10 kV to 27 kV (negative polarity). When employing voltages between 10 and 20 kV poorly defined peaks were registered. Increasing the voltage was possible to reduce the analysis time and improve the resolution of the analytes. Above 25 kV large differences were

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