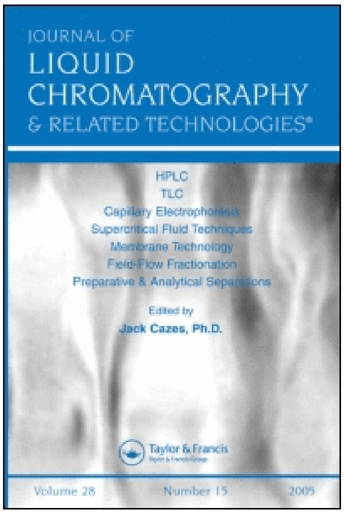

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HONEY CARBOHYDRATE ANALYSIS BY HPLC, WITH ELECTROCHEMICAL DETECTION, USING A NI-CR ALLOY ELECTRODE

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ABSTRACT

Honey carbohydrates were analyzed by high performance anion exchange liquid chromatography with constant potential amperometric detection at a nickel-chromium (80:20) alloy electrode. The sugars are oxidized by a surface catalyzed process proposed to involve Ni(II)/Ni(III) oxyhydroxides, which are formed at approximately 0.500 V (Saturated Calomel as reference electrode). The honey content of glucose, fructose, and sucrose is determined in less than 15 minutes per sample.

The high sensibility achieved with this alloy electrode allowed for the analysis of disaccharides and oligosaccharides usually present in honey as minor components. The analysis of these carbohydrates may be useful for the determination of the floral origin of honey.

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INTRODUCTION

Honey is a food product consumed by human beings since ancient times. It is a natural product of limited supply and relatively high price, which makes it a target for adulteration. Thus, honey consumers are generally concerned about honey quality, and have prompted government and food quality agencies to set honey authenticity parameters.

Carbohydrates are the major components of honey, comprising nearly 95-99% of the dry matter.[1] Fructose and glucose are the dominant fractions and account for almost 85-95% of the honeybee honey sugars.[2] However, other carbohydrates which are present as minor honey components, could be useful for the determination of floral origin because they may act as "fingerprints" of the floral source.[3,4] Moreover, various regulations require a minimum of reducing sugars and a maximum of sucrose content among other honey quality parameters. Therefore, carbohydrate analysis may be important as a honey quality parameter and floral origin determinations.

Many approaches for the identification and quantitation of carbohydrates in food products make use of high performance liquid chromatography (HPLC). However, the lack of a chromophore group in most of the carbohydrates constitutes a major difficulty for their direct UV-visible detection (a customary detection method), and requires the use of derivatizing agents, thus increasing the complexity of the analysis. Alternatively, refractive index detection can be used in the HPLC analysis of carbohydrates, but the lack of sensitivity of this detection method is widely recognized.

Electrochemical techniques have gained prominence in the HPLC analysis of sugars, especially since the introduction of the so-called "thin layer electrochemical cell."[5] Moreover, the pioneer work of Prof. D. Johnson and his group with pulsed amperometric methods at gold and platinum electrodes[6] has made the HPLC anion exchange analysis of carbohydrates the preferred analytical technique for a variety of complex matrices.[7] This methodology requires the continual pulsing of the working electrode potential to extreme oxidizing and reducing values to maintain a stable response, as the passivation of the electrode surface occurs due to the absorption of sugar oxidation products. Recently, a nickel-chromium alloy (Ni-Cr) has been shown to be useful as electrode material for the electrochemical detection of various carbohydrates[8] and amino acids.[9]

These studies proposed that the oxidation of those compounds proceeded through an electrocatalytic process involving Ni(II)/Ni(III) oxy/hydroxides. The advantages accrued to this electrode material were its high sensitivity (i.e. 500 fmol of glucose at a Signal-to-Noise ratio of 3), signal stability, and ease of implementation. We also considered important, the analysis of the performance of the Ni-Cr alloy electrode for the determination of carbohydrates in food products.

It is the aim of this work to study the capabilities of the nickel-chromium alloy electrode for the constant potential amperometric determination of carbohydrates in honey. It is shown that glucose, fructose, and sucrose can be easily analyzed without any complex pretreatment process, apart from dilution steps to lower fructose and glucose concentration, and a microfiltration procedure to avoid column fouling. Also, the feasibility of the analysis of some carbohydrates usually present in honey is demonstrated.

EXPERIMENTAL

Reagents

Standard carbohydrate (Sigma, USA) and sodium hydroxide (Merck, Argentina) solutions were prepared daily with HPLC quality water obtained from a WaterPro Mobile model 90901-01 (LABCONCO, USA). Approximately 1.90 g of fructose, 1.50 g of glucose, and 0.30 g of sucrose (weighed with a 0.1 mg precision) were dissolved in exactly 50 mL. One milliliter of this solution was then transferred to a 100 mL volumetric flask and the volume completed. Twenty microliters of the latter solution were injected in the HPLC for the determination of the standards peak heights. All reagents were analytical degree.

Honey samples were obtained from various hive sites in the south of the province of Córdoba (Argentina) during the 1998-1999 season. The samples were kindly provided by producers immediately after extraction, and they were refrigerated until use. Approximately 1.25 g of each honey sample (weighed with 0.1 mg precision) were dissolved with HPLC grade water in a 50 mL beaker and transferred quantitatively to a 25 mL volumetric flask where the volume was completed. Exactly 1 mL of that solution was then transferred to a 100 mL volumetric flask and diluted with HPLC grade water to the final volume. A small volume (e.g. 100 μL) of the latter solution was finally filtered through a 0.22 μm membrane filter, and 20 μL injected for carbohydrate analysis.

Equipment

Nickel-chromium (80:20) (Goodfellow, England) working electrodes for the cyclic voltammetric (CV) experiments, were prepared with 0.1 cm diameter wires embedded in Teflon shrinkable tubes. The working electrodes for the liquid chromatographic experiments were made with the same Ni-Cr wires embedded in Kel-F blocks of 0.5 x 1.0 x 1.0 inches. The electrodes were polished successively with 400, 600, and 0000 grit emery paper, and finished to a mirror surface with 1, 0.3, and 0.05 μm alumina particles suspended in water on a microcloth pad. They were sonicated, and then thoroughly washed with triply distilled water. A Saturated Calomel Electrode (SCE) reference electrode was used in the CV experiments.

Cyclic voltammetric experiments were performed with an EG&G PARC Model 273 computerized potentiostat. An EG&G PARC model 175 Universal Programmer was used as the waveform generator. The current-voltage (i-E) output was recorded with either the PC printer or with a Houston 2000 XY recorder. A conventional three electrode glass cell was used for the CV experiments.

The chromatographic system consisted of a model 307 pump (Gilson, France), a model 7125 injector (Rheodyne, USA) with a 20 µL injection loop, and a Hamilton RCX-10, 250 x 4.6 mm (Hamilton Co., USA) or a CarboPac PA-1, 250 x 4.6 mm (Dionex, USA). A home made potentiostat was used as an amperometric detector. The electrochemical signal was fed to a PC equipped with Peak Simple (SRI, USA) data processing software. The electrochemical cell for the flow experiments consisted of a home made working electrode, a stainless steel auxiliary electrode, and a SCE reference electrode. The dead volume of the flow cell was approximately 5 µL.

RESULTS AND DISCUSSION

Electrocatalytic Oxidation of Glucose

Previous studies performed in our laboratory showed that the voltammetric behavior of Ni-Cr electrodes in alkaline solutions is complex.[10] One oxidative and one reductive wave was observed during the first few potential scan cycles at a potential range from 0.00 V to 0.60 V. The oxidative peak was attributed to the oxidation of Ni(II) to Ni(III), whereas the reductive peak was assigned to the reduction of Ni(III) to Ni(II). The complexity of both the oxidative and reductive waves increased as the number of CV cycles increased.

The evolution of the i-E trace with time is shown in Figure 1-A. The potential was continuously scanned and the i-E trace recorded approximately every 20 minutes. The peak current and potential of the oxidative wave on the anodic trace (peak 1) increased with the number of CV cycles. A decrease of the peak current and electrochemical charge under the reductive wave (Figure 1-A, peak 2) was observed after about 50 CV cycles. Simultaneously, a shoulder (peak 3) developed on the left side of peak 2 during the negative going potential scan.

After approximately 500 CV cycles, this shoulder changed to a totally defined peak, and the reductive peak 2 was almost undetectable thereafter. The arrows in Figure 1-A indicate the evolution of the i-E trace with the number of CV cycles. A steady i-E response was obtained after approximately 600 CV cycles using the same potentiodynamic conditions (Figure 1-B).

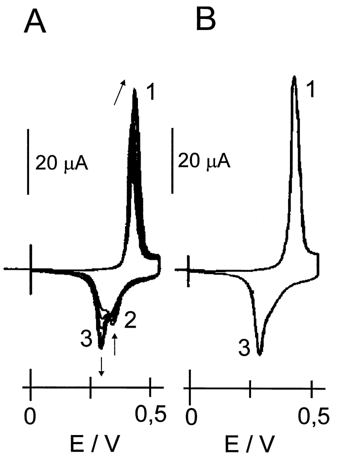

Figure 1. Cyclic voltammetric response of a Ni-Cr (80:20) alloy electrode in 0.10 M NaOH. Scan rate v=0.05 Vs^{-1}. (A) Evolution of the i-E trace of the electrode recorded nearly every 100 cycles. (B) Steady i-E response obtained after approximately 600 *cv* cycles.

An increment in the charge and peak current under the wave assigned to Ni(II) oxidation was observed when glucose was added to the background solution (Figure 2, trace B). This increment was glucose concentration dependent, as can be observed in Figure 2, traces C through F. On the other hand, a decrease in the charge and peak current under the wave assigned to Ni(III) reduction was obtained on the negative going potential scan. Other studies[8] proposed that the cyclic voltammetric behavior could be explained by an electrocatalytic mechanism (EC) where the electrogenerated catalyst, Ni(III), oxidizes glucose diffusing to the electrode surface from the bulk solution.

It was also shown that the Ni-Cr electrode remained active for glucose electrooxidation even after 21 days of continuous chromatographic analysis. Moreover, the Ni-Cr electrode was useful for the oxidation of sugar alcohols and mono- and disaccharides. These results suggested that it may be possible to implement the Ni-Cr alloy for constant potential amperometric detection of food carbohydrates after high performance anion exchange chromatographic separation.

HPLC Analysis of Honey Carbohydrates

It is known that complex mixtures of carbohydrates can be separated into their sugar components by anion exchange chromatography.[11] The separation is

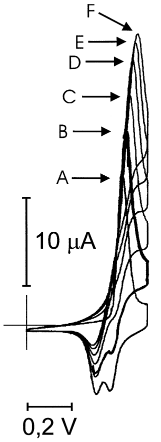

Figure 2. Cyclic voltammetric response of a Ni-Cr (80:20) alloy electrode in 0.10 M NaOH (trace A), and with different glucose concentrations: (B) 0.5 mM, (C) 1.0 mM, (D) 1.5 mM, (E) 2.0 mM, and (F) 2.5 mM. Scan rate v=0.05 Vs^{-1}.

possible because of the weakly acidic nature of these compounds, which requires the use of strongly alkaline mobile phases. The use of mobile phases with high pH also facilitated the implementation of some metals[6] and metal oxides[8,12] as electrocatalytic detectors.

A typical separation of mono and disaccharide standards on an anion exchange column, with constant potential amperometric detection at a Ni-Cr electrode, is shown Figure 3. The electrode responds quickly to the sugars injected so that the column resolution is maintained.

The analytical usefulness of the Ni-Cr alloy electrode is based on the linearity and reproducibility of its response to carbohydrate oxidation. The linearity of the Ni-Cr alloy electrode response for the main honey carbohydrates was studied here. The linearity of the electrode response to the concentration of sorbitol, glucose, ribose, and maltose was previously studied.[8] The response reproducibility of the Ni-Cr alloy electrode for the oxidation of glucose over a period of 21 days was analyzed in that study.

It was also shown there that excellent linearity and response reproducibility were attained with the Ni-Cr alloy electrode. The analytical parameters obtained for the calibration plots of glucose, fructose, and sucrose are listed in Table 1. Different intercepts (different αs) were obtained for the carbohydrates analyzed, which indicates that the sensitivity of the electrode is related to the degree of ionization of each carbohydrate.

The glucose, fructose, and sucrose content of honey samples were analyzed by HPLC with electrochemical detection after two dilution steps and a microfil-

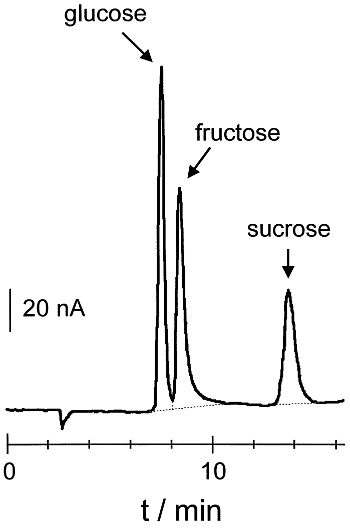

Figure 3. High performance anion exchange chromatogram of some carbohydrates usually found in honeys. Injection amount: 33 nmol glucose, 42 nmol fructose, and 4 nmol sucrose. Mobile phase 0.10 M NaOH, flow rate F=0.7 mL/min., T=298 K. Analytical column: Hamilton RCX-10.

tration. The dilution steps to lower the concentration of glucose and fructose to approximately 10^{-3} M were necessary due to the high content of these carbohydrates in honey. The weight percentage of each individual carbohydrate was calculated from its chromatographic peak height by using equation (1):

$$\mathrm{Ci}\% = 25\frac{H_i}{H_i^o}\frac{w_i^o}{w_s} \qquad (1)$$

where H_i^o and H_i are the chromatographic peak heights of the carbohydrate in the standard and in the sample, respectively, and w_i^o and w_s the weights of the carbo-

Table 1. Analytical Parameters for the Detection of Carbohydrates at the Ni-Cr Alloy Electrode

Compound	Log(α)*	β*	R	RSD
Glucose	4.725	0.935	1.000	1.9
Fructose	4.694	0.949	1.000	0.8
Sucrose	3.860	0.903	1.000	1.6

* Linear fit: $\log(S) = \log(\alpha) + \beta \log(C)$; concentration range analyzed: $1\times10^{-6}-1\times10^{-3}$ M

hydrate standard and the honey sample, respectively. This equation was derived by taking into account the dilution volumes as explained in the experimental section. The quantitation of carbohydrates in each honey sample was repeated five times to test the reproducibility of the method. The standard deviations obtained were always less than 3%, which shows the validity of the analysis. Moreover, the results obtained using the methodology described herein were in agreement with those obtained by traditional titration of reducing sugars using the Fehling reagent, as described in the specialized literature.[13]

The sensitivity demonstrated by the Ni-Cr alloy electrode may be useful for the determination of oligosaccharides usually present in honey as minor components. It has been proposed in the literature,[4] that the relative amount of these carbohydrates appears to be related to the floral origin of honey. Thus, these carbohydrates may act as “fingerprints” for the liquid chromatographic determination of the honey floral origin.

Characterization of unifloral honeys is usually performed by microscopical analysis, especially the identification and counting of pollen grains (melissopalynology). The HPLC analyses of floral origin suggested here will, thus, represent an advantage over the melissopalynologic methods, which are time consuming and require a high level of expertise.

The liquid chromatographic analysis of erlose, D(+)-melezitose, D(+)-treahlose, D(+)-turanose, and D(+)-raffinose was performed using the Ni-Cr alloy as working electrode. Figure 4 shows the liquid chromatographic separation of a mixture of these standards. The effectiveness of the chromatographic separation is indicated by the excellent resolution at a flow rate of 0.50 mL/min. Shorter retention times for D(+)-raffinose and D(+)-turanose (peaks D and E, respectively) were obtained at a higher flow rate of 1.0 mL/min. However, the resolution of the chromatogram was greatly diminished. Moreover, at a mobile phase flow rate of 1.0 mL/min., peak A is observed near the injection peak, making its quantitation difficult.

The catalytic oxidation of the above mentioned di- and oligosaccharides resulted in reproducible chromatographic responses. Symmetric peaks were obtained for all the carbohydrates studied, as can be seen from the chromatogram in Figure 4. Moreover, the Ni-Cr alloy electrode response remained stable for many hours with successive injections of these carbohydrates. The Ni-Cr alloy electrode could be used without surface cleaning for as long as 15 days, showing almost the same performance as that reported in a previous study.[8]

CONCLUSIONS

The Ni-Cr (80:20) alloy electrode can be used for the long-term constant-potential amperometric detection of the various carbohydrates usually present in honeys. The analysis is simple as it only requires dilution steps and microfiltra-

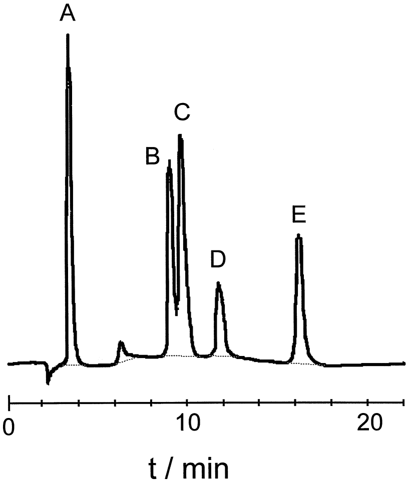

Figure 4. High performance anion exchange chromatogram of some carbohydrates usually found in honeys as minor components. (A) D(+)-treahlose, (B) D(+)-melezitose, (C) erlose, (D) D(+)-raffinose, and (E) D(+)-turanose. Injection amount: 20 nmol each carbohydrate. Mobile phase 0.05 M NaOH, flow rate F=0.5 mL/min., T=298 K. Analytical column: CarboPac PA-1.

tion. The electrode response is based on the catalytic oxidation of the sugars at the oxide-hydroxides layer formed on its surface at a working potential of approximately 0.50 V (ECS). The high sensibility achieved in the high performance anion exchange analysis of sugars with electrochemical detection at a Ni-Cr alloy electrode allows the determination of some carbohydrates usually present in honeys as minor components.

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