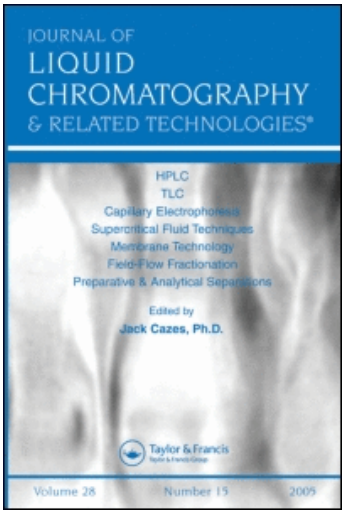

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Abstract: Pulsed amperometry is a powerful detection method for carbohydrates that does not require derivatisation of the sample, and this is particularly useful if coupled with high performance ionic chromatography (HPIC). Pulsed amperometric detection (PAD) utilises an amperometric cell with a gold working electrode and pulsed waveform of potentials and allows direct and selective determination of electroactive compounds containing amine, hydroxyl, or sulphur functions.

High performance ionic chromatography coupled with pulsed amperometric detection was used for the determination of glucose, fructose, sucrose, and lactose in chocolate based matrices. The sample, opportunely diluted and purified, was injected on a PA10 column and quantified using the external standard method.

Keywords: Sugars, HPIC, Chocolate, Pulsed amperometric detector

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INTRODUCTION

Sugars are raw materials originating within the EU that are profitable when the finished products are for export; control over the amounts used is considered highly important. The separation and determination of carbohydrates is of great interest in nutritional research, cellular biology, and biotechnology.[1] Carbohydrates, and glucose in particular, play an important role in physiological processes and great efforts have been made to develop sensitive methods for their determination in food and biological matrices.[2,3] There are numerous analytical methods that exploit various systems for detection.[4–9]

Over the last fifteen years liquid chromatography coupled with pulsed amperometric detection has revolutionised the separation and detection of polar aliphatic compounds.[10] Generally, these compounds generate weak signals and spectrophotometric methods require pre or post derivatisation.[11] The sensitivity of amperometric detection at constant potential, when possible, is low due to the electrode being poisoned.[12] Pulsed amperometric detection normally uses an electrode made of precious metal and a type of pulsed wave of appropriate potential to enable the "on-line" cleaning and reactivation of the electrode, thus heightening its sensitivity.[13,14] The outcome is the direct and selective detection of compounds containing electroactive amine or hydroxyl groups and also sulphur functions.[15]

Given the low acidity of carbohydrates, high performance anionic exchange liquid chromatography using a stationary alkaline resistant phase coupled with pulsed amperometric detection is a powerful analytical method that requires minimal handling of the sample and no pre or post column derivatisation.[16]

In this paper, high performance ionic chromatography coupled with pulsed amperometric detection was used for the determination of glucose, fructose, sucrose, and lactose in chocolate based food matrices.

EXPERIMENTAL

Reagents and Standards

Glucose dried in an oven at 100°C for 1 hour 99.8% pure (Merck, Darmstadt, Germany), fructose over 99% pure (Merck), sucrose dried in an oven at 100°C for 1 hour 99.5% pure (Merck), pure lactose monohydrate for biochemistry (Merck), HPLC grade methanol (Merck), aqueous solutions Carrez I at 24% (w/v) zinc acetate and 3% (w/v) acetic acid and Carrez II 15% (w/v) potassium ferrocyanide (Merck), certified LCG 7016 matrix (Novachimica, Milan, Italy), HPLC grade ultrapure water obtained by ELGA-PurelabUltra system (Steroglass, Perugia, Italy) were used.

A 500 mg/L standard solution of glucose, fructose, and lactose, and a 1000 mg/L standard sucrose solution were prepared. The solutions were filtered using a cellulose 0.2 μm filter and kept at 4°C in the dark. Three calibrating solutions were prepared for diluting the standard solutions to concentration intervals of 30 ÷ 70 μg/mL for glucose, fructose, lactose, and 60 ÷ 90 μg/mL for sucrose.

Equipment

An ionic chromatograph Dionex DX 600 (Sunnyvale, California, USA) with an isocratic pump IP25, a 50 μL loop, and a pulsed amperometric detector (Dionex) with gold working electrode, platinum controelectrode, and Ag/AgCl, KCl saturated reference were used. An EG50 cartridge to generate the eluent KOH from water, a decarbonating cartridge CR-TC (Dionex), and an auto sampler AS50 (Dionex) were also used. Chromeleon-Peaknet software (Dionex) was used for data collection and handling.

Preparation of Samples

The samples analysed comprised chocolate eggs and fruit filled chocolates supplied by the manufacturers. Every sample was homogenized, 1 g was carefully weighed in a conical flask of appropriate size, and 70 mL of water was added at 40°C. The mixture was sonicated for one hour at 40°C and shaken from time to time. It was quantitatively transferred into a 250 mL graduated flask, 5 mL of Carrez I solution added and, after shaking, 5 mL of Carrez II was added and brought up to volume with water. The solution was filtered with a pleated filter and 5 mL of filtrate diluted to 100 mL with water. The solution, purified on a column SPE Strata C18-U Phenomenex (Chemtek Analytica, Anzola, Italia) activated with methyl alcohol and filtered using a cellulose 0.2 μm filter, is ready to be injected into the chromatographic system. Each commercial sample was prepared three times and the certified matrix eleven times.

Analytical Procedure

The solution (50 μL) was injected and the components were separated using a CarboPac PA10 Dionex (250 mm × 4 mm) column with a polymer film stationary phase and a CarboPac PA10 Dionex (50 mm × 4 mm) precolumn. Isocratic elution was carried out at room temperature utilizing 50 mM potassium hydroxide as mobile phase, generated electrochemically at a flow rate of 1 mL/min. Amperometric detection was done using an appropriate pulsed waveform of potentials.

The working programme involved automatic injection twice of the calibrating solutions, then the injection of three samples followed again by the calibrating solutions.

RESULTS AND DISCUSSION

In this paper, high performance ionic chromatography coupled with pulsed amperometric detection for the determination of glucose, fructose, sucrose, and lactose in chocolate based food matrices was used.

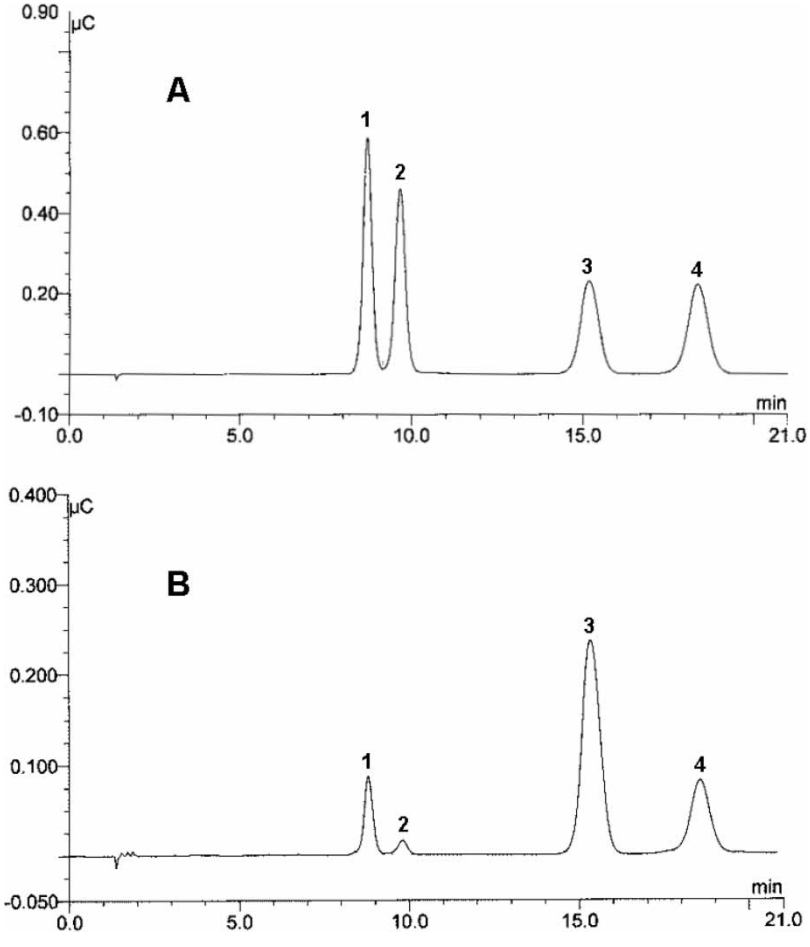

Figure 1. Chromatogram HPIC-PAD of (A) standard mixture, (B) fruit filled chocolate. Peaks: 1, glucose; 2, fructose; 3, sucrose; 4, lactose. Chromatographic conditions described in analytical procedure.

Figure 1 shows the chromatograms of a standard mixture and a sample of a fruit filled chocolate. As can be seen from the chromatograms, the conditions adopted and the efficiency of the stationary polymer film phase enabled the analytes to be well separated with an analysis time of around 20 minutes.

The identification of the analytes was achieved by comparison with the retention times of the standards.

The linearity of the concentration intervals as shown by plotting the areas ($\mu C \cdot min$) against concentrations ($\mu g/mL$) was good. This is consistent with the equations and the coefficients of determination of the calibration charges which were: $y = (0.0554) \times$ and $R^2 = 0.9998$ $(n = 3)$ $(t_{calc} > t_{crit}; 70.8 > 4.3)$ for glucose, $y = (0.0362) \times$ and $R^2 = 0.9998$ $(n = 3)$ $(t_{calc} > t_{crit}; 70.8 > 4.3)$ for fructose, $y = (0.0213) \times$ and $R^2 = 0.9998$ $(n = 3)$ $(t_{calc} > t_{crit}; 57.7 > 4.3)$ for sucrose, and $y = (0.0380) \times$ and $R^2 = 0.9997$ $(n = 2)$ $(t_{calc} > t_{crit}; 70.8 > 4.3)$[17] for lactose.

Replicability was assessed by analysing the certified matrix 11 times and verifying that the analytical results followed a normal distribution (Shapiro-Wilk test) and that there were no anomalous data (Dixon's test);[18,19] the standard deviation was 1.6% for sucrose and 1.2% for lactose.

Accuracy was assessed by comparing the results obtained (43.5 ± 0.7 g/100 g for sucrose; 7.8 ± 0.1 g/100 g for lactose; average $\pm$ SD) with the ones certified (44.8 ± 2.1 g/100 g for sucrose; 7.8 ± 0.7 g/100 g for lactose; average $\pm$ SD) and these agree well $(t_{calc} < t_{crit}; 0.2 < 4.3, n = 10, p = 0.95$ for sucrose and $t_{calc} < t_{crit}; 0 < 4.3, n = 10, p = 0.95$ for lactose).[20]

Detection limits in the conditions adopted were in the order of 0.04% w/w for all three sugars using a signal/noise ratio of 3.

Table 1. Amount obtained in samples of chocolate eggs and fruit-filled chocolates

Samples	glucose (g/100 g[a])	fructose (g/100 g[a])	sucrose (g/100 g[a])	lactose (g/100 g[a])
Chocolate egg 1			40.2	13.1
Chocolate egg 2			41.3	13.6
Chocolate egg 3			40.0	12.2
Chocolate egg 4			39.6	13.4
Fruit-filled chocolate 1	1.8	0.6	29.5	5.8
Fruit-filled chocolate 2	3.9	3.7	38.9	7.9

[a]Average of three analyses.

The method was applied to the analysis of four samples of chocolate eggs and two samples of fruit filled chocolates. The results obtained are given in Table 1 and were in line with the figures declared by the firms supplying the samples.

CONCLUSIONS

Pulsed amperometric detection after anionic exchange chromatographic separation, by an alkaline resistant polymer film stationary phase, was used for the determination of glucose, fructose, sucrose, and lactose in chocolate based food matrices supplied by manufacturers.

The analytes were separated isocratically using 50 mM of KOH electrochemically generated, and amperometric detection was done using an appropriate pulsed waveform of potentials, controlled by the same software used for data collection and handling.

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