Direct Mono- and Disaccharide Determinations in Foods and Beverages

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The mono- and disaccharide contents of coffee, sports drinks, fruit juices, and honey were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). The determination of free and total carbohydrates in coffee required less than ten minutes per injection. The low abundance small carbohydrates in honey were well resolved from the high concentrations of fructose and glucose (~35% each) using a recently introduced anion-exchange column with 4 µm resin particles.

Introduction:

Carbohydrates, especially mono- and disaccharides, are key ingredients of many foods and beverage products. The mono- and disaccharides often account for much of the product's caloric content as well as its sweetness, texture, and taste. Also, mono-and disaccharides are often a large percentage of the carbohydrate content that must be labelled in the United States [1] and many other countries. In addition, determination of the mono- and disaccharide content of food and beverage products is important for quality control and can indicate product adulteration and/or mislabelling. For example, the content of individual carbohydrates is indicative of a honey's origin and therefore a carbohydrate analysis can determine if the labelled origin is accurate. Honey from some countries is more valued than honey from others and those from lower priced origins have been fraudulently labelled as honey from a more highly valued origin. There are examples of products being adulterated with a sugar not typically present in the product to correct for a product deficiency such as a lack of sweetness. One such example is a recent report showing the carbohydrate content of pear juice from growing regions worldwide to be used for authentication, to reveal adulteration with lower cost sweeteners, and to determine if other juices, such as apple juice, have been adulterated with pear juice [2].

While there are numerous methods for carbohydrate analysis, over the last 30 years HPAE-PAD has become a standard technique for carbohydrate determinations of foods and beverages [3]. HPAE-PAD separates carbohydrates by converting them to oxyanions at high pH. These oxyanions are separated on a high-performance anion-exchange column, and are then detected by their oxidation at high pH on a gold working electrode with only a few percent of the carbohydrate oxidised for detection. Pulsed amperometric detection is sensitive and requires no analyte derivatisation. The carbohydrates are separated with a sodium or potassium hydroxide mobile phase (eluent). If desired, the potassium hydroxide eluent can be generated from deionised water by the instrument. This eluent generation precludes the analyst from having to prepare the eluent, eliminates eluent preparation errors, and enhances instrument to instrument and lab to lab method transfer. Some of the high-performance anion-exchange columns used for HPAE-PAD have recently been produced in formats containing 4 µm resin particles. This allows higher resolution carbohydrate separations with increased sample throughput. Newer chromatography systems for HPAE-PAD were designed to use columns with 4 µm resin particles together with electrolytic eluent generation for the determination of carbohydrates using hydroxide eluents.

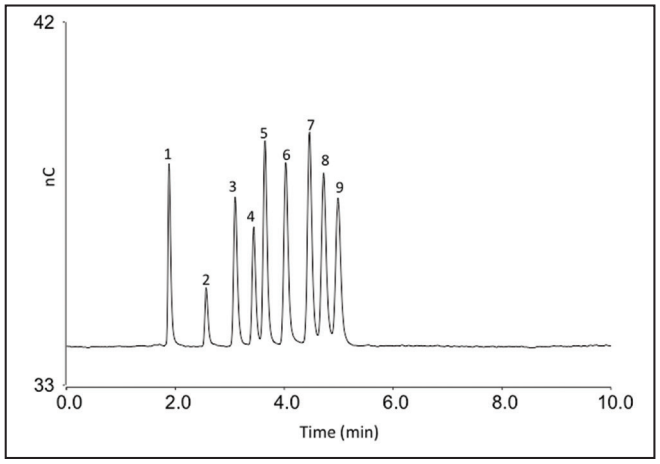

Figure 1. Separation of nine common sugars analysed on the CarboPac SA10-4µm column (4 x 250 mm) with its guard (4 x 50 mm) with the conditions used for instant coffee analysis described in the Experimental section. Peaks:1) mannitol 2) fucose 3) sucrose 4) arabinose 5) galactose 6) glucose 7) xylose 8) mannose 9) fructose.

In this article, three HPAE-PAD food and beverage applications are shown where the analytes of interest are monosaccharides, disaccharides, and other small carbohydrates. In less than ten minutes, the important carbohydrates in instant coffee were determined accurately, with a significant reduction in the analysis time required compared to the AOAC method [4]. The second application shows how the sucrose, glucose, and fructose contents

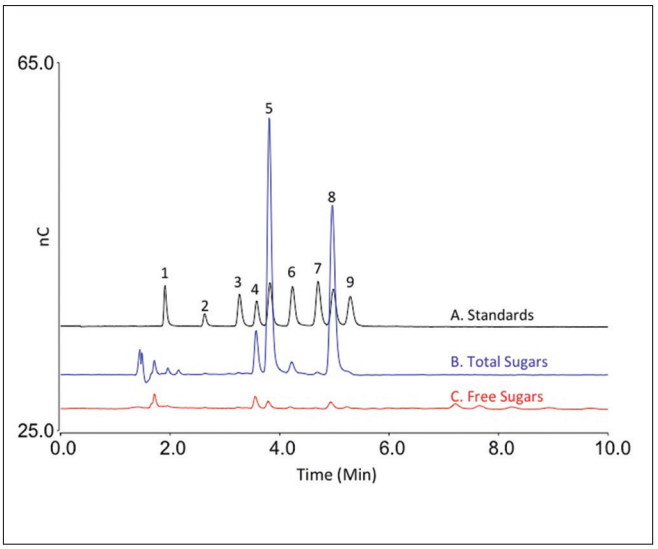

Figure 2. Free and total carbohydrates present in an instant coffee sample with conditions described in the Experimental section. Peaks:1) mannitol 2) fucose 3) sucrose 4) arabinose 5) galactose 6) glucose 7) xylose 8) mannose 9) fructose

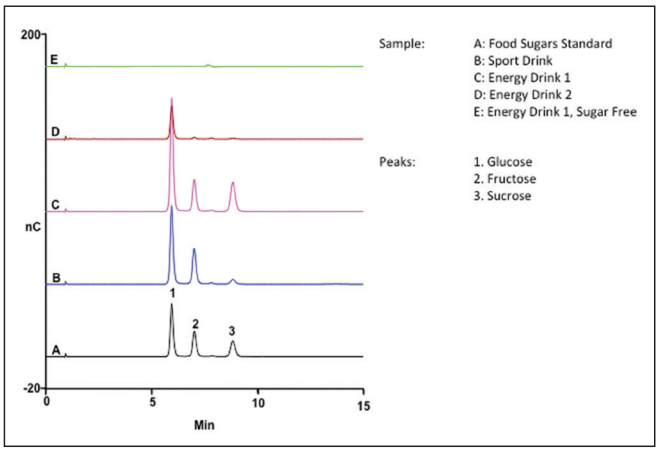

Figure 3: Analysis of energy drinks for glucose, fructose, and sucrose. Conditions are described in the Experimental section.

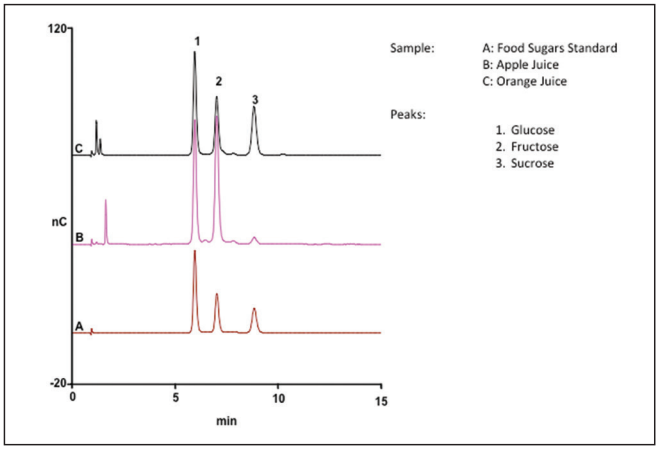

Figure 4: Analysis of fruit juices for glucose, fructose, and sucrose. Conditions are described in the Experimental section.

of common beverages can be assayed with a simple sample dilution and filtration prior to analysis. The recently introduced Thermo Scientific Dionex CarboPac PA210- 4 µm Fast column was used to profile the small carbohydrates in honey in the third application.

Experimental:

Materials:

The beverage samples were purchased from a local market. Carbohydrate standards were purchased from Sigma-Aldrich (St. Louis, MO), Fisher Chemical (Pittsburgh, PA), or Pfanstiehl (Waukegan, IL). The honey samples included a store-bought clover honey. The 'Beekeeper' and 'New Zealand Manuka' honeys were generous gifts of Kevin Thayer (beekeeper) and James Johnstone (from New Zealand). All water used for sample preparation and chromatography was deionised (DI) and had a resistivity of 18 mΩ-cm or higher. Samples that were filtered used a 0.2 µm Nalgene PES syringe filter (Thermo Scientific, Waltham, MA).

Equipment:

Samples were analysed on a Thermo Scientific Dionex Integrion system (Thermo Scientific, Sunnyvale CA) or a Thermo Scientific Dionex ICS-5000+ system along with a Thermo Scientific Dionex AS-AP autosampler. Both systems have eluent generation at backpressures up to 5000 psi, temperature control, an electrochemical detector, and an electrochemical cell. The Dionex ICS-5000+ system and the Dionex Integrion system included isocratic pumps with eluent degas. For 0.4 µL injections the autosampler was equipped with an injection valve that had a 0.4 µL internal injection loop.

Sample Preparation:

Instant coffee samples were prepared for free and total carbohydrates as described in AOAC Method 995.13 [4]. The free carbohydrate samples were prepared by dissolving 300 mg of instant coffee in 100 mL of DI water. The solution was treated with a Thermo Scientific Dionex OnGuard II RP cartridge as directed by the cartridge instructions. The filtrate was passed through a 0.2 µm syringe filter prior to injection. For total carbohydrates, 300 mg of instant coffee was dissolved in 50 mL of 1.0 M HCl and placed in a boiling water bath for 2.5 h, swirling every 30 min. After cooling to room temperature under tap water, the solution was diluted to 100 mL with DI water and filtered through folded filter paper (qualitative, fast). The sample was then treated with a Dionex OnGuard II Ag/H cartridge to remove chloride ion, as directed by the cartridge instructions, and passed through a 0.2 µm syringe filter prior to injection. Functional drinks and fruit juice samples were diluted 1 to 10,000 with DI water and filtered as described above. Honey samples were diluted 1 to 1000 (w/w) with DI water.

Chromatography Conditions:

All eluents were delivered using an electrolytic eluent generator. Prepared coffee samples (0.4 µL) were separated on a Thermo Scientific Dionex CarboPac SA10-4 µm column (4 x 250 mm) preceded by its guard column (4 x 50 mm) using 1 mM potassium hydroxide. The flow rate was 1.5 mL/min and the column temperature was 40°C. Carbohydrates were detected by PAD using a disposable gold on PTFE working electrode with a 0.062" gasket, Ag/AgCl reference electrode, and a four-potential waveform [5]. Prepared functional beverage and fruit juice samples (10 µL) were separated on a Thermo Scientific Dionex CarboPac PA20 column (3 x 150 mm) preceded by its guard column (3 x 30 mm) using 33 mM potassium hydroxide. The flow rate was 0.5 mL/min and the column temperature was 30°C. Detection conditions were the same as for coffee except that a 0.002" gasket was used. Diluted honey samples (10 µL) were separated on a Dionex CarboPac PA210-4 µm Fast column (4 x 150 mm) preceded by its guard column (4 x 50 mm) using a 48 mM potassium hydroxide. The flow rate was 0.8 mL/min and the column temperature was 30°C. Detection conditions were the same as for coffee except that a 0.002" gasket was used.

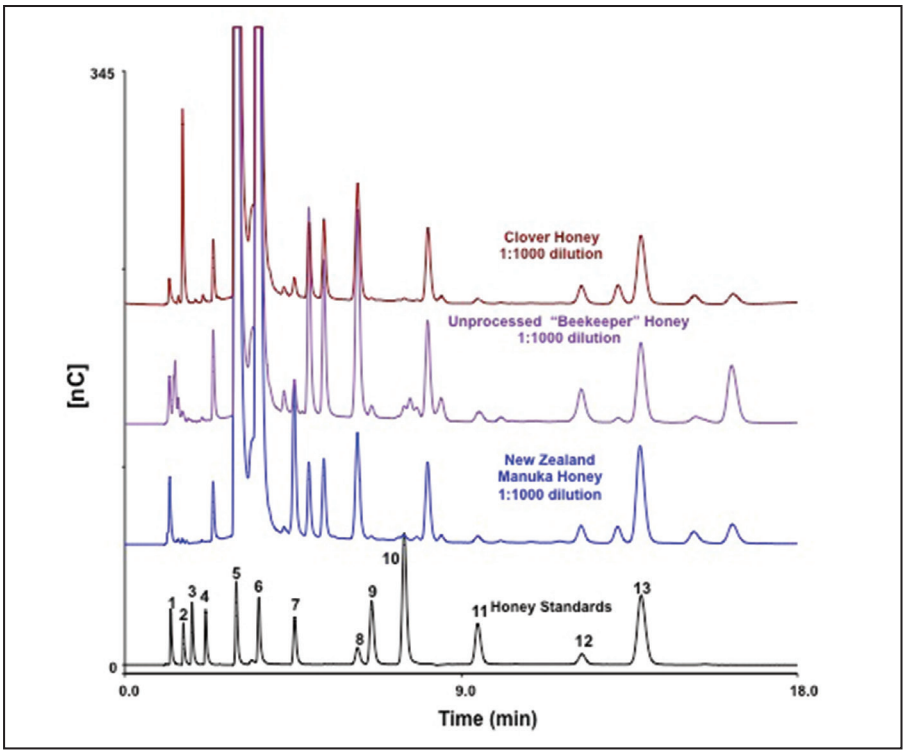

Figure 5: Carbohydrate profiles of honey samples. Conditions are described in the Experimental section. Peaks [concentration in standard (mg/L)]: 1) fucitol [1], 2) trehalose [2], 3) fucose [2], 4) rhamnose [2], 5) glucose [2], 6) fructose [3], 7) sucrose [5], 8) kojibiose [1], 9) melezitose [7], 10) gentibiose [8], 11) 1-kestose [2], 12) nigerose [3], 13) maltose [15]

Results and Discussion:

Coffee is enjoyed worldwide and its popularity has been growing. The green coffee bean is approximately 50% carbohydrate by weight, and the carbohydrates play a major role in the flavor profile of the brewed coffee. Measuring the carbohydrate content of the green coffee bean, roasted coffee, and instant coffee is one way to determine coffee quality, and for instant coffee, whether it has been adulterated. In 2014, Pauli et al. showed how HPAE-PAD could be used to detect adulteration of coffee with roasted soybean or wheat [6]. The AOAC International has approved an HPAE-PAD method using the Dionex CarboPac PA1 column for determining free and total carbohydrates in instant coffee [4]. The method requires over one hour per sample and a post-column addition of sodium hydroxide for analyte detection. After the introduction of the Dionex CarboPac SA10 column, a method using that column was reported that required less than 10 min per sample and no post-column sodium hydroxide addition [7]. Figure 1 shows the separation of coffee carbohydrates on the Dionex CarboPac SA10-4µm column. Mannitol, fucose, sucrose, arabinose, galactose, glucose, xylose, mannose, and fructose are resolved in less than six minutes. This separation delivers better resolution of the eight carbohydrates than published in reference seven, and was applied to the determination of free and total carbohydrates in instant coffee (Figure 2). Arabinose is the most prominent monosaccharide in the free carbohydrate analysis while galactose and mannose are the most prominent in the total carbohydrate analysis of the instant coffee. The major carbohydrates in both the free and total carbohydrate analyses of the instant coffee sample were quantified (Table 1). These determinations were validated by the good spike recovery achieved for each analyte in both types of analyses. Note the extremely low concentrations of glucose and fructose in Figure 2. Elevated concentrations of glucose and fructose are markers for roasted wheat and roasted soybean adulteration [6]. A second instant coffee sample (not shown) had a similar profile of free and total carbohydrates. This method will not resolve galactose and rhamnose as well as fructose and ribose. While rhamnose and ribose are two of the carbohydrates separated in AOAC Method 995.13, earlier work showed very low concentrations of rhamnose in the free and total carbohydrate analysis of instant coffee and no detectable ribose [7] and therefore this method is suitable for instant coffee determinations.

Table 1: Carbohydrate determination of an instant coffee sample.
There were three sample preparations and each sample was injected in triplicate.

Analyte	Analysis Type	Amount (mg/L)	Spiked Amount (mg/L)	Spike Recovery (%)
Arabinose	Free sugars	23.4	30.0	84.4
	Total sugars	86.6	100	83.5
Galactose	Free sugars	12.3	12.0	103
	Total sugars	633	650	100
Mannose	Free sugars	12.3	15.0	125
	Total sugars	612	600	110
Fructose	Free sugars	1.6	10.0	106
Glucose	Total sugars	21.8	20.0	86.5

The last decade has witnessed the emergence of functional beverages, which are beverages formulated to provide a specific benefit. Electrolyte replacement beverages, a.k.a. sports drinks, and energy drinks are two such beverages. These are typically sweetened with glucose, fructose, sucrose, or some combination of these three carbohydrates. Figure 3 shows an analysis of the glucose, fructose, and sucrose contents of four functional beverages. The samples were diluted, filtered, and injected. The three carbohydrates are well resolved and therefore easy to quantify. Note the variety of the ratios of the three analytes in these products as well as confirmation that the sugar free drink does not have any detectable glucose, fructose, and sucrose. The same method can be used to determine the carbohydrate content of two fruit juices (Figure 4). The peak at about 2 min in the apple juice sample is probably sorbitol, which is known to be a major component of apple juice's sweetness and will elute early from this column.

HPAE-PAD has been used for analysing the carbohydrate content of honey since 1990 [8]. The method has the required sensitivity to detect honey's small quantities of disaccharides, trisaccharides, and small

oligosaccharides in the presence of large amounts of glucose and fructose. The profile of honey's low concentration carbohydrates provides a 'signature' of the honey's origin. This analysis can also detect adulteration of honey with lower cost sweeteners. Figure 5 shows the separation of three honey samples and a standard containing some carbohydrates often found in honey. The separation uses the CarboPac PA210-4 µm Fast column, which was designed for high resolution separations of carbohydrates such as those found in honey using only a sodium hydroxide eluent. Note the different carbohydrate profiles of the three samples, especially with respect to the intensity of individual peaks. This is consistent with Swallow and Low's report that honeys have most of the same carbohydrates but differ in the relative concentrations of those carbohydrates [8].

Conclusion:

Three applications were presented; the carbohydrate determination of instant coffee, the analysis of beverages for glucose, fructose, and sucrose, and the profiling of low-abundance carbohydrates in honey. These show that HPAE-PAD is a fast, high resolution chromatography method for determining carbohydrates in food and beverages. For each application the carbohydrate determination is direct as no sample derivatisation is required for either analyte separation or detection.

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