Short note

Food & Beverage

Carbohydrates in Dutch coffee candies (Hopjes)

Introduction

Back in the 18th century in The Hague, the Dutch Baron Hendrik Hop left his cup of coffee, flavored with sugar and cream, on the stove. By the following morning, it had turned into a tasty caramelized substance with coffee flavor. Legend says this inspired the creation of the famous Dutch coffee candy named *Haagsche Hopjes*, or *Hopjes*, still enjoyed today [1].

The main components of this particular Dutch coffee candy are carbohydrates, consisting of over 90 g per 100 g of the product. Its main ingredients are sucrose (table sugar), and glucose syrup, which is a blend of glucose and glucose-oligomers with various chain lengths. Throughout the production process, these sugars are subjected to heat, leading to partial caramelization and the creation of more complex oligo- and polysaccharides. Additionally, dairy cream is included as another component, serving as a source of lactose within this candy type.

High Pressure Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD) is the method of choice for analysis of samples with a complex carbohydrate composition, like these Dutch coffee candies. The ALEXYS™ Carbohydrates Analyzer, shown in Figure 1, was utilized in combination with the new SweetSep™ AEX200 column to showcase the capabilities of the system.

Fig. 1. *ALEXYS Carbohydrate Analyzer.*

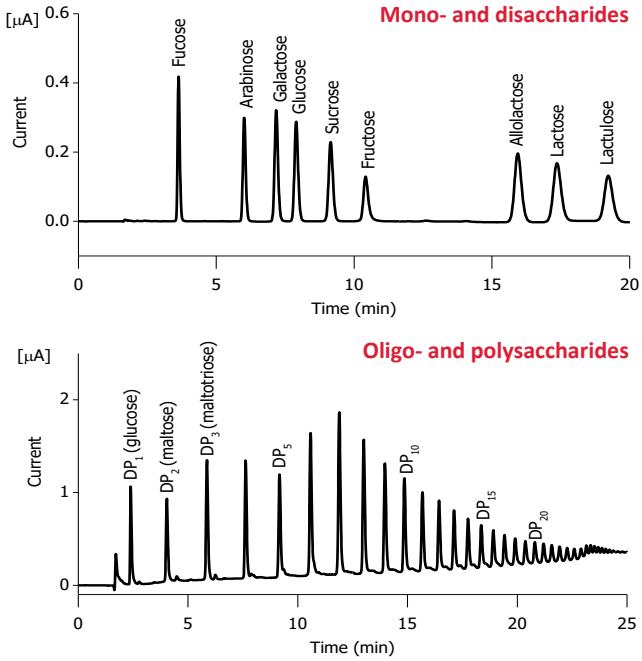

Fig. 2. Analysis of a 10 µM mix of mono- and disaccharides (top) and 200 ppm maltodextrin standard (bottom) in DI water, analyzed with LC-EC conditions according to Table 1.

Table 1. HPAEC-PAD conditions

HPLC	ALEXYS™ Carbohydrate Analyzer (Antec Scientific)
Columns	SweetSep™AEX200, 4x200mm column, 5µm SweetSep™AEX200, 4x50mm precolumn 5µm (Antec Scientific)
Flow rate	0.7 mL/min
Backpressure	200-250 bar
Injection	10 µL
Temperature	30°C for separation, 35°C for detection
Flow cell	SenCell Au WE, HyREF, AST setting 2
Potential waveform (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	0.2 - 0.8 µA
Mobile phase (mono-, disaccharides)	12 mM NaOH isocratic for 20 min, followed by column clean-up and equilibration
Mobile phase (oligo-, polysaccharides)	100 mM NaOH + 40 mM NaOAc to 100 mM NaOH + 450 mM NaOAc in a linear gradient at 0-30 min, followed by column clean-up and equilibration

Method

The analyses were performed on an ALEXYS Carbohydrate Analyzer with quaternary low pressure gradient mixing, consisting of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS6.1L autosampler, ET210 eluent tray for mobile phase blanketing, and Clarity data acquisition software. The SenCell with gold working electrode and HyREF (palladium) reference electrode using a 4-step PAD waveform was used for detection, and the SweetSep AEX200 analytical column with pre-column were used for the separation. Two different separation methods were applied to focus on either the mono- and disaccharides, or the oligo- and polysaccharides, see Table 1. The typical system performance parameters (linearity, reproducibility, detection limit) are reported in other application notes [2, 3].

Sample and standards preparation

Two different brands of the traditional Dutch coffee candy were analyzed:

- *Rademaker® Hopjes,* 'the one and only Rademaker Haagsche Hopjes' according to the label.
- *Holland Hopjes*, claiming to be based on the original recipe.

Candy samples were first crushed into small pieces and dissolved in DI water. Subsequently, the sample solutions were treated with Carrez clarification reagents to precipitate proteins and fat. After centrifugation the supernatant was filter over a 0.2 µm Polyethersulfone (PES) syringe filter resulting in a clear sample solution (20 gram product/L). This extract was diluted 10x for trace analysis of the minor sugar components present in the sample. The extract was diluted 100x an 1000x for quantification of the main sugars, and the 100x dilution was also used for profiling of the larger carbohydrates with a degree of polymerization DP > 3.

Mono-disaccharides method: for the single-point calibration a standard mix of 10 µM fucose, arabinose, galactose, glucose, sucrose, fructose, allolactose, lactose and lactulose in DI water was prepared. Sucrose was quantified on the basis of the 1000x dilution, and the other sugars were quantified on the basis of the 100x dilution. To confirm the peak identity of the sugars that were present in trace amounts, an additional 10x diluted sample was spiked with a concentration of 0.5 µM of the standards (data not shown).

Oligo– and polysaccharides method: for the single-point calibration a standard mix of 10 µM maltose and maltotriose in DI water was prepared. The components were quantified on the basis of the 100x dilution. For identification of the malto-oligo-saccharides (DP > 3) a 500 ppm maltodextrin and DP6 standard in water were prepared.

Table 2. Content (g/100g) of carbohydrates in coffee candy

Compound	Rademaker ® Hopjes	Holland Hopjes
Fucose	0.0004	0.005
Arabinose	0.005	0.008
Galactose	0.003	0.004
Glucose	0.7	3
Sucrose	51	47
Fructose	0.1	0.4
Lactose	0.03	0.2
Lactulose	n.d.	0.003
Maltose*	17	9
TOTAL sugars (DP<3)	69	60
Maltotriose (DP3)*	13	13
Content according product label		
Carbohydrates	92	94
Sugars	73	68

*) Quantified with the Oligo– and polysaccharides method.

Results

Sucrose is the primary ingredient in Dutch coffee candy, evident from its prominence as the principal peak in the chromatograms of the candy extracts (Fig. 3 and 4). Maltose and maltotriose were identified as the next most abundant carbohydrates (Table 2). Lactose, among the mono- and disaccharides detected, was present in small amounts in both samples. However, despite the low lactose content, the values are above the threshold for labelling the candies as 'lactose-free' products. Criteria: lactose < 0.01 g/100 g product [4].

A range of oligo- and polysaccharides were observed in both candies (Fig. 4). It is most likely a combination of the caramelization products formed during the heating process and malto-oligosaccharides originating from the glucose syrup. The obtained profiles for both candies show distinct differences. The profile of the Holland hopjes show more prominent DP4 - DP6 peaks. This is most probably due to a different source of glucose syrup used in this product.

The amount of sugars in the candies were quantified using a single point calibration method and the results are shown in Table 2. Notably, the total amount of sugars (maltotriose is not

Mono-, and disaccharides

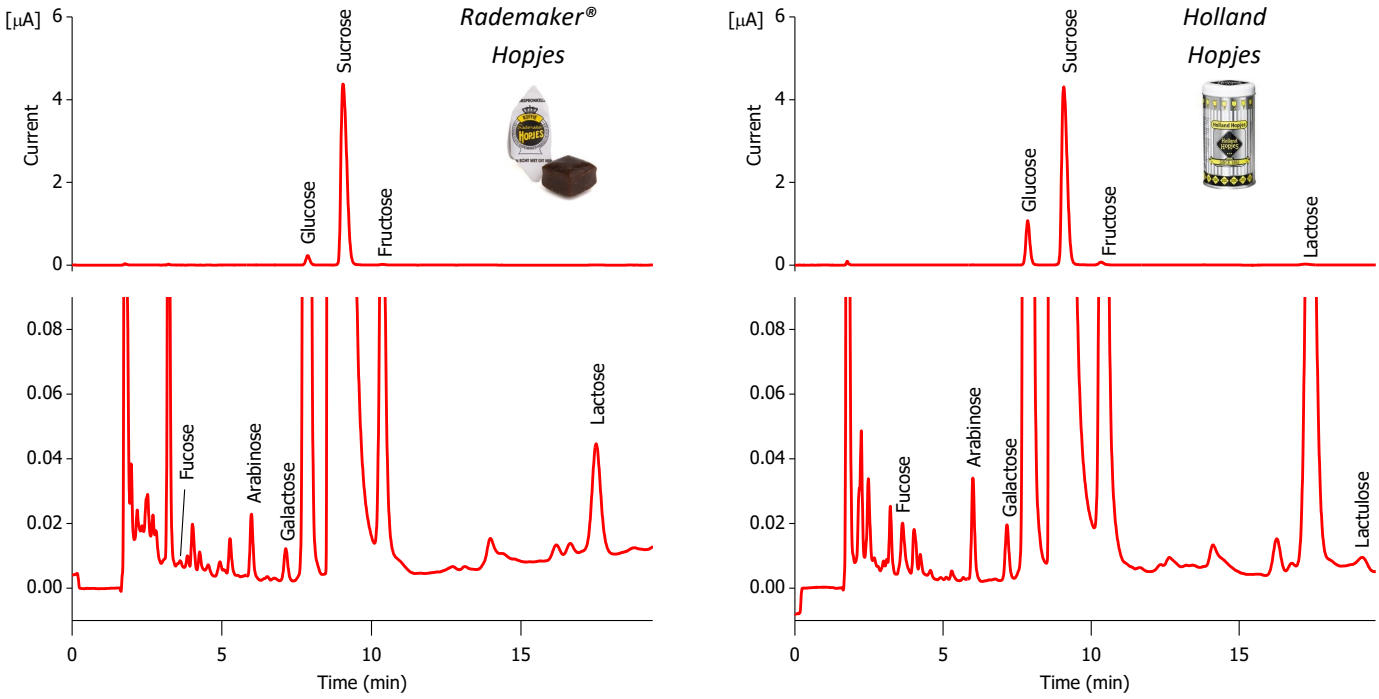

Fig. 3. Analysis of mono- and disaccharides in 2 types of Dutch coffee candy: Rademaker ® Hopjes (left) and Holland Hopjes (right). Tested extracts of samples based on 0.2 g product/L (top), or 2 g product/L (bottom). Conditions according to Table 1; range setting 200 µA (top) or 2 µA (bottom).

Oligo- and polysaccharides

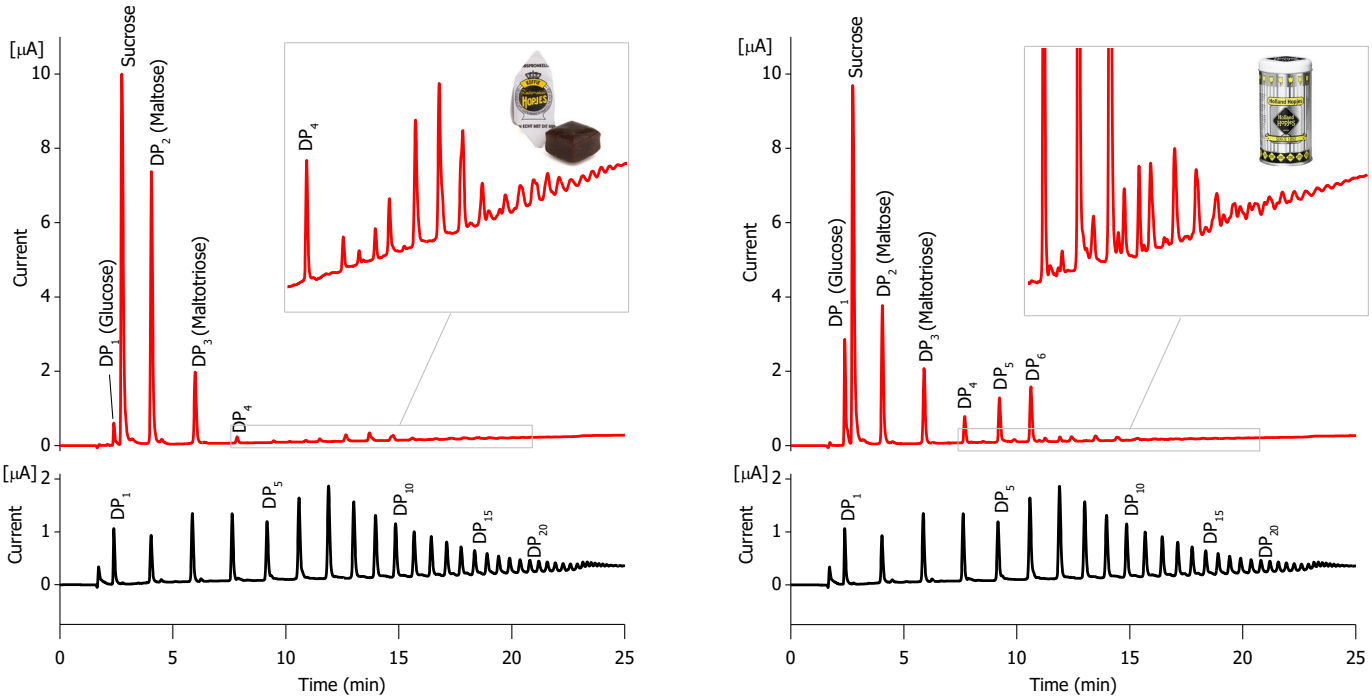

Fig. 4. Analysis of oligo-, and polysaccharides in 2 types of Dutch coffee candy: Rademaker ® Hopjes (left) and Holland Hopjes (right). The inset shows a zoom-in of the indicated area (same size for both chromatograms). Chromatogram of 200 ppm maltodextrin (black) given for comparison. Tested extract of samples contained 0.2 g product/L. Analytical conditions according to Table 1; range setting 10 µA.

considered a sugar) are a bit lower, but close to the 'sugars' content as stated on the product label of both candies. The difference may be related to the production process where sugars are caramelized into oligo– and polysaccharides.

Conclusion

The ALEXYS Carbohydrate Analyzer in combination with the new SweetSep™ AEX200 anion-exchange column offers a versatile solution for the sensitive HPAEC-PAD analysis of carbohydrates in various samples, such as food and dairy products. The presented example chromatograms obtained from Dutch coffee candy samples (Hopjes) demonstrate the ability of the SweetSep™ AEX200 column to separate a broad range of carbohydrates, from mono- up to polysaccharides, with a high resolution using a single column. By simply adapting the separation conditions (mobile phase composition and gradient profile), the AEX200 column can be used for the quantification of mono-, di- and trisaccharides, or for profiling of oligo- and polysaccharides.

References

1. Wikipedia, Hopjes, https://en.wikipedia.org/wiki/Hopje
2. Antec Scientific, Analysis of Maltodextrin in Syrups, Application note 220_027
3. Antec Scientific, Carbohydrates in honey, Application note 220_025
4. Antec Scientific, Lactose-free products, Application note 220_009

Ordering information

Detector only	
176.0035B	DECADE Elite SCC electrochemical detector
116.4321	SenCell 2 mm Au HyREF
Recommended ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
186.ATC00	
Separation columns	
260.0015	SweetSep™ AEX200, 4x50mm precolumn, 5µm
260.0010	SweetSep™ AEX200, 4x200mm column, 5µm
Software*	
195.0035	Clarity CDS single instr. incl. LC, AS module

*) The ALEXYS Carbohydrate Analyzer can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. For the DECADE Elite electrochemical detector only, control drivers are also available for Waters Empower™, Agilent OpenLab CDS, and Agilent OpenLab CDS Chemstation Edition. Please Contact Antec for more details.

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