The Sweet and Bitter Truth of Honey: Detecting Adulteration Using HPAEC-PAD

M. Eysberg, C. Marvelous, T. Mulder, Y. Tazini, H.J. Brouwer, J.P. Chervet
Antec Scientific, Alphen a/d Rijn, The Netherlands

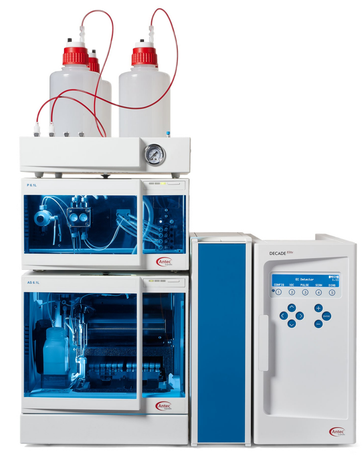
Introduction

Honey is a complex natural product composed primarily of carbohydrates, including mono-, di-, oligo-, and polysaccharides. Recent studies have highlighted the common occurrence of honey adulteration, with approximately 46% of imported honey samples failing to meet the regulatory standards. The main challenge in detecting fraudulent honey is the considerable natural variation in its composition, which makes it difficult to distinguish from authentic honey. High-performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is the analytical method of choice widely used in the detection of carbohydrates in honey. The use of HPAEC-PAD provides sensitivity and selectivity, allowing detection of carbohydrates at low concentrations. In this study, the new SweetSep™ AEX200 strong anion-exchange column is employed for high-resolution separation of the carbohydrates, enabling accurate carbohydrate profiling of honey.

Instrumentation

Dedicated metal-free HPAEC-PAD system consisting of:

- ET210 eluent tray, for sparging and blanketing of eluent with inert gas (N_2 or He).
- P6.1L quaternary LPG pump with 4 channel degasser.
- AS6.1L autosampler with cooling / heating (4°C up to 40°C)
- CT2.1 column oven / thermostat (5°C up to 85°C).
- SweetSep™ AEX200 4 x 200 mm column (pn 260.0010).
- DECADE Elite, electrochemical detector with dedicated flow cells (SenCell™ or FlexCell™) for carbohydrates.
- Data acquisition and instrument control via DataApex Clarity CDS or Thermo Scientific™ Chromeleon™ CDS.



ALEXYS™ Carbohydrate Analyzer (Antec Scientific)

Results

1. Analysis of sugars in natural honey

Profiling and quantification of sugars in honey can be used to assess floral origin, quality and adulteration of honey. Separation of 15 sugars commonly found in honey was achieved within 25 minutes with sufficient resolution (figure 1).

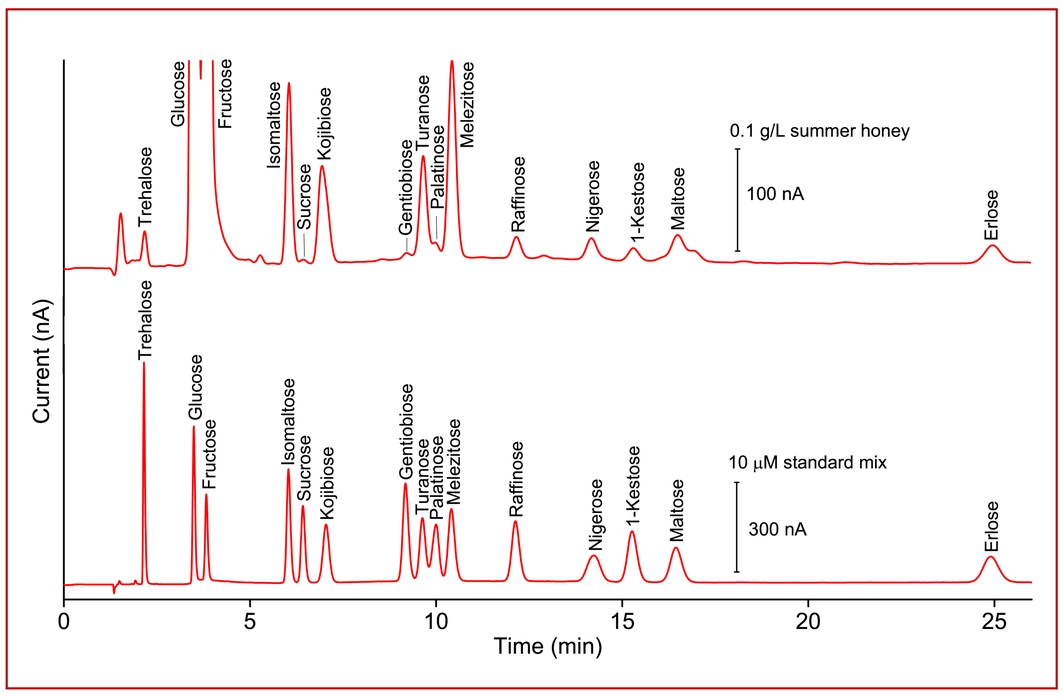


Figure 1. *Analysis of honey on SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL injection of a 0.1 g/L Swiss summer honey sample. Bottom: 10 µL injection of a 10 µM standard of 15 sugars commonly found in honey. Isocratic elution: 68 mM NaOH, 0.7 mL/min, 20°C. Sample prep: simple dilution & filtering over a 0.2 µM PES filter.*

Table 1. Quantification and adulteration criteria of honey samples

Parameters	*Pure honey criteria*	*Amount in sample*	*Within criteria?*
Sucrose	< 5 g/100 g	0.1 g/100 g	✓
Maltose	< 4 g/100 g	0.5 g/100 g	✓
Fructose + Glucose	> 45 g/100 g	54.5 g/100 g	✓
Fructose / Glucose	0.9 – 1.4	1.4	✓

2. Oligo- and polysaccharides in honey

HPAEC-PAD analysis of oligo- and polysaccharides offers a powerful approach for detecting low-level adulteration in honey based on cheap sweeteners such as corn or rice syrups. The method allows high-resolution separation of fructooligosaccharides from maltooligosaccharides, as well as separation of oligo- from polysaccharides, offering comprehensive carbohydrate profiling (figure 2 and 3).

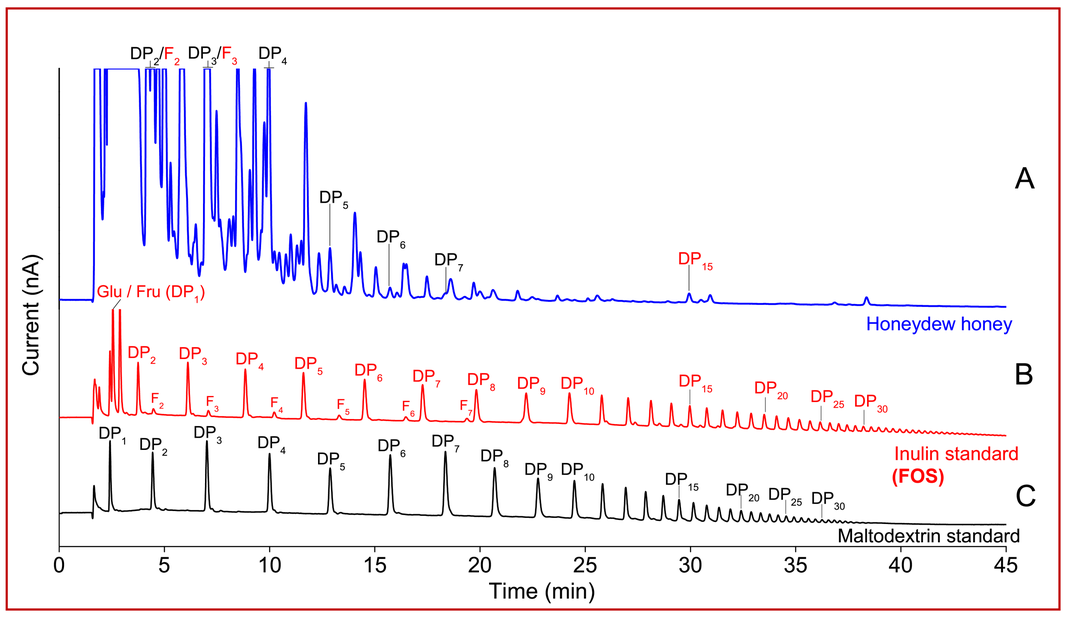


Figure 2. *Authentic honeydew honey with natural oligosaccharides, (A) 10 µL injection of honeydew honey sample, (B) 200 ppm inulin standard, and (C) 500 ppm maltodextrin standard (black line). Red peaks in (A) correspond to fructo-, black to malto-oligosaccharides. SweetSep™ AEX200 precolumn 4.0 mm ID x 50 mm + AEX200 column, 4.0 mm ID x 200 mm. Linear gradient from 100 mM NaOH + 25 mM NaOAc to 100 mM NaOH + 150 mM NaOAc from t = 0 to t = 20 min, followed by linear gradient to 100 mM NaOH + 450 mM NaOAc at t = 45 min.*

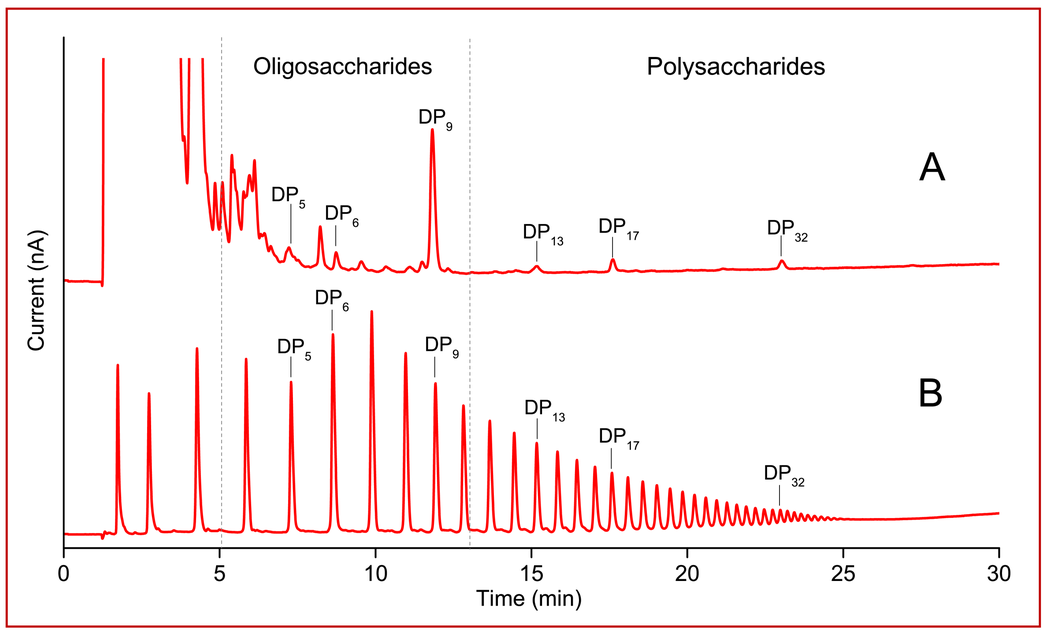


Figure 3. *Analysis oligo- and polysaccharides in (A) honey, (B) reference maltodextrin (dextrose equivalent, DE 4-7), SweetSep™ AEX200 column, 4.0 mm ID x 200 mm. Linear gradient from 100 mM NaOH + 40 mM NaOAc to 100 mM NaOH + 450 mM NaOAc from t = 0 to t = 30 min.*

3. Honey fraud and adulteration

Selective separation of oligo- and polysaccharides in honey enables the detection of adulteration, helping to prevent food fraud and protect both producers and consumers (figure 4).

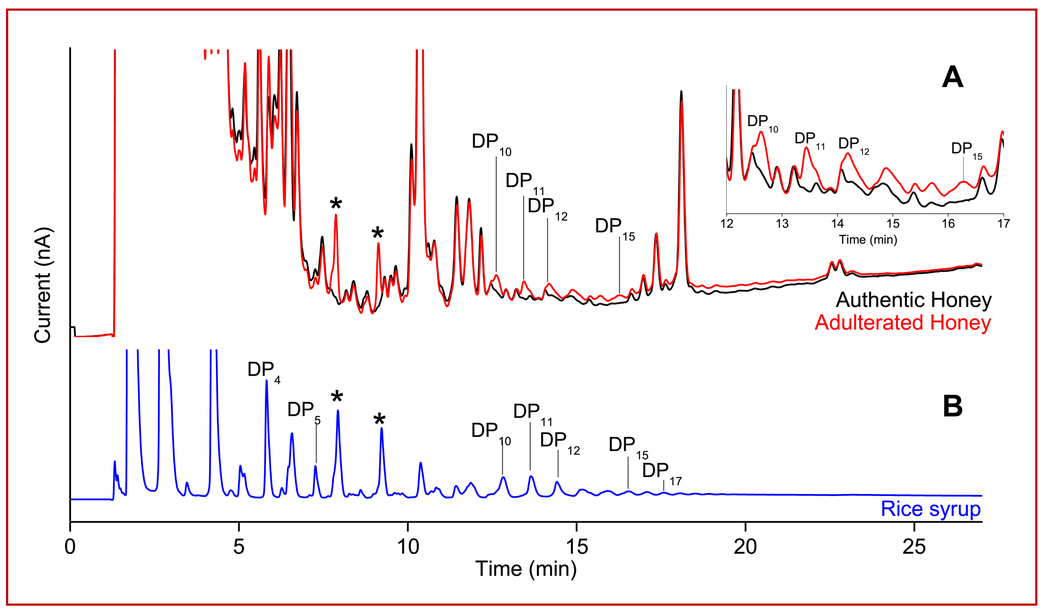


Figure 4. *(A) Overlay of chromatograms of a 10 µL injection of authentic avocado honey sample (black trace), and avocado honey sample adulterated with 1.1% rice syrup (red trace), and (B) 2.5 g/L rice syrup sample,. Peaks marked with (*) are from the rice syrup. Linear gradient from 100 mM NaOH + 40 mM NaOAc to 100 mM NaOH + 450 mM NaOAc from t = 0 to t = 30 min.*

Conclusions

A novel 4 x 200 mm anion-exchange column based on highly monodisperse 5 µm particles, SweetSep™ AEX200 (pn 260.0010), was utilized for the analysis of carbohydrates in honey. The presented data obtained with the Antec Scientific **SweetSep™ AEX200 column** in combination with the **ALEXYS Carbohydrate analyzer** demonstrate:

- **Fast, high-resolution separation** of mono-, di- and trisaccharides in honey.
- **Sensitive quantification** of carbohydrates down to femtomole levels.
- **High-resolution** separation of oligo– and polysaccharides.
- **Fraud/adulteration** detection down to 1%.

www.AntecScientific.com