2D separation and profiling of complex oligosaccharide mixtures

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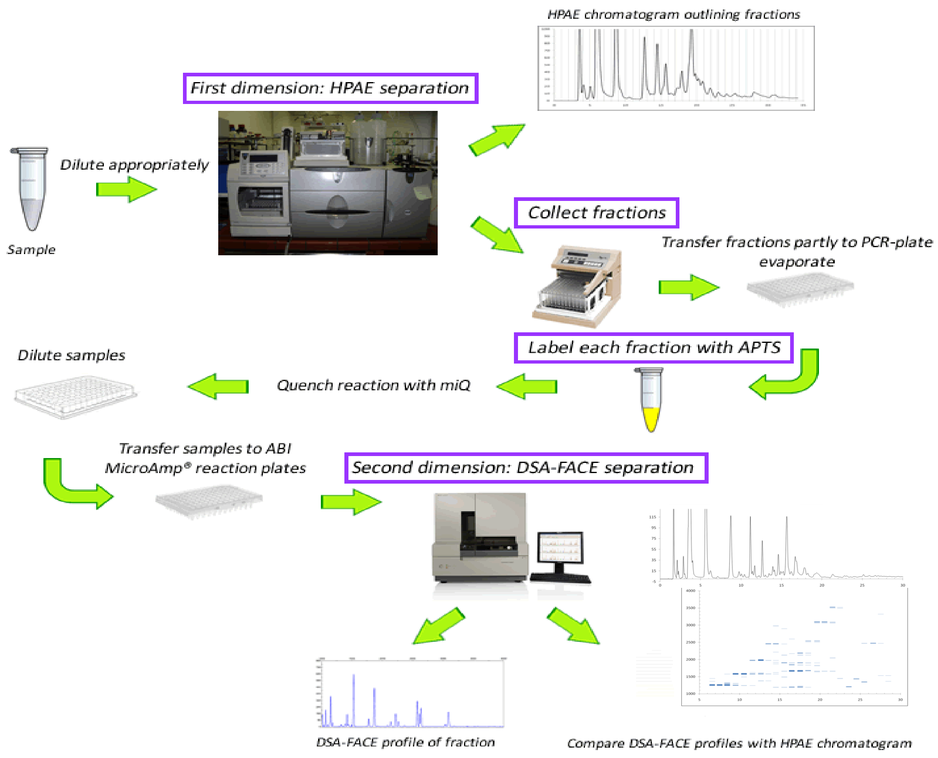
Oligosaccharides present in various natural sources can contain different linkages, susbstitutions and building blocks with the same mass. Oligosaccharide mixtures often contain these isomeric and isobaric structures, which causes their complexity. These components are difficult to separate and analyze with conventional analytical methods. The technique described below was developed to obtain a higher resolution.

1. The technique

High-performance (high-pH) anionic exchange chromatography (HPAEC) was coupled off-line to the carbohydrate fingerprinting method DSA-FACE (DNA sequencer-aided fluorophore-assisted carbohydrate electrophoresis).



The **first HPAEC-dimension** separates unlabeled oligosaccharides based on their size and charge, and collects these compounds in fractions. Separation is done using a Dionex ICS 3000 instrument. The flow is split by a threeway valve: one part is led to the detector for pulsed amperometric detection (PAD), and the remaining part is desalted and collected in microtiterplates. Part of the samples is evaporated and labeled by reductive amination with APTS (8-aminopyrene-1,3,6-trisulfonic acid).

In the **second dimension**, the fractions are analyzed by capillary electrophoresis (CE) using standard DNA sequencing equipment (four capillary ABI 3130 Genetic Analyzer) and the APTS-labeled oligosaccharides were detected using laser-induced fluorescence.

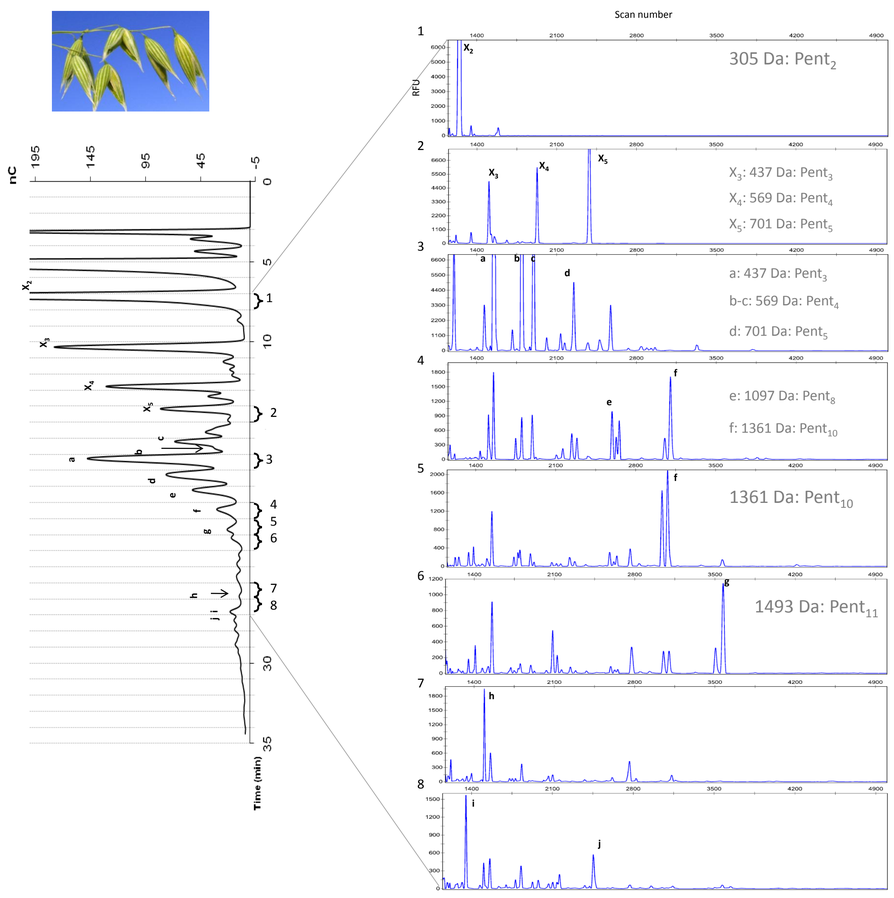
In parallel, some unlabeled fractions were analyzed with MALDI-TOF-MS.

2. Results obtained with xylan

Sample preparation

2% oat spelt xylan was treated with a commericial enzyme preparation to release **xylo-oligosaccharides.**

2D profile of xylan hydrolysate

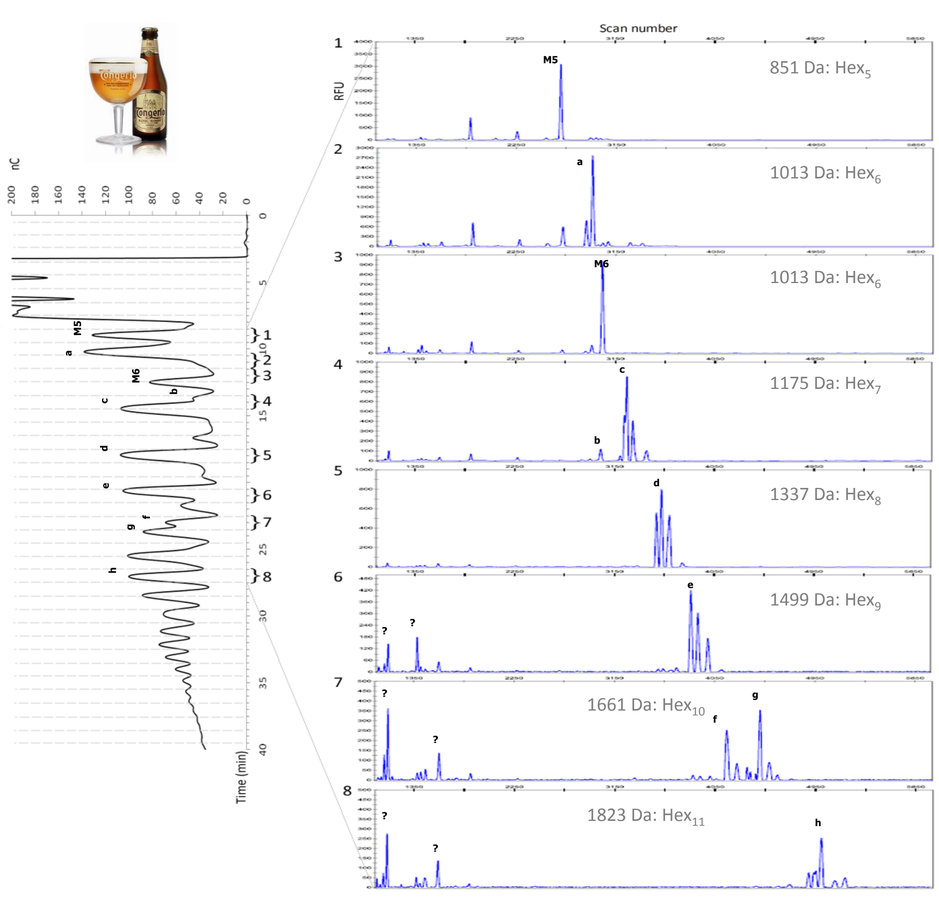


3. Results obtained with beer

Sample preparation

A Belgian beer (contains **isomalto-oligosaccharides**) was degassed and deproteinated.

2D profile of beer



4. Conclusions

To demonstrate the technique, the profiles of an enzymatic xylan hydrolysate (xylo-oligosaccharides) and a Belgian beer (isomalto-oligosacharides) were analyzed. Several fractions collected from the first dimension contain different components while only displaying one peak in the HPAE chromatogram. Using CE in the second dimension, these isomeric structures are resolved. Analysis in parallel of unlabeled fractions by MS confirms the mass of the structures. Negatively charged carbohydrates are bound stronger on the anion exchange column in the first dimension, but migrate at the front of the profile during CE in the second dimension. Linking of such peaks between the two dimensions is aided by the MS results.

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