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Profiling Fructosyloligosaccharide- and Galactosyloligosaccharide-containing Samples by HPAE-PAD

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ABSTRACT

Purpose: To develop and validate a high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) method for the determination of fructosyloligosaccharides (FOS) and galactosyloligosaccharides (GOS) in prebiotic samples.

Methods: The separation of individual oligosaccharides in FOS/GOS was achieved by HPAE using a Thermo Scientific™ Dionex™ CarboPac™ PA200 column with a sodium hydroxide/sodium acetate mobile phase. Oligosaccharides were detected using PAD with a gold working electrode.

Results: An HPAE-PAD method was successfully developed and validated for determination of FOS/GOS in prebiotic samples, including dietary supplements and infant formulas.

INTRODUCTION

HPAE-PAD is one of the most employed techniques for carbohydrate determinations in both routine monitoring and research applications. This technique has had great impact on the analysis of oligo- and polysaccharides, including prebiotics. Prebiotics are non-digestible ingredients that beneficially affect human health by selectively stimulating the growth of one or a limited number of bacteria in the colon.[1] Examples of prebiotics include FOS, GOS, and inulins. HPAE-PAD provides valuable insights for the selective determination of short-chain FOS and inulins present as prebiotic food ingredients as it can reveal the degree of polymerization (DP) and isomer distribution of the prebiotic. Determination of the DP of a particular prebiotic ingredient is critical for its intended use as it influences some intrinsic properties such as prebiotic activity, digestibility, caloric value, and sweetening power. In this work, the oligo- and poly-saccharide distributions of FOS and GOS samples were characterized by HPAE-PAD.

In addition, HPAE-PAD profiling was demonstrated as a method to determine the FOS or GOS content and the change in DP upon addition of probiotics. This allows evaluation of eventual synergic effect between prebiotics and probiotics and modulation of the strains involved to optimize the composition for the desired effect. The separation of individual oligosaccharides in FOS/GOS was achieved by HPAE using a high-performance anion-exchange column with a sodium hydroxide/sodium acetate mobile phase. The oligo- and poly-saccharides were detected directly using PAD with a gold working electrode. Therefore, no sample derivatization was required.

For more details please refer to Thermo Scientific application notes 1149 and 1151 in the Thermo Scientific AppsLab Library of Analytical Applications.[2,3]

MATERIALS AND METHODS

Prebiotic samples: Inulin (Chicory roots), inulin (Dahlia tubers), Jarrow's Inulin-FOS prebiotic dietary supplement, Bimuno®-GOS, European infant formula sample.

Probiotic sample: Yakult® probiotic drink

Sample Preparation: Mix 200 mg/L of Inulin-FOS/Bimuno-GOS sample with Yakult drink (10mg/mL) in 1:1 ratio. Incubate mixture for 48 h in a water bath maintained at 37 ºC. After incubation, transfer 12 mL to a 50 mL Amicon Ultra-15 Centrifugal filter device and cap. Centrifuge for 30 min at 5000 rpm and 20 ºC. Pass through a 0.2 µm filter before analysis.

Table 1. Method conditions

	FOS method	GOS method
Column	Dionex CarboPac PA200 + guard	
Eluent	-5 min: 100 mM NaOH/20 mM NaOAc, 0–15: 100 mM NaOH/20 mM NaOAc, 15–70: 100 mM NaOH/450 mM NaOAc, 70–70.1:100 mM NaOH/20 mMNaOAc, 70.1–75:100 mM NaOH/20 mM NaOAc, curve 5	-5 min: 100 mM NaOH/50 mM NaOAc, 0–45: 100 mM NaOH/150 mM NaOAc, 45–55:100 mM NaOH/500 mM NaOAc, 55–60:100 mM NaOH/50 mMNaOAc, curve 5
Temp.	30 ºC	
Flow rate	0.5 mL/min	
Inj. Vol.	10 µL	
Detection	PAD (Au) Disposable Waveform A (TN21)	

Data Analysis

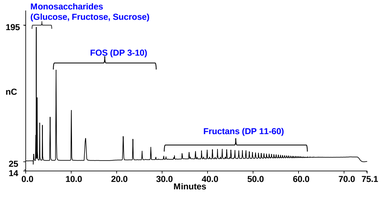
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software was used for all data acquisition and processing.

RESULTS

FOS Separation

Figure 1 shows a chromatogram of the Inulin-FOS sample showing the separation of low molecular weight monosaccharides from high molecular weight FOS and fructans. Inulin-FOS is a prebiotic dietary supplement containing inulins and FOS that help in promoting probiotic bacterial growth. Baseline separation in a single run was obtained using the gradient profile described in the conditions section. The separation was designed to be completed within 75 min. Using mild isocratic conditions at the beginning, glucose, fructose, and sucrose were resolved and then a linear gradient of NaOAc (20–500 mM) was applied to separate fructans with a DP ranging from 3 (1-kestose) to 60. Thus, separation of both low and high molecular weight fructans was achieved.

Figure 1. HPAE-PAD Separation of Jarrow's Inulin-FOS sample.

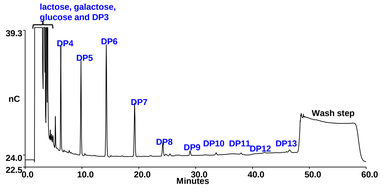


GOS Separation

Figure 2 is the chromatographic profile of the Bimuno GOS sample showing the separation of small sugars (lactose, galactose, and glucose) and major oligosaccharides. GOS has been reported to show different chain lengths, ranging from DP2 to DP10 with a terminal glucose.[13]

For Bimuno GOS sample, we observed peaks in the 45 min separation window that were tentatively identified as DP1-DP13. The DP peak area distribution is skewed toward the smaller carbohydrates, DP1 and DP2, thus making it difficult to observe all the peaks in the same chromatogram. We scaled the Bimuno GOS chromatogram to visualize DP4–DP13.

Figure 2. HPAE-PAD Separation of Bimuno-GOS sample.



The DP1 and DP2 peaks were identified based on the chromatogram of a mixture of glucose, galactose, and lactose. DP3 was identified by the chromatogram of maltotriose, a trisaccharide sugar consisting of three glucose molecules linked with α-1, 4 glycosidic bonds. Maltotriose elutes near the peak assigned as DP3. The assignment of the chromatographic peaks higher than DP3 was based on the generally accepted assumption that the retention time of a homologous series of carbohydrates increases as DP increases, and thus each successive peak represents a GOS that has one galactose more than the previous peak.

METHOD ACCURACY

Sample Recovery

Method accuracy was evaluated by measuring recoveries in spiked Inulin-FOS samples. As noted earlier, there are no true standards available for these oligosaccharide samples. Thus, we interchanged samples and standards

Figure 3. Chromatographic profile of A) unspiked inulin sample (chicory root) ; B) spiked inulin sample (+ 50 mg/L Inulin–FOS) and C) spiked inulin sample (+ 100 mg/L Inulin–FOS).

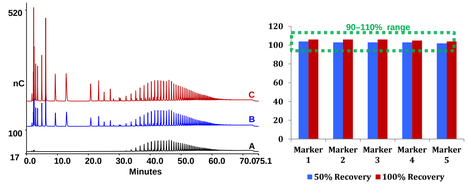
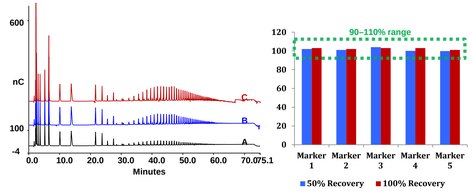
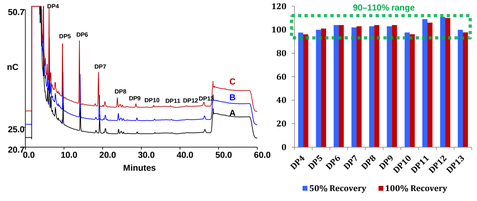


Figure 4. Chromatographic profile of A) unspiked Inulin-FOS sample (Prebiotic); B) spiked Inulin-FOS sample (+50 mg/L chicory inulin) and C) spiked inulin sample (+100 mg/L chicory inulin).



GOS recoveries were determined based on calibrations using Bimuno GOS. Two spiked concentration levels in the European-sourced infant formula sample spiked with Bimuno GOS were examined (See Figure 5).

Figure 5. Chromatographic profile of A) unspiked infant formula; B) spiked 1 infant formula and C) spiked 2 infant formula.



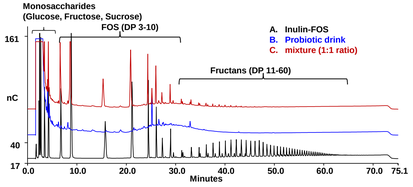
For the samples spiked and calibrated using Bimuno GOS, the recoveries ranged from 96.1–110%.

PROBIOTIC / PREBIOTIC INTERACTION

Addition of Probiotic to FOS

We used a probiotic drink, to assess the effect on chain length distribution, and amount of an added prebiotic. This probiotic drink contains live culture of 6.5 billion counts of Lactobacillus casei strain Shirota. HPAE-PAD chromatographic profiles allowed the comparison of prebiotic profile with and without incubation with probiotic. Figure 6 (below) shows the results of this experiment.

Figure 6. Chromatographic profile of A) prebiotic (Inulin FOS), B) probiotic drink and C) 1:1 mix of prebiotic/probiotic.

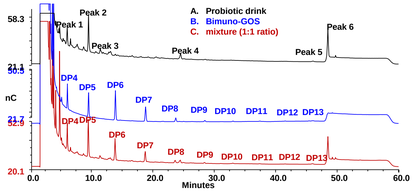


The above figure clearly demonstrates that the degradation of higher DP fructans is greater than the smaller FOS DP (≤ 10).

Addition of Probiotic to GOS

Similarly, Bimuno GOS sample was incubated with probiotic drink and analyzed using HPAE PAD. Figure 7 (below) shows the results of this experiment.

Figure 7. Chromatographic profile of A) probiotic drink, B) prebiotic sample (Bimuno-GOS) and C) 1:1 mix of prebiotic/probiotic.



As shown in Figure 7, six major peaks were observed in the chromatographic profile of probiotic sample, of which peaks 1, 2, 3 and 5 have the same RTs as DP4, DP5, DP6, and DP13 in the separation of the Bimuno GOS sample. Because the probiotic sample has other carbohydrate components such as corn dextrin, the five major peaks may be carbohydrates other than GOS. Table 2 lists the concentrations of the DPs calculated based on the individual calibration curves of the DP fractions of the Bimuno GOS (discussed above). With the exception of DP8, the concentrations for all the DPs decreased, indicating degradation of Bimuno GOS from the addition of probiotics.

Table 2. Comparison of concentration of individual DPs of Bimuno GOS profile with and without incubation with probiotic-containing Yakult.

DP (mg/g) →	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13
Probiotic drink	19.7	61.0	2.38	n.a.	n.a.	1.69	n.a.	n.a.	n.a.	1.84
Bimuno GOS	18.5	23.4	34.9	19.0	5.46	2.01	1.05	0.70	0.43	0.93
Probiotic and GOS (1:1mix)	27.5	54.6	35.1	18.3	5.90	2.03	1.00	0.68	0.39	2.19

These results suggest that HPAE-PAD is a powerful tool for profiling oligo- and poly-saccharides in complex mixtures and can provide significant information about interactions among bacteria and prebiotic fibers.

CONCLUSIONS

- The described HPAE-PAD method enables complete oligosaccharide profiling of fructans/FOS/GOS and determination of the fructan/FOS/GOS content in prebiotic samples.
- This method demonstrates good precision and accuracy. The recoveries of fructans/GOS in samples ranged from 90 to110% .
- HPAE-PAD profiling is a powerful tool to evaluate the interactions of probiotics and prebiotics. Moreover, it provides the possibility of evaluating an eventual synergic effect between prebiotics and probiotics and modulating the strains involved to optimize the composition to obtain the desired effect.

REFERENCES

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2. Thermo Scientific. Application Note 1149: Profiling Fructosyloligosaccharides (FOS)-containing samples by HPAE-PAD. Sunnyvale, CA, 2015. [Online] https://appslab.thermofisher.com/App/3085/an-1149-profiling-fructosyloligosaccharides-foscontaining-samples-by-hpaepad
3. Thermo Scientific Application Note 1151: Profiling Galactosyloligosaccharide (GOS)-containing Samples by HPAE-PAD. Sunnyvale, CA, 2015. [Online] https://appslab.thermofisher.com/App/3083/an-1151-profiling-galactosyloligosaccharide-gos-containing-samples-by-hpaepad

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