Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Simultaneous determination of mono-, di-, oligo- and polysaccharides via planar chromatography in 4 different prebiotic foods and 60 naturally degraded inulin samples

Simone Kirchert, Gertrud E. Morlock*

*Chair of Food Science, Institute of Nutritional Science, and Interdisciplinary Research Center (IFZ), Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392, Giessen, Germany*

ARTICLE INFO

*Article history:*
Received 1 June 2018
Received in revised form 7 July 2018
Accepted 12 July 2018
Available online xxx

*Keywords:*
High-performance thin-layer chromatography
Inulin
Fructooligosaccharides
Functional food
Quantification in food samples
Food inspection

ABSTRACT

Oligo- and polysaccharides, especially fructooligosaccharides and inulin, are increasingly used as food ingredients. They are reported to stimulate the growth of bifidobacteria and are therefore used as prebiotics. As mono-, di-, oligo- and polysaccharides can together be present in food, an HPTLC method was developed for analyzing all in a single development. This method was used for both, effective food screening and quantification on the same plate. After a minimal sample preparation (aqueous extraction, dilution and filtration) and application, the samples were separated on an HPTLC plate silica gel 60 with acetonitrile - water 4:1 (*V*/*V*), containing 3.6 mM natural product reagent A. For a good reproducibility of the separation, the control of the layer activity was recommended. For up to 20 samples in parallel, the HPTLC analysis took 45 min (2.3 min/sample) with running costs of ca. 7 Euro (0.35 Euro/sample). Determination coefficients of the calibration curves were obtained between 0.9980 and 0.9998. The high degree of automation proved the method to be robust and suited for routine analysis, especially in food control. The newly developed method was successfully tested with 4 different prebiotic food and 60 naturally degraded inulin samples. A minimal sample preparation along with the determination of the intact inulin and FOS allowed the evaluation of the natural inulin degradation profile, as shown for naturally degraded inulin samples. This fast analysis might also be of interest in other fields that study, for example, plant breeding, edible insects, functional feed and metabolic processes.



## 1. Introduction

Oligo- and polysaccharides, especially fructooligosaccharides (FOS) and inulin, are increasingly used as food ingredients [1–4]. FOS and inulin are fructose chains of different polymerization degrees ($n$ = 3–60) and fibers which are not metabolized by digestive enzymes. FOS are capable of masking off-flavors of high-intensity sweeteners and are used in combination with these. Additionally, FOS are used as an alternative sweetener because of its similar sweetness profile to sucrose and its moderate sweetness of 30–50% of sucrose, whereas inulin has only 10%. [2,5–7] It is allowed to label food products as sugar-free if only inulin and/or FOS have been added. This also makes them a marketing instrument [2,5–7].

Independent of the food matrix, the ingestion of inulin and/or FOS also stimulated the growth of bifidobacteria [2]. The increase of bifidobacteria led to an inhibition of potentially pathogenic bacteria like *Clostridium* species or enterobacteria. Unable to metabolize such saccharides, their colonialization was suppressed. Additionally, the acidic *Bifidus* flora avoided the spreading of putrefactive bacteria [2,8]. Such a composition change in gut microbiota underlined the use of some bifidobacteria as prebiotics, for example in pastries, dairy products and candies. Additional labels on food products which highlight the prebiotic effect are justified, if the dosage covers at least one third (≥1.5 g) of the daily dose. The minimum consumption quantity and consumption period have to be printed on the food package, since the prebiotic effect is only reported after a consumption period of at least 7 days. Such labelling prevents consumer deception [1,2].

Inulin and FOS are storage carbohydrates in many plants like wheat, sunchoke, asparagus, chicory root, blue agave and onions [1,9]. Vermins like wheat weevils and moths can ingest those car-

* Corresponding author.
*E-mail address:* Gertrud.Morlock@uni-giessen.de (G.E. Morlock).

https://doi.org/10.1016/j.chroma.2018.07.042
0021-9673/

bohydrates. After intake, these saccharides are metabolized and degradation products can be determined. Also submerse cultivation of fungi was used for inulinase production to degrade inulin [10,11].

HPTLC [12–19,20] and HPLC [16,21–23] methods are mainly available for mono-, di-, oligo- or polysaccharide analyses. Inulin analyses by HPLC required an enzymatic sample pre-treatment (hydrolysis of inulin with inulinase) [21,24], or a hot water [21–25] or microwave extraction [26] of inulin. For determination of inulin and FOS, mostly anion exchange adsorbents and refractive index detection was used [21,24,25]. Additionally for detection of FOS, also charged aerosol and evaporative light scattering detection were applied [22,26]. HPLC separation was performed with trifunctional amide [26], amine [24–26], ion exclusion [21] and carbohydrate columns (Prevail and CarboPac) [22,23]. HPLC with pulsed amperometric detection demonstrated the analysis of mono-, di-, oligo- and polysaccharides in a single sample run [23,27]. An alternative inulin determination via an electrochemical sensor [28] was quite specific for that particular analyte, but not able to screen for several analytes in parallel.

Using HPTLC [12–16,20,29], the intact inulin or FOS were analyzed on silica gel phases using two- or multi-step developments. In comparison to HPLC, the sample preparation for HPTLC was gentler because there was no need for hot water extraction or hydrolysis. Depending on the food matrix, thermal processings could produce decomposition products by forced degradation of oligo- and disaccharides or by isomerization of sugars. As shown for FOS in orange juice, changes in the polymerization degree were observed and the analyzed profile may be different than the original inulin profile of the samples [20].

In this study, a fast and streamlined HPTLC method was developed to detect inulin as well as mono- (glucose and fructose), di- (sucrose) and oligosaccharides (kestose, nystose and fructofuranosylnystose) in prebiotic food samples and naturally degraded inulin samples. As HPTLC is a matrix-robust separation technique, a minimal and gentle sample preparation along with the determination of intact inulin and FOS could be advantageous for evaluation of the natural inulin profile in food samples. As for the studied naturally degraded inulin samples, the evaluation of the natural degradation profile of inulin might also be of interest in other fields that study plant breeding, edible insects, functional feed and metabolic processes [30].

## 2. Materials and methods

### 2.1. Chemicals and reagents

D(+)glucose monohydrate (Glc, ≥99.5%), D(-)fructose (Fru, ≥99.5%), D(-)sucrose (Suc, ≥99.5%, p. a.), citric acid (≥99.5%, Ph. Eur.), 2-propanol (≥99.8%, p. a.), acetic acid (99–100%, p. a.), formic acid (>98%, p. a.), acetonitrile (>99% HPLC grade), acetone (≥99.9% HPLC grade), natural product reagent A (p. a.), potassium carbonate (99.9%) and molecular sieve (0.3 nm, grade 564, pearls) were obtained from Carl Roth, Karlsruhe, Germany. 1-Kestose (≥99.5%, HPLC), 1-nystose (≥99.5%, HPLC) and 1-fructofuranosylnystose (≥80%, HPLC) were from Wako Pure Chemical Industries, Neuss, Germany and inulin (<2% monosaccharides) was from Orafti HP by Beneo, Mannheim, Germany. Disodium phosphate (≥99.5%, p. a.) was bought from AppliChem, Darmstadt, Germany and *n*-butanol (99%, extra pure) from ACROS Organics, Geel, Belgium. Potassium hexacyanoferrate (II) trihydrate (for Carrez I; 99%, pure, Riedel-de Häen), boric acid (≥99.5%, ACS reagent), aniline (≥99.5%, ACS reagent), diphenylamine (≥98%, GC grade, Fluka), lactose-monohydrate (Lac, ≥99.5%, HPLC, Fluka), ammonium nitrate (99.9%, Fluka) and *p*-amino benzoic acid (≥99%) were purchased from Sigma-Aldrich, Seelze, Germany. Ethyl acetate (>99.8%) was purchased by Th. Geyer, Renningen, Germany, magnesium chloride hexahydrate (ACS) by AppliChem, Darmstadt, Germany and methanol (HPLC grade) from J. T. Baker, Deventer, The Netherlands. Zinc sulfate heptahydrate (for Carrez II; 99–104%, extra pure), *o*-phosphoric acid (85%, p. a.) and HPTLC plates silica gel 60 were obtained from Merck, Darmstadt, Germany. Doubled distilled water (named water in the following) was produced by distilling water twice with Destamat Bi 18E (Heraeus, Hanau, Germany). Naturally degraded inulin samples were obtained by the working group of Professor Zorn, Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Germany. A dietary protein chocolate shake (Aktivkost Multaben Figur, Diät-Shake, Schokolade), sugar reduced shortbread (Bahlsen Leibnitz Butterkeks, 30% weniger Zucker), a fibre enriched fruit juice (Amecke Mehrfrucht-Ballaststoff-Saft, prebiotisch) and a prebiotic infant formula (Milupa Aptamil Pre mit GOS/FOS) were obtained from the local Rewe Centre, Giessen, Germany.

### 2.2. Standard solutions

Standard mixture 1 (0.2% each in water) contained Fru, Glc, Suc and inulin. Standard mixtures 2 (1% each) and 3 (0.1% each) contained these four in citrate phosphate buffer (pH 6, 0.1 M sodium citrate and 0.2 M disodium phosphate solution). Standard mixture 4 (1% each in water) contained these saccharides plus 1-kestose, 1-nystose and 1-fructofuranosylnystose. Standard mixture 5 (0.05% each in methanol) contained all, but inulin. Standard mixture 6 contained all seven dissolved in water (1 mg/mL) and diluted 1:25 in methanol (40 ng/μL each). Lac was prepared analogously (40 ng/μL in methanol).

### 2.3. Sample preparation

#### 2.3.1. Optimization of the sample preparation

A food sample (exemplarily shortbread) was prepared in four variants. After homogenization, an aliquot (5 g) was weighted in a 100-mL flask (50 mg/mL), filled up to the mark with water. For comparison, an inulin solution (1 mg/mL) was treated similarly. Each of both solutions (shortbread and inulin) was heated for 20 min at 80 °C or 40 min at 100 °C and compared to an untreated reference. As it was not necessary for the inulin solution, only the shortbread solution was cleared by adding 0.5 mL of Carrez I (150 g/L) and Carrez II (300 g/L) solution each.

#### 2.3.2. Food sample extracts

For homogenized solid samples, 5 g each were weighted in a 100-mL flask and suspended in 30 mL water. For liquid samples, 10 mL each were transferred in a 100-mL flask. Each solution was cleared by adding 0.5 mL of each Carrez solution. All 100-mL flasks were filled up to the mark with water, filtered and diluted 1:10, 1:50 and 1:100 with methanol to cover the saccharide contents present over a wide range (about 1:70) in the samples. The dilution of 1:50 could be skipped to streamline the workflow, and instead, a lower (1:10 dilution) or higher volume (1:100 dilution) be applied. Infant formula and shortbread samples were spiked at two different levels, *i.e.* 0.2 and 13.5 g/100 g for shortbread as well as 0.2 and 10.5 g/100 g for infant formula.

#### 2.3.3. Naturally degraded inulin samples

Three different types of naturally degraded inulin sample supernatants were obtained from the Institute of Food Chemistry and Food Biotechnology. According to literature [31], the beetle *Sitophilus granarius* and the moth *Plodia interpunctella* were collected from their substrates (sunchoke and corn feeding), washed, quick-frozen (immersed in liquid nitrogen), pestled, extracted

Please cite this article in press as: S. Kirchert, G.E. Morlock, Simultaneous determination of mono-, di-, oligo- and polysaccharides via planar chromatography in 4 different prebiotic foods and 60 naturally degraded inulin samples, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.07.042