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Partially hydrolyzed guar gum characterization and sensitive quantification in food matrices by high performance anion exchange chromatography with pulsed amperometric detection—Validation using accuracy profile

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ABSTRACT

Interest concerning functional ingredients and especially dietary fibres has been growing in recent years. At the same time, the variety of ingredient accepted as dietary fibres and their mixing at low level in complex matrices have considerably complicated their quantitative analysis by approved AOAC methods. These reasons have led to the specific development of an innovative analytical method performed by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) to detect and quantify partially hydrolyzed guar gum (PHGG) in fruit preparation and dairy matrices. The analytical methodology was divided in two steps which could be deployed separately or in conjunction. The first, consists in a complete characterization of PHGG by size exclusion chromatography (SEC) with multi-angle light scattering and refractive index detection and HPAEC–PAD to determine its physico-chemical properties and galactomannans content, and the second step is the development of a new HPAEC–PAD method for PHGG direct quantification in complex matrices (dairy product). Validation in terms of detection and quantification limits, linearity of the analytical range, average accuracy (recovery, trueness) and average uncertainty were statistically carried out with accuracy profile. Overall, this new chromatographic method has considerably improved the possibility to quantify without fractionation treatment, low level of dietary fibres emerging from specific galactomannans, in complex matrices and many foodstuffs.

1. Introduction

Guar gum, a carbohydrate polymer neither digested nor absorbed in the human small intestine fits with the last CODEX definition of dietary fibres. Its constitutive polysaccharides are galactomannans obtained from the ground endosperm of guar beans (*Cyamoposis tetragonolobus* L.) after dehusking and milling [1]. Recently, partially hydrolyzed guar gum (PHGG) has become a molecule of interest as able to improve physiological effects observed with native guar without side effects [2]. For this purpose, researchers have developed an enzymatic process with a β-endo-mannanase to hydrolyze guar gum and produce after purification, drying and powdering a partially hydrolyzed guar gum (PHGG) [3].

In this study, we focused our attention on a specific ingredient supplied by Tayio Kagaku Co. Ltd. which uses a specific and patented process leading to a particularly low molecular weight PHGG. According to the last codex definition, this PHGG is a highly soluble dietary fibre (bulking agent, emulsification, fat reduction, prebiotic effects, etc.) [4,5]. In order to evaluate the fibres content (=galactomannans content) in the commercial ingredients, the official AOAC methods 985.29 and 2001.03 could be used, but for low level of fibres introduced in complex matrices, the aforementioned methods suffer a lack of sensitivity and interferences could lead to unsatisfactory results.

Since 1970, numerous papers have been dedicated to the study and characterization of galactomananns by chromatographic techniques. These publications were focused on the structure of the polysaccharides and their chemical composition [6–8]. For this purposes, hydrolysis procedures (enzymatic or chemic) were often investigated either to prepare small fraction of native galactomannans [9] or to release the constitutive monosaccharides and make a qualitative and quantitative determination [10,11].

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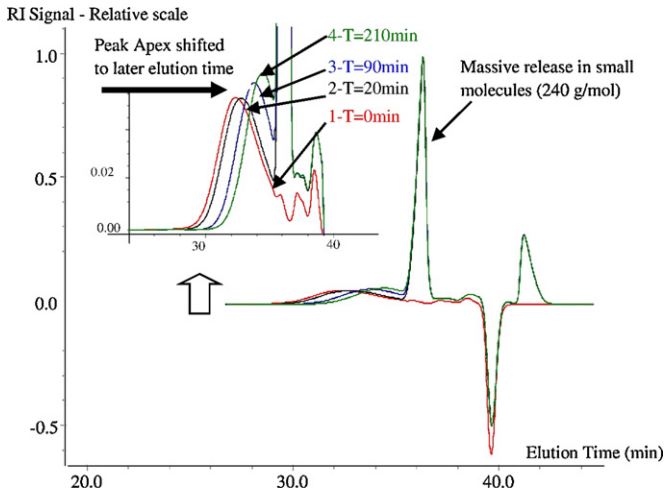

Fig. 1. SEC–RI profiles obtained on the PHGG ingredient after enzymatic hydrolysis with an endo-1,4-β-mannannase 1 – T = 0 min (red), 2 – T = 20 min (black), 3 – T = 90 min (blue), 4 – T = 210 min (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

High-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) [12,13] and size exclusion chromatography with multi angle laser light scattering detection (SEC–MALLS) [14,15] have also been demonstrated as powerful tools to characterize and analyze polysaccharides coming from different sources of seed including guar gum [16–18]. However, only few articles mentioned the possibility to use these techniques in conjunction for analytical issues [19]. This combination of specific techniques could allow on one hand an accurate macromolecular characterization and on the other hand the possibility to determine the polysaccharides content at low level in a complex matrix.

Furthermore, if the analysis of oligosaccharides by HPAEC–PAD without any pre-treatment or hydrolysis procedure [20,21] is widely described, papers describing the direct quantification of polysaccharides are rare. In the literature, previously to the polysaccharides quantification by HPAEC–PAD, fractionation steps and chemical or enzymatic hydrolysis are mainly used prior to a sensitive quantification [22].

With the growing interest on PHGG and a lack of specific and accurate methods for its direct quantification, it seemed very interesting and promising to design an innovative methodology using in conjunction HPAEC–PAD and SEC–MALLS techniques. This methodology validated in a statistical way allows the direct quantification of the PHGG by HPAEC–PAD in complex matrices like dairy products or fruit preparations at low level. If the related dietary fibres content is of interest, it could be calculated using the characterization procedure also presented in this paper

2. Analytical methodology

The first step of the study was to establish a characterization of the PHGG kindly provided by Taiyo Kagaku Co. Ltd., in terms of nature, structural parameters, osidic composition and galactomannans content, according SEC–MALLS and HPAEC–PAD analyses and by applying the calculation methodology described in AOAC 997.08 [23]. Then a specific chromatographic method for PHGG direct quantification in food matrices was developed, optimized and validated with a procedure designed in collaboration with an external consultant in Bio-statistics, Max Feinberg, this method consisted in the building of a graphical decision-making tool, called accuracy profile [24] (Fig. 1).

3. Experimental and chromatographic conditions

3.1. Reagents and reference chemicals

Five batches of PHGG in powder form were provided by Taiyo Kagaku Co. Ltd. The standard reagents arabinose, galactose, glucose and mannose were of HPLC grade (>99.5%) and purchased from Sigma–Aldrich. The endo-1,4-β-mannannase from *Bacillus* sp. was supplied by Megazyme International Ireland (EC 3.2.1.78, E-BMABS, 2000U). Sodium nitrate, absolute ethanol, sulfuric acid and barium hydroxide were of analytical grade and also supplied by Sigma–Aldrich. Sodium hydroxide (46–48%,w/w, or 17.8 M) and sodium acetate trihydrate were certified HPLC ionic chromatography grade and provided by Fisher Scientific. 18 MΩ ultrapure water was produced on MilliQ Integral System from Millipore. GHP membranes filters were obtained from Pall Gelman with different porosities, 0.10 μm, 0.22 μm and 0.45 μm. Anion exchange column CarboPac PA-1 (4 mm × 250 mm, Analytical) and size exclusion column OHPak SB 806 M HQ (8 mm × 300 mm, particle size 13 μm, maximum pore size 15,000 Å, exclusion limit 20,000,000 based on pullulans) were respectively obtained from Dionex and Shodex Co.

3.2. Instrumentation

Size exclusion chromatography with multi angle laser light scattering detection and refractive index detection (SEC–MALLS–RI) was performed with Ultimate 3000 equipment (Dionex, Sunnyvale, CA, USA). The chromatographic system setup consisted of 3 OHPak SB 806M HQ columns with linear mixed beds, serially connected, combined with a DAWN EOS MALLS photometer (Wyatt Technology, Santa Barbara, CA, USA) fitted with a helium–neon laser (λ = 690 nm) and a K_5-flow cell, followed by a Optilab rEX RI detector fitted with the same light source (Wyatt Technology, Santa Barbara, CA, USA). Data acquisition was performed using ASTRA software Version 5.3.4.14 (Wyatt Technology, Santa Barbara, CA, USA). The molecular weight calculations were realized with the Debye–Zimm formalism and a refractive index increment fixed at $\delta n/\delta c$ = 0.146 mL/g [18].

High performance anion-exchange chromatography–pulsed amperometric detection (HPAEC–PAD) was performed using an ICS 3000 equipment containing a gradient pump GP50 with a degas module, autosampler AS50 and electrochemical detector ED50 (Dionex, Sunnyvale, CA, USA). Separation of carbohydrates was carried out on a CarboPac PA-1 guard column (4 mm × 50 mm) and a CarboPac PA-1 anion-exchange column (4 mm × 250 mm) and 10–25 μL were injected. The flow rate was 1.0 mL/min and carbohydrates were detected by pulsed amperometric detection (PAD) with a gold working electrode and a Ag/AgCl-pH reference electrode, using the standard quadruple potentials waveform with the following potentials and durations: E_1 = +0.10 V (t_1 = 400 ms), E_2 = −2.00 V (t_2 = 20 ms), E_3 = +0.6 V (t_3 = 10 ms) and E_4 = −0.1 V (t_4 = 70 ms). The chromatographic equipment was controlled by Chromeleon® Software version 6.80 and the chromatograms integrated and processed with the same software.

3.3. PHGG structural characterization

3.3.1. Sample preparation

A solution of PHGG ingredient with a concentration of 5 mg/mL was prepared in 100 mM $NaNO_3$ previously filtered on GHP 0.1 μm membrane filter (Pall Gelman) for SEC–MALS–RI analysis. An aliquot of this solution was submitted to an alcoholic precipitation at 80% with absolute ethanol to isolate the polysaccharidic material from the solution and eliminate the small molecules (free sugars, salts, etc.). The precipitate was then centrifuged at 10,000 × g, 25 °C during 10 min and solubilized in 100 mM $NaNO_3$ prior to