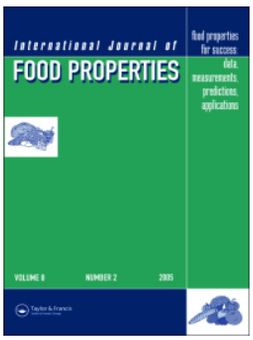

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A HPLC-ESI-Q-ToF-MS Method for the Analysis of Monomer Constituents in PHGG, Gum Arabic And Psyllium Husk Prebiotic Dietary Fibre Supplements

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ABSTRACT

"Soluble" type dietary fibre contains polysaccharides with monomer constituents that play a major role in lowering the glycemic index of food ("low-GI") and improving colonic health through gut microbiome fermentation. Soluble fibres such as partially hydrolysed guar gum (PHGG), gum Arabic and psyllium husk are of particular interest in gastrointestinal research, exploring their effects in motility disorders such as gastroparesis, constipation, diarrhea and irritable bowel syndrome (IBS) is important. The primary aim of this research was to develop a rapid pre-column derivatized HPLC-ESI-Q-ToF-MS method for the identification and quantification of monomer sugar constituents in commercial dietary fibre supplements of PHGG, gum Arabic and psyllium husk. Polysaccharides in the samples were hydrolysed and derivatized using 1-phenyl-3-methyl-5-pyrazolone (PMP). A rapid 21 min HPLC-ESI-Q-ToF-MS method separated nine different PMP-labelled monomer sugars. Five different sugars (i.e., Galactose, Arabinose, Rhamnose, Mannose and Xylose) were identified and quantified. Standard curve linearity was excellent ($R^2 > 0.999$), with good intra-laboratory precision (< ±5% SD for concentrations, ≤ ±0.02 min peak shifting for retention times). The average three-level (50%, 75% and 100%) spiking recoveries for the analytes was acceptable (96.22–109.49%). Positive scanning mode ESI-MS/MS was used to generate $[M + H]^+$ precursor ions and three (*m/z*) product ion fragments to provide identity confirmation. The concentrations of monomer sugars in gum Arabic and psyllium husk dietary fibres were consistent with literature reports. In PHGG, the concentration of galactose was unexpectedly higher than mannose, indicating that the commercial hydrolysis process of guar gum during PHGG manufacturing affected monomer composition. The method presented here provides the basis for the standardisation and labelling of commercial PHGG, gum Arabic and psyllium husk supplements. Future research will need to explore the monomer variability in other prebiotic soluble fibres and their efficacy in the production of beneficial short-chain fatty acids (SCFA) in the colonic environment.

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Introduction

Dietary fibre is a critical component in a healthy and balanced diet.[1–3] Dietary fibres are defined by Food Standards Australia New Zealand (FSANZ) as "The fraction of edible parts of plants or their extracts, or synthetic analogues, that are resistant to digestion and absorption in the small intestine,

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

usually with complete or partial fermentation in the large intestine. This includes polysaccharides, oligosaccharides (degree of polymerisation >2) and lignans".[4] There are two primary types of dietary fibre, "soluble" and "insoluble," with variable physiological effects.[5]

Dietary fibre of the water "soluble" type is a glycemic index lowering (low-GI) macronutrient in the upper gastrointestinal tract and plays a major role in the lower gastrointestinal tract (the colon) as a prebiotic.[6] Short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate are released into the colon by gut microbiome fermentation of soluble fibre polysaccharides, especially by gram-positive *Bifidobacterium*. The release of SCFA has been proven to lead to better colonic health, reduced inflammation, insulin sensitivity, renal function and bodyweight control, especially in patients with Type-1/Type-2 diabetes.[7–17] The laxative effects of soluble fibres are variable with respect to constipation, diarrhea, and irritable bowel syndrome (IBS),[18–21] and these effects depend largely on chemical structure and rheological behavior.[22,23]

Diets lacking in soluble fibre play speculated to play a notable role in gut dysbiosis (imbalance of the gut microbiome), causing constipation and diarrhea with complex cellular and molecular pathways being involved.[24] A recent study has reported that people with gastrointestinal motility disorders such as gastroparesis suffer from slow transit constipation at higher rates (64.7%) compared to the normal population (28.1%), but these patients avoid consuming dietary fibres due to detrimental symptom effects.[25,26] In order to find possible solutions for this situation, the rheological characteristics and the clinical suitability of certain soluble fibres such as the "low-viscosity" type partially hydrolysed guar gum (PHGG) and gum Arabic fibres and the commonly prescribed "high-viscosity" type psyllium husk fibre have been studied by our group.[27,28]

The chemistry of constituent polysaccharides in different soluble dietary fibres are known to vary greatly not only in chain length, but also in monomer unit prevalence and composition. The variations in the constituent monomer components of soluble fibre polysaccharides are known to produce varied physiological and metabolic effects, affecting the proportions of SCFA release in the colon.[29,30] In addition to this, some people with gastrointestinal disorders suffer from sugar intolerances and rare genetic disorders such as glucose-galactose malabsorption.[31] Therefore, a meticulously validated method for the analysis of monomer constituents in the polysaccharides found in PHGG, gum Arabic and psyllium husk needed to be developed. The analytical method validation of these soluble fibre supplements would also provide manufacturers with a useful tool for commercial batch processing, standardisation and labelling.

The analytical quantification of monomer sugar components in polysaccharides is a time consuming, laborious and potentially expensive process. Phytochemical commercial supplements containing biologically-active molecules such as phenols and flavonoids (< 1200 Da) can be easily extracted using organic/inorganic solvent combinations. Such compounds have detectable chromophores in the UV-Vis fluorescence range (200–750 nm) when analysed using mass spectrometry (MS) and commonly available carbon-18 (C18) columns. Polysaccharides are much more difficult to extract due to their larger sizes (> 1200 Da), size-similarity and co-solubility with proteins (> 1200 Da) in aqueous phases.[32] In addition to this, monomer sugars such as glucose, mannose and others possess epimers (aldohexoses) or stereoisomers with no chromophores in the UV-Vis fluorescence range.

Various analytical techniques such as high-performance liquid chromatography (HPLC),[33–38] gas chromatography mass spectrometry (GC-MS) and gas chromatography flame-ionization detection (GC-FID),[39–43] capillary-gel electrophoresis (CE)[44,45] and thin-layer chromatography[46–49] have been employed in the detection and analysis of monomer sugar constituents in complex matrices such as mushrooms, plants and herbs. Specialized detection techniques such as high-performance liquid chromatography refractive index (HPLC-RI)[50,51] or high-performance liquid chromatography charged aerosol detection (HPLC-CAD)[38] or hydrophilic interaction chromatography evaporative light scattering detection (HILIC-ELSD)[52,53] can be used without the need to derivatize monomer sugar samples, but such detectors are highly specialized for sugar analysis and expensive to operate exclusively in laboratory settings. The most sensitive technique among all of the above is HPLC, which can assist in the separation of neutral, acidic and basic monomer constituents in complex samples such as dietary fibre supplements.[54]

Pre-column derivatization (or labelling) of monomer components creates chromophores, ensuring rapid detection and analysis.[35,43,55] Excellent derivatization methods in literature have described the analysis of monomer sugars in the fungi *P. umbellatus*[33] and the algae *S. fusiforme*[34] using the highly-sensitive HPLC ESI-MS/MS (Electrospray-Ionization Mass Spectrometry) technique. The derivatization agent 1-phenyl-3-methyl-5-pyrazolone (PMP) is well suited for the simultaneous analysis of acidic (uronic acids), neutral and basic monomer sugars due to its ability to derivatize under mild conditions, causing no inadvertent isomerization during sample preparation.[56–58] Derivatization also improves sensitivity and column binding due the increased hydrophobicity of the PMP-derivative in comparison to the original monomer. When a suitable column such as the charged surface hybrid (CSH C18) column is employed, rapid analyte separation can be achieved for PMP-labelled monomers. In addition to these advantages, PMP-labelling of monomers produces derivatives that are detectable at 245 nm (UV-Vis range).

An ideal high-performance liquid chromatography (HPLC) method of a phytochemical sample or commercial dietary supplement possess these four characteristics in simultaneous combination: A large number of monitored target analytes (> 5 analytes), a short run time (< 60 min), a repeatable and reliable extraction method (n > 3 replicates), and an inexpensive and easily accessible method of analysis. HPLC methods reported in literature are either rapid (< 60 min), using specialized instrumentation and techniques (HPLC-RI, HPLC-CAD, HILIC-ELSD)[38,50–52] or have very long run-times (> 60 min)[33,34] using the comparatively cheaper and more versatile technique HPLC-ESI-MS/MS.

For the all the stated reasons, a rapid pre-column derivatized HPLC-ESI-Q-ToF-MS method for the analysis of monomer constituents in PHGG, gum Arabic and psyllium husk prebiotic dietary fibre supplements was developed. Method validation parameters such as accuracy, intra-laboratory precision, linearity, detection limit and stability were measured. The chemical structures of the major polysaccharides found in PHGG, gum Arabic and psyllium husk are shown in Figure 1, Figure 2 and Figure 3 respectively.

Materials and methods

Instrumentation

A Waters SYNAPT G2-Si Q-ToF (Quadrupole Time-of-Flight) UPLC (Ultra Performance Liquid Chromatography) system (Waters Corporation, Milford, MA, USA) coupled to a hybrid quadrupole mass spectrometer was used for rapid method development and validation. Analyte separation was achieved using an ACQUITY™ UPLC CSH (Charged Surface Hybrid) C18 Column (130 Å, 1.7 µm,

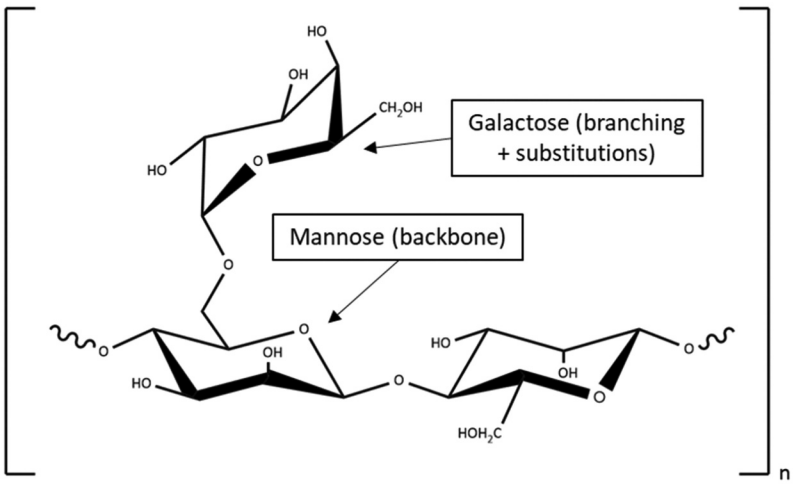

Figure 1. Structure of the main guar gum polysaccharide (galactomannan). PHGG is the enzymatic hydrolysed derivative of guar gum.

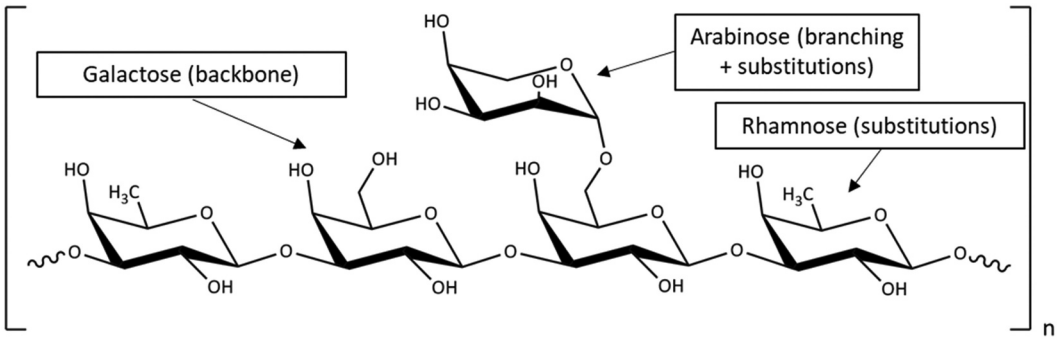

Figure 2. Structure of the main gum Arabic polysaccharide (arabinogalactan).

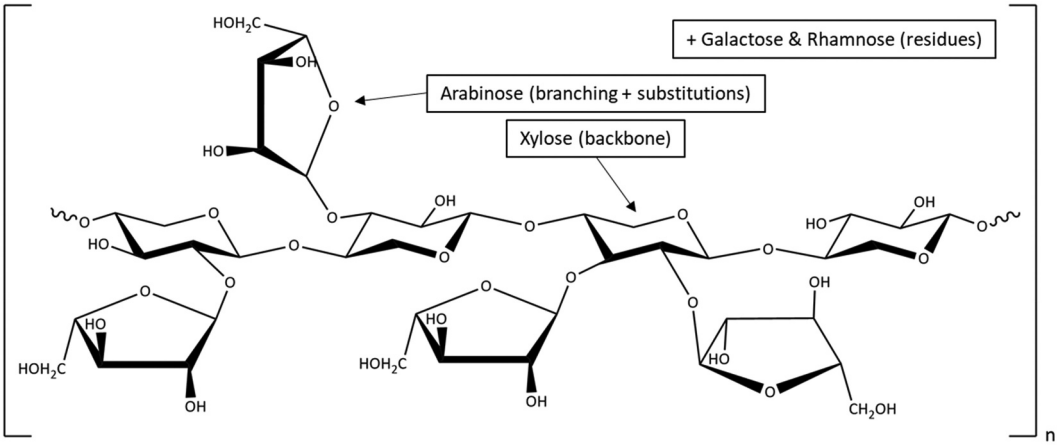

Figure 3. Structure of the main psyllium husk polysaccharide (arabinoxylan).

2.1 mm X 100 mm packing) coupled with ion mobility separation. Injection was performed using a Waters ACQUITY™ UPLC H-Class autosampler. A positive electrospray ionization ((+)/(-) ESI-MS /MS) scanning mode and argon collision gas were used.

All samples, reagents and analytical standards used in extraction, hydrolysis and derivatization were weighed out using a Mettler Toledo Analytical Balance ME204 (Mettler Toledo, Port Melbourne, VIC, Australia). A Vacuubrand PC 3001 Vario™ speedy vacuum concentrator with a CVC 3000 controller (BrandTech Scientific, Essex, CT, USA), a Dynamica Velocity 14R refrigerated centrifuge (Dynamica Scientific, Livingston, UK), liquid nitrogen storage, and other assorted laboratory equipment including glassware were used during extraction and sample preparation. All experiments were performed in the PC2 (Physical Containment Level 2) laboratory facilities at the School of Medicine, Campbelltown Campus, Western Sydney University (WSU, Sydney, Australia).

Reagents, Chemicals, and Samples

Reagent grade ethanol (95%), acetone (95%), n-butanol (98%), chloroform (98%), ammonia (4 M in methanol), trifluoroacetic acid (TFA) (99%), glacial acetic acid (100%), phosphorous pentoxide (99%) and formic acid (98%) were obtained from Sigma-Aldrich (St Louis, MO, USA). De-ionized water used during extraction and reverse-phase chromatography (> 18 MΩ.cm) was obtained from a Milli-Q® Advantage A10 system with a Q-POD™ from Merck Millipore (Darmstadt, Germany). HPLC grade

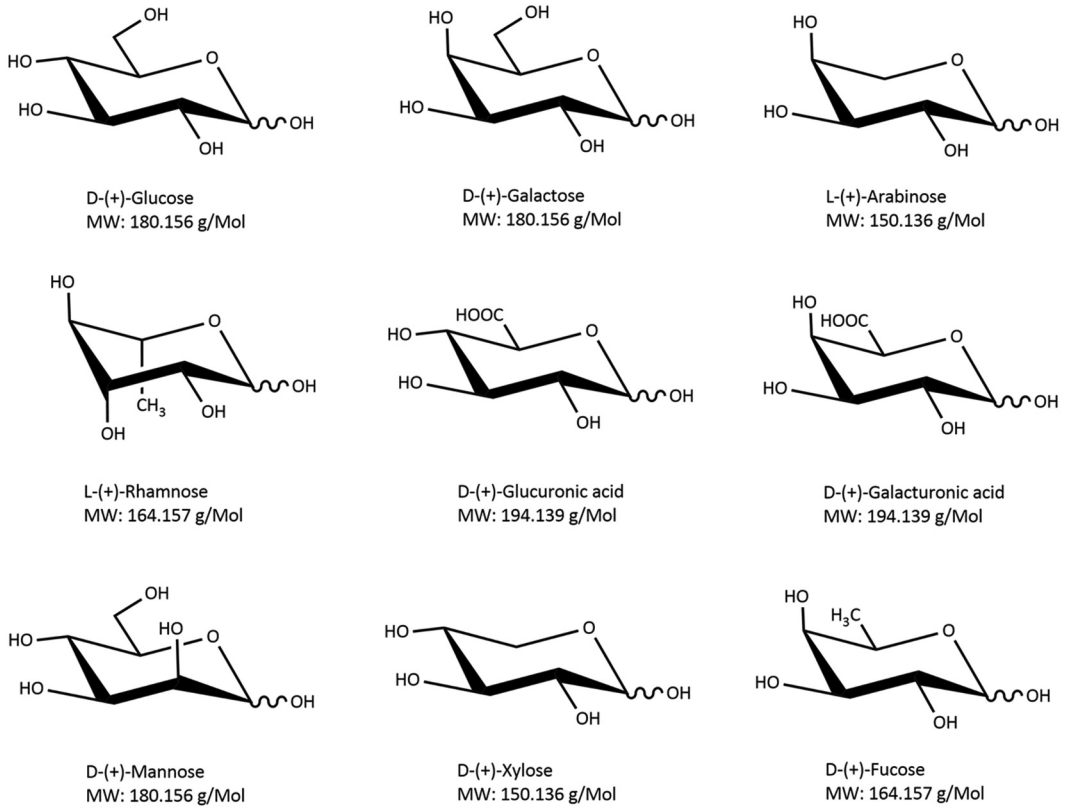

Figure 4. Structures of the nine monomer sugars (chair form) purchased as analytical standards for method validation.

acetonitrile solvent was purchased from Honeywell (Honeywell International, NC, USA). The nebulizing gas argon, and other gases, including nitrogen, used in analytical method development and MS analysis were provided by Coregas (Coregas, Sydney, NSW, Australia).

The derivatization reagent 1-Phenyl-3-methyl-5-pyrazolone (PMP) (99.0%), also known as edaravone, was obtained from Sigma-Aldrich (St Louis, MO, USA). The analytical standards of the monomer sugars glucose, galactose, arabinose, rhamnose, glucuronic acid, galacturonic acid, mannose, xylose and fucose (all ≥ 99.0%) were purchased from Sigma-Aldrich (St Louis, MO, USA). The chemical structures of the monomer sugar standards are shown in Figure 4. The partially hydrolysed guar gum (PHGG) (80%) brand name Sunfiber™ (Taiyo International, Minneapolis, MN, USA), gum Arabic (80%) (New Directions Australia, Australia) and psyllium husk (80%) (SF Health Foods, Australia) dietary fibre supplements were purchased from local suppliers in Australia.

Extraction and Purification of Polysaccharide

Polysaccharide extraction was based on methods reported in literature.[42,59] Samples (1 g ± 0.001 g each) of the powdered dietary fibre supplement were finely ground using a mortar and pestle and sieved (≤ 200 μm) in a 250 mL conical flasks. 30 mL of de-ionized water was added, and each flask was sealed with two layers of aluminum foil and autoclaved for 1.5 h at 121 ℃. Water soluble polysaccharides were extracted into the supernatant. The flask were then cooled to room temperature 25 ℃ for 30 min. The supernatant was then carefully pipetted out into 50 mL Eppendorf tubes and centrifuged at 4000 rpm (2683 × *g*) for 10 min. The aqueous supernatant was then precipitated with 120 mL 95% ethanol (supernatant: ethanol (1:4)) in a 250 mL conical flask with slow addition and

stirring. The precipitate was then filtered in a Buchner flask (with a 0.45 μm Whatman paper) and washed with acetone. The "wet" precipitate was then sealed in a 50 mL Eppendorf tube and placed in a refrigerator (4.0 ℃) for 24 h to complete the precipitation reaction. The precipitate was then removed and pelleted in a centrifuge at 4000 rpm (2683 × *g*) for 40 min. The crude polysaccharide pellet was then dissolved in de-ionized water (10 mg/mL). Protein in the crude polysaccharide pellet was removed by the addition of ¼ volume of Sevag reagent (n-butanol: chloroform (1:4)) using the Sevag method for protein removal in polysaccharide samples.[60] The protein gel layer was removed, and the process was repeated twice. The de-proteinated polysaccharide aqueous layer was then desiccated for 48 h using phosphorus pentoxide. After desiccation, the polysaccharide pellet was freeze-dried for using liquid nitrogen and stored at 4.0 ℃.

Hydrolysis, Derivatization and Preparation of Calibrated Stock Solution

Analytical standards (2 mg ±0.1 mg each) of the glucose, galactose, arabinose, rhamnose, glucuronic acid, galacturonic acid, mannose, xylose and fucose were carefully weighed into clean 10 mL glass vials. 5 mL of ammonia was then pipetted into each vial and the stock solution (400 μg/mL) was sonicated for 10 min at 40 ℃ and cooled at room temperature (25 ℃) for 5 min. The stock was then serially diluted to 80 μg/mL using 4 mL of ammonia. This monomer stock solution was then PMP-labelled and derivatized using an existing method described in literature.[33,34]

The derivatization procedure is described as follows. 100 μL of analytical calibrated stock (80 μg/mL) was added to a clean 10 mL glass vial. Table 1 shows the serial dilutions of the initial stock for the generation of the calibration curve. 100 μL of PMP was then added and the solution was mixed. The calibrated stock solution was allowed to react on a hot plate for 30 min at 40 ℃, cooled to room temperature and neutralized with 20 μL of 1% glacial acetic acid. This was followed by the addition of 1.5 mL of de-ionized water and 1.5 mL of chloroform. The immiscible two-layer solution was shaken vigorously, and the cap was opened repeatedly to release any gas pressure formed during mixing. The top aqueous layer (1.72 mL) and bottom chloroform layer (1.5 mL) solution were allowed to rest for 10 min under a fume hood. The aqueous layer, containing the PMP-labelled monomers was then carefully pipetted out into a 1.7 mL Eppendorf tube for vortexing (1 min) and micro-centrifugation at 12000 rpm (1610 *g*) for 5 min. The aqueous layer was diluted by ¼ (0.5 μg/mL -> 0.025 μg/mL) and then transferred into clean slit HPLC vials for overnight refrigeration storage (4 ℃) and mass spectrometry (MS) analysis. The derivatization reaction for mannose is shown in Figure 5.

Hydrolysis and Derivatization of Purified Polysaccharide

1.2 mg (±0.1 mg) of PHGG polysaccharide, 2.1 mg (±0.2 mg) of gum Arabic polysaccharide and 1.1 mg (±0.1 mg) of psyllium husk polysaccharide were carefully weighed out into three separate 1.7 mL Eppendorf tubes. 700 μL of 4 M TFA was added to each tube and sealed at room temperature for 15 min. 175 μL of de-ionized water was then added. The sample tubes were then sealed and placed in a 120 ℃ boiling water bath for 2 h to allow hydrolysis to occur. At the 2-hour time point, a further 350 μL of de-ionized water was added to each tube and then placed back in the

Table 1. Serial dilutions for the construction of the calibration curve.

Concentration (μg/mL)	Stock Volume (μL)	Ammonia Volume (μL)
2.0	43.00	57.00
1.5	32.25	67.25
1.0	21.50	78.50
0.5	10.75	89.25
0.25	5.40	94.60
0.10	2.15	97.85

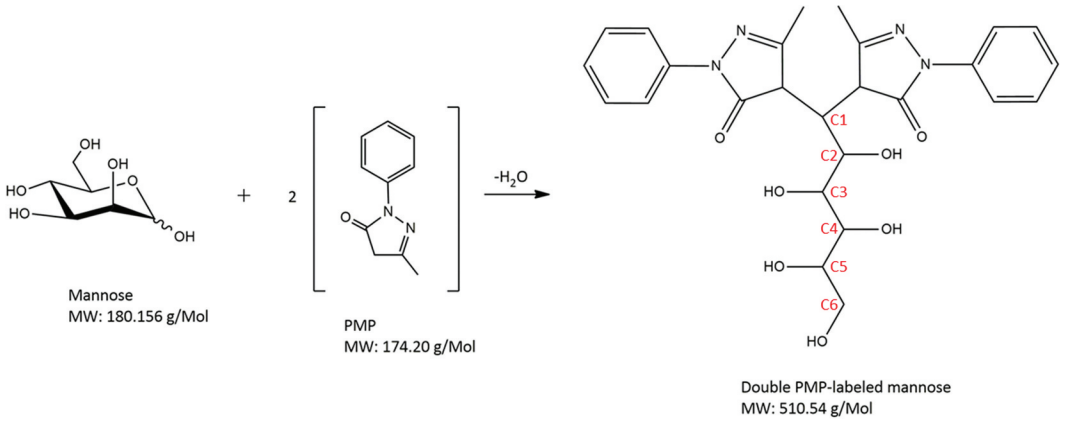

Figure 5. Derivatization reaction of mannose with PMP resulting in the PMP-labelled mannose adduct (chromophore). Carbon positions are labelled C1, C2 etc., on the PMP-labellled mannose derivative.

water bath for 1 h. At the 3-hour time point, the sample tubes were removed from the water bath, cooled back to room temperature (25 ℃) and micro-centrifuged at 12000 rpm (1610 *g*) for 10 min. The samples were then evaporated overnight (4 h) in 1.7 mL Eppendorf tubes using a vacuum concentrator.

The samples were then dissolved in 1 mL of ammonia and vortexed for 2 min. 10 µL of stock was transferred into a clean glass vial and 990 µL of ammonia was then added to produce the sample stock solution (1/100 serial dilution). The derivatization procedure for the polysaccharide samples is the exactly as described in the previous section, except in this case, the water-layer was not diluted by ¼ in the penultimate step of the procedure, before the transfer of the layer into a clean slit HPLC vial.

Recovery Solution Preparation

To determine the extraction efficiency of monomers in this method, careful amounts of each dietary polysaccharide sample were weighed out in the manner described in the previous section. 10 µL of a sample-specific stock solution (i.e., containing monomers only found in each dietary fibre polysaccharide sample) was added to create the 100% spike level. 5 µL of stock solution was added for the 50% spike level and 7.5 µL of stock solution was added for the 75% spike level. The spiking solutions were then evaporated using a nitrogen gas manifold in the fume hood. Hydrolysis and derivatization were performed as described in the previous section. Seven replicates were used for each spike level to obtain average recoveries.

HPLC-ESI-Q-ToF-MS Conditions and Data Analysis

The PMP-labelled monomers were separated using a CSH C18 column attached in series with the ToF (Time of Flight) detector. The autosampler injection volume for each sample was 1.0 µL. The mobile phase flow rate was set at 0.5 mL/min. The sample pre-injection temperature was set at 4 ℃, with the column temperature set at 35 ℃. The weak and strong volumes for the autosampler wash in-between injections was 600 µL and 200 µL, respectively. Each run was 21.0 min long including a 1.5 min wash. The mobile phase gradient program for the method is shown in Table 2. All standards and samples were injected in triplicate.

The ESI (Electro-spray Ionization) source conditions were set with the desolvation gas (nitrogen) at 800 L/h, the source block temperature at 120 ℃ and the desolvation temperature at 500 ℃. The collision gas flow (argon) was set at 0.15 mL/min. Pressure was maintained in the column with the

Table 2. Mobile phase gradient program for the LC-ESI-MS/MS method.

Time (min)	% Water (A) (with 0.1% *v/v* formic acid)	% Acetonitrile (B) (with 0.1% *v/v* formic acid)
Initial	95.0	5.0
1.50	95.0	5.0
12.50	75.0	25.0
16.50	70.0	30.0
17.50	5.0	95.0
19.50	95.0	5.0
21.00	95.0	5.0

high-pressure warning limit set at 15000 psi. The MS inter-scan delay was set at 0.005 s with both polarity mode delay and enhanced inter-scan delay set at 0.02 s. The extractor voltage was set at 3 V, and the cone gas flow (L/h) was set to OFF status. The capillary voltage for the positive (+) ESI scanning mode was 0.50 kV.

Multiple reaction monitoring (MRM) dwell times (0.050 s for all reaction channels) were auto-calculated by the in-built MassLynx™ v4.2 software and data points of interest were carefully selected for the peak determination. Any *m/z* ions below 1200 Da in size were scanned. In total, four reaction channels for the ions *m/z* 525.21, *m/z* 511.23, *m/z* 495.24 and *m/z* 481.23 were monitored down to *m/z* 175.09. Tuning for optimal cone voltages and collision energies was performed by the IntelliStart™ microfluidics system built into the SYNAPT G2-Si mass spectrometer. In the MRM of all four channels, the cone voltage was set at 30 V, and the collision energy was set at 25 eV. Multiple *m/z* product ions were identified for each target analyte for identity confirmation in both the analytical standard and the samples. The European Commission Directorate for Agriculture guidelines of a minimum of two product ions from a precursor ion with matching intensities between standard and sample peaks were to be met for all target analytes.[61]

Initial analysis of MS fragmentations and chromatographic data was performed using the MassLynx™ software v4.2 (Waters Corporation, Milford, MA, USA). Integrated peak area values and retention times were obtained from chromatographic data using the in-built TargetLynx™ software v4.2. All method validation parameters such as the standard curve, analyte concentrations, LOD, LOQ and spiked recoveries of target analytes were calculated using Microsoft Excel (Office 2016).

Results

MS Identity Confirmation and Precision

Of the nine monomer analytes scanned for in the samples, only five were found among the dietary fibre polysaccharides. Method validation parameters including linear range, linearity, precision, LOD, LOQ and stability calculated from $n = 7$ replicates are shown in Table 3. Excellent linearity ($R^2 > 0.999$) for all analytes are observed within their respective linear ranges.

The precision RSD for the analytes found in the samples are ≤ 5%, which is excellent. Glucose, glucuronic acid, galacturonic acid and fucose were not present in any of the three polysaccharide samples, therefore the precision measurements for these analytes was not possible. For all the standards and samples, the extraction and instrumental standard deviation (SD) are the two primary contributors to the relative standard deviation (RSD) observed in the analyte sample concentrations and peak retention times. The method precision shown in Table 3 is an aggregate of both the extraction and instrumental precision, with the instrumental precision contributing to one-third of the SD. Analytes found within the sample were stable for 72 h when stored at 4 ℃, wrapped in aluminum foil (≤ 2% degradation in peak area). The stability of analytes present only in the calibrated stock solution, and not within any of the three samples was higher at 96 h (in the mixed standard).

Table 3. Precision of quantitation.

Analyte	Linear Range (μMol/L) [d]	Linearity (R^2)	Precision [a]		LOD (mg/g) [b]	LOQ (mg/g) [c]	Stability (h)
			Amount (mg/g) ± % RSD	RT (min) ± SD			
Glucose	0.56–11.10	0.9990	N.P.	12.92 ± 0.01	N.P.	N.P.	96
Galactose (*)	0.56–11.10	0.9995	425.03 ± 0.69	12.83 ± 0.02	8.86	29.53	72
Arabinose (**)	0.67–13.32	0.9992	658.82 ± 2.10	13.05 ± 0.02	41.42	138.07	72
Rhamnose (**)	0.61–12.18	0.9991	31.91 ± 1.32	12.62 ± 0.01	1.26	4.20	72
Glucuronic acid	0.52–10.30	0.9991	N.P.	13.04 ± 0.01	N.P.	N.P.	96
Galacturonic acid	0.52–10.30	0.9994	N.P.	13.15 ± 0.02	N.P.	N.P.	96
Mannose (*)	0.56–11.10	0.9993	269.04 ± 1.52	12.06 ± 0.02	12.24	40.79	72
Xylose (***)	0.67–13.32	0.9991	723.89 ± 1.03	13.18 ± 0.01	22.47	74.91	72
Fucose	0.61–12.18	0.9990	N.P.	13.32 ± 0.02	N.P.	N.P.	96

(*) Validated in PHGG, (**) Validated in gum Arabic, (***) Validated in psyllium husk, (N.P.) Not present in samples. (a) Average, standard deviation (SD) and relative standard deviation (RSD) calculated from $n = 7$ replicates injected in triplicate. (b) The limit of detection (LOD) is three times the standard deviation (SD) for each analyte in the corresponding validation sample. (c) The limit of quantitation (LOQ) is ten times the standard deviation (SD) for each analyte in the corresponding validation sample. (d) The calibration range for all target analytes was 0.1–2.0 μg/mL, shown here in μMol/L units, accounting for the different molecular weights of individual monomers.

For all five analytes found in the samples, the relative intensity ratios of three product ions from the $[M + H]^+$ precursor *m/z* ion were measured. The most useful product ions were formed by C2-C3 (*m/z* 373) and C5-C6 (*m/z* 271) cleavages, and by $[M + H - PMP - 2H_2O]^+$ and $[M + H - PMP - 3H_2O]^+$ fragmentations which varied depending on the precursor mass. In Figure 6, which compares the MS/MS breakdowns for galactose in the mixed standard and the PHGG sample, it can be observed that the *m/z* 175 $[PMP + H]^+$ fragment is of higher relative intensity than the precursor ion $[M + H]^+$. Such a phenomenon occurs due to the breakdown of the precursor into the major PMP fragment during MS/MS. Fragments from the C4-C5 cleavage (*m/z* 241), the C2-C3 cleavage (*m/z* 217), and the C1-C2 cleavage (*m/z* 187) were also observed, but the ionic masses for these *m/z* fragments were common across all monomer sugars and not distinct, therefore, they were ignored for the purpose of identity confirmation. It can be observed in Table 4 that all *m/z* fragments used for identity confirmation are well within the tolerances described by the European Commission Directorate for Agricultural guidelines,[61] providing good identity confirmation for monomers found in the polysaccharide samples.

Chromatographic Data, Recoveries and Analyte Concentrations

Chromatograms for the calibrated stock solution, including the four scanned MRM channels and the total ion chromatogram (TIC) are shown in Figure 7. Representative chromatograms for the PHGG, gum Arabic and psyllium husk method validation samples are shown in Figure 8. As reported in Table 5, good average recoveries were observed for the five monomer analytes that were quantified in three method validation samples (96.22–109.49%). The 100% spike level produced slightly higher recoveries than the 50% and 75% levels, but this can be attributed to the higher proportion of glassware adsorption of analytes at the 50% and 75% spike levels.[62] Average recovery RSD values were ≤ 10% for most target analytes. The

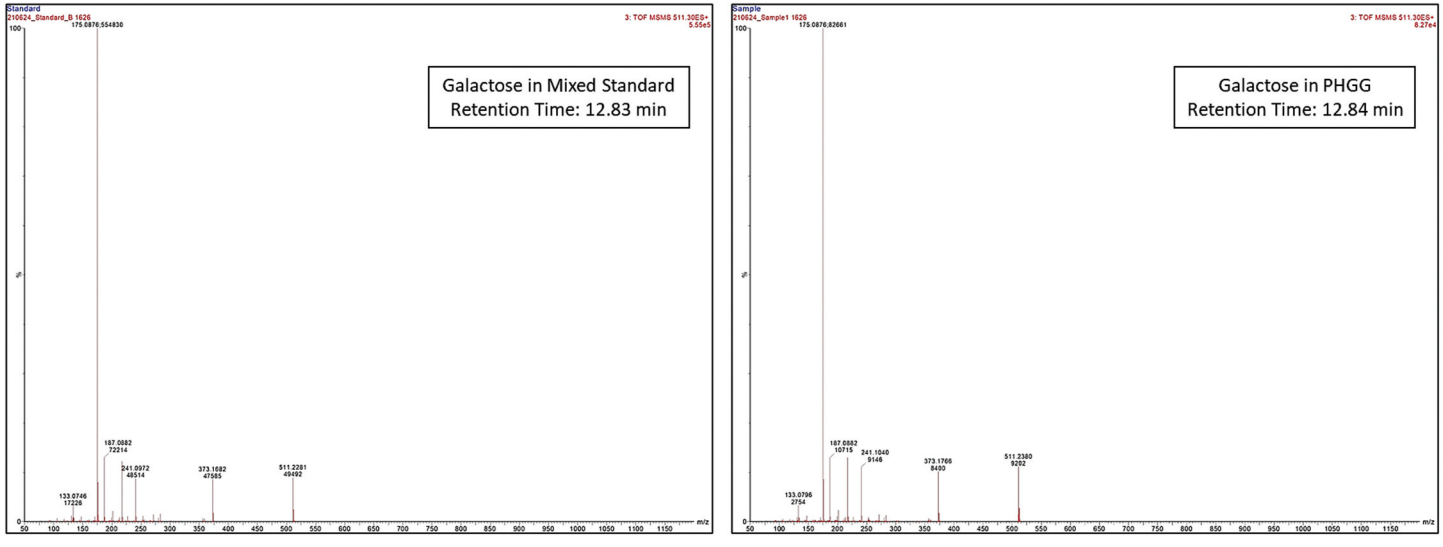

Figure 6. Comparison of MS/MS spectra of the galactose peak in the mixed standard (calibrated stock solution) and the galactose peak in the PHGG supplement.

Table 4. Labelled MRM fragmentations of analytes in tested dietary fibre supplements.

		Relative Intensity			Tolerances		
Analyte	Precursor & Product Ion Fragmentations [a, b]	*m/z*	Standard (%)	Sample (%)	Relative Difference ±(%) [d]	Permitted Tolerance ±(%) [e]	Pass/ Fail
Glucose	$[M + H]^+$	511.2183	100	N.P.	-	Base peak	N/A
Galactose (*)	$[M + H]^+$	511.2281	100	100	-	Base peak	N/A
	C2-C3 cleavage – H_2O	373.1682	96.1	91.3	4.8	20	Pass
	$[M + H - PMP - 3H_2O]^+$	283.1098	17.6	11.3	6.3	30	Pass
	Previous – C5-C6 cleavage [c]	271.1122	16.1	12.8	3.3	30	Pass
Arabinose (**)	$[M + H]^+$	481.2203	100	100	-	Base peak	N/A
	C2-C3 cleavage – H_2O	373.1682	127.5	130.1	2.6	20	Pass
	$[M + H - PMP - 2H_2O]^+$	271.1122	62.5	45.2	17.3	20	Pass
	$[M + H - PMP - 3H_2O]^+$	253.0989	62.9	52.7	10.2	20	Pass
Rhamnose (**)	$[M + H]^+$	495.2361	100	100	-	Base peak	N/A
	C2-C3 cleavage – H_2O	373.1682	98.5	103.5	5.0	20	Pass
	$[M + H - PMP - 2H_2O]^+$	285.1261	52.6	49.8	2.8	20	Pass
	$[M + H - PMP - 3H_2O]^+$	267.1159	74.7	78.1	3.5	20	Pass
Glucuronic acid	$[M + H]^+$	525.2108	100	N.P.	-	Base peak	N/A
Galacturonic acid	$[M + H]^+$	525.2108	100	N.P.	-	Base peak	N/A
Mannose (*)	$[M + H]^+$	511.2380	100	100	-	Base peak	N/A
	C2-C3 cleavage – H_2O	373.1766	100.7	83.4	17.3	20	Pass
	$[M + H - PMP - 3H_2O]^+$	283.1172	20.0	17.1	2.9	25	Pass
	Previous – C5-C6 cleavage [c]	271.1122	30.6	25.9	4.7	25	Pass
Xylose (***)	$[M + H]^+$	481.2203	100	100		Base peak	N/A
	C2-C3 cleavage – H_2O	373.1766	101.7	107.3	5.6	20	Pass
	$[M + H - PMP - 2H_2O]^+$	271.1122	57.0	52.7	4.3	20	Pass
	$[M + H - PMP - 3H_2O]^+$	253.1058	54.0	60.1	6.1	20	Pass
Fucose	$[M + H]^+$	495.2361	100	N.P.	-	Base peak	N/A

(*) Validated in PHGG, (**) Validated in gum Arabic, (***) Validated in psyllium husk, (N.P.) Not present in samples, (N/A) Not applicable. (a) The precursor ion for each analyte shown as $[M + H]^+$. (b) Carbon atoms to be labelled C1, C2 etc., from the terminal carbon, top to bottom, as shown in Figure 5, with carbon cleavage, PMP and H_2O fragmentations occurring as indicated in the table. (c) $[M + H - PMP - 3H_2O - C6]^+$ where the terminal $2H_2O$ molecules re-attach on carbons C2 and C3. (d) Relative difference = [intensity of sample – intensity of analytical standard) / (intensity of analytical standard)] × 100. (e) Maximum permitted tolerance from the European Commission Directorate for Agricultural guidelines.[61]

recovery RSD for galactose in PHGG was slightly higher at 10.63%, but this is still an acceptable value (≤ 15%). The concentration of the five analytes measured in the three dietary fibre polysaccharide samples are shown in Table 6. As expected, galactose and mannose was present in PHGG, galactose, arabinose and rhamnose was present in gum Arabic, arabinose and xylose were present in psyllium husk. Galactose and rhamnose residues were detected in psyllium husk, but the concentration of these residues were well below the LOQ.

Discussion

A rapid pre-column derivatized 21 min method was developed for the quantification of five monomer sugar constituents in PHGG, gum Arabic and psyllium husk commercial supplements. During extraction optimization, solvent combinations such as methanol/water and dichloromethane/water were tested, but ethanol/water combinations consistently produced the highest amounts of precipitate. Besides its greater yield, ethanol is more suitable as a food-grade extraction solvent when compared against methanol or acetonitrile. During the gradient program optimization, several analytical columns with C18 and amide stationary phases were tested, but the CSH C18 column consistently produced optimal analyte resolution, separation and the shortest method run times. The MS/MS conditions for the identification and confirmation of the selected monomer sugars was optimized and developed on an ad hoc basis, for the HPLC-ESI-Q-ToF instrument without relying exclusively on literature sources.

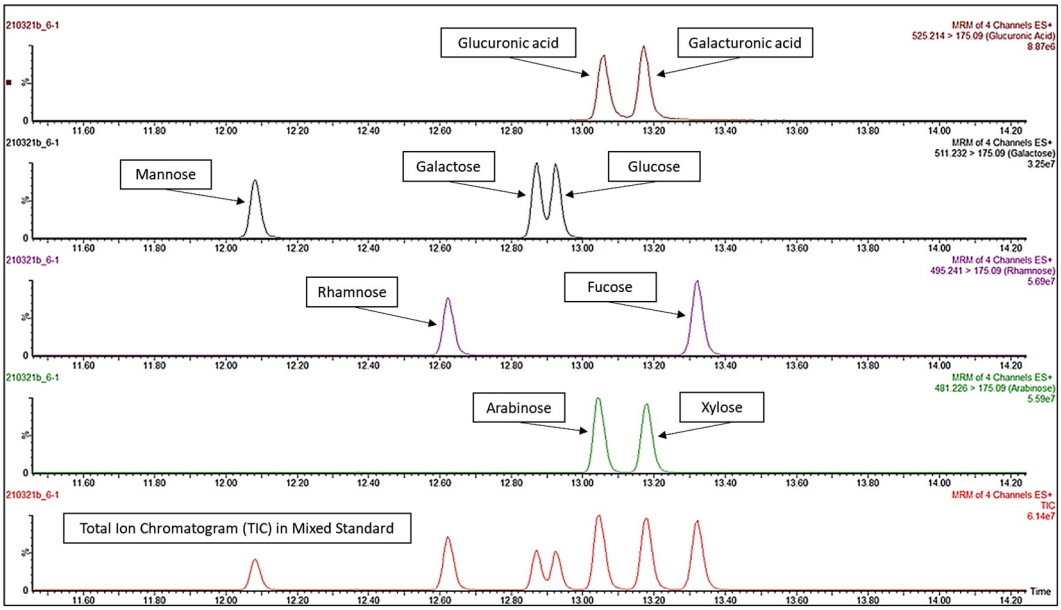

Figure 7. Representative HPLC-ESI-MS/MS chromatograms of the target analytes in mixed standard (calibrated stock solution). MRM channels at *m/z* 525, *m/z* 511, *m/z* 495 and *m/z* 481 shown along with total ion chromatogram (TIC).

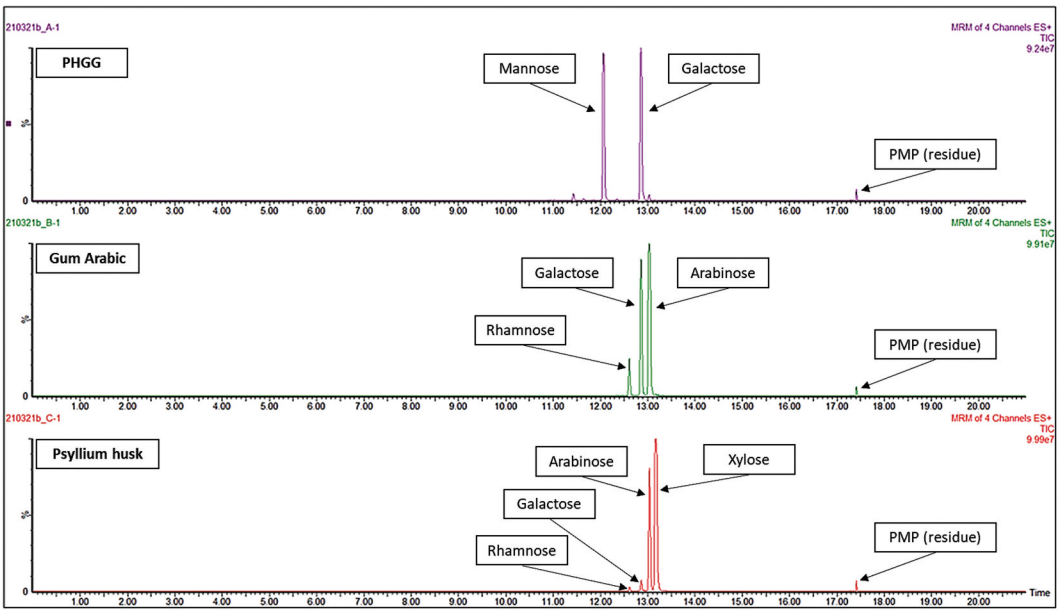

Figure 8. Representative HPLC-ESI-MS/MS chromatograms for analytes found in the purified polysaccharide of PHGG, gum Arabic and psyllium husk samples. Note the common PMP residue at 17.42 min in all three samples. The run is 21 min long, including a 1.5 min terminal wash phase.

When compared to recent pre-column derivatized HPLC-ESI-ToF-MS/HPLC-MS methods, the method presented here is more than three times faster,[33,34] using the HPLC-ESI-Q-ToF-MS technique for the quantitation of five monomers, and the identification and separation of nine monomers. The analyte recoveries (96.22–109.49%) of the quantitated monomers in PHGG, gum Arabic and psyllium husk also

Table 5. Table of analyte recoveries.

	Spike Levels [a]							
	Level 1 (50%)		Level 2 (75%)		Level 3 (100%)		Cumulative Results	
Analyte	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Average Recovery [b]	% RSD
Galactose (*)	95.73	13.15	92.85	9.66	100.07	9.08	96.22	10.63
Arabinose (**)	100.54	5.25	110.13	5.75	117.80	6.39	109.49	5.80
Rhamnose (**)	103.09	4.63	110.40	9.12	112.50	4.42	108.66	6.05
Mannose (*)	101.46	13.20	109.11	10.14	115.92	6.02	108.83	9.78
Xylose (***)	92.45	6.94	103.50	1.94	100.10	2.59	98.68	3.82

(*) Validated in PHGG, (**) Validated in gum Arabic, (***) Validated in psyllium husk. (a) % Recovery ± % RSD calculated from $n = 7$ replicates injected in triplicate. (b) Average recovery of all three spiking levels ± % RSD.

Table 6. Concentration of target analytes in samples.

	Analyte Concentration (mg/g) ± SD [a]				
Analyte	Galactose	Arabinose	Rhamnose	Mannose	Xylose
PHGG (*)	425.03 ± 2.95	N.P.	N.P.	269.04 ± 4.08	N.P.
Gum Arabic (**)	141.19 ± 2.24	658.82 ± 13.81	31.91 ± 0.42	N.P.	N.P.
Psyllium husk (***)	< LOQ	135.64 ± 2.18	< LOQ	N.P.	723.89 ± 7.49

(*) Validated sample PHGG, (**) Validated sample gum Arabic, (***) Validated sample psyllium husk, (N.P.) Analyte was not present in the validation sample, (LOQ) Analyte was present in sample but below limit of quantitation. (a) Analyte concentration calculated from $n = 7$ replicates injected in triplicate.

compares favorably against reported un-derivatized methods. In addition to this, the large number (nine) of monomer analytes identified and separated here can be used as the basis for the development and validation of polysaccharides in dietary fibre supplements other than PHGG, gum Arabic and psyllium husk. Therefore, the method presented here represents a considerable saving in run time, mobile phase use and other costs and expenses for manufacturers looking to standardise polysaccharide constituents in batches of commercial dietary fibre supplements. Standardisation and labelling by manufacturers will provide adequate clarity about product efficacy and allergen warnings to consumers purchasing commercial prebiotic dietary fibre supplements.

As expected, mannose and galactose were identified in the partially hydrolysed guar gum polysaccharide (galactomannan). What was unexpected was the higher amount of galactose (425.03 mg/g) in comparison to mannose (269.04 mg/g). This result is curious, since guar gum, the dietary fibre from which PHGG is enzymatically derived, is known to have a 1:2 ratio of galactose to mannose.[63] This result suggests that the enzymatic hydrolysis of guar gum by Endo-β-D-mannanase breaks the mannose backbone more frequently than the galactosyl side chain residue. The conventional assumption is that the proportional monomeric composition of guar gum is very similar to PHGG, but this result seems to suggest otherwise. In gum Arabic, arabinose (658.82 mg/g) predominated greatly over the concentration of galactose (141.19 mg/g) and rhamnose (31.91 mg/g) residues. This result is unsurprising since gum Arabic polysaccharide arabinogalactan is extensively branched and these branches primarily consist of arabinosyl side chains.[64] Residues containing galacturonic and glucuronic acid have been reported in gum Arabic, but neither of these uronic residues were found in the dietary fibre supplement. Psyllium husk is less extensively branched and primarily consists of a xylose backbone with some residues of arabinose and galactose. Xylose (723.89 mg/g) is present in large quantities, accompanied by noticeable arabinose (135.64 mg/g). Smaller residues of galactose and rhamnose were detected as shown in Figure 8, but both these monomers were below the LOQ. This result conforms with what has been reported in literature regarding the structure of arabinoxylan.[65]

In theory, any potential variability in the monomer chemical composition of PHGG (galactomannan), gum Arabic (arabinogalactan) and psyllium husk (arabinoxylan) polysaccharides found in the analysed dietary fibre supplements are bound to have an effect on their gut microbiome fermentation ability and

subsequent release of short-chain fatty acids (SCFA) in the caecum section of the colon.[66,67,68] Therefore, the variability of monomer composition in commercial dietary fibre polysaccharides might have significant implications for manufacturer claims of action against gastrointestinal disorders such as constipation, diarrhea and IBS.

There are some limitations to the method presented here. Only five monomers were quantified in the dietary fibre supplements of PHGG, gum Arabic and psyllium husk. There may have been other monomer residues (fructose, erythrose, lyxose etc.) present in the samples that were not analysed in this method. Furthermore, method validation for different dietary supplements using this method as a basis might require further analyte identification, separation and method development. Another unavoidable limitation of this method is the need for the additional derivatization step during sample preparation, but fortuitously, that step is a very short one (~1-2 hours).

Conclusion

In summary, a rapid 21.0 min HPLC-ESI-Q-ToF method with MS/MS was developed and validated for the identification and quantitation of five monomer analytes found in PHGG, gum Arabic and psyllium husk commercial dietary fibre supplements. Expected monomer units were identified and quantified in PHGG (galactose, mannose), gum Arabic (arabinose, galactose, rhamnose) and psyllium husk (arabinose, xylose). Although some other residues were reported in existing literature, they were either not present or below the limit of quantitation (LOQ). The developed method provides manufacturers with a tool for batch standardisation, labelling and allergen warning screening in PHGG, gum Arabic and psyllium husk commercial dietary fibre supplements. The compositional variability of monomers in the analysed supplements has interesting implications for SCFA release efficacy in the colon. The developed method can also be used as the basis or starting point for identifying and quantifying monomer sugar components in other commercial soluble dietary fibre supplements.

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Disclosure Statement

The authors declare that no financial interest, benefit or other conflicts have arisen from the direct applications of this research.

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References

[1] Office of Nutrition and Food Labeling. Science Review of Isolated and Synthetic Non-Digestible Carbohydrates; Center for Food Safety and Applied Nutrition, Food and Drug Administration, US Department of Health and Human Services: College Park, MD, USA, 2016. Available online: https://www.fda.gov/food/food-labeling-nutrition/science-review-isolated-and-synthetic-non-digestible-carbohydrates/ (accessed on 1 July 2021).

[2] Veronese, N.; Solmi, M.; Caruso, M. G.; Giannelli, G.; Osella, A. R.; Evangelou, E.; Maggi, S.; Fontana, L.; Stubbs, B.; Tzoulaki, I. Dietary Fiber and Health Outcomes: An Umbrella Review of Systematic Reviews and meta-analyses. *Am. J. Clin. Nutr* 2018, *107*(3), 436–444. DOI: 10.1093/ajcn/nqx082.

[3] Hervik, A. K.; Svihus, B. The Role of Fiber in Energy Balance. *J. Nutr. Metab*. 2019, *2019*, 4983657. DOI: 10.1155/2019/4983657.

[4] National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes; Australian Government Department of Health: Canberra, Australia, 2017. Available online: https://www.nhmrc.gov.au/about-us/publications/nutrient-reference-values-australia-and-new-zealand-including-recommended-dietary-intakes/ (accessed on 31 March 2022).

[5] Lattimer, J. M.; Haub, M. D. Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients*. 2010, *2* (12), 1266–1289. DOI: 10.3390/nu2121266.

[6] Markowiak, P.; Slizewska, K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*. 2017, *9*(9), 1021. DOI: 10.3390/nu9091021.

[7] Ruppin, H.; Bar-Meir, S.; Soergel, K. H.; Wood, C. M.; Schmitt, M. G. Absorption of Short-Chain Fatty Acids by the Colon. *Gastroenterology*. 1980, *78*(6), 1500–1507. DOI: 10.1016/s0016-5085(19)30508-6.

[8] Vinolo, M. A.; Rodrigues, H. G.; Nachbar, R. T.; Curi, R. Regulation of Inflammation by Short Chain Fatty Acids. *Nutrients*. 2011, *3*(10), 858–876. DOI: 10.3390/nu3100858.

[9] Parada Venegas, D.; De la Fuente, M. K.; Landskron, G.; Gonzalez, M. J.; Quera, R.; Dijkstra, G.; Harmsen, H. J. M.; Faber, K. N.; Hermoso, M. A. Short Chain Fatty Acids (Scfas)-mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front. Immunol* 2019, *10*, 277. DOI: 10.3389/fimmu.2019.00277.

[10] den Besten, G.; van Eunen, K.; Groen, A. K.; Venema, K.; Reijngoud, D. J.; Bakker, B. M. The Role of short-chain Fatty Acids in the Interplay between Diet, Gut Microbiota, and Host Energy Metabolism. *J. Lipid Res* 2013, *54*(9), 2325–2340. DOI: 10.1194/jlr.R036012.

[11] Reider, S. J.; Moosmang, S.; Tragust, J.; Trgovec-Greif, L.; Tragust, S.; Perschy, L.; Przysiecki, N.; Sturm, S.; Tilg, H.; Stuppner, H., et al. Prebiotic Effects of Partially Hydrolyzed Guar Gum on the Composition and Function of the Human Microbiota-Results from the PAGODA Trial. *Nutrients*.2020, *12*(5), 1257. DOI: 10.3390/nu12051257.

[12] Baxter, N. T.; Schmidt, A. W.; Venkataraman, A.; Kim, K. S.; Waldron, C.; Schmidt, T. M. Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *mBio*. 2019Jan29, *10*(1), e02566–18. DOI: 10.1128/mBio.02566-18.

[13] Tamargo, A.; Cueva, C.; Alvarez, M. D.; Herranz, B.; Moreno-Arribas, M. V.; Laguna, L. Physical Effects of Dietary Fibre on Simulated Luminal Flow, Studied by in Vitro Dynamic Gastrointestinal Digestion and Fermentation. *Food Funct* 2019, *10*, 3452–3465. DOI: 10.1039/c9fo00485h.

[14] Chambers, E. S.; Byrne, C. S.; Morrison, D. J.; Murphy, K. G.; Preston, T.; Tedford, C.; Garcia-Perez, I.; Fountana, S.; Serrano-Contreras, J. I.; Holmes, E., et al. Dietary Supplementation with inulin-propionate Ester or Inulin Improves Insulin Sensitivity in Adults with Overweight and Obesity with Distinct Effects on the Gut Microbiota, Plasma Metabolome and Systemic Inflammatory Responses: A Randomised cross-over Trial. *Gut*.2019, *68*(8), 1430–1438. DOI: 10.1136/gutjnl-2019-318424.

[15] Glover, D. A.; The effects of dietary supplementation with Gum Arabic on blood pressure and renal function in subjects with Type 2 diabetes mellitus. 2012 (Doctoral dissertation, Cardiff University).

[16] Hernandez, M. A. G.; Canfora, E. E.; Jocken, J. W. E.; Blaak, E. E. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients*. 2019, *11*(8), 1943. DOI: 10.3390/nu11081943.

[17] Babiker, R.; Elmusharaf, K.; Keogh, M. B.; Saeed, A. M. Effect of Gum Arabic (Acacia Senegal) Supplementation on Visceral Adiposity Index (VAI) and Blood Pressure in Patients with Type 2 Diabetes Mellitus as Indicators of Cardiovascular Disease (CVD): A Randomized and placebo-controlled Clinical Trial. *Lipids Health Dis*. 2018, *17* (1), 56. DOI: 10.1186/s12944-018-0711-y.

[18] Cuomo, R.; Cuomo, R.; Zito, F. P.; Savino, I. G.; Savino, I. G.; Sarnelli, G.; Cuomo, R. Partially Hydrolyzed Guar Gum in the Treatment of Irritable Bowel Syndrome with Constipation: Effects of Gender, Age, and Body Mass Index. *Saudi J. Gastroenterol* 2015, *21*(2), 104–110. DOI: 10.4103/1319-3767.153835.

[19] El-Salhy, M.; Ystad, S. O.; Mazzawi, T.; Gundersen, D. Dietary Fiber in Irritable Bowel Syndrome (Review). *Int. J. Mol. Med* 2017, *40*(3), 607–613. DOI: 10.3892/ijmm.2017.3072.

[20] Niv, E.; Halak, A.; Tiommny, E.; Yanai, H.; Strul, H.; Naftali, T.; Vaisman, N. Randomized Clinical Study: Partially Hydrolyzed Guar Gum (PHGG) versus Placebo in the Treatment of Patients with Irritable Bowel Syndrome. *Nutr. Metab. (Lond)*. 2016, *13*(1), 10. DOI: 10.1186/s12986-016-0070-5.

[21] Moayyedi, P.; Quigley, E. M.; Lacy, B. E.; Lembo, A. J.; Saito, Y. A.; Schiller, L. R.; Soffer, E. E.; Spiegel, B. M.; Ford, A. C. The Effect of Fiber Supplementation on Irritable Bowel Syndrome: A Systematic Review and meta-analysis. *Am. J. Gastroenterol* 2014, *109*(9), 1367–1374. DOI: 10.1038/ajg.2014.195.
[22] McRorie, J. W., Jr. Evidence-Based Approach to Fiber Supplements and Clinically Meaningful Health Benefits, Part 1: What to Look for and How to Recommend an Effective Fiber Therapy. *Nutr. Today* 2015, *50*(2), 82–89. DOI: 10.1097/NT.0000000000000082.
[23] McRorie, J. W., Jr.; McKeown, N. M. Understanding the Physics of Functional Fibers in the Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions about Insoluble and Soluble Fiber. *J. Acad. Nutr. Diet* 2017, *117*(2), 251–264. DOI: 10.1016/j.jand.2016.09.021.
[24] Singh, R.; Zogg, H.; Ro, S. Role of microRNAs in Disorders of Gut-Brain Interactions: Clinical Insights and Therapeutic Alternatives. *J. Pers. Med* 2021, *11*(10), 1021. DOI: 10.3390/jpm11101021.
[25] Zikos, T. A.; Kamal, A. N.; Neshatian, L.; Triadafilopoulos, G.; Clarke, J. O.; Nandwani, M.; Nguyen, L. A. High Prevalence of Slow Transit Constipation in Patients with Gastroparesis. *J. Neurogastroenterol. Motil* 2019, *25*(2), 267–275. DOI: 10.5056/jnm18206.
[26] Parkman, H. P.; Yates, K. P.; Hasler, W. L.; Nguyan, L.; Pasricha, P. J.; Snape, W. J.; Farrugia, G.; Calles, J.; Koch, K. L.; Abell, T. L., et al. Dietary Intake and Nutritional Deficiencies in Patients with Diabetic or Idiopathic Gastroparesis. *Gastroenterology*.2011, *141*(2), 486–498, 498 e481–487. DOI: 10.1053/j.gastro.2011.04.045.
[27] Suresh, H.; Ho, V.; Zhou, J. Rheological Characteristics of Soluble Fibres during Chemically Simulated Digestion and Their Suitability for Gastroparesis Patients. *Nutrients*. 2020Aug17, *12*(8), 2479. DOI: 10.3390/nu12082479.
[28] Suresh, H.; Zhou, J.; Ho, V. The Short-Term Effects and Tolerability of Low-Viscosity Soluble Fibre on Gastroparesis Patients: A Pilot Clinical Intervention Study. *Nutrients*. 2021Nov28, *13*(12), 4298. DOI: 10.3390/nu13124298.
[29] Fak, F.; Jakobsdottir, G.; Kulcinskaja, E.; Marungruang, N.; Matziouridou, C.; Nilsson, U.; Stalbrand, H.; Nyman, M. The physico-chemical Properties of Dietary Fibre Determine Metabolic Responses, short-chain Fatty Acid Profiles and Gut Microbiota Composition in Rats Fed Low- and high-fat Diets. *PLoS One*. 2015, *10*(5), e0127252. DOI: 10.1371/journal.pone.0127252.
[30] Gamage, H.; Tetu, S. G.; Chong, R. W. W.; Bucio-Noble, D.; Rosewarne, C. P.; Kautto, L.; Ball, M. S.; Molloy, M. P.; Packer, N. H.; Paulsen, I. T. Fiber Supplements Derived from Sugarcane Stem, Wheat Dextrin and Psyllium Husk Have Different in Vitro Effects on the Human Gut Microbiota. *Front Microbiol* 2018, *9*, 1618. DOI: 10.3389/fmicb.2018.01618.
[31] Wright, E. M. I. Glucose galactose malabsorption. *Am J Physiol-Gastrointestinal Liver Physiol* Nov 1, 1998, *275*(5), G879–82. DOI: 10.1152/ajpgi.1998.275.5.G879.
[32] Shi, L. Bioactivities, Isolation and Purification Methods of Polysaccharides from Natural Products: A Review. *Int. J. Biol. Macromol* 2016, *92*, 37–48. DOI: 10.1016/j.ijbiomac.2016.06.100.
[33] Guo, N.; Bai, Z.; Jia, W.; Sun, J.; Wang, W.; Chen, S.; Wang, H. Quantitative Analysis of Polysaccharide Composition in Polyporus Umbellatus by HPLC-ESI-TOF-MS. *Molecules*. 2019, *24*(14), 2526. DOI: 10.3390/molecules24142526.
[34] Wu, X.; Jiang, W.; Lu, J.; Yu, Y.; Wu, B. Analysis of the Monosaccharide Composition of water-soluble Polysaccharides from Sargassum Fusiforme by High Performance Liquid chromatography/electrospray Ionisation Mass Spectrometry. *Food Chem* 2014, *145*, 976–983. DOI: 10.1016/j.foodchem.2013.09.019.
[35] Ai, Y.; Yu, Z.; Chen, Y.; Zhu, X.; Ai, Z.; Liu, S.; Ni, D. Rapid Determination of the Monosaccharide Composition and Contents in Tea Polysaccharides from Yingshuang Green Tea by Pre-Column Derivatization HPLC. *J. Chem* 2016, *2016*, 1–5. DOI: 10.1155/2016/6065813.
[36] Xu, W.; Liang, L.; Zhu, M. Determination of Sugars in Molasses by HPLC following Solid-Phase Extraction. *Int. J. Food Prop* 2014, *18*(3), 547–557. DOI: 10.1080/10942912.2013.837064.
[37] Weiß, K.; Alt, M. Determination of Single Sugars, Including Inulin, in Plants and Feed Materials by High-Performance Liquid Chromatography and Refraction Index Detection. *Fermentation*. 2017, *3*(3), 36. DOI: 10.3390/fermentation3030036.
[38] Barzen-Hanson, K. A.; Wilkes, R. A.; Aristilde, L. Quantitation of Carbohydrate Monomers and Dimers by Liquid Chromatography Coupled with high-resolution Mass Spectrometry. *Carbohydr. Res* 2018, *468*, 30–35. DOI: 10.1016/j.carres.2018.08.007.
[39] Black, I.; Heiss, C.; Azadi, P. Comprehensive Monosaccharide Composition Analysis of Insoluble Polysaccharides by Permethylation to Produce Methyl Alditol Derivatives for Gas Chromatography/Mass Spectrometry. *Anal. Chem* 2019, *91*(21), 13787–13793. DOI: 10.1021/acs.analchem.9b03239.
[40] Xia, Y. G.; Sun, H. M.; Wang, T. L.; Liang, J.; Yang, B. Y.; Kuang, H. X. A Modified GC-MS Analytical Procedure for Separation and Detection of Multiple Classes of Carbohydrates. *Molecules*. 2018, *23*(6), 1284. DOI: 10.3390/molecules23061284.
[41] Ruiz-Matute, A. I.; Hernandez-Hernandez, O.; Rodriguez-Sanchez, S.; Sanz, M. L.; Martinez-Castro, I. Derivatization of Carbohydrates for GC and GC-MS Analyses. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci* 2011, *879*(17–18), 1226–1240. DOI: 10.1016/j.jchromb.2010.11.013.

[42] Zhang, L.; Reddy, N.; Koyyalamudi, S. R. Isolation, Characterization, and Biological Activities of Polysaccharides from Medicinal Plants and Mushrooms. *Studi. Nat. Prod. Chem* 2014Jan1, *42*, 117–151. DOI: 10.1016/b978-0-444-63281-4.00005-7.
[43] Jones, T. M.; Albersheim, P. A Gas Chromatographic Method for the Determination of Aldose and Uronic Acid Constituents of Plant Cell Wall Polysaccharides. *Plant Physiol* Jun 49, 1972, *6*(6), 926–936. DOI: 10.1104/pp.49.6.926.
[44] Guttman, A.; Pritchett, T. Capillary Gel Electrophoresis Separation of High-mannose Type Oligosaccharides Derivatized by 1-aminopyrene-3, 6, 8-trisulfonic Acid. *Electrophoresis*. 1995, *16*(1), 1906–1911. DOI: 10.1002/elps.11501601314.
[45] Rizelio, V. M.; Tenfen, L.; da Silveira, R.; Gonzaga, L. V.; Costa, A. C.; Fett, R. Development of a Fast Capillary Electrophoresis Method for Determination of Carbohydrates in Honey Samples. *Talanta*. 2012, *93*, 62–66. DOI: 10.1016/j.talanta.2012.01.034.
[46] Bischel, M. D.; Austin, J. H.; Kemeny, M. D.; Hubble, C. M.; Lear, R. K. Separation and Identification of Acid Polysaccharides by thin-layer Chromatography. *J. Chromatogr. A*. 1966Jan1, *21*, 40–45. DOI: 10.1016/S0021-9673(01)91258-0.
[47] Iinuma, F.; Hiraga, Y.; Kinoshita, T.; Watanabe, M. Simultaneous Fluorometric and Colorimetric Detection of Carbohydrates on Silica Gd Plates Using o-Aminobenzenesulfonic Acid. *Chem. Pharm. Bull*. 1979, *27*(5), 1268–1271. DOI: 10.1248/cpb.27.1268.
[48] Gauch, R.; Leuenberger, U.; Baumgartner, E. Quantitative Determination of Mono-, di-and Trisaccharides by thin-layer Chromatography. *J. Chromatogr. A*. 1979Jun 1, *174*(1), 195–200. DOI: 10.1016/S0021-9673(00)87050-8.
[49] Bounias, M. N-(1-Naphthyl) Ethylenediamine Dihydrochloride as a New Reagent for Nanomole Quantification of Sugars on thin-layer Plates by a Mathematical Calibration Process. *Anal. Biochem* 1980Aug 1, *106*(2), 291–295. DOI: 10.1016/0003-2697(80)90523-0.
[50] Tihomirova, K.; Dalecka, B.; Mezule, L. Application of Conventional HPLC RI Technique for Sugar Analysis in Hydrolysed Hay. *Agron. Res* 2016, *14*(5), 1713–1719.
[51] Sławińska, A.; Jabłońska-Ryś, E.; Stachniuk, A. High-Performance Liquid Chromatography Determination of Free Sugars and Mannitol in Mushrooms Using Corona Charged Aerosol Detection. *Food Anal. Methods*. 2020, *14*(2), 209–216. DOI: 10.1007/s12161-020-01863-8.
[52] Crha, T.; Pazourek, J. Rapid HPLC Method for Determination of Isomaltulose in the Presence of Glucose, Sucrose, and Maltodextrins in Dietary Supplements. *Foods*. 2020, *9*(9), 1164. DOI: 10.3390/foods9091164.
[53] Thacker, J. B.; Schug, K. A. Quantitative Determination of Fructose, Glucose, and Sucrose in Hard Ciders and Apple Juice by LC–MS/MS. *Sep. Sci. Plus*. 2020, *3*(7), 286–293. DOI: 10.1002/sscp.202000033.
[54] Wang, Q. C.; Zhao, X.; Pu, J. H.; Luan, X. H. Influences of Acidic Reaction and Hydrolytic Conditions on Monosaccharide Composition Analysis of Acidic, Neutral and Basic Polysaccharides. *Carbohydr. Polym* 2016, *143*, 296–300. DOI: 10.1016/j.carbpol.2016.02.023.
[55] Wang, W.; Optimization of HPLC Detection of PMP Derivatives of Carbohydrates. 2017 (Masters dissertation, Clemson University).
[56] Bailly, C.; Hecquet, P. E.; Kouach, M.; Thuru, X.; Goossens, J. F. Chemical Reactivity and Uses of 1-phenyl-3-methyl-5-pyrazolone (PMP), Also Known as Edaravone. *Bioorg. Med. Chem* 2020, *28*, 115463. DOI: 10.1016/j.bmc.2020.115463.
[57] Honda, S.; Iwase, S.; Makino, A.; Fujiwara, S. Simultaneous Determination of Reducing Monosaccharides by Capillary Zone Electrophoresis as the Borate Complexes of N-2-pyridylglycamines. *Anal. Biochem* 1989Jan 1, *176*(1), 72–77.
[58] Shen, X.; Perreault, H. Characterization of Carbohydrates Using a Combination of Derivatization, high-performance Liquid Chromatography and Mass Spectrometry. *J. Chromatogr. A*. 1998Jun 19, *811*(1–2), 1–2, 47–59. DOI: 10.1016/S0021-9673(98)00238-6.
[59] Jeong, S. Characteristics of anti-complementary Biopolymer Extracted from Coriolus Versicolor. *Carbohydr. Polym* 2004, *55*(3), 255–263. DOI: 10.1016/j.carbpol.2003.09.012.
[60] Jones, A. S.; Marsh, G. E. The Deproteinisation of Nucleoproteins. *Biochim. Biophys. Acta*. 1954Jan 1, *14*, 559–566. DOI: 10.1016/0006-3002(54)90236-X.
[61] Byrne, D. Commission Decision of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Result. *Off J Eur Commun* 2002, *1*(L221), 8–36.
[62] Eichholz, G. G.; Nagel, A. E.; Hughes, R. B. Adsorption of Ions Dilute Aqueous Solutions on Glass and Plastic Surfaces. *Anal. Chem* 1965Jun1, *37*(7), 863–868. DOI: 10.1021/ac60226a020.
[63] Yoon, S. J.; Chu, D. C.; Raj Juneja, L. Chemical and Physical Properties, Safety and Application of Partially Hydrolized Guar Gum as Dietary Fiber. *J. Clin. Biochem. Nutr*. 2008, *42*(1), 1–7. DOI: 10.3164/jcbn.2008001.
[64] Nie, S.-P.; Wang, C.; Cui, S. W.; Wang, Q.; Xie, M.-Y.; Phillips, G. O. A Further Amendment to the Classical Core Structure of Gum Arabic (Acacia Senegal). *Food Hydrocolloids*. 2013, *31*(1), 42–48. DOI: 10.1016/j.foodhyd.2012.09.014.
[65] Qaisrani, T. B.; Qaisrani, M. M.; Qaisrani, T. M. Arabinoxylans from Psyllium Husk: A Review. *J. Environ. Agric. Sci* 2016, *6*, 33–39.

[66] Fu, X.; Li, R.; Zhang, T.; Li, M.; Mou, H. Study on the Ability of Partially Hydrolyzed Guar Gum to Modulate the Gut Microbiota and Relieve Constipation. *J. Food Biochem* 2019, *43*(2), e12715. DOI: 10.1111/jfbc.12715.

[67] Wang, Y.; Liu, Y.; Ivusic Polic, I.; Chandran Matheyambath, A.; LaPointe, G. Modulation of Human Gut Microbiota Composition and Metabolites by Arabinogalactan and *Bifidobacterium Longum* Subsp. Longum BB536 in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). *J. Funct. Foods*. 2021, *87*, 104820. DOI: 10.1016/j.jff.2021.104820.

[68] Pereira, G. V.; Abdel-Hamid, A. M.; Dutta, S.; D'Alessandro-Gabazza, C. N.; Wefers, D.; Farris, J. A.; Bajaj, S.; Wawrzak, Z.; Atomi, H.; Mackie, R. I., et al. Degradation of Complex Arabinoxylans by Human Colonic Bacteroidetes. *Nat. Commun* 2021, *12*(1), 459. DOI: 10.1038/s41467-020-20737-5.