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Fractionation and physicochemical characterization of psyllium gum

Qian Guo [a], Steve W. Cui [b,*], Q. Wang [b], J. Christopher Young [b]

[a] *Food Science Department, University of Guelph, Guelph, Ont., Canada N1G 2W1*
[b] *Food Research Program, Agriculture and Agri-Food Canada, Guelph, Ont., Canada N1G 5C9*

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Abstract

Psyllium gum has been used in the landscape industry as an environmental friendly natural binder. This research is focused on the basic chemical and some physical properties of psyllium gum. Psyllium husk was extracted with hot water (80 °C), and with 0.5 M NaOH, 1.2 M NaOH and 2.0 M NaOH solutions (at room temperature), respectively, to produce a series of psyllium gum fractions labeled as water extractable (WE), 0.5 M alkali extractable ($AES_{0.5}$), 1.2 M alkali extractable ($AES_{1.2}$), and 2.0 M alkali extractable ($AES_{2.0}$). The alkaline extracted solutions were neutralized, during which, a gel-like precipitate was observed in the 0.5 M NaOH extracted solution, which was then separated by centrifugation to give a soluble fraction ($AES_{0.5}$) and a gel fraction ($AEG_{0.5}$). The yields of fractions WE, $AES_{0.5}$ and $AEG_{0.5}$ were 18.6%, 8.7%, and 61.4%, respectively, based on the dry weight of husk. The combined yields from the 1.2 and 2.0 M NaOH extracted fractions were less than 1.5%. Monosaccharide analysis revealed that WE, AES and AEG contained mainly xylose and arabinose. Substantial amounts (~15%) of uronic acids were found in the WE and $AES_{0.5}$ fractions compared to $AEG_{0.5}$, which only contains neutral sugars. Methylation analysis proved that WE and $AEG_{0.5}$ basically contain $1 \rightarrow 4$) and $1 \rightarrow 3$) linked β-D-xylopyranosyl residues in the main chain. The side chains are primarily composed of terminal arabinose and xylose connected to the main chain by *O*-3 and/ or *O*-2 linkage. In contrast, the backbone chain of $AES_{0.5}$ fraction was mainly composed of $1 \rightarrow 4$ linked β-D-xylopyranosyl residues and the side chains only contained arabinosyl residues where were linked only through the *O*-3 position of the $1 \rightarrow 4$ linked β-D-xylopyranosyl residues. Further study was carried out to evaluate the particle size of the molecules in solutions by dynamic light scattering (DLS). It was found that these fractions were very soluble in 1 M NaOH solutions, and the fraction $AES_{0.5}$ and $AEG_{0.5}$ were stable in such alkaline conditions, but slight degradation occurred for WE with time. Fraction WE also dissolved well in water, however, aggregation formed over storage. The apparent hydrodynamic diameters (d_h) determined by DSL were over 100 nm for all three fractions. Theses gums are of high molecular weight and can be expected to be good viscosity enhancers. The current study provided new structural information on psyllium gum, which will be helpful for explaining its unique functional properties.

Keywords: Psyllium; Polysaccharides; Fractionation; Methylation analysis; Dynamic light scattering (DLS)

1. Introduction

Psyllium, the seed from *Plantago*, is widely distributed throughout the temperate region of the world and has high economic value. Traditionally, psyllium has been used as a laxative agent and a dietary fibre supplement (Cui, 2001). Therefore, most studies to date have been focused on the healthy benefits of psyllium (Belknap, Davidson, & Smith, 1997; Hayden, Mcguirk, West, & Carey, 1998; Kris-Etherton et al., 2002; Olson, Anderson, Becker, & Anderson, 1997; Pérez-Olleros, García-Cuevas, Ruiz-Roso, & Requejo, 1999; Taylor et al., 1999; Zaman, Manzoor, Zaki, Aziz, & Gilani, 2002). Recently, psyllium has been innovatively used in the landscape industry as an environmental friendly natural binder possibly due to its high content of polysaccharide gum. Knowledge of the basic chemical and physical properties of psyllium gum is helpful to understand the binding mechanism and stability of psyllium gum in the field. In the literature, there are only a few papers exploring

* Corresponding author. Tel.: +1 519 780 8028; fax: +1 519 829 2600.
E-mail address: cuis@agr.gc.ca (S.W. Cui).

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the structural and rheological properties of psyllium (Haque, Richardson, Morris, & Dea, 1993; Kennedy, Sandhu, & Southgate, 1979; Samuelsen, Hanne, Smestad, Brull, & Thomas-Oates, 1999). These papers were also focused on the health benefit related to structural and rheological properties of psyllium. Some conflicts exist between some of the published studies. Thus, the current research is focused on the basic chemical and some physical properties of psyllium gum. In this investigation, psyllium husk was extracted with hot water (80 °C), and with 0.5 M NaOH, 1.2 M NaOH, and 2.0 M NaOH solutions (at room temperature 22 °C), respectively, to produce a series of fractions of psyllium gum. The structural characteristics of the different fractions were studied by methylation analysis. Further study was carried out to estimate the particle size of the molecules of different fractions in solutions by dynamic light scattering.

2. Materials and methods

2.1. Materials

Psyllium husk was provided by Envirobond Product Corporation, (Kitchener, ON, Canada). All chemicals were of reagent grade.

2.2. Chemical and monosaccharide analysis of psyllium husk

Protein content was analyzed by NA2100 Nitrogen and Protein Analyzer (Strada Rivoltana, Milan, Italy) using the factor of 6.24 to convert measured nitrogen to protein. Lipid was determined by extraction with hexane using a 2050 Soxtec Avanti auto extract unit connecting with an auto control unit and a drive unit (Foss Tecator, Sweden). Ash and moisture were examined according to the AOAC methods (Anonymous, 2000). Mineral analysis was done by another independent lab (Laboratory Services, University of Guelph, Canada).

The total sugar analysis of psyllium husk was conducted by modifying the method of Mopper et al. (1992). The sample was hydrolyzed in 1 M H_2SO_4 at 100 °C for 3 h and diluted 10 times. The diluted samples were passed through a 0.45 μm filter and injected to a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex-5500, Dionex Corporation, Canada). Separations were achieved with isocratic eluent (100 mM NaOH) on a CarboPac PA1 column (250 × 4 mm I.D., Dionex Corporation, Canada) and a guard column (3 × 25 mm, Dionex Corporation, Canada) at a flow rate of 1.0 mL/min. The column system was cleaned after each analysis with 300 mM NaOH for 30 min. A post-column delivery system of 600 mM NaOH with a flow-rate of 1.0 mL/min was added to the HPAEC-PAD system. The instrument was controlled and data were processed using Dionex AI 450 software (Dionex Corporation, Canada). The amount of total sugar was the sum of monosaccharides in psyllium husk.

2.3. Extraction and fractionation of psyllium gum

Psyllium husk was extracted by a sequential extraction procedure. First, 5 g psyllium husk was dispersed in 1000 mL distilled water at 80 °C for 2 h under constant stirring, and the dispersion became a homogenous gel. The dispersion was then centrifuged (Sorvall RC5C, Mondel Scientific Co., Ltd., USA) at 21,000*g* for 60 min, to separate the gel and the solution. The solution was concentrated to one fourth of the original volume by vacuum evaporation (Buchi Ltd. Vacuum evaporator, Germany) and dialysed at room temperature for 48 h with deionised water, and then freeze dried (Model 4451 F, Labconco Ltd., USA) to obtain fraction WE. The gel phase was dissolved in 0.5 M NaOH solution at room temperature for 2 h, and the solution and a small amount of residue were separated. The residue was used for further extraction. The alkaline extract was neutralized with 2 M HCl. During the neutralization process, a large amount of gel-like precipitate was observed and separated by centrifugation from the soluble fraction. The soluble fraction was treated in a similar manner as fraction WE to obtain the fraction $AES_{0.5}$. The gel fraction was homogenized (BDC 1850, Caframo Ltd., Canada) for 1 h and washed three times with distilled water, and dialyzed at room temperature for 48 h, then freeze dried to obtain the fraction $AEG_{0.5}$. Finally, the residue was extracted with 1.2 M NaOH and then 2.0 M NaOH solutions at room temperatures, respectively, to produce corresponding fractions of psyllium gum. The alkaline extractions were neutralized with 2 M HCl after extraction. After neutralizing, only solutions were obtained, which were designated as $AES_{1.2}$ and $AES_{2.0}$, respectively. The details of extraction and fractionation procedure are summarized in Fig. 1. This procedure was repeated three times and the results were averaged to obtain the yields of different fractions.

2.4. Monosaccharide and uronic acid analysis of fractions

Monosaccharide composition of different fractions from psyllium husk was determined by hydrolyzing samples in 1 M H_2SO_4 at 100 °C for 3 h followed by HPAEC-PAD analysis as mentioned above. The amount of total sugar in different fractions was taken as the sum of the monosaccharides in each fraction.

Uronic acid content was determined by an enzymic-high performance liquid chromatographic method modified from Matsuhashi's method (Matsuhashi, Inoue, & Hatanaka, 1992). Samples (10 mg) were dissolved in 0.5 mL of 50 mM sodium acetate buffer at room temperature and 0.5 mL of driselase (Fluck 44585, Sigma, Canada) was added. After being incubated for 48 h at 36 °C, the mixtures were diluted and filtered through 0.45 μm filters. The samples were quantitatively analyzed by HPAEC-PAD and responses compared with known amounts of the galacturonic acid and glucuronic acid standards. Separations were conducted on a CarboPac PA1 column

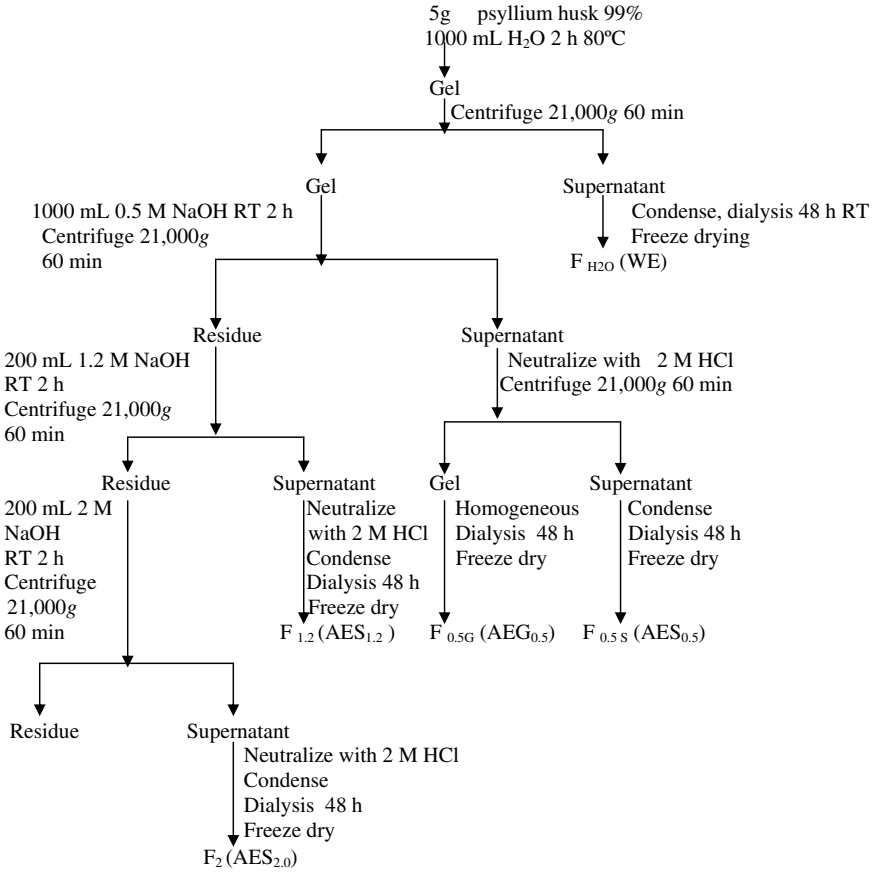

Fig. 1. Procedure for psyllium gum extraction and fractionation from psyllium husk.

(250 × 4 mm I.D., Dionex Corporation, Canada) and eluted isocratically with the eluent 150 mM NaOH at a flow rate of 1.0 mL/min. The column system was cleaned after each analysis with 500 mM NaOH for 30 min. The instrument was controlled and data were processed using Dionex AI 450 software (Dionex Corporation, Canada). The amount of total uronic acid was the sum of galacturonic acid and glucuronic acid in the fractions.

2.5. Methylation and GC–MS for the fractions

Methylation analysis of psyllium gum was conducted according to the method of Ciucanu and Kerek (1984) with slight modification. The dried samples (WE, $AES_{0.5}$, $AEG_{0.5}$) were dissolved in anhydrous dimethyl sulphoxide, sonicated at 50 °C for 6.5 h and then heated at 85 °C for 2 h with constant stirring, followed by stirring at room temperature overnight to obtain a clear solution. Dry sodium hydroxide (20 mg) was added, and the mixture was stirred for 3 h at room temperature. The mixture was stirred for another 2.5 h after addition of 0.3 mL methyl iodide. The methylated polysaccharide was then extracted with 1.5 mL methylene chloride. The methylene chloride extract was passed through a sodium sulphate column (0.5 × 15 cm) to remove water, and then evaporated by a stream of nitrogen. The dried methylated polysaccharide was hydrolyzed in 0.5 mL of 4.0 M trifluoroacetic acid (TFA) in a sealed test tube at 100 °C for 6 h and TFA was removed by evaporation under a stream of nitrogen and the concentrate dissolved in 0.3 mL distilled water. The hydrolysate was reduced by using 5 mg sodium borodeuteride and acetylated with acetic anhydride (0.5 mL) for 2 h. Borate was removed by repeated additions and evaporations first of 19:1 methanol–acetic acid then methanol alone. The resultant partially methylated alditol acetates (PMAA) were passed through a sodium sulphate column again to remove water. Aliquots of PMAA were injected on to a GCQ ion trap GC–MS system (ThermoQuest Finnigan, San Diego, CA) fitted with a SP-2330 (Supelco, Bellefonte, PA) column (30 m × 0.25 mm, 0.2 μm film thickness, temperature programmed from 160 to 210 °C at 2 °C/min, and then 210 to 240 °C at 5 °C/min, helium flow rate 1 ml/min).

2.6. Examination of hydrodynamic radius of the particles by dynamic light scattering

The dynamic light scattering (DLS) measurement were performed on two water soluble factions, WE and $AES_{0.5}$ in water, 0.5 M NaOH and 1.0 M NaOH solutions, respectively. Since fraction $AEG_{0.5}$ was not soluble in water, measurements were done in 0.5 M NaOH and

1.0 M NaOH solutions only. WE, $AES_{0.5}$, $AEG_{0.5}$ NaOH solutions (0.1%, w/w) were prepared by magnetic stirring until clear solutions were obtained at room temperature. WE and $AES_{0.5}$ water solutions (0.02%, w/w) were prepared by heating at 80 °C for 3 h and then cooled to room temperature. All of the solutions were filtered through 1.0 μm syringe filters (Chromatographic Specialties Inc., Brockville, ON, Canada) three times to remove dust interference. This particular pore size (1.0 μm) was chosen because it was the smallest size that those sample solutions could pass through without excessive forces and loss of too much samples. The sample recovery after the filtration was 90–95% as measured by colorimetric methods (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

DLS was performed using a Brookhaven Light Scattering instrument including a precision goniometer, a photomultiplier, and a 128-channel BI-9000AT digital autocorrelator (Brookhaven Instruments, Holtsvile, NY). A 35 mW helium neon laser (Melles Griot Laser Group, Carlsbad, CA) with a wavelength of 632.8 nm as the light source. Sample solutions were contained in a quartz scattering cell (25 mm in diameter). The scattering light was measured at the scattering angle $q = 90°$ and room temperature (25 °C). The data were recorded and calculated using Brookhaven DLS software. All sample solutions were kept at room temperature and tests were performed on the second and third day.

The apparent hydrodynamic diameter (d_h) was calculated by applying the Stokes–Einstein relation:

$$R_h = kT/6\pi\eta D$$

where D is the diffusion coefficient; η is the solvent viscosity; k is the Boltzmann constant; and T is the absolute temperature. The distribution of the diffusion coefficient D is connected to the decay rate Γ by the definition $\Gamma = q^2D$,q is the scattering vector.

3. Results and discussions

3.1. Chemical and monosaccharide analysis of psyllium husk

Table 1 shows the results of chemical composition of psyllium husk. The result of monosaccharide analysis is shown in Table 2. The mineral analysis result is shown in Table 3. According to these results, the main content of psyllium husk was polysaccharide. Thus, the extraction of polysaccharide from psyllium husk directly should be effective.

Table 1
Chemical composition of psyllium husk

Moisture (%)	Lipid (%)	Protein (%)	Total ash (%)	Soluble ash (%)	Total carbohydrate (%)
6.83 ± 0.04	0.00 ± 0.11	0.94 ± 0.00	4.07 ± 0.02	2.62 ± 0.03	84.98 ± 4.26

*All the percentage is calculated by dry weight.

3.2. Extraction and fractionation of psyllium husk

The yields of different fractions are shown in Table 4. The main fractions of psyllium husk were WE, $AEG_{0.5}$, and $AES_{0.5}$, and the total yield of these three fractions was nearly 90%. The total yield of $AES_{1.2}$ and $AES_{2.0}$ was less than 1.5%. Thus, the methylation analysis and hydrodynamic diameter study were focused on the three main fractions. The solubility and extractable property of three main fractions obtained from this procedure could be interpreted into water soluble fraction (WE), alkaline extractable but not water soluble fraction ($AEG_{0.5}$), and alkaline extractable and water soluble fraction ($AES_{0.5}$). The reason why $AES_{0.5}$ was not extracted by hot water at the first place could be that $AES_{0.5}$ was entrapped in the cell wall. $AES_{0.5}$ was released after the alkaline solution destroyed the cell wall.

3.3. Monosaccharide and uronic acid analysis

The total sugar and total uronic acid of different fractions are also shown in Table 4. The monosaccharide composition of the fractions from psyllium husk is shown in Table 5. Although six different monosaccharides were found in various fractions, main constituents were arabinose and xylose. The compositional results suggest that these fractions may have different structural features, but they share a similar main structure. It could be proposed that all these fractions had xylose as backbone and the main subchain included arabinose. In other words, these fractions were mainly arabinoxylans according the high contents of arabinose and xylose. This result is consistent with previous studies (Edwards, Chaplin, Blackwood, & Dettmar, 2003; Kennedy et al., 1979; Samuelsen et al., 1999). The fraction, $AES_{0.5}$, was not extractable by water at first might be because this fraction was entrapped in the matrix of the psyllium husk due to the interaction between its sub-chains with the other polysaccharide in the husk. The details of the structures of the fractions are discussed further according to the results of methylation and GC–MS analysis.

A substantial amount of uronic acids (~15%) was found in both WE and $AES_{0.5}$, but zero for $AEG_{0.5}$. This result showed that fraction $AEG_{0.5}$ contained neutral sugars only, whereas WE and $AES_{0.5}$ contained acidic sugars. This result may solve one discrepancy in the literature. Kennedy et al. (1979) reported that psyllium was acidic; however, Fisher et al. (2004) suggested that psyllium polysaccharide was neutral. Based on our results, this difference may be due to the extraction methods used, which method may extract a different fraction from the husk, and caused different results for psyllium polysaccharides in the literature.

3.4. Methylation and GC–MS for the fractions

Fisher et al. (2004) suggested that xylose was present only in pyranose ring form, whereas arabinose was solely

Table 2
Monosaccharide analysis of psyllium husk

Monosaccharide analysis (%)						Total (%)
Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose	
1.50 ± 0.10	21.96 ± 1.29	3.76 ± 0.24	0.64 ± 0.17	56.72 ± 2.97	0.40 ± 0.02	84.98 ± 4.26

Table 3
Mineral analysis of psyllium husk

Contents	Psyllium husk
Calcium (μg/g)	1500
Magnesium (μg/g)	150
Phosphorous (μg/g)	140
Potassium (μg/g)	8500
Sodium (μg/g)	640
Sulphur (μg/g)	23

Table 4
Yield, total sugar, and uronic acid of psyllium husk fractions

Fraction	Yield (%)	Total sugar (%)	Uronic acid (%)
WE	18.6 ± 0.3	83.2 ± 0.2	15.9 ± 0.2
$AEG_{0.5}$	61.4 ± 1.2	98.1 ± 0.2	0 ± 0
$AES_{0.5}$	8.7 ± 0.3	85.9 ± 0.2	14.7 ± 0.2
$AES_{1.2}$	0.8 ± 0.1	97.2 ± 0.1	0 ± 0
$AES_{2.0}$	0.5 ± 0.1	98.5 ± 0.3	0 ± 0
Residue	8.3 ± 0.2	53.0 ± 0.3	1.9 ± 0.1

in the furanose form in psyllium gum. We presumed that to be the case here; the results of methylation and *GC–MS* analysis for the three main fractions, WE, $AEG_{0.5}$, and $AES_{0.5}$, are presented in Table 6.

The highly branched nature of the three fractions is evident from methylation data. The non-reducing terminal units of fractions WE, $AEG_{0.5}$, and $AES_{0.5}$ were 36.9%, 38.7%, and 21.9%, respectively. The ratio between apparent terminal unit and branching point was approximate one from our methylation data (Table 6) (WE = 1.05; $AEG_{0.5}$ = 1.01; $AES_{0.5}$ = 0.9). This is consistent with the fact that the number of branch point of polysaccharide approximately equals the number of terminal units minus one. Among the backbones, the branching point of three fractions (WE, $AEG_{0.5}$, and $AES_{0.5}$) were 60%, 74%, and 32%, respectively (Table 6). The data from methylation analysis also suggested that the side chain structure of WE was more complicated than $AEG_{0.5}$: it has more types and longer side chains than $AEG_{0.5}$. Even though $AEG_{0.5}$ had the most branching point, the side chains were more regular compared with that of WE. This structural variation may explain the different gelling capacities of the three fractions. WE had long and irregular side chains and contained around 15% acidic sugars, which could be the reason for the high solubility and poor gel forming properties. The higher amount of regular short side chains of $AEG_{0.5}$ and its high molecular weight could be the reasons for better gel forming properties. This type of gel was considered as a fibrous gel (Haque et al., 1993). $AES_{0.5}$ had the simplest side chain structure compared with the other two fractions. The ratios between arabinose and xylose of these three fractions from methylation data (WE = 1:4.2; $AEG_{0.5}$ = 1:2.3; $AES_{0.5}$ = 1:3.1) are correlated with the results of monosaccharide analysis, which were 1: 4.3, 1:2.9, and 1:3.9 for We, $AEG_{0.5}$, and $AES_{0.5}$, respectively (Table 5).

In terms of the structural features of WE, the formation of →2,4)-Xyl*p* and →3,4)-Xyl*p* linkages indicated branching through *O*-2 and *O*-3 positions, respectively. However, the presence of →2,3,4)-Xyl*p* linkage was considered as a single branch point due to the possibility that the lone free hydroxyl group on these residue escaped the methylation process (Fischer et al., 2004). The →4,6)-Gal*p* and →2,3)-Man*p* linkages are denoted in the side chains based on the available literature (Edwards et al., 2003; Fischer et al., 2004; Kennedy et al., 1979; Samuelsen et al., 1999). The linkages of 1,4-*O*-Ac_2-2,3,5-*O*-Me_3-arabinitol and 1,5-*O*-Ac_2-2,3,4-*O*-Me_3-xylitol indicated that terminal arabino-furanosyl and xylo-pyranosyl residues existed in this fraction (Table 6). The interior linkages of →-2)-Ara*f* →3)-Ara*f*, →3)-Xyl*p*, and →4)-Xyl*p* could be attributed to the side chain or in the main chain. Considering the available literature (Edwards et al., 2003; Fischer et al., 2004; Kennedy et al., 1979; Samuelsen et al., 1999), the linkages of →2)-Ara*f* and →-3)-Ara*f* could be attributed to the side chain, whereas the →4)-Xyl*p* linkage is mostly in the main backbone chain. Kennedy et al. (1979) and Samuelsen et al. (1999) assigned the →3)-Xyl*p* linkage to the main chain. However, Fisher et al. (2004) and Edwards et al. (2003) claimed that this linkage should be in the branched chains. These discrepancies cannot be easily resolved as the samples reported in these studies were from

Table 5
Monosaccharide composition of psyllium gum fractions (%)

Fraction	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose
WE	9.86	15.97	2.63	0	68.94	2.26
AEG 0.5	0.76	24.52	1.82	0	71.16	1.74
AES 0.5	1.89	21.78	3.55	0.49	68.63	3.66
AES 1.2	1.93	20.70	5.36	16.30	51.28	4.43
AES 2.0	2.97	16.24	9.59	34.99	27.66	8.55

Table 6
Glycosyl-linkage compositions of fractions, WE, $AEG_{0.5}$, and $AES_{0.5}$

Fraction	Residue linkage	Corresponding derivatives	Peak area percentage (%)
WE	T-Ara*f*	1,4-*O*-Ac_2-2,3,5-*O*-Me_3-arabinitol	16.9
	T-Xyl*p*	1,5-*O*-Ac_2-2,3,4-*O*-Me_3-xylitol	20.0
	→2)-Ara*f*	1,2,4-*O*-Ac_3-3,5-*O*-Me_2-arabinitol	1
	→3)-Ara*f*	1,3,4-*O*-Ac_3-2,5-*O*-Me_2-arabinitol	1.1
	→3)-Xyl*p*	1,3,5-*O*-Ac_3-2,4-*O*-Me_2-xylitol	11.0
	→4)-Xyl*p*	1,4,5-*O*-Ac_3-2,3-*O*-Me_2-xylitol	12.6
	→2,4)-Xyl*p*	1,2,4,5 -*O*-Ac_4-3-*O*-Me-xylitol	10.0
	→3,4)-Xyl*p*	1,3,4,5-*O*-Ac_4-2-*O*-Me-xylitol	13
	→2,3,4)-Xyl*p*	1,2,3,4,5-*O*-Ac_5-xylitol	12.3
	→4,6)-Gal*p*	1,2,3,5-*O*-Ac_4-3,6-*O*-Me_2-galactitol	1
	→2,3)-Man*p*	1,4,5,6-*O*-Ac_4-2,3-*O*-Me_2-mantitol	1.1
$AEG_{0.5}$	T-Ara*f*	1,4-*O*-Ac_2-2,3,5-*O*-Me_3-arabinitol	20.6
	T-Xyl*p*	1,5-*O*-Ac_2-2,3,4-*O*-Me_3-xylitol	18.1
	→3)-Ara*f*	1,3,4-*O*-Ac_3-2,5-*O*-Me_2-arabinitol	9.7
	→3)-Xyl*p*	1,3,5-*O*-Ac_3-2,4-*O*-Me_2-xylitol	13.3
	→2,4)-Xyl*p*	1,2,4,5-*O*-Ac_4-3-*O*-Me-xylitol	3.3
	→3,4)-Xyl*p*	1,3,4,5-*O*-Ac_4-2-*O*-Me-xylitol	25.3
	→2,3,4)-Xyl*p*	1,2,3,4,5-*O*-Ac_5-xylitol	9.7
$AES_{0.5}$	T-Ara*f*	1,4-*O*-Ac_2-2,3,5-*O*-Me_3-arabinitol	21.9
	→3)-Ara*f*	1,3,4-*O*-Ac_3-2,5-*O*-Me_2-arabinitol	2.5
	→4)-Xyl*p*	1,4,5-*O*-Ac_3-2,3-*O*-Me_2-xylitol	51.3
	→3,4)-Xyl*p*	1,3,4,5-*O*-Ac_4-2-*O*-Me-xylitol	24.3

different species. For psyllium polysaccharides from *Plantago ovata* F., the →3)-Xyl*p* linkage was more likely in the side chain, as evidenced by NMR data from Fisher et al.'s study, which supported the sequence of Ara*f*-(1 → 3)-Xyl*p*-(1 → 3)-Ara*f* side chain. Although Kennedy et al. denoted →3)-Xyl*p* as part of the main chain structure, they did not provide solid evidence. However, Samuelsen et al. studied the other specie, *Plantago major* L., and found both →3)-Xyl*p*, and →4)-Xyl*p* existed in the main chain (Samuelsen et al., 1999). In this study, psyllium polysaccharides were partially hydrolysed. The resulting major oligosaccharides were analyzed by HPAEC, methylation analysis and concluded that the (→3)-Xyl*p*, and →4)-Xyl*p* linkages could be randomly or repeatedly distributed along the backbones (Samuelsen et al., 1999). It seems necessary to carry out more carefully planned studies to verify these structural features reported in the literature. According to monosaccharide profiles and methylation analysis on the three main fractions in the current study, our sample is different from the samples used in the literature. The high ratio of →3)-Xyl*p* linkage (11%, Table 6) indicates that this structural feature could be present in the backbone chain; 2D NMR spectroscopic analysis on the sample is underway and will be reported in the following study.

The structural feature of fraction $AEG_{0.5}$ was quite different, even though it had similar linkage patterns as the fraction WE, as shown in Table 6. According to the same principle mentioned above, →3)-Xyl*p*, →2,4)-Xyl*p*, →3,4)-Xyl*p*, and →2,3,4)-Xyl*p* were denoted in the main chain and →3)-Ara*f* was assigned to the side chain. There is evidence of the presence of both terminal xylose and arabinose in $AEG_{0.5}$ fraction. These side chains connected with the main chain through the branching points, i.e., *O*-2 and *O*-3 positions of the →4)-Xyl*p* residues. However, the linkage →2)-Ara*f*, →4,6)-Gal*p* and →2,3)-Man*p* were not found in $AEG_{0.5}$ by methylation analysis. Therefore, the side chain of $AEG_{0.5}$ was much simpler and shorter than that of WE. Only 2,4)-Xyl*p*, →3,4,)-Xyl*p*, and →2,3,4)-Xyl*p* linkages were found in the backbone chain. It suggested that the →4)-Xyl*p* linkage of the backbone chain was highly branched and each residue had at least one side chain. The other type of linkage, i.e., 1 → 3) Xyl*p*, was also present, however, the distribution of these different linkages along the backbone chain remains unknown.

The structural feature of $AES_{0.5}$ was simple compared with the other two fractions. It had only one type of terminal unit: T-Ara*f*. The →3,4)-Xyl*p* linkage indicated the only branch point at *O*-3 of the 4)-Xyl*p* residue in the $AES_{0.5}$ fraction. The linkage of →3)-Ara*f* was assigned to the side chain. In addition, no →3)-Xyl*p* linkage was detected in this fraction. Therefore, the structure of $AES_{0.5}$ could be described as: arabinose randomly or regularly connected with 1–4 linked xylose backbone through *O*-3 position. The side chain could be a single arabinose unit or multiple arabinoses linked by 1 → 3 linkage.

In summary, the proposed structures for these fractions are presented in Fig. 2, and the possible linkage features were shown but not the ratios between linkages. The structural features of these three fractions will be further investigated by a subsequent nuclear magnetic resonance (NMR) spectroscopic analysis.

3.5. Estimation of apparent hydrodynamic radius of fractions

Dynamic light scattering operated at a 90 degree angle was used to estimate the molecular/particle size of each

fraction in water and in dilute NaOH solution. The true hydrodynamic diameter can only be obtained by extrapolating the apparent hydrodynamic diameters to zero angle and zero concentration. There are a number of computational methods existing in the literature for the analysis of DLS data including those based on different algorithms, such as cumulants, histogram analysis, constrained regularization (CONTIN), and the non-negatively constrained least-squares method (NNLS). A comparison of these computational methods was conducted by Stork and Ray (1985). The cumulants method was the most common method used in the early years for DLS. It can provide accurate results for distributions which have only small values of high-order moments. In other words, the cumulants method is only suitable for converged distribution. Accurate values of the average and variance can be provided by the constrained method usually; hence, this method has been widely used. However, it tends to smooth over distributions where there is significant noise in the data. The NNLS method is mostly suitable for multimodal distributions with narrow, widely spaced peaks. It can estimate the accurate average and variance of such experimental data and has been successfully applied to polysaccharide solutions with multiple size distributions (Wang, Huang, Nakamura, Burchard, & Hallett, 2005).

The three fractions had different behaviour in the water and aqueous alkaline solutions. They showed broad multimodal size distributions from both NNLS and CONTIN analysis; the hydrodynamic diameter ranged from 10 nm to 330 nm. As an example, the size distributions of WE freshly prepared in water and aqueous alkaline solutions were presented in Fig. 3. The mean hydrodynamic diameter obtained from CONTIN and NNLS analysis are close to each other and the results from NNLS analysis for all the samples were summarised in Table 7. Notably, the size distribution of freshly prepared WE in water was very similar to that in 1 M NaOH solution, but slightly different from that in 0.5 M NaOH (Fig. 3). The mean d_h of WE in freshly prepared water, 0.5 M NaOH and 1.0 M NaOH solutions are almost identical (~150 nm), indicating WE disperses well in water as in alkaline solution. The size of WE in water increased with time during the three day storage (d_h from 151.2 to 436 nm) as a result of formation of aggregates. The d_h slightly decreased with time in alkaline solutions and it decreased more in 1.0 M NaOH, possibly due to molecular degradation. The d_h of fraction $AES_{0.5}$ in

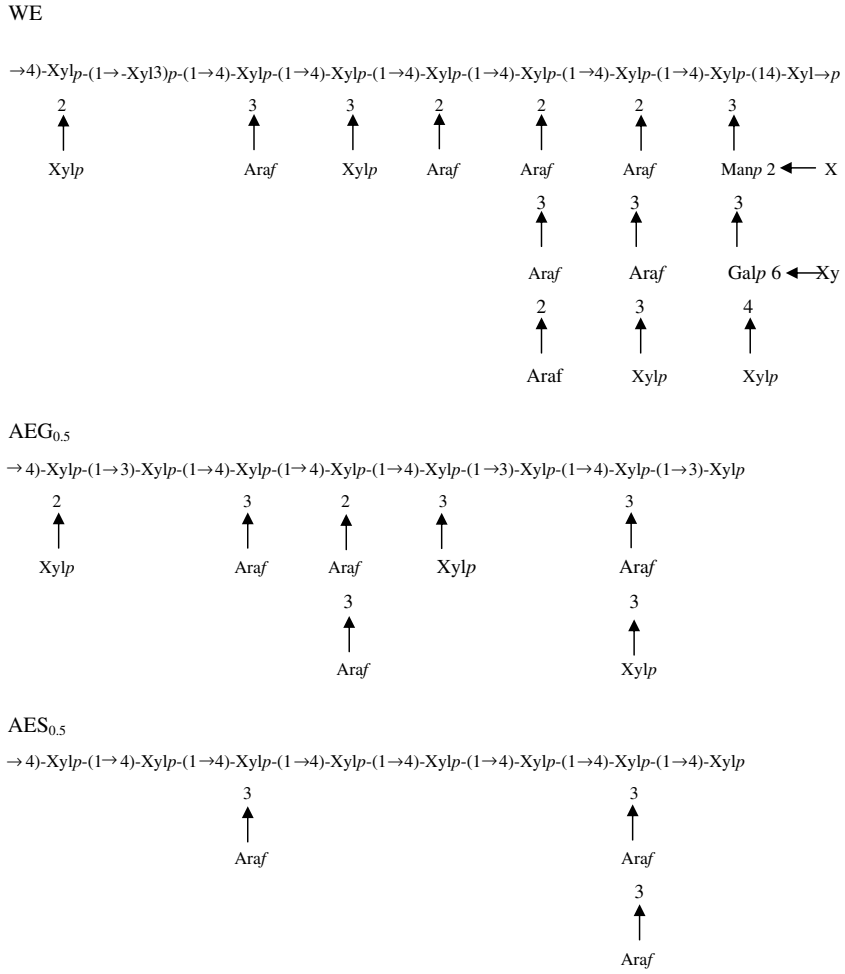

Fig. 2. Main structural features of the fraction WE, $AEG_{0.5}$ and $AES_{0.5}$.

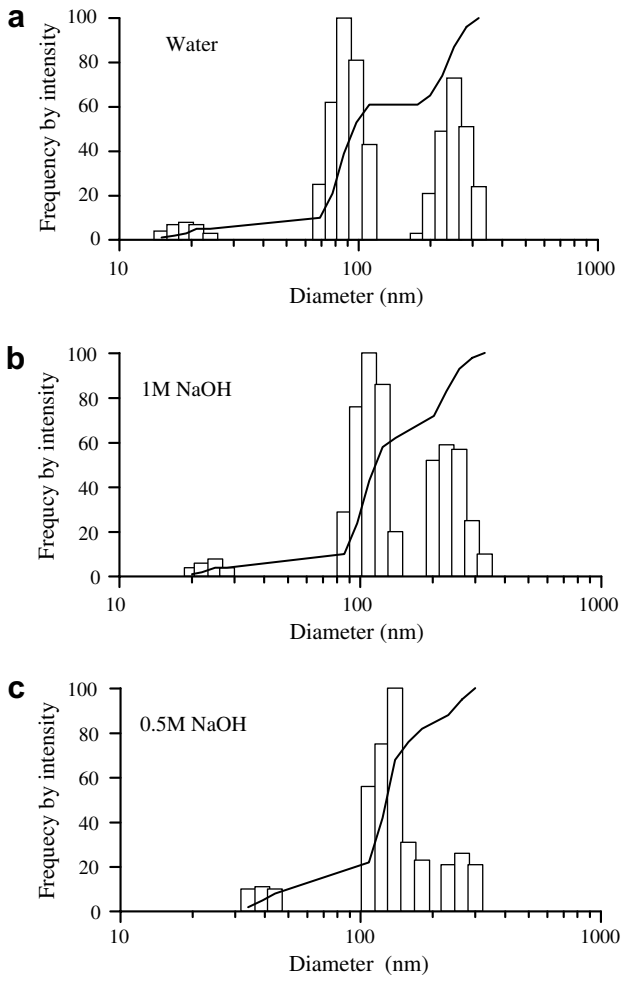

Fig. 3. Size distribution of fraction WE freshly prepared calculated by NNLS in different solutions (a) water; (b) 0.5 M NaOH; (c) 1 M NaOH.

freshly prepared water solution are significantly higher than those in alkaline solutions. This indicates that $AES_{0.5}$ formed aggregates, or aggregated more in water compared to in alkaline solutions. The d_h increased from 184 to 461 nm in water after three days storage, but remained relatively unchanged. in the alkaline solutions. $AEG_{0.5}$ was not soluble in water and showed similar behaviour in the alkaline solution as $AES_{0.5}$.did. The d_h of $AEG_{0.5}$ and $AES_{0.5}$ in 0.5 M NaOH were similar to those in 1.0 M NaOH solutions. The above results indicated that fractions of WE and $AES_{0.5}$ aggregated in water solutions during storage. It is consistent with the well-known fact that polysaccharides tend to aggregate in aqueous solution. Dilute alkaline solution could dissolve the three fractions well and avoided the aggregation. However, for WE, it appeared that slight degradation occured with time in the alkaline solution. Fractions $AES_{0.5}$ and $AEG_{0.5}$, were stable in dilute alkaline solution (less than 1.0 M NaOH) up to three days, thus 1.0 M NaOH can be used to extract polysaccharide from psyllium husk to achieve the effective extraction.

4. Conclusion

The polysaccharides extracted sequentially from psyllium husk can be seperated into three main fractions-WE, $AEG_{0.5}$, and $AES_{0.5}$, and the total yield of these three fractions was nearly 90%. These three fractions had different solubilities. WE was water soluble; $AES_{0.5}$ was alkaline extractable and water soluble; $AEG_{0.5}$ was alkaline extractable but not water soluble. Monosaccharide analysis revealed that WE, $AES_{0.5}$ and $AEG_{0.5}$ contained mainly xylose and arabinose. Substantial amounts (~15%) of uronic acids were found in WE and $AES_{0.5}$, but not in $AEG_{0.5}$. The data obtained by methylation and GC–MS was not sufficient to fully elucidate these fractions' chemical structures, but they allow us to formulate a hypothesis. The three fractions had different structural features: WE and $AEG_{0.5}$ basically contain $1 \rightarrow 4)$ and $1 \rightarrow 3)$ linkages in the main chain and the side chains are connected to the main chain via *O*-3 and/ or *O*-2 linkage. The main chain of $AES_{0.5}$ was 1→4)-Xyl*p* linkage and the side chains were linked only through *O*-3 position. The WE fraction had the most complicated side chains; in contrast, $AES_{0.5}$ had the simplest side chain structure. The structural features of these three fractions are different from those reported in previous studies (Fischer et al., 2004; Kennedy et al., 1979). These differences may be due to the different psyllium sources, and also could be due to different extraction and fractionation methods used. In addition, the results from DLS suggested that fractions $AEG_{0.5}$ and $AES_{0.5}$ were stable in dilute alkaline solution, but fraction WE could degrade in alkaline conditions. $AES_{0.5}$ and WE formed aggregates in aqueous solution during storage. However, the d_h of $AEG_{0.5}$ and $AES_{0.5}$ in 0.5 M and 1.0 M NaOH solutions were similar when freshly prepared and remained almost the same after three days, indicating high stability of the two fractions in alkaline conditions. The current study provided new structural information of psyllium gum which will be helpful for explaining its unique functional properties.

Table 7
Dynamic diameter of psyllium husk fractions

	Diameter (nm) (dissolved in H_2O)			Diameter (nm) (dissolved in 0.5 M NaOH)			Diameter (nm) (dissolved in 1 M NaOH)		
	Fresh	2nd day	3rd day	Fresh	2nd day	3rd day	Fresh	2nd day	3rd day
WE	151 ± 1	201 ± 1	436 ± 19	150 ± 3	140 ± 1	122 ± 1	148 ± 12	111 ± 3	105 ± 4
$AEG_{0.5}$	N.D	N.D	N.D	118 ± 6	129 ± 19	116 ± 12	121 ± 3	123 ± 12	122 ± 7
$AES_{0.5}$	184 ± 12	206 ± 4	461 ± 10	115 ± 8	105 ± 5	101 ± 10	95 ± 12	111 ± 2	102 ± 2

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