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## 1                    **Structural features and rheological behavior of a water-soluble** 2                    **polysaccharide extracted from the seeds of *Plantago ciliata* Desf.**

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### 16 17    **Abstract**

18    A water-soluble polysaccharide (PSPC) was extracted from the seeds of *Plantago ciliata*  
19    Desf., a spontaneous Algerian Saharan plant by a hot aqueous extraction then purified by  
20    successive ethanolic precipitations. The final extraction yield for PSPC was close to 18.6%  
21    (w/v). PSPC was then investigated regarding its global composition, structural features and  
22    rheological properties. PSPC is a neutral arabinoxylan, composed of a  $\beta$ -(1,3)/ $\beta$ -(1,4)-D-xylan  
23    backbone with side chains of Xylp, and Araf residues attached in O-2 and O-3 positions. The  
24    macromolecular characteristics of PSPC in water was determined by SEC/MALLS, with a  
25    high molecular weight (Mw) of 700 kDa, a low polydispersity index (PDI) of 1.47 and an  
26    intrinsic viscosity ( $\eta$ ) close to 157 mL/g. PSPC showed a pseudoplastic behavior in semi-  
27    dilute media and the critical overlay concentration (C\*) was estimated around 0.32-0.37%  
28    (w/v). This current research has supplied original structural information on a new  
29    arabinoxylan which could be particularly useful as a novel source of soluble fiber belonging  
30    to psyllium.

31    **Key words:** Arabinoxylan; *Plantago ciliata*; Rheology

## 33 1. Introduction

34 *Plantago* L. (Plantaginaceae) includes about 275 herbs and sub-shrubs, widely distributed all  
35 over the world [1,2] and very ethnobotanically associated with the various tribes [3,4], such as  
36 *P. asiatica* [5,6], *P. lanceolate* [7,8], *P. major* [9-11], and *P. ovata* [12]. Many *Plantago* seeds  
37 have great economic value used as food ingredients, drug, and approved as raw materials for  
38 pharmaceutical industries, like Serutan®, Konsyl®, Metamucil®, Cilium®, etc. They are  
39 used, as example, for treating constipation [13], diabetes, hypercholesterolemia [14] or as  
40 anticarcinogenic adjuvants in colon and breast cancers [15,16]. Regarding the literature,  
41 polysaccharides are the main active constituent of *Plantago* seeds [3]. Even if these  
42 polysaccharides are often described as heteroxylan, significant variations of structures were  
43 described in the literature and were associated to biotic/abiotic parameters and plant  
44 physiological changes [17]. This structural heterogeneity is not innocuous and can greatly  
45 affect the physico-chemical, biological and/or rheological properties of the polymers [18]. A  
46 heteroxylan obtained from *P. major* L. seeds, constituted of  $\beta$ -(1,4)-D-Xylp main chain with  
47 many side chains as  $\beta$ -D-Xylp,  $\alpha$ -L-Araf,  $\alpha$ -L-Araf-(1,3)- $\beta$ -D-Xylp,  $\alpha$ -D-GlcpA-(1,3)- $\alpha$ -L-Araf  
48 and 1,4- $\alpha$ -D-GalpA linked to O-2 or O-3 positions, showed interesting anti-complement  
49 properties [19], in addition to several activities *i.e.* anti-diarrheal, anti-coagulant and wound  
50 healing [20-23]. A polysaccharide obtained from *P. ovata* seeds, described as a highly  
51 branched arabinoxylan [24-26], was composed of a linear  $\beta$ -(1,4)-linked D-Xylp backbone,  
52 with several lateral chains such as  $\alpha$ -D-GalpA-(1,3)-linked- $\alpha$ -L-Araf-(1,4)- $\beta$ -D-Xylp and  $\alpha$ -L-  
53 Araf-(1,3)-linked- $\beta$ -D-Xylp-(1,4)- $\beta$ -D-Xylp [27]. The mucilaginous material from *P. ovata*  
54 had weak gelling properties, generally similar to those of xanthan and also showed  
55 hypocholesterolemic and hypoglycemic effects [24,25,28]. Another arabinoxylan, constituted  
56 of a (1,4)-linked D-Xylp backbone including both of (1,2,4)-linked and (1,3,4)-linked D-Xylp  
57 residues, was isolated from *P. asiatica* seeds. This polymer exhibited a pseudoplastic  
58 behavior, a low gelling capacity [29] and could induce the maturation of murine dendritic  
59 cells [30,31]. More recently, the structure of a heteroxylan from *P. notata* seeds was  
60 characterized [32]. It was composed of a  $\beta$ -(1,3)/ $\beta$ -(1,4)-D-Xylp backbone, which highlighted  
61 the structural variation of heteroxylan from this plant genus even in the main skeleton.  
62 *P. ciliata* Desf. locally named Lelma, is an annual desert plant which grows on sand dunes  
63 [33]. Water-soluble decoction as well as medicinal creams prepared from the seeds are used  
64 for cicatrizing injuries and treating inflammatory problems [34]. To our knowledge, no  
65 available information has been reported about water-soluble polysaccharides extracted from

66 *P. ciliata* seeds. These polymers might be involved for explaining these traditional uses in  
67 Algerian ethnomedicine. Thus, the present paper deals with the extraction, purification and  
68 structural characterization of a water-soluble polysaccharide from *P. ciliata* seeds (PSPC).  
69 Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography coupled to Mass  
70 Spectrometry (GC-MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) were used to  
71 define the structural features of PSPC. The rheological behavior of PSPC was also monitored  
72 to better comprehend the formulation of this polysaccharide as hydrogel.

## 73 **2. Material and experimental methodology**

### 74 **2.1. Plant material and chemicals**

75 *P. ciliata* plants were harvested in their full maturity from the Oued Nechou region, Ghardaïa-  
76 Province (Algeria), during April 2017. The ears of each plant were cut out and dried at room  
77 temperature for three weeks in the dark. The seeds were then manually isolated from the dry  
78 ears and stored in kraft paper bags at room temperature. All chemicals were purchased from  
79 Sigma-Aldrich and were of analytical grade.

### 80 **2.2. Polysaccharides extraction procedure**

81 Water-soluble polysaccharides of *P. ciliata* were extracted by hot maceration of unground  
82 seeds (211 g) in ultra-pure water (5% w/v) at 60 °C for 4 h under moderate stirring (450 rpm).  
83 The highly viscous dispersion was sequentially filtered through (i) a fine mesh strainer to  
84 eliminate the non-soluble residues, (ii) under vacuum using a Büchner equipped with a fine  
85 mesh filter to remove the residual debris. The residues were solubilized again in ultra-pure hot  
86 water (60 °C) at 50 g/L under moderate stirring (450 rpm). The suspension was filtered and  
87 submitted again to the same sequence as described above. The polysaccharides in the total  
88 filtrate were precipitated by adding three volumes of cold ethanol (96%, -20 °C) under gentle  
89 stirring (300 rpm). The pellet was recovered after centrifugation (10000 g, 4 °C, 15 min),  
90 resuspended in ultra-pure water at 25 g/L, precipitated again with cold ethanol and  
91 centrifuged following the same procedure (three times). The final pellet was washed with  
92 acetone two times on a frit glass of porosity 3 (16-40 µm) under vacuum. Finally, the  
93 enriched-polysaccharides fraction obtained from *P. ciliata* seeds (called PSPC) was dried at  
94 50 °C for 48 h, and then crushed into a fine powder (< 3 mm) by a mechanical blender.

### 95 **2.3. Chemical determination**

96 The composition of **PSPC** was determined by colorimetric assays. Total sugars were  
97 determined by the procedure of Dubois et al. [35] using Xyl as standard. Neutral sugars were  
98 measured by the sulfuric resorcinol assay using Xyl as standard [36] and uronic acids content  
99 was quantified by the *m*-hydroxybiphenyl method using GalA as reference [37]. Uronic acids  
100 and neutral sugars concentrations were calculated using the correction method proposed by  
101 Montreuil et al. [38]. Proteins content were estimated by the micro-Bradford assay using  
102 bovine serum albumin as reference [39] and total phenolic compounds were determined by  
103 the Folin-Ciocalteu procedure using gallic acid as standard [40].

#### 104 **2.4. FTIR spectroscopy**

105 The FTIR spectrum of PSPC was performed using a VERTEX 70 FTIR instrument with ATR  
106 A225 diamante. Fifty scans were measured at 18 °C (referenced against air) ranging 500 to  
107 4000 cm<sup>-1</sup>. The data were analyzed with OPUS 7.2 software.

#### 108 **2.5. Monosaccharides and glycosyl-linkage composition analysis by GC/MS**

109 Two micrograms of PSPC were chemically hydrolyzed by 2 M trifluoroacetic acid in a dry  
110 bath (10 g/L, 120 °C, 90 min). The hydrolysate was shortly evaporated under nitrogen steam  
111 at 60 °C. The residual traces of acid were removed by adding 1 mL methanol (three times).  
112 The derivatization step of released monosaccharides was carried out following the protocols  
113 adapted by Pierre et al. [41] and Pierre et al. [42] using BSTFA: TMCS (99:1) under stirring  
114 for 2 h at room temperature. After evaporation of reagents, trimethylsilyl-*O*-glycosides  
115 residues were solubilized in dichloromethane (10 g/L) and diluted at various concentrations  
116 (0.5 to 10 g/L). The standards (L-Rha, D-Rib, L-Fuc, L-Ara, D-Xyl, D-Man, D-Gal, D-Glc, D-  
117 GlcA, D-GalA, D-GalN, D-GalN) were subsequently prepared following the same method.  
118 The analysis was performed by GC/MS coupled to Electronic Impact (EI) using an Agilent  
119 6890 series GC system coupled to an Agilent 5973 network mass selective detector. One  
120 microliter of the sample and/or standards were injected into an OPTIMA-1MS column (30 m,  
121 0.32 mm, 0.25 µm) with a split ratio of 50:1. The helium flow rate used was 2.3 mL/min, with  
122 a pressure of 8.8 psi. The initial temperature was set for 3 min at 100 °C. The first  
123 temperature rise of 8 °C/min was made up at 100 °C for 3 min. Thus, second rise of  
124 temperature of 8 °C/min was programmed up to 200 °C and maintained for 1 min. Finally, a  
125 rise of temperature from 5 °C/min to 215 °C (runtime 19 min 50) was recorded. The  
126 ionization was performed by electronic impact (EI, 70 eV). The target ion was set between 40

127 to 800 m/z, and the trap temperature was maintained at 150 °C. The injector temperature was  
128 programmed at 250 °C.

129 In order to methylate PSPC for glycosyl-linkage analysis, an adapted method of Peña et al.  
130 [43] was performed. Pellets of solid NaOH/DMSO were prepared following the procedure  
131 described by Ciucanu and Kerek [44]. Briefly, PSPC in DMSO and NaOH/DMSO was  
132 methylated using iodomethane then hydrolyzed using TFA (2 M, 90 min, 120 °C). The  
133 reduction and acetylation of methylated residues were performed using sodium borodeuteride  
134 and ethyl acetate. Partially-*O*-methylated alditol acetate (PMAA) derivatives were analyzed  
135 by GC/MS after solubilization in dichloromethane. Five microliters were injected on the same  
136 apparatus as described above in splitless mode. The initial temperature was set at 80 °C for 2  
137 min. Then, a first rise of 30 °C/min until 170 °C was programmed, followed by a second one  
138 of 4 °C/min until 240 °C. This final temperature was maintained for 20 min.

## 139 **2.6. NMR Spectroscopy**

140 Two grams of PSPC was hydrolyzed in 200 mL of 20% H<sub>2</sub>SO<sub>4</sub> aqueous solution (30 min, 50  
141 °C, 400 rpm). The hydrolysate (PSPCH) was then neutralized using few drops of NaOH 10  
142 M. Sodium sulfate salts were removed by ultrafiltration (Amicon stirred cell Millipore-8400,  
143 cut-off Mw 3000 Da) until obtaining a very low permeate conductivity level (< 10 µS). The  
144 final retentate was then concentrated two times then freeze-dried. For improving NMR  
145 analysis, a substitution of deuterium exchangeable protons was performed. Hundred  
146 milligrams of both PSPC and PSPCH were dissolved in 1 ml of D<sub>2</sub>O (99.9% D) and freeze-  
147 dried (3 times). PSPC and PSPCH solutions (15 g/L) were finally analyzed at 80 °C using a  
148 400 MHz Bruker Avance spectrometer equipped with a BBFO probe. The NMR experiments  
149 were applied with a spectral width of 3000 Hz with the following acquisition parameters, *i.e.*  
150 (i) for <sup>1</sup>H experiments, recovery = 5 s (for a complete return after a 90° pulse), number of  
151 scans > 60, acquisition mode = 2 s, pulse 90° = 8 µsec; (ii) for <sup>13</sup>C experiments, recovery = 2  
152 s, number of scans > 16 000, acquisition mode = 0.34 s, pulse = 7 µsec, accumulation >10 h.  
153 Two-D spectrum was applied using heteronuclear single-quantum coherence (HSQC).

## 154 **2.7. Molecular weight determination by HPSEC-MALLS**

155 The macromolecular magnitudes of PSPC were identified by High-Pressure Size Exclusion  
156 Chromatography (HPSEC) fitted with three detectors on line, *i.e.* (i) a Multi-Angle Laser  
157 Light scattering (MALLS), a He-Ne laser at 690 nm and a K5 cell (50 µL) (HELEOSII Wyatt

158 Technology Corp., USA), a Differential Refractive Index (DRI) (RID10A Shimadzu, Japan)  
159 as well as a viscosimeter (Viscostar II, Wyatt Technology Corp., USA). The columns  
160 (OHPAK SB-G guard column, OHPAKSB806 and 804 HQ columns (Shodex)) were eluted  
161 with LiNO<sub>3</sub> (0.1 M) at a volume flow of 0.7 mL/min. The solvent was filtered with a 0.1 μm  
162 filter unit (Millipore), degassed and then filtered in an upstream column of 0.45 μm. PSPC  
163 solution (2 g/L) was prepared in 0.1 M LiNO<sub>3</sub> under stirring for 48 h at room temperature.  
164 The solution was then injected through a 500 μL full loop. The data were analyzed involving  
165 Astra 6.1 software package and a dn/dc of 0.15 mL/g.

## 166 2.8. Rheological measurements

167 PSPC solutions were prepared either in ultra-pure water or in a saline solution of NaCl (0.5  
168 M) at concentrations ranging from 0.25 to 5% (w/v). After 72 h at 4 °C under gentle stirring,  
169 the rheological analysis was carried out using a rheometer AR-2000 (TA Instrument, Great  
170 Britain, Ltd) equipped with a 40 mm cone-plate geometry (54 microns gap) equipped with a  
171 Peltier heating system for accurate control. For each analysis, 700 μL of solution was used  
172 and a thin layer of hexadecane was added to avoid evaporation during measurements. Steady-  
173 shear flow properties (0.25-5.0% (w/v)) were investigated at 25 °C with a shear rate ( $\dot{\gamma}$ ) from  
174 0.001 to 1000 s<sup>-1</sup>. The shear stress ( $\tau$ ) and apparent viscosity ( $\eta$ ) were recorded as a function  
175 of shear rate ( $\dot{\gamma}$ ) in different conditions (ultra-pure water and in NaCl, as described above).  
176 The critical overlap concentration (C\*) representing the limit between non-entangled system  
177 and entangled network was measured in water at 25 °C and expressed using the Williamson  
178 model (Eq. 1). C\* was calculated using the log-log plot of the specific viscosity ( $\eta_{sp}$ ) vs the  
179 concentration of polysaccharide.

180

$$181 \quad \eta = \frac{\eta_0}{(1+(\lambda\dot{\gamma})^{1-n})} \quad (1)$$

182 *Where  $\eta$  is the apparent viscosity (Pa.s),  $\eta_0$  is the zero shear viscosity (Pa.s),  $\lambda$  is the*  
183 *transition time (s),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>) and  $n$  is the flow index (dimensionless).*

184

185 *Dynamic frequency sweep measurements were performed to determine and quantify the*  
186 *viscous and elastic responses of polysaccharidic solutions. The basis of these tests is the*  
187 *application of a sinusoidal strain of frequencies (rad/s or Hz) to the system and measuring its*

188 corresponding strain. For dynamic viscoelastic systems, the strain (%) and the stress (Pa) were  
 189 out of phase. The linear viscoelastic region has been determined to confirm the constant strain  
 190 before the oscillatory test. The complex viscosity of PSPC (0.25-5.0% (w/v)) was measured at  
 191 25 °C in a constant strain of 20% (or in linear viscoelastic range), through an oscillatory  
 192 process and frequency ranging from 10<sup>-2</sup> to 10 Hz (0.063-62.83 rad.s<sup>-1</sup>). The elastic modulus  
 193 G' (storage modulus), viscous modulus G'' (loss modulus) and the loss tangent (tan δ = G''/G')  
 194 (damping factor) as a function of frequency (Hz or rad.s<sup>-1</sup>) were continuously evaluated during  
 195 the rheological analysis.

### 196 3. Results and discussion

#### 197 3.1. Chemical composition of PSPC

198 PSPC was obtained by hot-water extraction, followed by multiple ethanolic precipitations  
 199 with a final yield close to 18.6% based on dried seeds of *P. ciliata*. The fraction was mainly  
 200 composed of neutral sugar (> 80%) and it was low contaminated by proteins and phenolic  
 201 compounds (< 1%) (Table 1). This value of 18.6% (w/w) was higher than those reported by  
 202 Benaoun et al. [32] who obtained an extraction yield of 4.66% from *P. notata* seeds, a semi-  
 203 arid plant species collected in the same region. Guo et al. [45] obtained similar results for  
 204 water polysaccharides extracted from Psyllium (*P. ovata*) seeds husk (18.6%). PSPC was  
 205 classically composed of neutral sugars and some uronic acids as described for other *Plantago*  
 206 species in the literature. The uronic acid content was close to the values described for *P.*  
 207 *notata* seeds (4.9%) [32] and *P. ovata* Forsk. seeds husk (5%) [24]; but lower than for *P.*  
 208 *asiatica* L. seeds (16.62%) and various fractions of *P. depressa* seeds (PDSP-1, PDSP-2,  
 209 PDSP-3 and PDSP-4 with 19.5%, 12.4%, 10.1% and 19.7%, respectively) [46, 47]. These  
 210 changes in biochemical compositions could be attributed to area and species differences [46].

211  
 212 **Table 1.** Biochemical and monosaccharide compositions of PSPC extracted from *P. ciliata*  
 213 seeds.

| Extraction yield (% w/w) | Total sugar (% w/w) | Neutral sugar (% w/w) | Uronic acid (% w/w) | Proteins (% w/w) | Phenolic compounds (% w/w) | Monosaccharides (mol%) <sup>a</sup> |     |     |      |
|--------------------------|---------------------|-----------------------|---------------------|------------------|----------------------------|-------------------------------------|-----|-----|------|
|                          |                     |                       |                     |                  |                            | Xyl                                 | Ara | Rha | GalA |
| 18.6                     | 86.5 ± 4.32         | 63.3 ± 3.17           | 7.96 ± 0.39         | 0.35 ± 0.02      | 0                          | 78                                  | 18  | 3   | 1    |

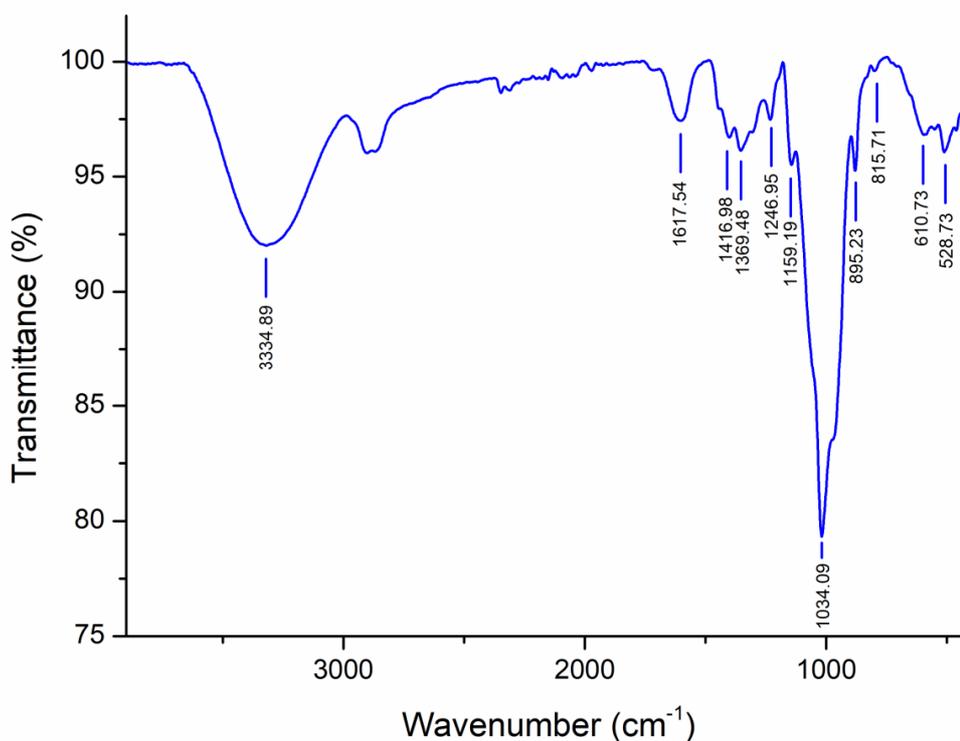
214 <sup>a</sup>Monosaccharides composition measured by GC/MS-EI. Xyl: Xylose; Ara: Arabinose; Rha: Rhamnose; GalA:  
 215 Galacturonic acid.

216

## 217 3.2. Structural characterization

### 218 3.2.1. FT-IR spectrum

219 FTIR spectrum of PSPC showed the presence of various functional groups (Fig. 1). A wide  
220 intense peak at  $3334.89\text{ cm}^{-1}$  was attributed to hydroxyl stretching vibration of both  
221 carbohydrates and water [48] whereas the -CH band of aliphatic bending groups was detected  
222 approximately at  $2900\text{ cm}^{-1}$  [49]. The peaks near to  $1034.09\text{-}1246.95\text{ cm}^{-1}$  were assigned to C-  
223 O-C and C-O glycosidic band vibrations, which suggested the presence of carbohydrates  
224 [50]. The strong absorption peak near  $1046.1\text{ cm}^{-1}$  corresponded to the stretching vibration of  
225 carbohydrates (pyranose ring) [4]. The absorption bands at  $1617.54$  and  $1416.98\text{ cm}^{-1}$  were  
226 attributed to carboxylate groups of uronic acid residues [51]. The absorption band at  $895.23$   
227  $\text{cm}^{-1}$  could be attributed to the presence of  $\beta$ -linked carbohydrates [52], and possibly  $\beta$ -(1,4)-  
228 D-xylan regarding the literature and some polysaccharides extracted from *Plantago* seeds  
229 [32,53].



230

231 **Fig. 1.** Infrared spectra of PSPC extracted from *P. ciliata* seeds.

232

### 233 3.2.2. Monosaccharides composition (GC/MS)

234 PSPC was mainly composed of Xyl (78%) and Ara (18%) (Table 1). With a 4:1 ratio of  
235 Xyl:Ara, PSPC was probably an arabinoxylan which was in accordance with the literature for  
236 other polysaccharides extracted from *Plantago* seeds. The purified PLP-3 polysaccharide  
237 fraction obtained from *P. asiatica* seeds after hot-water extraction was an arabinoxylan (1.4:1  
238 ratio) notably composed of Xyl (55.9%) and Ara (39.6%) but also Rha (1.85%), Glc (0.83%)  
239 and Gal (1.83%) [46]. Guo et al. [45] characterized an arabinoxylan from a psyllium gum  
240 composed of Xyl:Ara with a ratio close to 4.3:1. More recently, Benaoun et al. [32] identified  
241 a heteroxylan from *P. notata* seeds rich in Xyl (77.4%) and Ara (7.58%) but also Rha  
242 (9.20%), Gal (2.58%), Glc (1.00%) and GalA (2.21%), probably due to contaminant  
243 polysaccharides [29,54].

### 244 3.2.3. Methylation and GC-MS analysis

245 PSPC was sequentially methylated, hydrolyzed, reduced and acetylated to produce partially  
246 PMAA which were then analyzed by GC/MS. The results are detailed in Table 2 (and  
247 Supplementary data, Fig. 1). These findings revealed the presence of various residues, *i.e.*  
248 (1,3)-linked Xylp (55.1%), terminal Xylp (11.5%), (1,3)-linked Araf (7.8%), (1,2,5)-linked  
249 Araf (6.0%), (1,2,4)-linked Xylp (3.6%), terminal Araf (3.0%), (1,3,4)-linked Xylp (2.2%),  
250 (1,2,3,4)-linked Xylp (2.1%), (1,2,3,5)-linked Araf (1.8%), (1,4)-linked Xylp (0.3%) and (1,2)-  
251 linked Xylp (0.3%). The results were in accordance with the overall monosaccharides  
252 composition described above after TMS derivatization (Table 1) with a ratio Xyl (75.1%):Ara  
253 (18.6%) close to 4:1. In addition, both terminal and branched total residues % were close  
254 (14.6% and 15.7%, respectively). PSPC was an arabinoxylan mainly composed of  $\beta$ -(1,3)-  
255 linked D-Xylp but also some  $\beta$ -(1,4)-linked D-Xylp according to the following pattern  $[\rightarrow 3)\text{-}\beta$ -  
256 D-Xylp-(1 $\rightarrow$ ]<sub>a</sub>-[ $\rightarrow$ 4)- $\beta$ -D-Xylp-(1 $\rightarrow$ ]<sub>b</sub>. The presence of primary branched sugar residues  
257 (1,2,4)-linked Xylp (3.6%) and (1,3,4)-linked Xylp (2.2%) showed that PSPC main chain  
258 could be branched throughout O-2 or O-3 positions, respectively of (1,4)-linked Xylp  
259 backbone, probably by (1,3)-Araf and few (1,5)-Araf. The non-reducing ends in PSPC are  
260 probably T-Xylp (11.5%), and T-Araf (3.0%). These statements were consistent with previous  
261 reports on heteroxylan and/or arabinoxylan extracted from *Plantago* sp. seeds [45,55].  
262 Benaoun et al. [32] described a heteroxylan composed of  $\beta$ -(1,3)-D-Xylp and  $\beta$ -(1,4)-D-Xylp,  
263 including various side chains in O-2 or O-3 positions, in addition to T-Xylp or T-Araf. The  
264 significant content of (1,3)-linked Araf (7.8%) attributed to side chains was already observed

265 by other authors [45,55,56]. Note that (1,3)-linked Xylp were also already observed in the side  
 266 chains [55,57].

267

268

269 **Table 2.** Partially methylated alditol acetates (PMAAs) and glycosidic linkages of PSPC.

| PMAA <sup>a</sup>            | % <sup>b</sup> | Linkage type <sup>c</sup> |
|------------------------------|----------------|---------------------------|
| 2,3,4-Me <sub>3</sub> -Ara   | 3.0            | Araf-(1→                  |
| 2,4-Me <sub>2</sub> -Ara     | 7.8            | →3)-Araf-(1→              |
| 2,3-Me <sub>2</sub> -Ara     | trace          | →5)-Araf-(1→              |
| 2-Me-Ara                     | trace          | →3,5)-Araf-(1→            |
| 3-Me-Ara                     | 6.0            | →2,5)-Araf-(1→            |
| Ara                          | 1.8            | →2,3,5)-Araf-(1→          |
| <i>total</i>                 | <b>18.6</b>    |                           |
| -----                        |                |                           |
| 2,3,4-Me <sub>3</sub> -Xyl   | 11.5           | Xylp-(1→                  |
| 3,4-Me <sub>2</sub> -Xyl     | 0.3            | →2)-Xylp-(1→              |
| 2,4-Me <sub>2</sub> -Xyl     | 55.1           | →3)-Xylp-(1→              |
| 2,3-Me <sub>2</sub> -Xyl     | 0.3            | →4)-Xylp-(1→              |
| 4-Me <sub>1</sub> -Xyl       | 2.2            | →3,4)- Xylp-(1→           |
| 3-Me <sub>1</sub> -Xyl       | 3.6            | →2,4)- Xylp-(1→           |
| Xyl                          | 2.1            | →2,3,4)- Xylp-(1→         |
| <i>total</i>                 | <b>75.1</b>    |                           |
| -----                        |                |                           |
| Galactitol acetate fragments | 1.8            | nd                        |
| <i>total<sup>d</sup></i>     | <b>1.8</b>     |                           |
| -----                        |                |                           |
| Rhamnitol acetate fragments  | 4.5            | nd                        |
| <i>total</i>                 | <b>4.5</b>     |                           |

All analyses were run in duplicate and the relative standard deviations were less than 5%.

<sup>a</sup> 2,3,4-Me<sub>3</sub>-Xyl=2,3,4-tri-*O*-methyl-xylitol-acetate, etc.

<sup>b</sup> % of peak area of *O*-methylated alditol acetates relative to total area, determined by GC-MS.

<sup>c</sup> Based on derived *O*-methyl alditol acetates.

<sup>d</sup> corresponded both for Galp and GalpA

nd: not determined

270

### 271 3.2.4. NMR spectrum

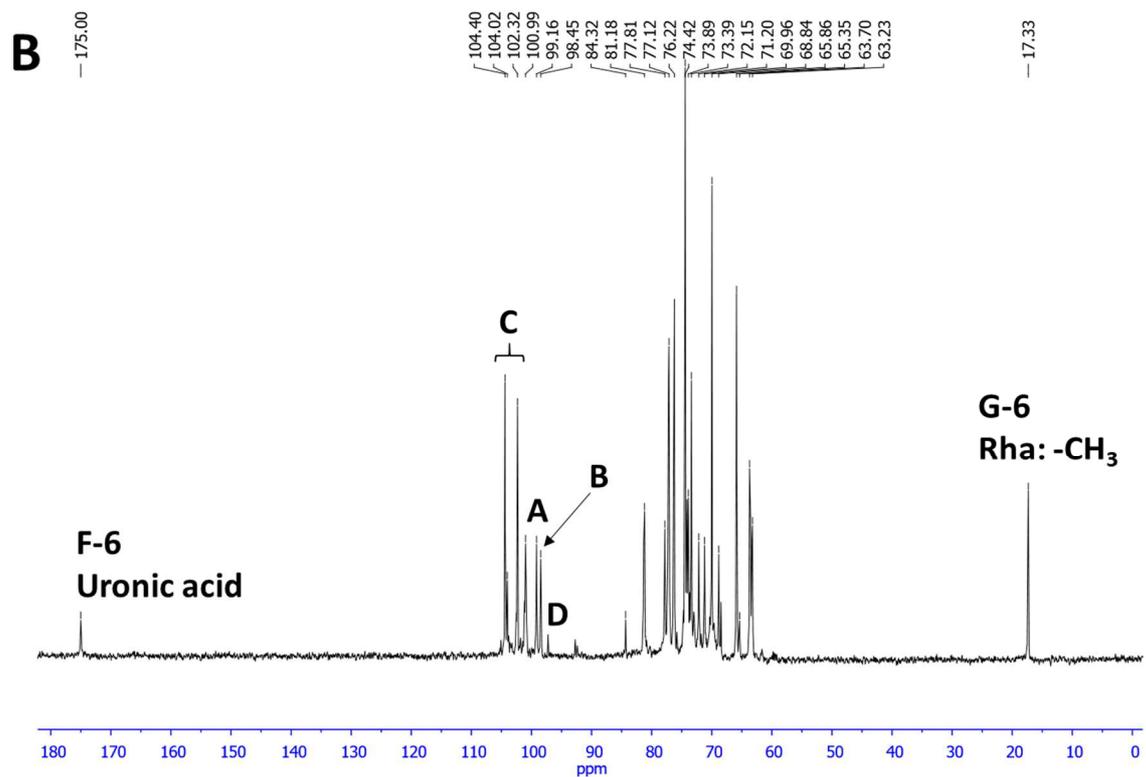
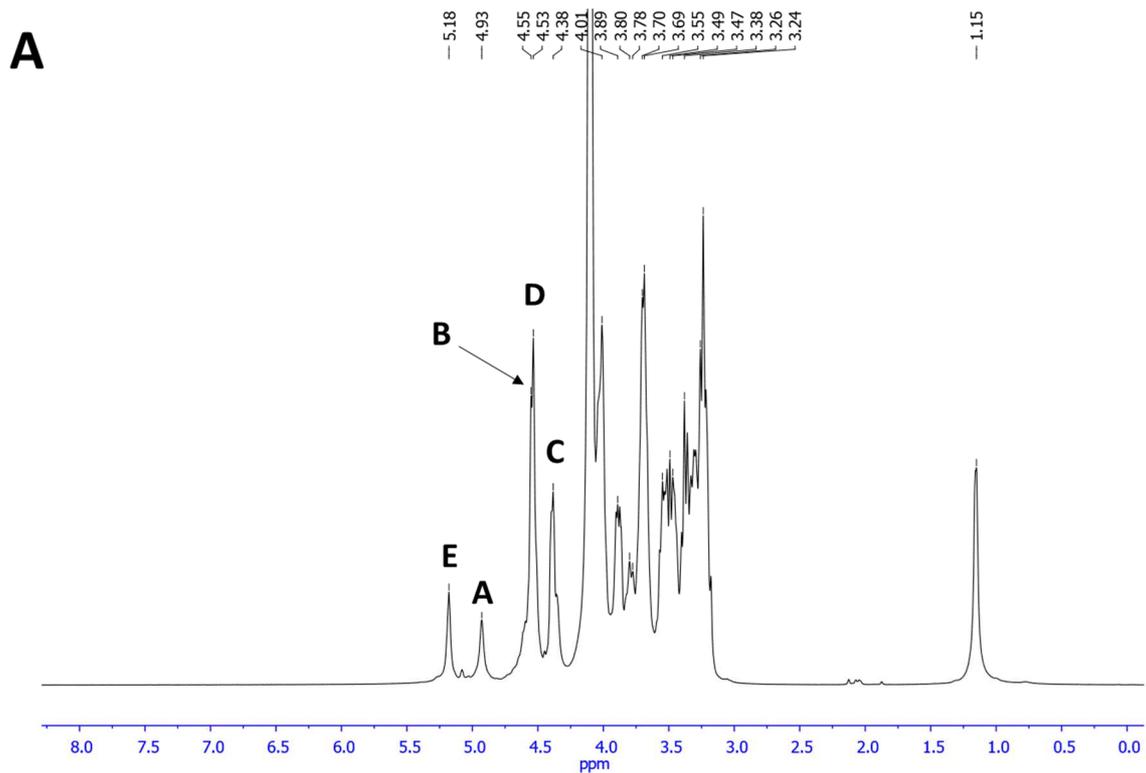
272 NMR spectroscopy was used to confirm GC/MS results and give a complement to the  
 273 structural features of PSPC. PSPC being highly viscous, a partial acidic (PSPCH) hydrolysis  
 274 was used to increase its solubility in D<sub>2</sub>O and enhance the quality of NMR spectra. To our  
 275 knowledge, this kind of hydrolysis allows identifying the main xylan backbone by removing  
 276 some branching residues such as terminal arabinose and terminal uronic acid units [19,32,56].  
 277 Both <sup>1</sup>H and <sup>13</sup>C NMR one-dimensional (1D) spectra (Fig. 2) and HSQC (Fig. 3) show the

278 complexity of PSPCH. Numerous assignments of PSPCH were determined according to  
279 chemical shifts described in the literature, *i.e.* (i) a neutral arabinoxylan from *P. ovata* Forsk  
280 seed husk consisted of a  $\beta$ -1,4-xylan backbone, branched in *O*-2 and *O*-3 by T-Xylp or a L-  
281 Araf- $\alpha$ -(1,3)-D-Xylp- $\beta$ -(1,3)-L-Araf trisaccharide, respectively [55]; (ii) an arabinoxylan from  
282 an Indian variety of sorghum consisting of  $\beta$ -(1,4)-linked D-Xylp, with few substitutions in *O*-  
283 3 or both of *O*-2 and *O*-3 by  $\alpha$ -(1,3)-L-Araf residues [58]; (iii) a highly-branched heteroxylan  
284 from *P. notata* seeds made up of  $\beta$ -(1,3)-D-Xylp and  $\beta$ -(1,4)-D-Xylp, with various side chains  
285 in *O*-2 and *O*-3 positions such as  $\alpha$ -L-Araf-(1,3)- $\beta$ -D-Xylp and  $\beta$ -D-Xylp-(1,2)- $\beta$ -D-Xylp, as  
286 well as T-Xylp or T-Araf [32]; (iv) a heteroxylan from *P. major* L. seeds was shown to have  
287  $\beta$ -(1,4)-Xylp, carrying short side chains such as  $\beta$ -D-Xylp,  $\alpha$ -L-Araf,  $\alpha$ -L-Araf-(1,3)- $\beta$ -D-Xylp  
288 and  $\alpha$ -D-GalpA-(1,3)- $\alpha$ -L-Araf attached to *O*-2 or *O*-3 positions [19]; (v) a mucilage from the  
289 seeds husk of *P. ovata* Forsk which was composed of both  $\beta$ -(1,3) and  $\beta$ -(1,4)-D-Xylp  
290 residues, the latter being substituted with 2-*O*-( $\alpha$ -D-GalpA)- $\alpha$ -L-Rha groups [59]. <sup>1</sup>H and <sup>13</sup>C  
291 NMR spectra of PSPCH highlighted the existence of a main backbone of  $\beta$ -(1,3)/ $\beta$ -(1,4)-D-  
292 xylan [62]. Beta-anomeric region (4.38-4.93 ppm) of the <sup>1</sup>H NMR spectrum (Fig. 2A) was  
293 attributed to anomeric protons of  $\beta$ -D-linked-Xylp. Figure 2 (A and B) showed specific H-  
294 1/C-1 signals from  $\beta$ -D-Xylp residues at 4.93/99.16, 4.55/98.45 and 4.53/97.16 ppm which  
295 could correspond to  $\beta$ -(1,3)-D-Xylp,  $\beta$ -(1,4)-D-Xylp residues and terminal  $\beta$ -D-Xylp units  
296 branched to *O*-3 position of the main backbone, respectively [19,32,62,63,67]. In <sup>13</sup>C NMR  
297 (Fig. 2B), the peaks corresponding to C-3 and C-4 (81.18 and 75.81 ppm respectively) were  
298 assigned to  $\beta$ -(1,3,4)-linked Xylp. The signal at 4.38 ppm (Fig. 2A) corresponded to the  
299 anomeric protons of branched  $\beta$ -(1,4)-D-Xylp and/or branched  $\beta$ -(1,3)-D-Xylp [32,64]. The  
300 shift for lateral chains branched in *O*-2 of the xylan backbone was identified ranging from  
301 73.66 to 77.20 ppm. Thus, core-chain units may be also substituted by arabinose side chains  
302 [64]. Proton chemical shift around 5.18 ppm (Fig. 2A), which was very close to the 5.2-5.4  
303 ppm region, was assigned to anomeric protons of  $\alpha$ -(1,2)-L-Araf and also  $\alpha$ -(1,3)-L-Araf  
304 [60,61]. Regarding the literature, the anomer signals from terminal L-Araf linked to  $\beta$ -(1,3)- or  
305 (1,4)-D-Xylp residues (around 109 ppm) was not detected (Fig. 2B) [19,66]. However, the  
306 substitution of the main backbone by Araf units in *O*-2 or *O*-3 was identified at 63.23 ppm of  
307 C-5 [56,67]. Two weak signals at 1.150 ppm (Fig. 2A) and 175.1 ppm (Fig. 2B) were  
308 assigned to the methyl groups of Rha and C-6 of GalpA respectively. These assignments were  
309 supported by other reports [32,68]. Note that the linkage types for Rha and GalpA residues  
310 were not determined by methylation analysis, but NMR results suggested the possible  
311 presence of terminal  $\alpha$ -D-GalpA probably attached to  $\alpha$ -L-Rhap in *O*-2 positions [19,65].

312 Overall, the various assignments were in accordance with the 2D heteronuclear correlation  
313 spectrum of PSPC. The HSQC spectrum (Fig. 3) showed only 5 correlations  $^1\text{H}/^{13}\text{C}$  between  
314 99 and 110 ppm identified as  $\beta$ -configuration anomeric carbons. These intersections of  
315 chemical shifts named A-1 ( $\delta$  4.71/103.52), H-1 ( $\delta$  4.52/ 101.82), B-1 ( $\delta$  4.42/ 101.15), I-1 ( $\delta$   
316 4.47/ 101.82), C-1 ( $\delta$  4.72/ 102.67) and D-1 ( $\delta$  4.67/ 103.24) were assigned to the C-1(H-1)  
317 of  $\beta$ -(1,3)-linked Xylp,  $\beta$ -(1,2,4)-linked Xylp,  $\beta$ -(1,4)-linked Xylp,  $\beta$ -(1,2,4)-linked Xylp,  $\beta$ -  
318 (1,3,4)-linked Xylp and T-Xylp, respectively [46,57]. The disappearance of T-Araf (or linked)  
319 intersections in the 5.1-5.8 ppm region was probably due to acid hydrolysis as described in the  
320 literature [19,56]. Based on methylation analysis (see section 3.2.3), NMR spectroscopy (1D  
321 and 2D) and regarding the literature, PSPCH, and thus PSPC, should be considered as an  
322 arabinoxylan whose the backbone was probably composed of  $\beta$ -(1,3)-linked Xylp, including  
323 also  $\beta$ -(1,4),  $\beta$ -(1,2,4)- and  $\beta$ -(1,3,4)-linked Xylp. Further experiments such as enzymatic  
324 hydrolysis should be performed to determine the alternance pattern [59]. PSPCH structure  
325 showed the presence of terminal  $\beta$ -Xylp possibly linked to *O*-3, as well as  $\alpha$ -L-Araf residues  
326 probably substituted to *O*-2 and *O*-3 of  $\beta$ -D-Xylp residues of the backbone. This specificity of  
327 structure could be due to biotic and/or abiotic conditions [57].

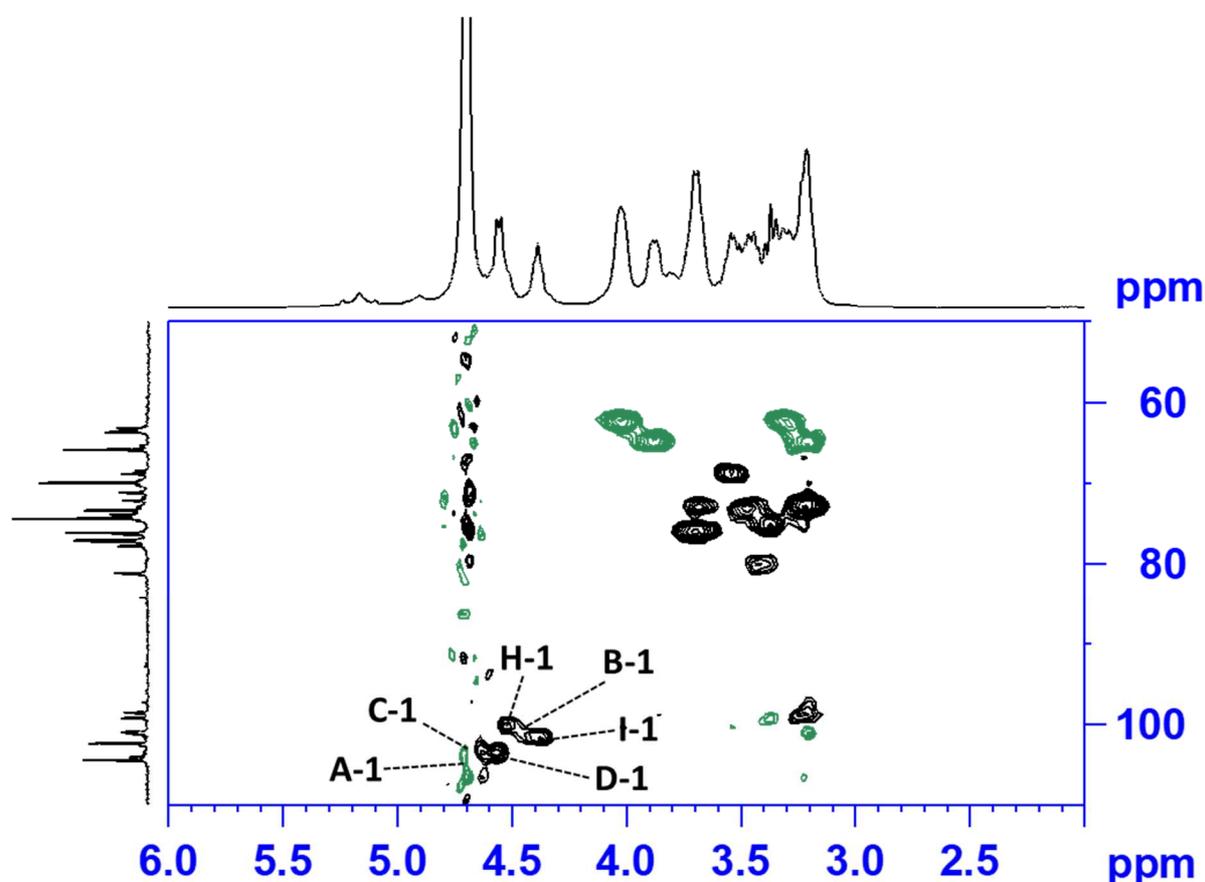
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330

331 **Fig. 2.** (A)  $^1\text{H}$  NMR, (B)  $^{13}\text{C}$  NMR spectra of PSPCH from *Plantago ciliata* L. seeds.  
 332 Analyses were recorded at 15 g/L in  $\text{D}_2\text{O}$ . A, B, C, D, E, F and G residues respectively  
 333 correspond to  $\beta$ -(1,3)-linked D-Xylp,  $\beta$ -(1,4)-linked D-Xylp,  $\beta$ -(1,3,4)-linked D-Xylp, terminal  
 334  $\beta$ -D-Xylp, branched  $\alpha$ -(1,2) or (1,3)-linked L-Araf, terminal  $\alpha$ -D-GalpA and  $\alpha$ -(1,2)-linked L-



336  
 337 **Fig. 3.**  $^1\text{H}/^{13}\text{C}$ -HSQC spectrum (anomeric region) of PSPCH from *Plantago ciliata* L. seeds.  
 338 Analyses were recorded at 15 g/L in  $\text{D}_2\text{O}$ . A, B, C and D residues respectively correspond to  
 339  $\beta$ -(1,3)-linked D-Xylp,  $\beta$ -(1,4)-linked D-Xylp,  $\beta$ -(1,3,4)-linked D-Xylp and terminal  $\beta$ -D-Xylp.  
 340 H and I residues correspond to  $\beta$ -(1,2,4)-linked D-Xylp.

341

### 342 3.3. Physico-chemical analysis

#### 343 3.3.1. Molecular weight (SEC/MALLS)

344 The size exclusion chromatography (SEC) coupled with multi-angle laser light scattering  
 345 (MALLS) analysis was carried out to determine the macromolecular characteristics of PSPC  
 346 (Table 3). First, the **weight-average** molecular weight (Mw) and number-average molecular  
 347 weight (Mn) of PSPC were 700 kDa and 477 kDa respectively. These values were lower than  
 348 those reported by Benaoun et al. [32], for a water-soluble heteroxylan extracted from *P.*  
 349 *notata* with a Mw of 2300 kDa. Similarly, a heteroxylan extracted from *P. asiatica* L. seeds  
 350 also showed a high Mw (1894 kDa) [30]. Regarding the literature, water-soluble

351 polysaccharides extracted from the seeds of *Plantago* species are in general described for their  
 352 significant Mw, often up to several million Dalton [4,27,30,32,69]. The low value of PDI  
 353 (Mw/Mn = 1.47) for a natural polysaccharide showed the presence of a relatively  
 354 homogeneous polymer. The intrinsic viscosity value  $[\eta]$  of PSPC, which highlights the  
 355 capacity to change the water mobility, was 157 mL/g. This value depends on molecular  
 356 weight, conformation of polymer chains or solvent nature [70]. PSPC intrinsic viscosity was  
 357 different in comparison to other similar arabino and/or heteroxylyan extracted from the seeds  
 358 of *P. major* [20], *P. ovata* [71], *P. asiatica* [72] or *P. notata* [32] with 1424, 560, 368 and 715  
 359 mg/L respectively. Note that these values were larger for heteroxylyan presenting the greater  
 360 values of Radius of Gyration (Rg). The slope from the double log plot of Rg as function of  
 361 Mw gave a value around 0.6 (data not shown) [73]. Ranging 14 to 16.5 mL for integration  
 362 (Table 3; Supplementary data, Fig. 2), various relationships corresponding to a random coil  
 363 conformation for a polysaccharide in water can be verified, *e.g.*  $[\eta] = 0.038 M^{0.62}$  and  $R_h =$   
 364  $f(M^{0.54})$ . Finally, the theoretical critical concentration  $C^*$  of PSPC, which can be obtained by  
 365  $k_s/[\eta]$  (with  $k_s = 0,5-1$  for coil polysaccharides in water) could range from 3.2 to 6.4 g/L.

366

367 **Table 3.** Macromolecular characteristics of PSPC.

| Mw <sup>a</sup> (kDa) | Mn <sup>b</sup> (kDa) | PDI <sup>c</sup> | Rg <sup>d</sup> (nm) | Rh <sup>d</sup> (nm) | $[\eta]^e$ (mL/g) |
|-----------------------|-----------------------|------------------|----------------------|----------------------|-------------------|
| 700                   | 477                   | 1.47             | 59                   | 24.7                 | 157               |

368

369 <sup>a</sup>Mw: **Weight-average** molecular weight measured by SEC-MALLS,

370 <sup>b</sup> Mn: Number average molecular weight was measured by SEC-MALLS,

371 <sup>c</sup> PDI: Polydispersity index Mw/Mn,

372 <sup>d</sup>Rg and Rh: Average gyration and hydrodynamic radii were measured by SEC-MALLS,

373 <sup>e</sup> $[\eta]$ : Intrinsic viscosity was measured by SEC.

374

### 375 3.3.2. Rheological behavior of PSPC

376 The flow curves at 25 °C for various concentrations of PSPC are given in Fig. 4. PSPC in  
 377 water showed a classical pseudoplastic behavior since the apparent viscosity dropped with  
 378 increasing shear rate due to the alignment of long chain to the flow direction. At low shear  
 379 rates, the hydrogen bonding and polymer entanglement increase the viscosity of PSPC  
 380 solutions [74]. These statements were consistent with the literature concerning other hetero-  
 381 and/or arabinoxylyan extracted from *Plantago* species [32,29,75]. The effect of the ionic  
 382 strength and temperature on PSPC solutions are shown in Fig. 5. Classically, increasing the  
 383 temperature reduce weak energy bonds interactions between molecules and/or intermolecular

384 entanglements thanks to the generation of energy and thermal agitation [76]. The use of NaCl  
385 slightly changed the apparent viscosity of PSPC which it is interesting regarding its weak  
386 polyelectrolyte character (low amount of uronic acids) [78]. The ionic strength could limit  
387 low-energy interactions between the polymer chains. The elastic ( $G'$ ) and viscous ( $G''$ ) moduli  
388 increased with rising concentrations and angular frequencies (Fig. 6), both in water and NaCl  
389 solution. PSPC solutions exhibited a viscous liquid-like behavior for  $\omega < 1 \text{ rad.s}^{-1}$  since the  
390  $G''$  values were higher than  $G'$ . For  $\omega > 1 \text{ rad.s}^{-1}$ , the trend changed since the  $G'$  values were  
391 higher than for  $G''$ , highlighting a strong contribution of the elastic deformation. At low  
392 angular frequencies, PSPC has enough time to relax to a more stable molecular state  
393 disentanglement, thus the dissipating flow is mainly characterized by the loss modulus ( $G''$ ).  
394 For high frequencies, the elastic deformation of the interleaved network significantly  
395 increases and thus also the storage modulus ( $G'$ ) suggesting a higher elastic contribution to the  
396 gel structure [29,78]. Calculating the loss tangent ( $\tan \delta = G''/G'$ ) (damping factor) as a  
397 function of angular frequency ( $\text{rad.s}^{-1}$ ), which ranged from 2.8 to 0.01 for increasing  $\omega$ ,  
398 clearly showed the high viscoelastic feature of PSPC and typical weak-gel structure in water  
399 or NaCl solutions, as described in the literature [32,55,75,79].

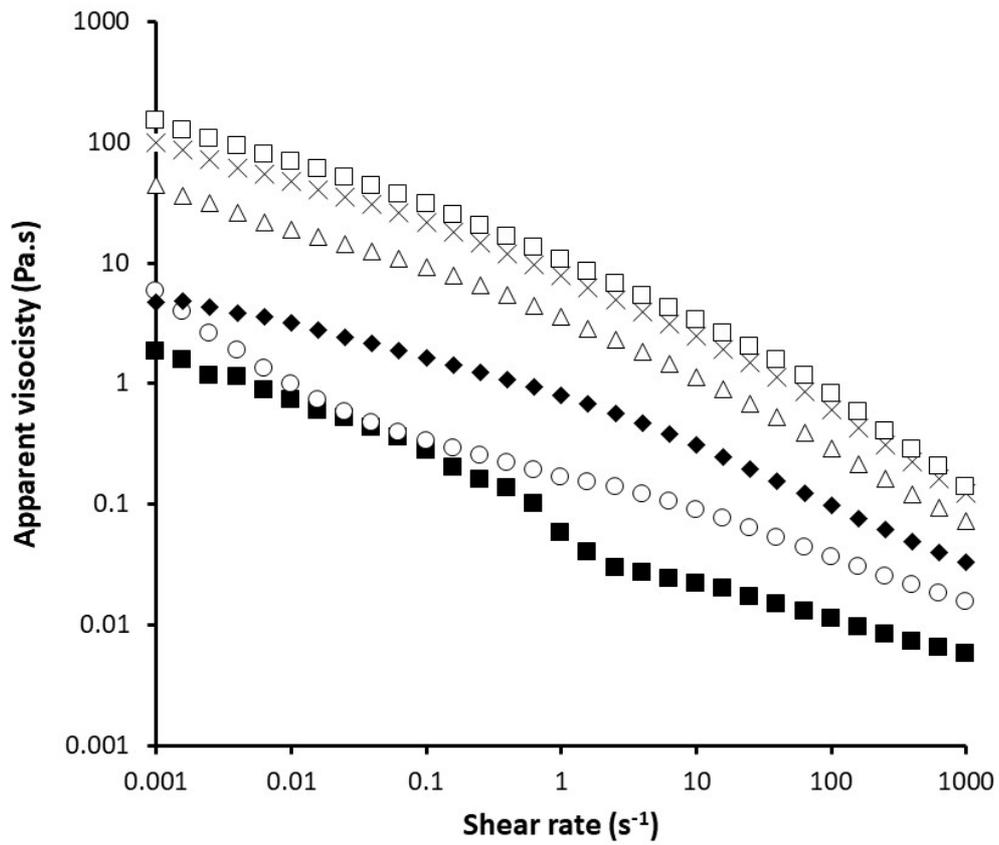
400 The critical concentration ( $C^*$ ) of PSPC in water at 25 °C, which separates the dilute and  
401 semi-dilute regimes, corresponded to the intersection point of the two regression lines ranging  
402 from 0.1-0.35% and 0.5-1.50% (Fig. 7).  $C^*$  was found around 3.2-3.7 g/L. This value was  
403 consistent with the theoretical one estimated in Section 3.3.1 (3.2 to 6.4 g/L). Note that the  
404 practical value of  $C^*$  and intrinsic viscosity  $[\eta]$  allow the accurate determination of  $k_s$   
405 regarding Eq. 2. The specific constant for PSPC was 0.5-0.58 and thus in accordance with  
406 some values described in the literature for coil polysaccharides [80-82] confirming the overall  
407 consistency of rheological and SEC/MALLS data.

408

$$409 \quad C^* = \frac{k_s}{[\eta]} \quad (2)$$

410 *Where  $k_s$  is the specific constant for each type and conformation of polymer in solution, which*  
411 *ranges from 0.5 to 4 depending on the polysaccharide nature and conformation in water;  $[\eta]$*   
412 *is the intrinsic viscosity and  $C^*$  is the overlap concentration.*

413

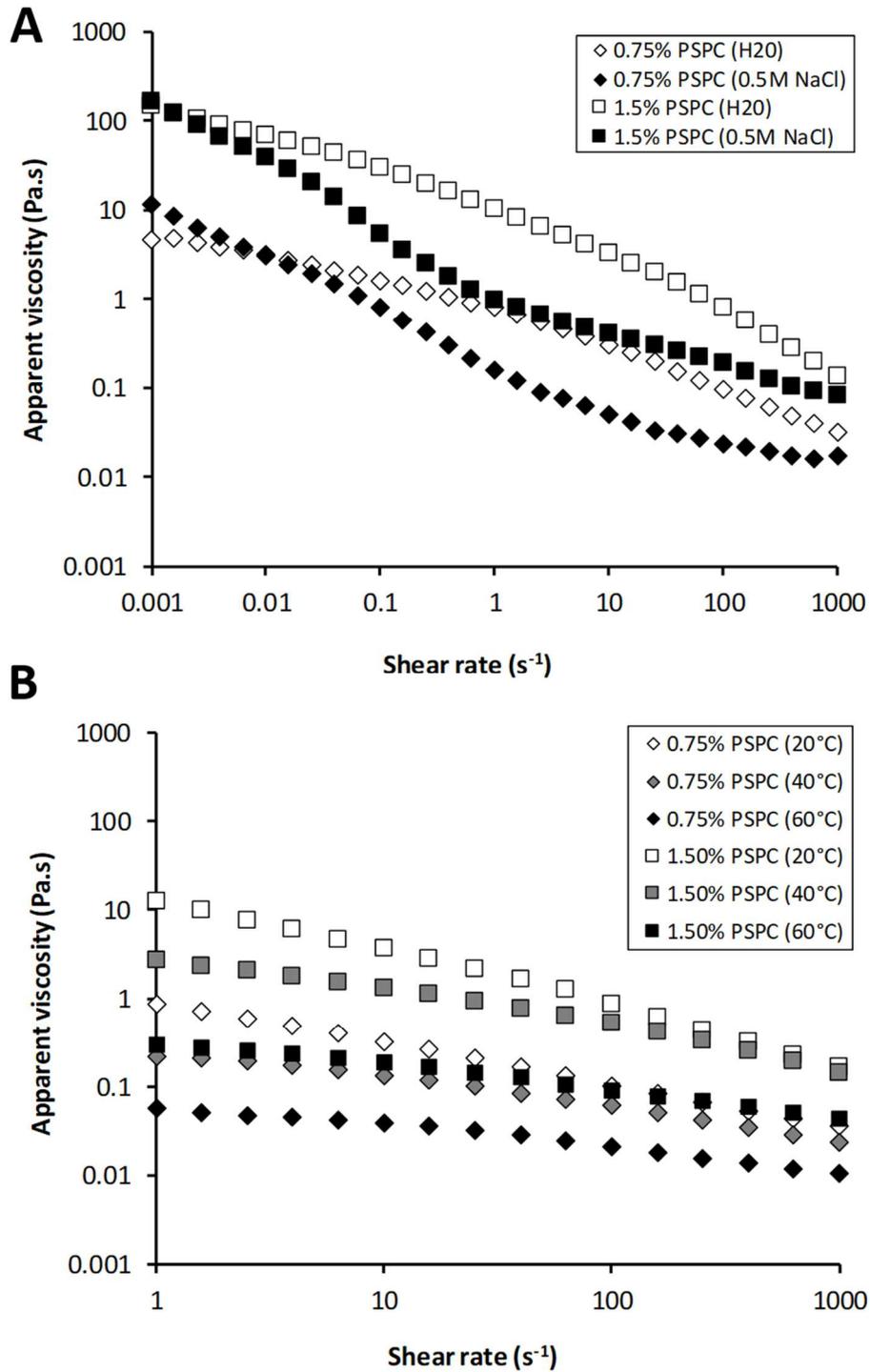


414

415 **Fig. 4.** Effect of PSPC concentration on apparent viscosity at 25 °C in aqueous solution. (■),  
 416 (○), (◆), (×) and (□) respectively correspond to 0.25, 0.50, 0.75, 1, 1.25 and 1.50% (w/v)  
 417 of PSPC in water.

418

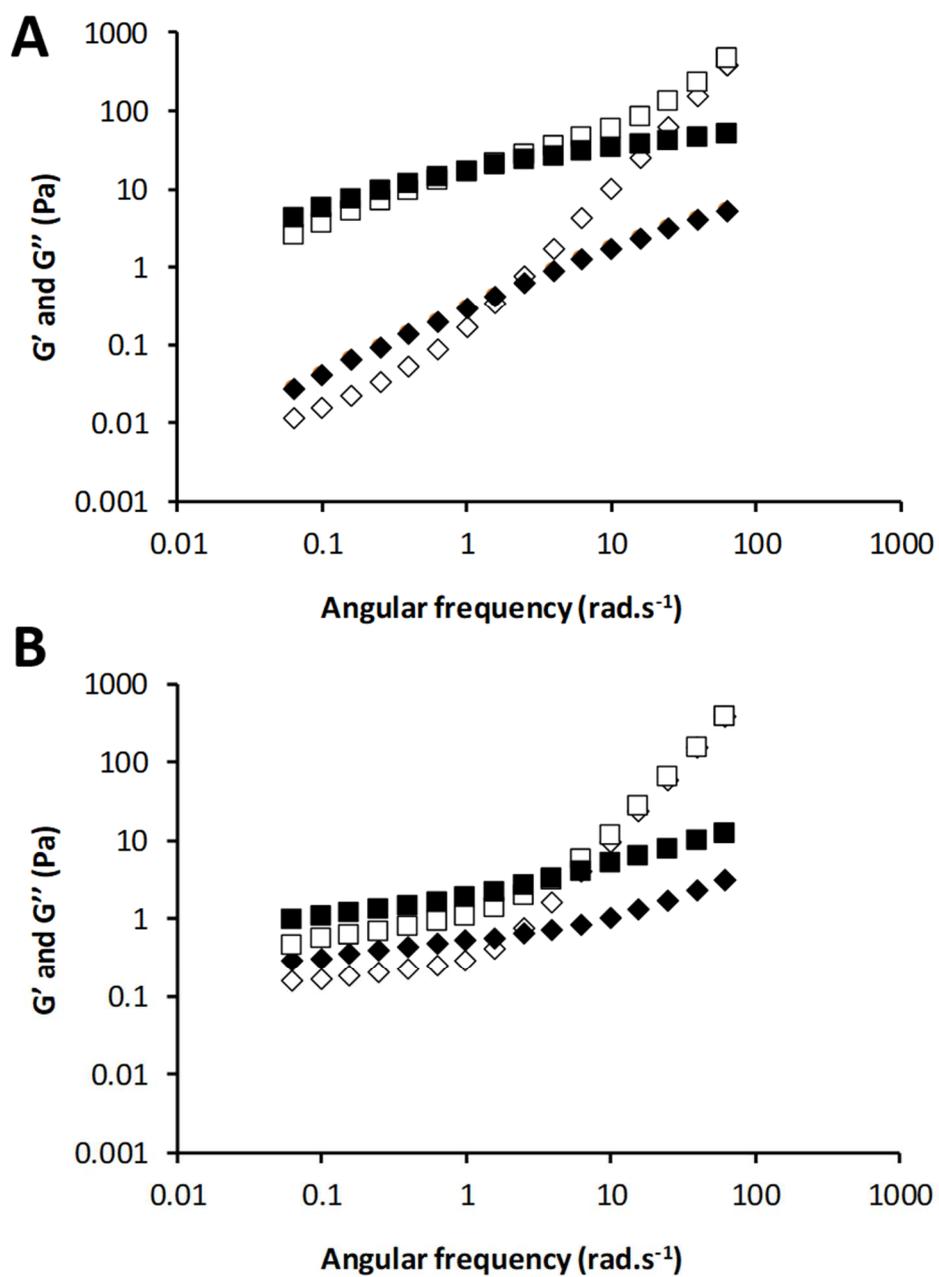
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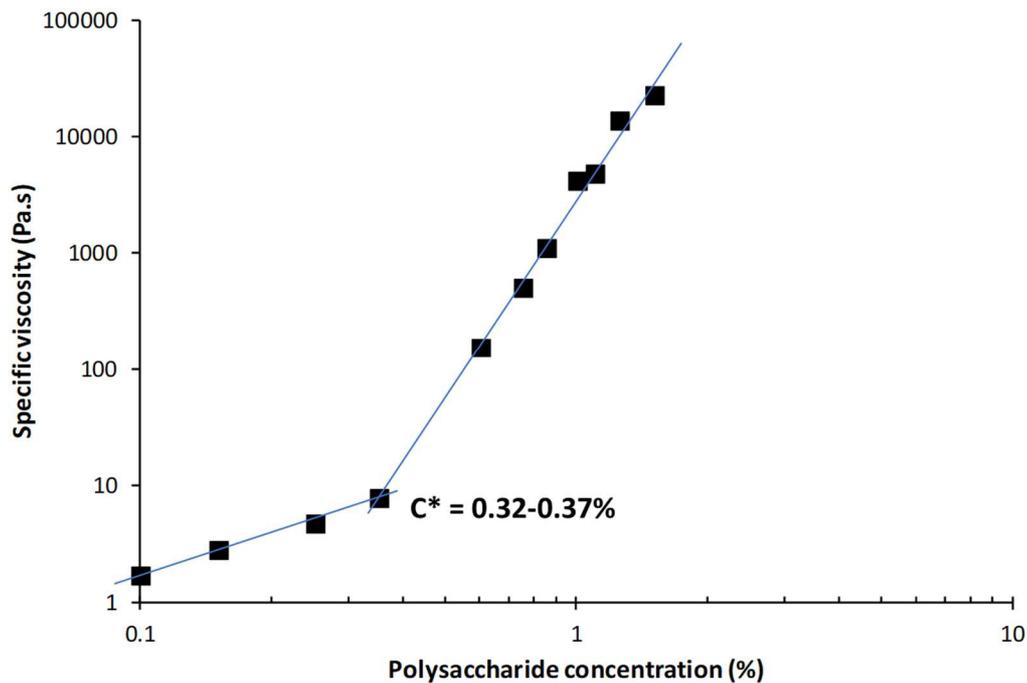
423 **Fig. 5.** Influence of (A) 0.5 M NaCl (25 °C) and (B) temperature (20, 40 and 60 °C) on  
424 apparent viscosity of both (diamond) 0.75% and (square) 1.50% PSPC solutions (w/v).

425



426

427 **Fig. 6.** Storage ( $G'$ , in white) and loss ( $G''$ , in black) moduli for (diamond) 0.75% and  
 428 (square) 1.50% PSPC concentrations (w/v) of (A) aqueous and (B) 0.5 M NaCl solutions at 25  
 429 °C.



430

431 **Fig. 7.** Overlap concentration ( $C^*$ ) from the log-log plot of  $\eta_{sp}$  vs PSPC concentration (%,

432 w/v) in water at 25 °C.

#### 433 4. Conclusion

434 The structural features and rheological behavior of a water-soluble fraction (PSPC) extracted  
 435 from *P. ciliata* seeds were determined. PSPC was an arabinoxylan mainly composed of  $\beta$ -  
 436 (1,3)-linked D-Xylp residues including also  $\beta$ -(1,4),  $\beta$ -(1,2,4)- and  $\beta$ -(1,3,4)-linked Xylp with  
 437  $\alpha$ -L-Araf residues probably substituted to O-2 and O-3 and terminal D-Xylp linked to O-3 of  
 438 the main backbone. Further experiments using as instance specific xylanases should be  
 439 performed to fully elucidate the branched structures and backbone repetition. With a Mw  
 440 close to 700 kDa and an intrinsic viscosity ( $\eta$ ) about 157 mL/g, PSPC showed a coil  
 441 conformation in aqueous and saline solutions, which was also confirmed through the overlap  
 442 concentration  $C^*$  of 0.32-0.37% (w/v) and the calculation of the specific constant  $k_s$  (0.5-  
 443 0.58). As already reported for other arabino- and/or heteroxylan from *Plantago* species, PSPC  
 444 showed a pseudoplastic behavior and surprisingly a strong viscoelastic property, which placed  
 445 it among polysaccharide leaders able to make a weak-gel structure in water or saline  
 446 solutions.

447

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