Gelation of cereal β-glucan at low concentrations

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ABSTRACT

Viscosity of cereal β-glucan during digestion is considered to be a vital factor for its health effects. Thus, studies on solution properties and gelation are essential for understanding the mechanisms of the β-glucan functionality. The aim of this study was to investigate the effect of the dissolution temperature on gelation of cereal β-glucan at low concentrations that are relevant for food products. The rheological properties of oat and barley β-glucans (OBG and BBG) using three dissolution temperatures (37 ˚C, 57 ˚C and 85 ˚C) at low concentration (1.5% and 1%, respectively) were studied for 7 days. Additionally, the β-glucans were oxidised with 70 mM H_2O_2 and 1 mM $FeSO_4 \times 7H_2O$ as a catalyst, to evaluate the consequence of oxidative degradation on the gelation properties. The study showed that dissolution at 85 ˚C did not result in gelation. The optimal dissolution temperature for gelation of OBG was 37 ˚C and for gelation of BBG 57 ˚C. At these temperatures, also the oxidised OBG and BBG gelled, although the gel strength was somewhat lower than in the non-oxidised ones. Gelation was suggested to require partial dissolution of β-glucan, which depended on the molar mass and aggregation state of the β-glucan molecule. Therefore, the state of β-glucan in solution and its thermal treatment history may affect its technological and physiological functionality.

KEYWORDS

β-glucan; gelation; oxidation; dissolution temperature

1 INTRODUCTION

Mixed linkage β-glucan or (1→3)(1→4)-β-D-glucan (β-glucan) is a major non-starch polysaccharide in both oat and barley, where the contents vary from 3% to 7% and from 3% to 11%, respectively (Cui & Wood, 2000). Oat and barley β-glucans have been sufficiently shown to have health effects and there are health claims for their ability to lower blood cholesterol levels and postprandial glucose response (EFSA, 2010, 2011a, b; FDA, 1997, 2005). The effect of β-glucan on postprandial glucose response is related to its ability to increase luminal viscosity, which reduces digestive activity and hinders nutrition absorption (Wood, 2010). However, the exact mechanism for its cholesterol lowering effect is still unknown even though it has been suggested to be linked to the increased viscosity, as well (EFSA, 2010, 2011b; Wolever et al., 2010). The increased intestinal viscosity has been proposed to hinder the absorption of bile acids, thus leading to their excretion, which in turn, would result in increased synthesis of new bile acids from cholesterol (EFSA, 2010; Othman et al. 2011). Additionally, the increased viscosity of intestinal digest has been linked to the decreased absorption of dietary cholesterol (Othman et al., 2011).

The structure of β-glucan is composed of consecutive (1→4)-β-linked segments of mostly three (cellotriosyl, DP3) or four (cellotetraosyl, DP4) glucose units, although also longer segments are found. These linear cellulose-like segments are linked via (1→3)-β-linkages, which causes bending of the molecule and enhances water-solubility. For the ability to form viscous solutions, the solubility of β-glucan, which is affected by the structural factors such as the ratio of (1→4)-β- to (1→3)-β-linkages and ratio of cellotriosyl units to cellotetraosyl units (DP3:DP4 ratio), is vital (Izydorczyk & Biliaderis, 2000; Wood, 2010). Additionally, the molar mass and concentration of β-glucan affect the viscosity formation (Lazaridou, Biliaderis, & Izydorczyk, 2003; Tosh, Wood, Wang, & Weisz, 2004b; Vaikousi, Biliaderis, & Izydorczyk, 2004)

It has been suggested that gelation of β-glucan is also affected by both the concentration and molar mass, which influence the probability of the molecules to encounter and the mobility of the molecules, respectively (Böhm & Kulicke, 1999). There are two proposed mechanisms for the formation of a β-glucan gel network. The first is based on the long cellulosic segments, which can interact to form junction zones (Fincher & Stone, 1986). However, these longer segments in the β-glucan structure are only few, and thus, it is not likely that this is the only mechanism occurring. The second – and more probable – mechanism is based on the repeated cellotriosyl segments in the structure that give rise to the gel network (Böhm & Kulicke, 1999). Barley β-glucan has higher DP3:DP4 ratio than oat β-glucan (Tosh, Brummer, Wood, Wang, & Weisz, 2004a), which supports this theory as barley β-glucan has been shown to have a higher gelation rate.

Previous studies have shown that cereal β-glucan is able to form gel structures but gelation has been shown to require quite high β-glucan concentration when compared to the concentrations that would be relevant in food products. Lazaridou et al. (2003) reported the critical concentration for gelation to be 3.5% and 4.4% for oat β-glucans with a molar mass of 35 000 g/mol and 110 000 g/mol, respectively. Also acid hydrolysed oat and barley β-glucans (with a molar mass of 40 000-70 000 g/mol) have been shown to gel at a concentration of 6% (Tosh, Wood, & Wang, 2003). Lazaridou & Biliaderis (2004) showed gelation of oat and barley β-glucans at low concentrations (1%) through repeated freeze-thaw cycles (cryogelation), and thus, indicated that gelation may take place in frozen products. However, there is no knowledge on gelation of cereal β-glucan at low concentrations without freeze-thaw cycles.

The rheological properties of β-glucan may be altered during processing and storage of foods. Besides enzymatic and acid hydrolysis, also oxidation has been shown to cause degradation of β-glucan (Faure, Andersen, & Nyström, 2012; Kivelä, Gates, & Sontag-Strohm, 2009a; Kivelä, Nyström, Salovaara, & Sontag-Strohm, 2009b; Kivelä, Henniges, Sontag-Strohm, & Potthast, 2012; Mäkelä, Sontag-Strohm, & Maina, 2015, Mäkelä et al., 2016). The initiation of β-glucan oxidation

79 can occur in the presence of reactive oxygen species (ROS), the hydroxyl radical (·OH) being the
80 most reactive one. The hydroxyl radicals can originate from hydrogen peroxide (H_2O_2)
81 decomposition catalysed by transition metals (Haber & Weiss, 1934). Additionally, lipid radicals
82 have been shown to be able to cause oxidation of β-glucan (Wang, Mäkelä, Maina, Lampi, &
83 Sontag-Strohm, 2016). Oxidation of β-glucan leads to a decrease in molar mass and consequently to
84 a loss of viscosity, which may threaten the physiological and technological functionality of β-
85 glucan (Kivelä et al., 2009a; Lazaridou & Biliaderis, 2007; Wood, 2010). However, for gelation the
86 degradation of β-glucan can be considered as a benefit, since the smaller molar mass molecules
87 have higher mobility, and thus, may form interactions faster (Böhm and Kulicke, 1999; Tosh et al.,
88 2004b).

89 The aim of this study was to investigate the gelation of oat and barley β-glucans at low
90 concentration induced by different dissolution temperatures. The oat and barley β-glucans were
91 compared in relation to the structural differences of these β-glucans. Another objective was to study
92 how the gelation phenomenon changes when β-glucan is oxidised, as oxidation has been considered
93 to decrease molar mass and viscosity, which on the other hand may lead to gelation.

94

95 **2 MATERIALS AND METHODS**

96 **2.1 Preparation of the samples**

97 Barley β-glucan (BBG, High Viscosity, purity > 94 %) and oat β-glucan (OBG, High Viscosity,
98 purity > 94 %) were purchased from Megazyme (Ireland). 1.25% (w/w) BBG and 1.875% (w/w)
99 OBG solutions were prepared by wetting the sample with 99.5% ethanol (AA ethanol, Altia Oy,
100 Finland) prior to the dissolution with MilliQ water. The dissolution of barley and oat β-glucans was
101 done at 37 ˚C (BBG37 and OBG37), 57 ˚C (BBG57 and OBG57) and 85 ˚C (BBG85 and OBG85)
102 for 2 hours with constant stirring. After 2 hours the samples were allowed to cool down and the

evaporated water was compensated by adding MilliQ water to obtain the desired concentration. Stirring was then continued for an hour at room temperature.

Three replicates of the non-oxidised and oxidised samples were prepared from each sample solution (BBG37, BBG57, BBG85, OBG37, OBG57, OBG85). The oxidation was initiated by adding 70 mM hydrogen peroxide (30% H_2O_2, Merck, Germany) and 1 mM iron (II) sulphate heptahydrate ($FeSO_4 \times 7H_2O$, Merck, Germany) as a catalyst. MilliQ was added to adjust the concentration of the BBG and OBG samples to 1% (w/w) and 1.5% (w/w), respectively. The non-oxidised samples were diluted to the same concentration with MilliQ.

From each sample 3 moulds (cylindrical plastic moulds, ø35 mm, 3 g of sample per each) were prepared for the oscillatory measurements. The rest of the samples were stored in test tubes for the viscosity measurements. All samples were covered to prevent drying during storage at room temperature.

2.2 Viscosity measurement

The viscosity (flow curve) was measured at 20 °C with Haake RheoStress 600 rheometer (Thermo Electron GmbH, Germany). A cone and plate geometry was used with a 35 mm diameter and 2° cone angle. A stepwise rotation program with a shear rate ranging from 1 to 100 s^{-1} and 100 to 1 s^{-1} was used for all the samples. The viscosity of the samples was measured on day 1, day 4 and day 7, and the shear stress curves and viscosity values at 14 s^{-1} were compared.

2.3 Dynamic oscillation measurement

The storage modulus (G') and loss modulus (G'') were measured with Haake RheoStress 600 rheometer (Thermo Electron GmbH, Germany). The measurements were conducted at 20 °C with a parallel plate and plate geometry using a 35 mm plate. The oscillation frequency ranged from 0.01 to 10 Hz and the strain was 0.4 in all the measurements (the strain sweep was used to ensure that the

126 analysis was carried out within the linear viscoelastic range of the samples). The samples were
127 measured on days 1, 4 and 7.

128 **2.4 Fluorescent microscopy**

129 The samples were stained with calcofluor (Calcofluor White, Megazyme, Ireland) and for this
130 purpose 10 g/l stock solution of calcofluor was prepared freshly by dissolving it in 100 mM sodium
131 carbonate (pH 10, Merck, Germany). The β-glucan samples were mixed with the calcofluor stock
132 solution (1:1). Both the stock solution and the samples were protected from light prior to analysis.
133 The imaging of the stained samples was conducted using a microscope (Axio Scope.A1, Carl Zeiss
134 MicroImaging GmbH, Germany) coupled with an illuminator (HXP-120, Carl Zeiss MicroImaging
135 GmbH, Germany).

136 **2.5 Statistical analyses**

137 The results were calculated as an average of three replicate samples and the results are reported as
138 averages ± standard error of mean (SEM). Statistical analyses were accomplished with Statistical
139 Package for the Social Science (SPSS Statistics version 24, IBM, USA), using the one-way analysis
140 of variance (ANOVA) with a post-hoc LSD test. A logarithmic transformation of the viscosity data
141 was applied prior to the statistical analysis because of the >10-fold differences in the values.
142 Differences were considered as significant at $P<0.05$.

143

144 **3 RESULTS AND DISCUSSION**

145 **3.1 Viscosities and hysteresis of barley and oat β-glucans dissolved at different temperatures**

146 In this study, the possible entanglements and structure formation in the β-glucan samples were
147 investigated using shear stress curves. When the shear stress is plotted as a function of the shear
148 rate, a hysteresis loop is obtained for materials that encounter structural changes due to the flow

149 (Mewis & Wagner, 2009). The changes can be either reversible (thixotropy), when the viscosity
150 recovers with some lag-time, or they can be irreversible.

151 Clear hysteresis was observed in BBG37, BBG57 and OBG37 (Fig 1a, b and c), and in the case of
152 BBG37 and OBG37 the oxidised sample showed a larger hysteresis loop. Joly & Mehrabian (1976)
153 described the hysteresis loop as an indicator of the structural breakdown and more precisely the
154 large hysteresis loop results from a significant structural breakage and smaller hysteresis loops from
155 a small breakdown. Thus, in this study BBG37, BBG57 and OBG37 were observed to have some
156 structural changes that caused hysteresis during the measurement.

157 For the samples that did not show hysteresis, only viscosities were measured at three time points
158 (day 1, day 4, day 7) (Table 1). The samples dissolved at 85 ˚C were viscous solutions and the
159 viscosity loss of BBG85 and OBG85 was about 94% and 78% on day 1, respectively, and about
160 98% and 94% on day 7. Thus, slower decrease in viscosity was observed in OBG than in BBG. This
161 corresponds well with the results of Faure et al. (2012), which showed faster formation of hydroxyl
162 radicals in BBG than in OBG during the first 6 h of oxidation and similar contents of hydroxyl
163 radicals in both BBG and OBG after 24 h of oxidation. The difference in the effectiveness of
164 hydrogen peroxide to oxidatively degrade BBG85 and OBG85 was already shown in our former
165 study (Mäkelä et al., 2016), where significantly higher M_w decrease was observed in BBG85 when
166 the oxidative degradation of BBG and OBG were compared. According to Wang, Maina, Ekholm,
167 Lampi, & Sontag-Strohm (2016), this difference was caused by a variation in the phytate content of
168 these commercial β-glucans.

169 In OBG57 no hysteresis was observed (Fig. 1d) and the viscosity of the non-oxidised sample did
170 not change significantly (P=0.54) with time (360 mPas on day 1 and 440 mPas on day 7 measured
171 at 14 s^{-1}) (Table 1). Although the viscosity of the non-oxidised OBG57 and OBG85 were similar
172 (P=0.87) on day 1 (360 mPas and 330 mPas, respectively), the behaviour of the oxidised samples

was somewhat different. The viscosity of OBG85 decreased continuously during the 7-day oxidation, which resulted in a significant difference (P=0.00) in the viscosities of day 1 and day 7 samples (71 mPas and 20 mPas, respectively). Instead, in OBG57 the viscosity first decreased but stayed constant (P=0.54) after the first oxidation day (100 mPas on day 1 and 130 mPas on day 7). Possibly, some structure formation may have occurred in the oxidised OBG57, which then compensated the effect of the molar mass decrease on the viscosity. Even though gel formation was not expected in OBG57 based on the shear stress measurement, this was still confirmed by the oscillatory measurement, because of the viscosity behaviour suggesting some structure formation. In the oscillatory measurement, G' of the non-oxidised OBG57 was 0.52 Pa and G'' was 2.8 Pa on day 7 (Table 2, Figure 2), which confirmed that no gelation occurred. Thus, the behaviour difference of OBG57 and OBG85 is suggested to be caused by the formation of some entanglements in the OBG57 samples. Usually the entanglements are formed when the critical overlap concentration C* is reached as reviewed by Saha & Bhattacharya (2010). However, in this case the concentration is similar in OBG57 and OBG85 and hence the reason for the higher viscosity in OBG57 is more likely the junction zones caused by the lower dissolution temperature.

In BBG57 and OBG37, the non-oxidised sample had higher viscosity (about 15-fold and 1.4-fold on day 7 at shear rate of 14 s^{-1}, respectively) than the oxidised sample (Table 1). Interestingly, the oxidised BBG37 had about 2.5-fold higher viscosity compared to the non-oxidised BBG37. However, both samples were highly heterogeneous and consisted of large particles that were floating in a watery continuous phase (Figure 3). This may have caused some error during the measurements, which was also supported by the high standard error for this sample. Consequently, despite the large hysteresis loop observed in the samples (Fig 1a), they were not used in oscillatory measurements and it was obvious that the sample did not form continuous gel network. In rheological measurements the particles can interfere if their size is not small enough compared to the height of the gap in the plate and plate geometry.

198

199 **3.2 Gelation behaviour of oat and barley β-glucans**

200 Based on the shear stress measurements, BBG57 and OBG37 were proposed to have some
201 entanglements or formation of a gel network, since they showed hysteresis (Figure 1b and c). The
202 oscillatory measurements were done at three different time points (day 1, day 4 and day 7) and the
203 mechanical spectra are shown for day 1 and day 7 samples (Fig 4 and 5 for BBG57 and OBG37,
204 respectively). The mechanical spectra show the storage modulus (G') and loss modulus (G'') as a
205 function of frequency. The storage modulus reflects the elastic properties of the material and for an
206 ideal elastic solid the measured shear stress would be in-phase with the applied strain (Mitchell,
207 1980). The loss modulus describes the viscous properties of the material and for an ideal liquid there
208 would be 90˚ phase difference in applied strain and measured shear stress. For viscoelastic materials
209 the phase difference is between 0˚ and 90˚.

210 Both BBG57 and OBG37 showed gel-like behaviour in the oscillatory measurements (Fig 4 and 5).
211 The gel strength of the non-oxidised sample (Fig 4a and 5a) was higher compared to the oxidised
212 one (Fig 4b and 5b) in both BBG57 and OBG37 but in BBG57 the difference was more
213 pronounced. Based on the storage moduli, the elasticity of the non-oxidised BBG57 and OBG37 did
214 not differ significantly (P=0.16), since G' was 38 and 32, respectively, on day 7 at 1 Hz. However,
215 the oxidised BBG57 formed a significantly weaker (P=0.00) gel than the oxidised OBG37 (4 Pa
216 compared to 21 Pa measured on day 7 at 1 Hz, respectively). This therefore showed that the high
217 mobility of the β-glucan molecules due to low molar mass after oxidation did not enhance gelation.

218 In this study, the molar masses of OBG and BBG were different (361 000 g/mol and 495 000 g/mol,
219 respectively), which has to be considered when comparing the results. The rigidity of the gel is
220 affected by the density of the junction zones during the formation of the gel network, and this is
221 influenced by both the concentration and the molar mass (Böhm & Kulicke, 1999). Preliminary

studies indicated that gel formation did not occur with 1% OBG, which was most likely due to its lower molar mass. Consequently, to compensate the lower molar mass, the concentration of OBG was increased to 1.5%, while for BBG the concentration was 1%. The gel strengths were similar when comparing the G' values in the optimal dissolution temperature of each β-glucan (37 ˚C for OBG and 57 ˚C for BBG). Böhm & Kulicke (1999) indicated that for increasing the gel strength, the concentration is more effective than the molar mass. Thus, despite being lower in molar mass, OBG had similar gel strength to BBG due to its higher concentration.

A correlation between the increase in DP3:DP4 ratio and the increase in gelling ability has been reported (Böhm & Kulicke, 1999; Cui, Wood, Blackwell, & Nikiforuk, 2000; Tosh et al., 2004a). Thus, in this study BBG was hypothesised to gel more than OBG, since the DP3:DP4 ratio in barley β-glucan has been shown to be higher than in oat β-glucan (2.7–3.6 and 1.7–2.4 in barley and oat β-glucan, as reviewed by Wood (2010)). However, these results showed that the gelation was similar in both BBG57 and OBG37 and no structure-related difference was observed with low concentrations when using the optimised dissolution temperatures for each β-glucan.

At high concentrations the molecules are more prone to interact because of the higher density and closer proximity (Böhm & Kulicke, 1999). Thus, it is reasonable that the gelation tendency at high concentrations follows the regularity of the structure, since the initiation of a gel network formation is not restricted by the lack of encounter. However, in this study the concentration was low and the DP3:DP4 ratio likely could not significantly affect the gelation. Therefore, the gelation of β-glucan at low concentrations is hypothesised to be driven by partial dissolution of the β-glucan molecules. Based on BBG85 and OBG85, it seems that when β-glucan is totally dissolved, gel formation does not occur at these low concentrations. However, with lower temperatures the samples are shown to gel, most likely because partially dissolved β-glucans act as nucleation sites for gelation. Junction zones – also described as well-ordered domains – are needed in order to form β-glucan gels (Böhm & Kulicke, 1999). Usually the formation of junction zones is considered to be favoured when there

247 is high amount of DP3 segments in the β-glucan structure. However, since in this study barley β-
248 glucan did not show more gelation than oat β-glucan, possibly the junctions of the gel network were
249 not formed only by the cellotriose units but also by the undissolved parts of β-glucan. According to
250 the behaviour of BBG37, which had large particles suspended in a watery medium, it can be
251 concluded that too low temperature leads to insufficient dissolution, and thus, the molecules are
252 closely packed in the solution and unable to form a large continuous gel network that can entrap
253 water.

254 Interestingly, the optimal dissolution temperatures differed significantly as OBG gelled at 37 ˚C
255 while BBG at 57 ˚C, and additionally the dissolution temperature range leading to gelation was
256 wider for OBG. The optimal dissolution temperature was verified by testing temperatures near 37
257 ˚C and 57 ˚C for OBG and BBG, respectively, to see which temperature gave the strongest gel.
258 These tests showed that BBG gelled only at 57 ˚C but with OBG some gelation was observed at all
259 tested dissolution temperatures ranging from 35 ˚C to 50 ˚C (data not shown). However, the
260 strongest OBG gels were obtained when the dissolution temperature was 37–40 ˚C. The reason for
261 the differences in the optimal dissolution temperatures of OBG and BBG is not known and we
262 hypothesise that the state of the molecule after dissolution has a significant role in the formation of
263 a gel network at low concentrations. Thus, the temperature difference can be considered to reflect
264 differences in the susceptibility of the β-glucans to dissolution. Based on the higher optimal
265 dissolution temperature of BBG compared to OBG, it seems that BBG requires more energy to
266 sufficiently open the structure. The temperatures (37 ˚C and 57 ˚C) are possibly optimal to ensure
267 partial dissolution, thus resulting in nucleation sites that enhance gelation. One possible factor that
268 determines the optimal dissolution temperature for gelation is the molar mass. When dissolving β-
269 glucan, higher temperature may be required in order to dissolve the molecules with high molar
270 mass, since there are more interactions between the molecules and more energy is needed to break
271 these interactions. Additionally, structural features such as DP3:D4 ratio also contribute to the

optimal dissolution temperature by affecting the aggregation of molecules, and hence, the solubility. Izydorczyk, Macri, & MacGregor (1998) extracted barley β-glucan at 40 ˚C and 65 ˚C and showed higher DP3:DP4 ratio in β-glucan extracted at higher temperature. This finding was considered to be linked to the lower solubility of the β-glucan with higher DP3:DP4 ratio, most likely due to intermolecular interactions resulting from structural regularity. It is therefore likely that due to a lower DP3:DP4 ratio the OBG powder used in our study had less aggregates than BBG powder, and thus, the partial dissolution of OBG structure needed for the gelation occurred at lower temperature.

The β-glucan extracts that can be used in food formulation vary in molar mass and purity. There is a wide variation in reported molar masses in different studies: e.g. 180 000–2 700 000 g/mol for oat β-glucan (Autio, Myllymäki, Suortti, Saastamoinen, & Poutanen, 1992; Beer, Wood, & Weisz, 1997; Cui et al. 2000; Johansson et al. 2000; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Sundberg et al. 1996) and 450 000–2 500 000 g/mol for barley β-glucan (Beer et al. 1997; Cui et al. 2000; Gómez, Navarro, Manzanares, Horta, & Carbonell, 1997). The current study on gelation of the non-oxidised and oxidised β-glucans gives an interesting field for further studies. As the health effects of β-glucan are generally linked to its viscosity in small intestine, the finding that OBG can actually gel even at low concentrations at physiological temperature (37 ˚C) indicates that a combination of β-glucan structure and dissolution temperature can be optimised to enhance physiological functionality. However, more studies are needed to understand the factors enhancing the gelation, and how these factors are linked to processing and physiological functionality.

4 CONCLUSIONS

The physicochemical properties of β-glucan are important for its health benefits. Though the benefits have mainly been related to enhancement of viscosity *in vivo*, often conflicting results have been obtained when investigations to correlate molar mass, concentration and extractability have

been carried out. This indicates that there may be other factors that enhance or hinder physiological functionality. The results from this study indicate that even at low concentration under the optimal conditions β-glucan can gel, implying that in addition to physicochemical properties, the physical state of β-glucan molecules and factors such as thermal treatment history, may contribute to the solution properties of β-glucan. How this is related to physiological functionality, requires further investigation.

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CAPTIONS

Table 1. Viscosities of 1% (w/w) barley β-glucan (BBG) and 1.5% (w/w) oat β-glucan (OBG) dissolved at different temperatures. Oxidised samples were treated with 70 mM H_2O_2 and 1 mM $FeSO_4 \times 7H_2O$. Measurements were conducted at 20 ˚C.

Table 2. Storage and loss moduli (G' and G'', respectively) of 1% (w/w) barley β-glucan dissolved at 57 °C (BBG57) and 1.5% (w/w) oat β-glucan dissolved at 37 °C (OBG37) and 57 °C (OBG57). Samples for these oscillatory measurements were chosen based on the viscosity measurements. Oxidised samples were treated with 70 mM H_2O_2 and 1 mM $FeSO_4 \times 7H_2O$. Measurements conducted at 20 ˚C.

Figure 1. Shear stress curves of 1% (w/w) barley (a, c, e) and 1.5% (w/w) oat (b, d, f) β-glucans dissolved at 37 °C (a, b), 57 °C (c, d) and 85 °C (e, f). Curves for non-oxidised samples shown with dark grey and for oxidised (70 mM H_2O_2, 1 mM $FeSO_4 \times 7H_2O$) samples with light grey. Measurements were conducted at 20 ˚C after 7 days of storage at room temperature.

Figure 2. Frequency sweeps (0.4 strain, 20 ˚C) of non-oxidised (a) and oxidised (b) OBG57 (1.5%, w/w) on day 1 and day 7.

Figure 3. a) The visual structure of the non-oxidised BBG37 (1%, w/w) on day 7 showing large particles in a watery medium. b) Fluorescent microscopy picture showing the structure of the non-oxidised BBG37 (1%, w/w) on day 7.

Figure 4. Frequency sweeps (0.4 strain, 20 ˚C) of non-oxidised (a) and oxidised (b) BBG57 (1%, w/w) on day 1 and day 7.

Figure 5. Frequency sweeps (0.4 strain, 20 ˚C) of non-oxidised (a) and oxidised (b) OBG37 (1.5%, w/w) on day 1 and day 7.

Table 1

Sample material	Dissolution temperature	Gelation time	Viscosity[a] (mPas) Non-oxidised	Oxidised
BBG	37 ˚C	Day 1	1300 ± 900	2100 ± 400
		Day 4	1600 ± 600	1500 ± 500
		Day 7	810 ± 170	2000 ± 700
	57 ˚C	Day 1	530 ± 10	76 ± 18
		Day 4	740 ± 20	120 ± 20
		Day 7	770 ± 30	50 ± 2
	85 ˚C	Day 1	290 ± 10	16 ± 2
		Day 4	300 ± 10	6.7 ± 0.2
		Day 7	290 ± 10	6.4 ± 0.6
OBG	37 ˚C	Day 1	660 ± 60	340 ± 90
		Day 4	950 ± 90	840 ± 270
		Day 7	1400 ± 200	1000 ± 300
	57 ˚C	Day 1	360 ± 20	100 ± 10
		Day 4	390 ± 50	110 ± 10
		Day 7	440 ± 50	130 ± 20
	85 ˚C	Day 1	330 ± 0	71 ± 8
		Day 4	330 ± 10	30 ± 3
		Day 7	340 ± 10	20 ± 1

[a]The average viscosities at 14 s^{-1}.

Table 2

Sample material	Dissolution temperature	Gelation time	Treatment	G' [a] (Pa)	G'' [b] (Pa)
BBG	57 ˚C	Day 1	Non-oxidised	12 ± 1	8.7 ± 0.6
			Oxidised	1.2 ± 0.4	0.31 ± 0.07
		Day 4	Non-oxidised	26 ± 1	7.4 ± 1.4
			Oxidised	2.7 ± 0.9	0.46 ± 0.11
		Day 7	Non-oxidised	38 ± 5	13 ± 4
			Oxidised	3.8 ± 0.8	0.79 ± 0.20
OBG	37 ˚C	Day 1	Non-oxidised	10 ± 2	6.3 ± 1.1
			Oxidised	12 ± 3	4.9 ± 1.2
		Day 4	Non-oxidised	27 ± 3	11 ± 2
			Oxidised	22 ± 2	13 ± 4
		Day 7	Non-oxidised	32 ± 6	12 ± 2
			Oxidised	21 ± 1	14 ± 5
	57 ˚C	Day 1	Non-oxidised	1.1 ± 0.5	3.9 ± 1.2
			Oxidised	0.23 ± 0.17	1.5 ± 0.7
		Day 4	Non-oxidised	0.84 ± 0.31	3.5 ± 0.9
			Oxidised	0.057 ± 0.014	0.48 ± 0.26
		Day 7	Non-oxidised	0.52 ± 0.14	2.8 ± 0.6
			Oxidised	0.15 ± 0.14	0.50 ± 0.18

[a]The average storage moduli at 1 Hz.

[b]The average loss moduli at 1 Hz.

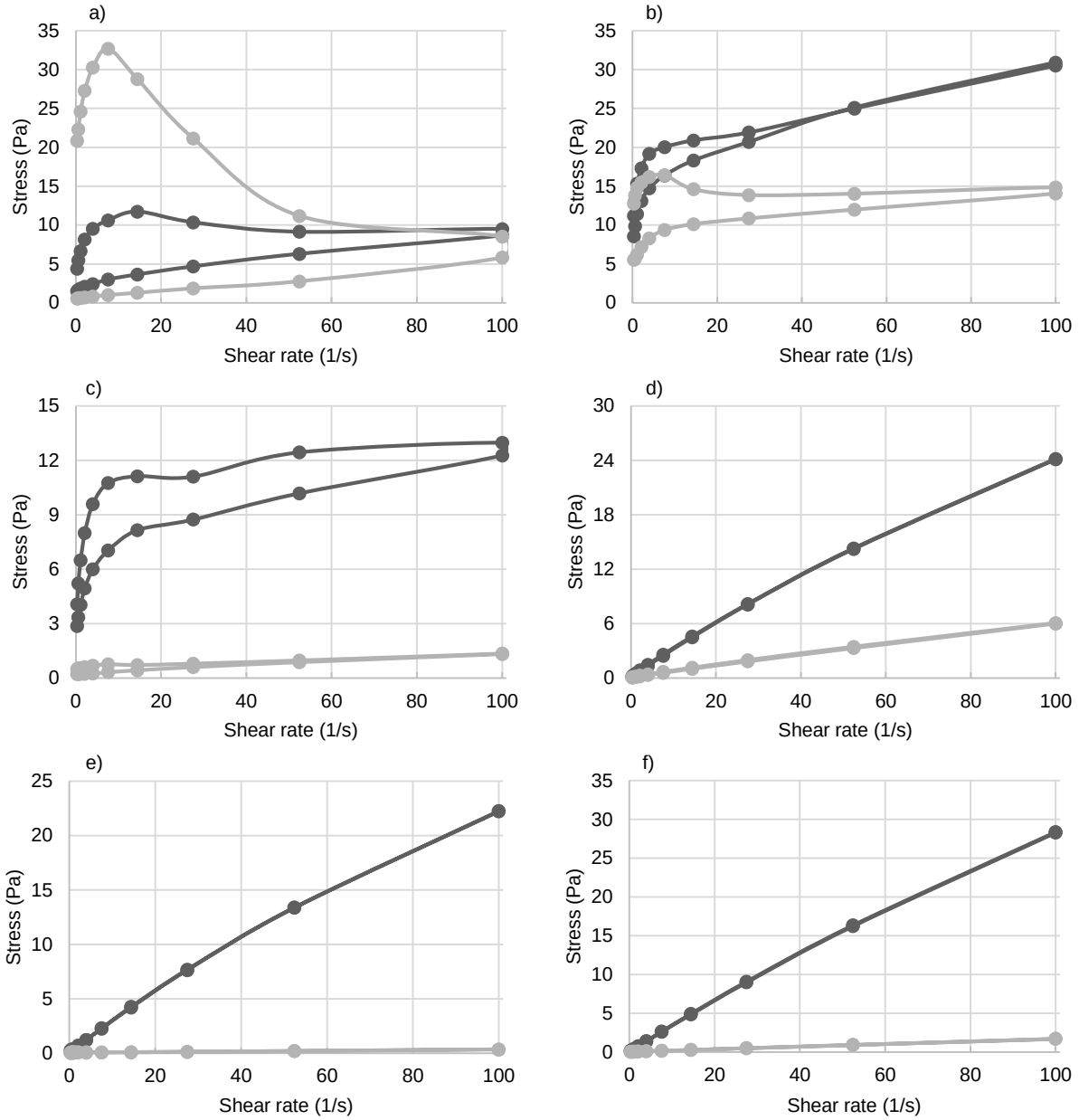

a)
Stress (Pa)
Shear rate (1/s)
b)
Stress (Pa)
Shear rate (1/s)
c)
Stress (Pa)
Shear rate (1/s)
d)
Stress (Pa)
Shear rate (1/s)
e)
Stress (Pa)
Shear rate (1/s)
f)
Stress (Pa)
Shear rate (1/s)

Figure 1

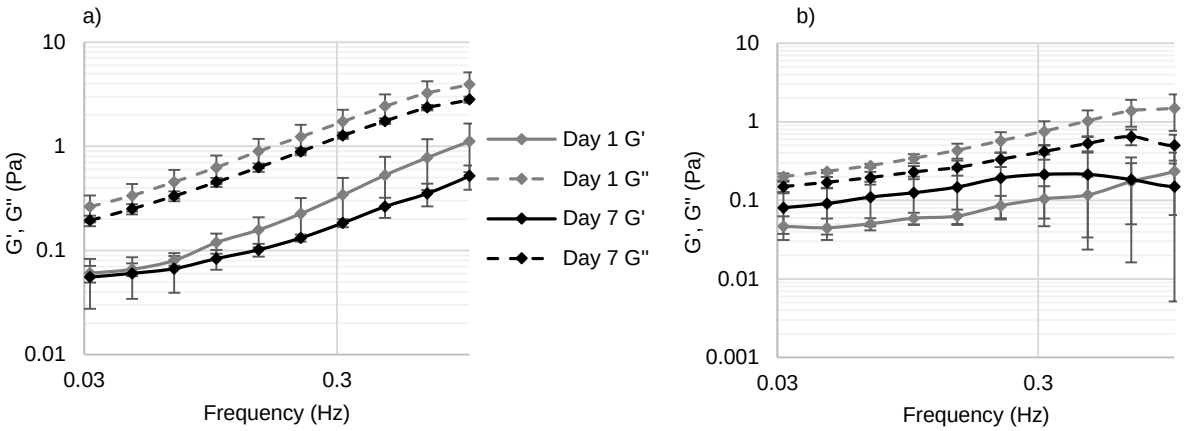
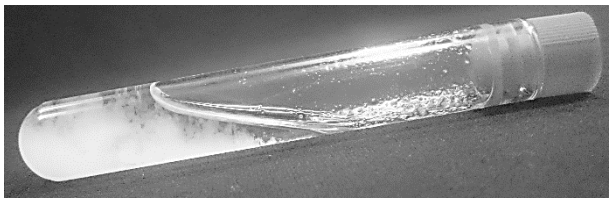

a)
10
1
0.1
0.01
G', G'' (Pa)
0.03
0.3
Frequency (Hz)
Day 1 G'
Day 1 G''
Day 7 G'
Day 7 G''
b)
10
1
0.1
0.01
0.001
G', G'' (Pa)
0.03
0.3
Frequency (Hz)

Figure 2

a)



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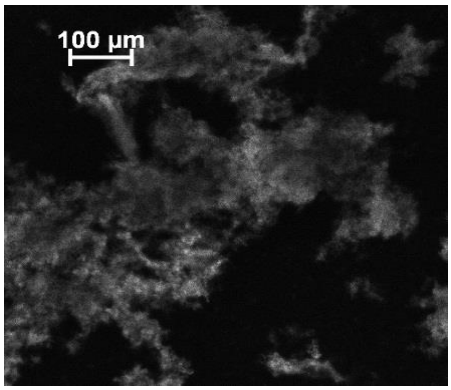

Figure 3.

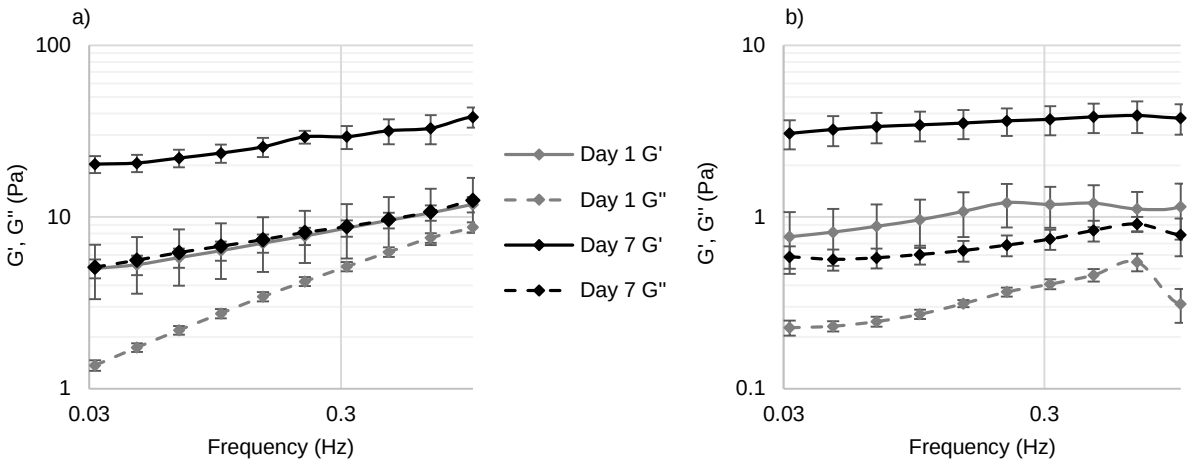
a)
100
10
1
G', G'' (Pa)
0.03
0.3
Frequency (Hz)
Day 1 G'
Day 1 G''
Day 7 G'
Day 7 G''
b)
10
1
0.1
G', G'' (Pa)
0.03
0.3
Frequency (Hz)

Figure 4

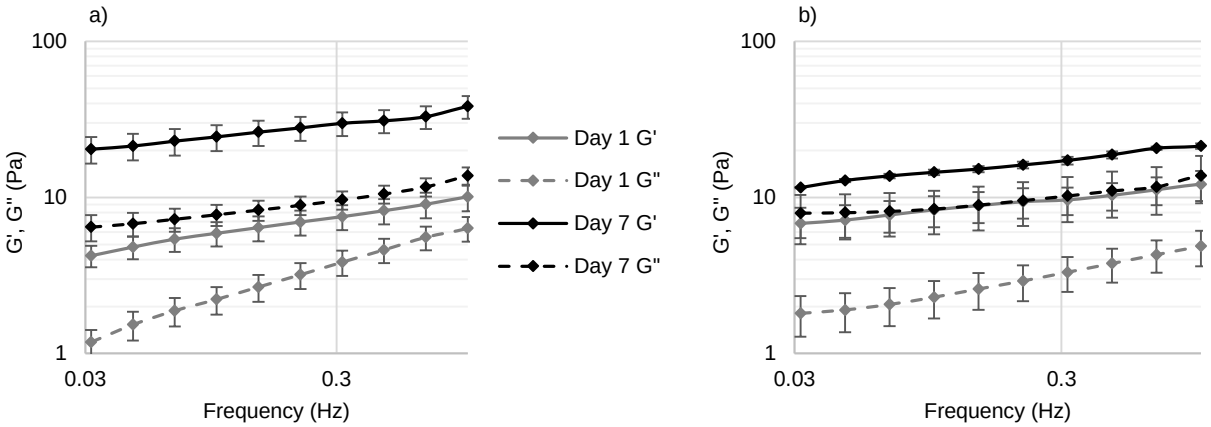
a)
100
10
1
G', G'' (Pa)
0.03
0.3
Frequency (Hz)
Day 1 G'
Day 1 G''
Day 7 G'
Day 7 G''
b)
100
10
1
G', G'' (Pa)
0.03
0.3
Frequency (Hz)

Figure 5