Freeze-thaw Properties of β-glucan Gels

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Abstract The network structure of β-glucan polymers and the presence of water have significant effects on the properties of β-glucan gels produced by freeze-thaw cycles. The properties of the gel are influenced by its fine structure, molecular size, mass fraction, number of freeze-thaw cycles, and temperature of the β-glucan. To characterize β-glucan freeze-thaw gels, we used low-field nuclear magnetic resonance (LF-NMR) to measure water proton transverse relaxation in aqueous β-glucan solutions during storage. The results indicated that microphase separation occurred during cryogelation, and three water components were identified in the cryostructure. The spin-spin relaxation time was analyzed on the basis of chemical exchange and diffusion exchange theory. The location of each water component was identified in the porous microstructure of the cryogel. The pore size measured from scanning electron microscopy (SEM) images agreed with the pore size estimated from relaxation time. The formation of cryogel was confirmed by rheological. The results suggested that LF-NMR can monitor the polysaccharide cryogelation process by the evolution of spin-spin relaxation characteristics.

Keywords: β-glucan, cryogelation, LF-NMR, water distribution, SEM, rheology

1. Introduction

Nowadays, thanks to its function to lower cholesterol [1], reduce the risk of type 2 diabetes [2,3], enhance gut health[4], and improve immunity [5], β-glucan has been getting increasing attention, which can be extracted from cereals such as oat, barley, rye, and wheat. β-glucan is a linear polysaccharide of d-glucopyranosyl residues linked by β-(1→3) and β-(1→4) glycosidic bonds [6], whose molecular weight is reported to be between 60 and 3000 kDa[7]. Together with lichenase (EC 3.2.1.73), the oat β-glucan will release oligosaccharide fragments, which are regarded as the building blocks of an intact β-glucan chain. There exist two major oligosaccharides segments in the hydrolysate, namely, 3-O-β-cellobiosyl-d-glucose (DP3) and 3-O-β-cellotriosyl-d-glucose (DP4) [8,9,10]. The polysac charide structure, solution viscosity, and gelation behavior mainly contribute to the healthcare effects of oat β-glucan. Therefore, it's quite imperative to thoroughly understand how the β-glucan polysaccharide works in different aqueous environments. With a sufficiently high concentration, β-glucan may change from a solution form to a gel form. There are many studies indicating how aggregate structures of oat β-glucan can be in the solution and in the form of a gel, with static and dynamic light scattering [15,16], rheometry [17,18], atomic force microscopy [19], and confocal microscopy [20]. In different aqueous solutions, the effects of molecular weight, structure and concentration of oat β-glucans on its

aggregation state are different. For example, the β-glucans with low molecular weight is more likely to form aggregates and gels [21,22,23,24] than the one with high molecular weight. Moreover, the gelation rate of β-glucan increases with the increase of the polysaccharide concentration in the solution. Time-domain 1H NMR has been widely applied to the study of polysaccharide solution and gel [25,26,27]. Meanwhile, more useful information on polymer dynamics and aggregation structure can be obtained through the transverse relaxation time, T_2 [28]. Adding polysaccharide to water will usually aggrandize aqueous proton transverse relaxation rate, $1/T_2$. The proton frequently exchange between water and polysaccharide hydroxyl groups, which has a significant effect on the transverse relaxation mechanism of polysaccharide aqueous solution and gel. It is possible to calculate [29,30,31] the water proton transverse relaxation time with the help of a two-site exchange model. Investigations were made to study the hydration of chitosan and the water tightly coordinated with the polysaccharide in chitosan hydrogel. Therefore, it is possible to elucidate the microstructure of native starch granule and the gelatinization of starch through the NMR relaxation and diffusion methods. In the work described in this article, different water components was identified by low-field nuclear magnetic resonance (LF-NMR) and the description of its residue's microscopic structure was made. In addition, the formation process of oat β-glucan gel was verified by rheological method, providing a theoretical basis for the practical application of β-glucan in food industry and the fine processing of oat and

Highland barley, eventually making a contribution to the development of grain science. Moreover, the oat β-glucan gel can also be used as a sustained-release drug carrier. In contrast to the conventional pharmaceutical preparations, these gel carriers have many advantages, such as long treatment period, relatively low drug delivery frequency, little gastrointestinal stimulation, non-toxic side effects, few fluctuations in drugs peak period, no oral administration no drug release in the front of the stomach and small intestine, repeated drug use on gastrointestinal mucosa stimulation and reduced systemic side effects [32].

2. Materials and Methods

2.1. Isolation and Purification of β-glucan

Oat β-glucan was extracted from the Weiduyou 1 oat cultivar and barley β-glucan was extracted from the Highland barley cultivar using the method described by Lazaridou et al [33] with minor modification. Briefly, the bran was treated with hot 80% ethanol in water, then washed with absolute ethanol. After air-drying, the bran was used for β-glucan extraction with 52°C water, involving a thermostable amylase and a pan creatin digestion. The β-glucan was precipitated with ethanol, and the precipitate was solubilized with water and lyophilized.

2.2. Partial hydrolysis with acid

Mild acid hydrolysis was used to obtain low molecular weight polysaccharide, as described by Wood et al [12]. Two grams of β-glucan was dissolved in 200 mL of deionized water. The solution was heated to 70°C with stirring. Concentrated HCl was added to 0.1 M final. Samples were hydrolyzed at 85°C for 30, 60, 90 min, quickly cooled to room temperature, and neutralized with NaOH solution. Two volumes of 100% ethanol were added to precipitate the partially hydrolyzed oat β-glucan. The precipitates were solubilized in water, then desalted by dialysis in tubing with a molecular weight cut off 10 kDa. The dialyzed solutions were lyophilized.

β-glucan samples with different molecular weights (Table 1) were used to make 4% (w/w) aqueous solutions. The solutions were prepared in the same manner as solubilization for HPSEC-MALLS analysis, except the solvent was deionized water. Two mL of each β-glucan solution was transferred into a NMR sample tube with an internal diameter of 15 mm. The tubes were sealed with PTFE plugs and stored in a refrigerator at -18°C for 21 h. Then the tubes containing β-glucan samples were moved into an incubator and allowed to thaw at 25°C for 3 h. The samples were tested after each of five freeze-thaw cycles, and then tested after every three additional cycles for 11 total freeze-thaw cycles. For rheology analysis, 5 mL of β-glucan solution was transferred into a cylindrical mold with an internal diameter of 36 mm. The solutions were treated with repeated freeze-thaw cycles and tested after 0, 1, 2, 3, 4, 5, 8 and 11 freeze-thaw cycles.

Table 1 Chocolate formulations studied in this work

Sample	Hydrolysis(min)	β-glucan content(%)	Mw (kDa)	$Ln\eta_r/c$
OG0	0	84.40	136	0.73
OG30	30	86.04	123	0.65
OG60	60	87.10	68	0.32
OG90	90	89.40	51	0.22
BG0	0	80.17	439	3.06
BG30	30	84.07	268	1.68
BG60	60	86.16	132	0.70
BG90	90	87.83	67	0.47

2.3. Preparation of β-glucan Frozen Gel

Various molecular weight (mass fraction is 4%) oat and barley β-glucan solutions were prepared in an 85°C water bath with stirring for 3 h. After the solutions had cooled to room temperature, they were placed in a refrigerator at 18°C, then frozen for 21 h, and thawed at room temperature for 3 h, process time for a freezing and thawing cycle, according to the request of test sample solution for the corresponding number of freezing and thawing. The prepared samples were labeled as OG0 and BG0.

2.4. Methylation Analysis

Sample processing steps: (1) β-glucan methylation with CH$_3$I; (2) hydrolysis with 2 mol/L trifluoroacetic acid at 121°C for 1.5 h; (3) post-hydrolysis with sodium borohydride β-glucan reduction; (4) acetylation at 100 °C for 2.5 h; (5) quantitative determination of partially methylated sugar alcohol acetate with a 7890B/5977A GC-MS equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The initial temperature of the column was 140 °C for 2 min, then the temperature was raised to 320°C at 6°C/min for another 3 min. Partially methylated sugar alcohol acetate was characterized by GC-MS and quantified using a gas chromatograph equipped with a hydrogen flame ionization detector [34].

2.5. LF-NMR Measurements

A MiniMR NMR spectrometer (Niumag, China) operating at 23 MHz for 1H resonance was used to perform the LF-NMR experiments. All the NMR tubes containing samples were placed in a water bath at 32°C and kept for at least 15 min. Then the samples were transferred into the NMR probe with a constant temperature of 32°C. The spin-spin relaxation time, T_2, was obtained using a Carr-Purcell-Meiboom-Gill (CPMG) sequence with a 90°-180° pulse and spacing of 0.1 ms. The 90° pulse was 18 μs, and the number of echoes was 18000. It produced a sampling space of about 4.23 s. Samples prepared under different conditions were assayed with at least three replicates. Each sample was repeatedly scanned eight times with a delay of 10 s. Signal collection and analysis were performed with Niumag NMR analysis software (Niumag, China). The relaxation signal decay curves were fitted with an exponential equation.

$$M(t) = \sum_i A_i \exp\left(-\frac{t}{T_{2i}}\right) + A_0$$

Where A_i is the echo amplitude of the ith component at time t and T_{2i} is the corresponding spin-spin relaxation time. A_0 is the noise of the curve.

2.6. Principal Component Analysis of LF-NMR

Principal component analysis module in SPSS18.0 was used to analyze the LF-NMR relaxation time distribution data of 4% oat and highland barley β-glucan gel systems with different mass fractions and with different molecular weights, after obtaining the score data of principal components, the principal components with eigenvalues greater than 1 were retained, and the score graph drawn by Origin9.0 and analyzed accordingly.

2.7. Dynamic Rheometry

The rheological properties of the β-glucan solution and cryogel were investigated with a Physica MCR-301 rheometer (Anton Paar, Austria) at 25°C using parallel plate geometry (PP25/P2, 25 mm diameter, 1 mm gap). The solution or cryogel was transferred from the cylindrical mold to the lower plate of the rheometer before the test. G′ (storage modulus), G″ (loss modulus), and tan δ (G″/G′) were obtained from oscillatory measurements with 0.1% strain and frequency from 0.1 to 10 Hz. Analysis was carried out with at least three replicates.

2.8. Scanning Electron Microscopy

The β-glucan solutions and cryogel were lyophilized and a thin layer of the sample was cut carefully with a sharp blade. The microstructure of a cross section of the sample was obtained with a JSM-7500 F scanning electron microscope (JEOL, Japan). A small piece of cross section was fixed onto an aluminum stub with double-sided conductive tape and sputter-coated with gold. Then it was observed with SEM at an acceleration voltage of 3 kV.

3. Results and Discussion

3.1. Analysis of Glycosidic Linkage

Figure 1 shows gas chromatography-mass spectrometry measurements of partially methylated glycolic acetate of β-glucan. Figure 1(a): the mole percent of OG0 (Glcp)1→ is 1.60%, the mole percent of OG0 →3(Glcp)1→ is 27.10%, and the mole percent of OG0 →4(Glcp)1→ is 71.30%. Figure 1 (b), the mole percent of →(Glcp)1→ to BG0 is 2.22%, the mole percent of (Glcp)3→ to 1 is 27.68%, and the mole percent of (Glcp)4→ to 1 is 70.10%. From these data, we calculated that the ratio of bonds (1→4) to (1→3) in oat β-glucan was 2.63, and the ratio of bonds (1→4) and (1→3) in highland barley β-glucan was 2.53.
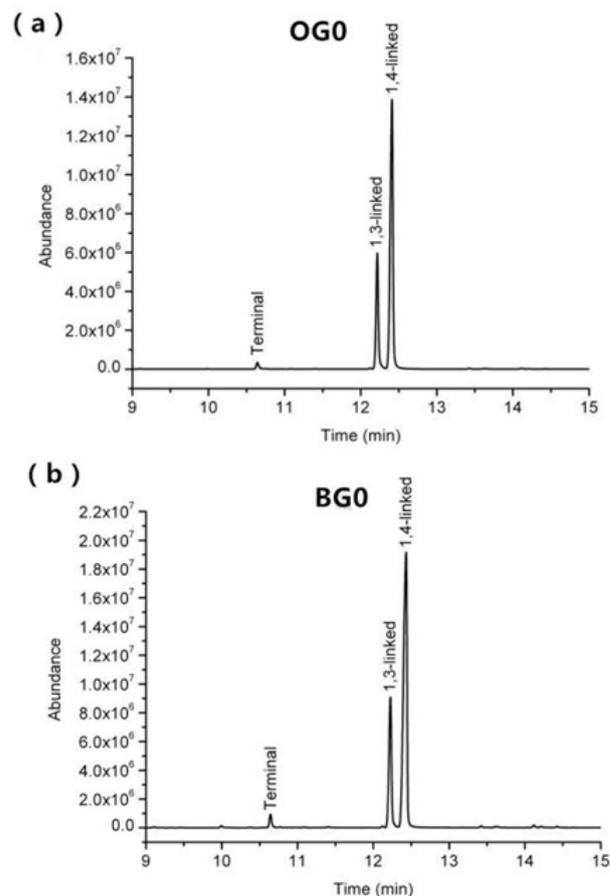

Figure 1. GC-MS chromatograms of partially methylated alditol acetates of OG0 (a) and BG0 (b)

3.2. LF-NMR Measurements

We measured spin-lattice relaxation time (longitudinal relaxation time) and spin-spin relaxation time (transverse relaxation time), both of which reflect the combination of water and matrix. The transverse relaxation time T_2 has a higher sensitivity in characterizing the fluidity of different moisture and determining the type of moisture with respect to the longitudinal relaxation time T_1. In general, the shorter the relaxation time T_2, the better the binding of water to the matrix, and the longer the relaxation time T_2, the weaker the combination of water and matrix. Proton density M_2 represents the relative moisture content of different transverse relaxation times.

Figure 2 (a) and (b) shows the transverse relaxation time distributions of different mass fractions OG0 and BG0 (see Table 1 in Methods) after freezing and thawing eight times. The gel system of β-glucan shows three sets of proton relaxation peaks, T_{21}, T_{22} and T_{23}. T_{21} corresponds to the water located in the physical cross-linking zone formed between the β-glucan molecules, and the transverse relaxation time is short; T_{22} corresponds to the moisture located in the gel skeleton, and the transverse relaxation time is relatively long; T_{23} corresponds to the water in the macropores formed by the melting of ice crystals with the longest transverse relaxation time. In the gel system, the diffusion between water molecules in different microenvironments is greatly affected by the gel network, and the diffusion exchange between the microenvironments is blocked, thus producing multiple

T_2 distributions, i.e., multiple peaks (Figure 2). The relaxation time T_{23} corresponding to the peak of the largest proportion in the gel is closely related to the mass fraction. As the mass fraction increases, the corresponding relaxation time decreases, indicating a change in the moisture state and the environment. This process can be qualitatively understood as the relationship between the concentration of β-glucan and the transverse relaxation time of water. When the β-glucan concentration is high, the more active hydrogen can be exchanged in the system,

and affect the transverse relaxation time of water. Moreover, because the relaxation time of the active hydrogen on the sugar chain is small, the transverse relaxation time of the water protons that undergo chemical exchange becomes smaller with the increase of the polysaccharide mass fraction. Therefore, with a short relaxation time, the gel network formed by the higher mass fraction of β-glucan in aqueous solution becomes denser, which hinders the diffusion of water molecules and reduces the lateral relaxation time [35].
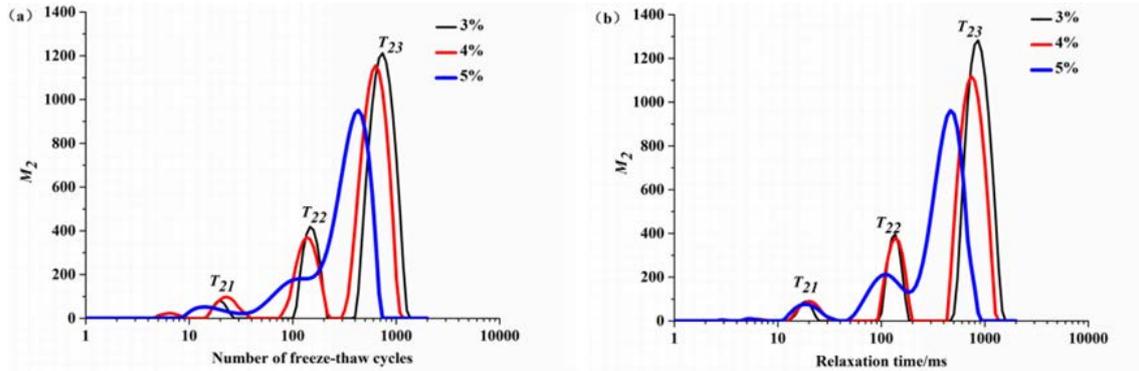

Figure 2. T_2 distribution of OG0 (a) and BG0 (b) with different concentrations after eight freeze-thaw cycles
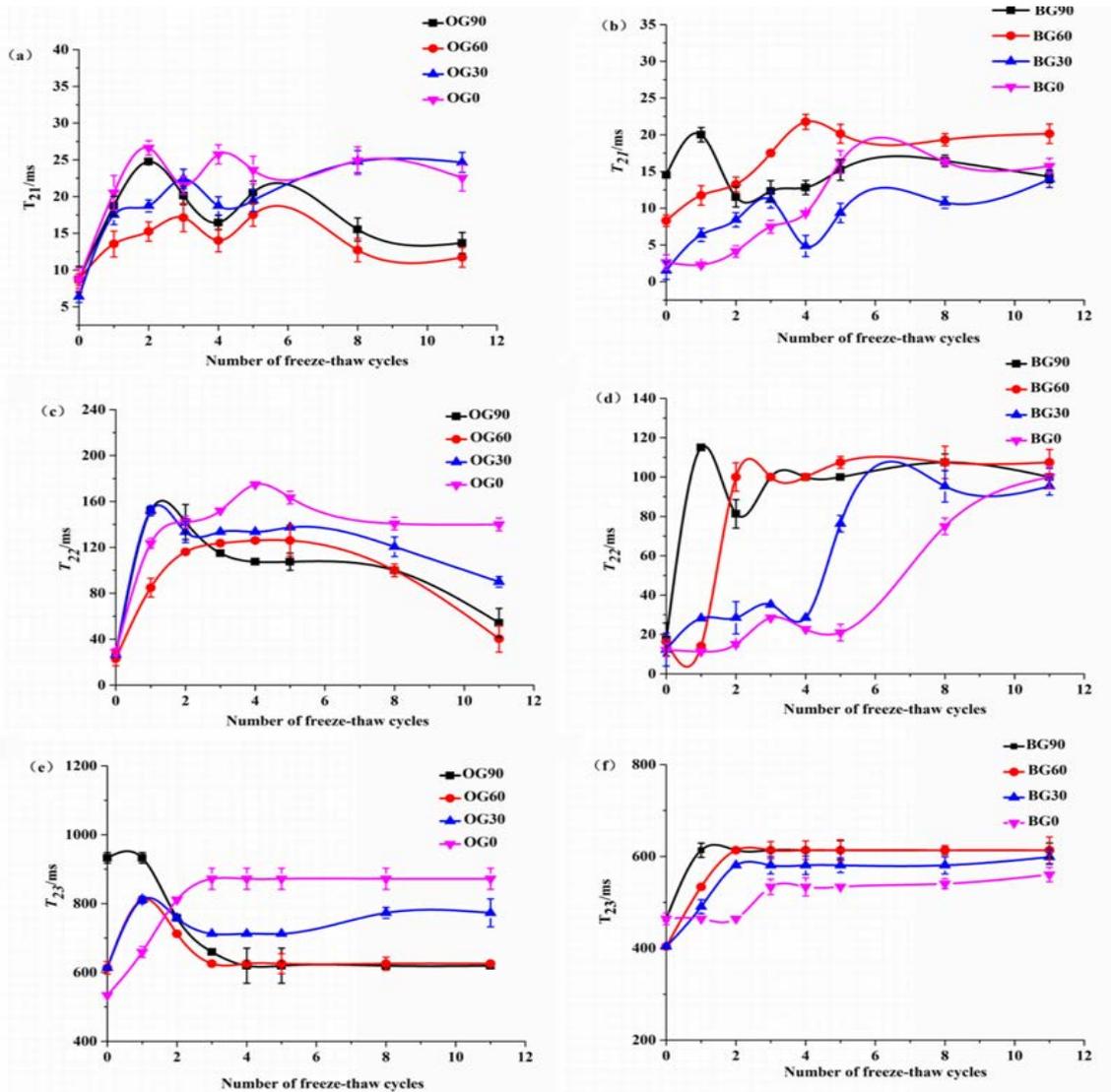

Figure 3. Effects of freeze-thaw cycles on T_2 of 4% OG (a, c, e) and BG (b, d, f) with different molecular weights

The 4% concentration of β-glucan in all the solutions was above the critical overlap concentration, belonging to the concentrated solution regime [36]. In concentrated solutions, β-glucan chains overlap and are entangled, thus, they are more likely to form aggregates. As shown in Figure 3, the T_{21} of different molecular weight OG and BG polymers gradually rose during the freeze-thaw cycles, a condition that indicated formation of gel structure. The fast relaxation component (component 1) had a spin-spin relaxation time, T_{21}, of about 5 ms for OG and 1-15 ms for BG. The inert protons on oat β-glucan were not expected to contribute to component 1 because the CPMG pulse sequence can hardly collect the rapid decay signal. Component 1 can be reasonably considered as stemming from water molecules trapped within the cross-links formed by consecutive DP3 units and stable physical entanglement points of β-glucan chains. Once the cross-links and stable physical entanglements were formed by freeze-thaw cycles, they became stable structures and could not be altered easily by further freeze-thaw treatment. Therefore, T_{21} remained essentially constant during the cryogelation process, even though the values have some fluctuation. The spin-spin relaxation of this group of water protons is mainly modified by chemical exchange with labile protons of polysaccharide in the cross-links and stable entanglement points. The relatively small value of T_{21} reflects the low mobility of oat β-glucan chains in the network skeleton. To some extent, these water molecules can be regarded as an integral part of the network skeleton structure.

Component 2 of different molecular weight β-glucan changed as follows: The spin-spin relaxation time, T_{22}, distributed from 35 to 160 ms for OG and 10 to 120 ms for BG, which means higher mobility of this group of water protons than for component 1. Thus, component 2 is considered as water confined in the interstitial space between β-glucan chains. β-glucan aqueous solutions experience a slow freezing process at -18°C. During freezing, ice crystals gradually grow larger and the concentration of β-glucan increases in the liquid phase, thereby facilitating interaction between β-glucan molecules. In the subsequent thawing process, the rise in temperature enhances the dynamics of β-glucan interactions; meanwhile, the temperature is still below the freezing point for a period of time, and the ice crystals remain in a solid state, keeping a high concentration of β-glucan in the liquid phase. The freeze-thaw cycles result in the formation of water pools surrounded by thin walls of concentrated cryogel, similar to a cell structure. Water molecules trapped in the interstitial space between aggregates of oat β-glucan chains are assigned to component 2. The spin-spin relaxation time, T_{22}, first increases and then shows a declining trend with increasing freeze-thaw cycles. The increase of T_{22} may be an indication of more open association of β-glucan aggregates, and the decrease of T_{22} is probably caused by a denser gel phase compressed by ice crystals. At the least, the change of T_{22} indicates the porosity and heterogeneity during the cryogelation. There was no clear relationship between the molecular weight of β-glucan and the corresponding T_{22}. But β-glucan of lower molecular weight was more likely to form a gel.

Figure 3(e) shows the variation of spin-spin relaxation time of component 3, T_{23}, which reflects the water in the pores formed by the melting of ice crystals in the gel. The initial values of T_{23} approached the values in fresh β-glucan solutions, thus, component 3 was considered as bulk water in the aqueous systems. The T_{23} of each molecular weight at different stages changed as follows: Freeze-thaw treatment caused a rapid increase of T_{23} at the first stage, which meant fewer labile protons on the polysaccharide were available to exchange with bulk water protons. Fewer labile protons cause an increase in T_{23} because T_{23} is mainly determined by chemical exchange. Higher mobility produces more smaller-sized β-glucan entering the gel microphase and causes a further rise in T_{23}. T_{23} begins to present a significant rise after about four freeze-thaw cycles, and the rise is more significant for lower molecular weight samples.

With more freeze-thaw treatments, the β-glucan concentration in bulk water gradually increases. Except for OG0, freeze-thaw treatment caused a slight increase in T_{23} of OG30, OG60, OG90 at the first stage. The T_{23} of OG0 kept rising when it was frozen and thawed two to four times. In the process of freezing and thawing 2-4 times, T_{23} basically reached equilibrium; after four freeze-thaw cycles, the T_{23} of various glucans was in balance. The smaller the molecular weight, the smaller the T_{23} of OG, and the smaller the molecular weight, the larger the T_{23} of BG. This difference is caused mainly by the differences in molecular weight and molecular structures of the two polysaccharides. The initial values of T_{23} approached the values for fresh oat β-glucan solutions, so component 3 was considered as bulk water in the aqueous systems. It is surprising that T_{23} is lower in the aqueous system containing small-sized β-glucan at the beginning of freeze-thaw treatment. Although the lower molecular weight β-glucan aqueous system appeared less viscous, the mobility of water therein seemed to be lower than the water mobility in the more viscous solution having larger-sized oat β-glucan. A likely reason for this abnormal result lies in the self-aggregation of oat β-glucan; β-glucan of smaller size is more prone to aggregate than larger polysaccharide molecules. The aggregation may result in a suspension or a network, which causes a decrease in the mobility of oat β-glucan molecules and the slight lowering of the T_{23} of water interacting with these polysaccharide aggregates after the first several freeze-thaw cycles. The leveling of T_{23} (Figure 3e) and T_{21} (Figure 3a) indicates that the cryostructure has changed little after 10-13 freeze-thaw cycles.

As shown in Figure 4a and Figure 4b, the spin density, A_{21}, of component 1 gradually increased with increasing number of freeze-thaw cycles, a result that reflected an increase in cross-links and stable physical entanglements. Small-sized BG molecules form a network skeleton earlier compared with their larger counterparts. Smaller BG seemed to form more network skeleton during cryogelation. Clearly, after 11 freeze-thaw cycles, BG0 produced a network skeleton significantly smaller than the other small-sized samples. It is generally considered that barley β-glucan with a small size has higher mobility of chains, facilitating the formation of cryogel structure. The formation and increase of cryostructure deduced from the presence of component 1 and increase in A_{21} agree with the results obtained with traditional methods.
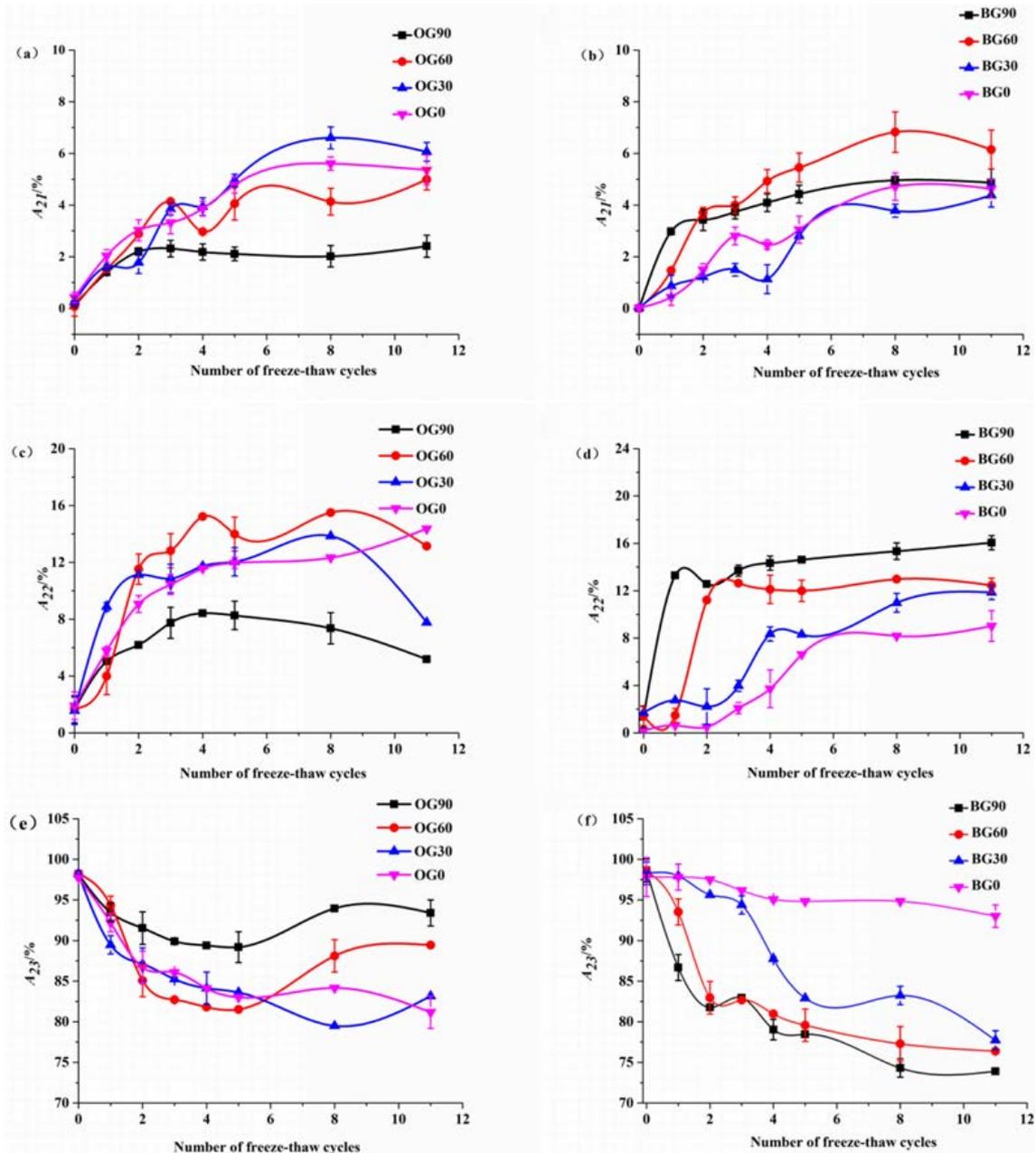
Figure 4. Effects of freeze-thaw cycles on peak area proportion of 4% OG (a, c, e) and BG (b, d, f) with different molecular weights

As shown in Figure 4c and Figure 4d, spin density, A_{22}, increased with the number of freeze-thaw cycles, reflecting the increase in the cryogel microphase. A_{22} showed an inverse relationship with the molecular weight of oat β-glucan at all cycles; demonstrates that oat β-glucan of small size produces more cryogel microphase compared with larger polysaccharides. This result has been verified by others. A_{22} increased sharply and then leveled, indicating that the quantity of cryogel microphase approaches a maximum and remains constant after eight freeze-thaw cycles. A_{21} also showed the same trend, and eight could be considered as a critical number of freeze-thaw cycles.

After 11 freeze-thaw cycles, the spin density, A_{23}, of the bulk water decreased from nearly 98% to about 80% for OG and from 100% to about 75% for BG in β-glucan samples with decreasing molecular weight. The reduction of bulk water ratio was due to the increase of cryogel microphase and increasing amount of water entrapped in

the gel microphase. That is, the bulk water gradually converted into entrapped water during cryogelation.

3.3. Principal Component Analysis

Figure 5 shows the scores of OG(a) and BG(b) with different molecular weights after eight freeze-thaw cycles. Each circle represents the overall characteristics of a particular molecular weight gel. The differences in gel samples of different molecular weights can be scored by principal component analysis. The spacing distances in Figure 5 reflect the fact that the greater the difference in molecular weight β-glucan gel, the greater the difference in gel properties. Principal component analysis can better distinguish gel samples of different molecular weights. For OG and BG, the β-glucan gel samples that were hydrolyzed for 0 min and 30 min were concentrated on the left side of the PC1 axis; the β-glucan gel hydrolyzed for 60 min and 90 min was concentrated on the right side of

the PC1 axis, indicating that there was a critical molecular weight between the hydrolyzed β-glucan samples for 30 min and 60 min. Although there was no significant difference on the PC2 axis, there was a significant difference in the PC1 axis, indicating that molecular weight has a significant effect on the formation of β-glucan gel [37].
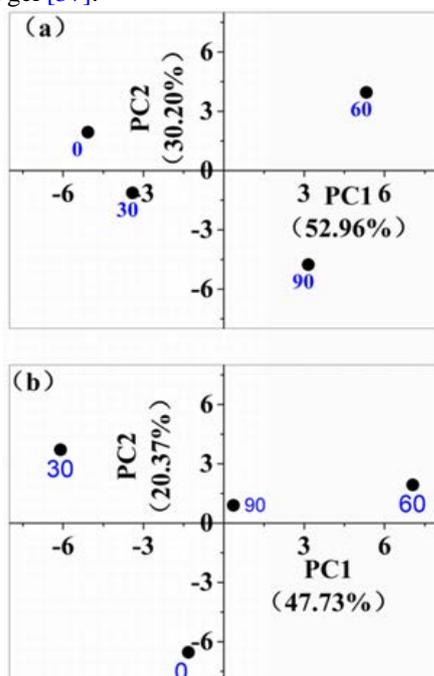

Figure 5. PCA scores plots (PC1, PC2) for 4% OG (a) and BG (b) with different molecular weight β-glucan after eight freeze-thaw cycles

3.4. Analysis of Rheological Properties

The effect of an applied stress on a fluid can be expressed in two ways, namely elasticity and viscosity, which denote G' and G", respectively. G' is the storage modulus or elastic modulus, which reflects the ability of a polymer to deform with the change in external force. G" is the loss modulus or the viscous modulus (Pa) which reflects the energy loss caused by the internal or inter-molecular stretching of a polymer when the external force changes. Tan δ = G"/G' (δ is the loss angle) is an index of the viscoelastic properties of the system. Generally, tan δ = 1 is the limit. The larger the tan δ, the more dominant the viscous component, showing more liquid solid properties [38].

Figure 6 shows the viscoelastic properties of gels formed by different molecular weight OG(a) and BG(b) at 4% concentration and after eight freeze-thaw 8 cycles. The values of G' and G" have correlate with the molecular weights of the β-glucan gels; the smaller the molecular weight, the larger the values of G' and G". Although G' of the BG0 gel increased with the increase in oscillation frequency, the G' and G" of the other molecular weight β-glucan gels did not change with increased oscillation frequency. This difference may be because the molecular weight of BG0 is larger. The molecular weight of the hydrolyzed BG was small, and its gel form has better gel properties compared with unhydrolyzed BG0. Thus, the viscosity behavior of the fluid decreases and the

characteristics of elastic behavior enhances with a decrease in the molecular weight of β-glucan and further confirmed that β-glucan having a smaller molecular weight is more likely to form a gel.
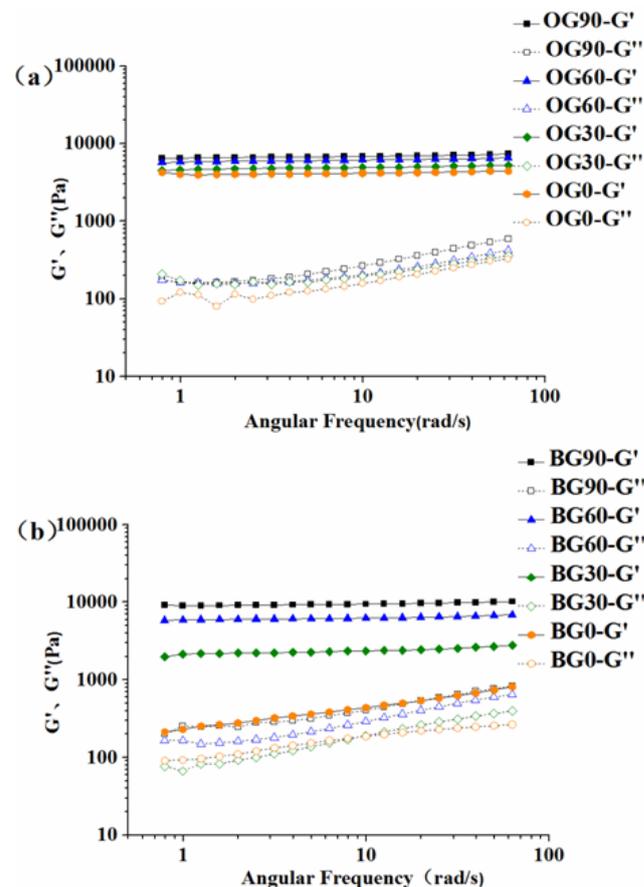

Figure 6. Rheology of 4% OG (a) and BG (b) gels with different molecular weight β-glucans after eight freeze-thaw cycles.

3.5. Scanning Electron Microscopy

In β-glucan aqueous solutions, water forms a large number of hydrogen bonds with the hydroxyl groups of the polysaccharide, and the hydrated β-glucan macromolecule moves in an aqueous solution and forms a circular, helical, or double helix structure by folding and crimping. Then, by stretching, a certain part of the molecules is arranged in a straight line to form a sugar chain and a large number of β-glucan molecules are bonded at different points to form a three-dimensional network filled with water molecules to form a gel. Figure 7 shows SEMs of different molecular weight oat β-glucan and barley β-glucan gels. Clearly, β-glucan gel is a type of three-dimensional network, and the porous structure formed has a certain regularity related to molecular weight. Samples with smaller molecular weights have higher mobility and diffusivity, and it is easier to form a three-dimensional network. In addition, the small molecule β-glucan chains contain a small proportion of inactive fragments, and the degree of intramolecular action is low, hence the probability of effective collision between chains increases, and the chances of collision and entanglement between molecules increase.
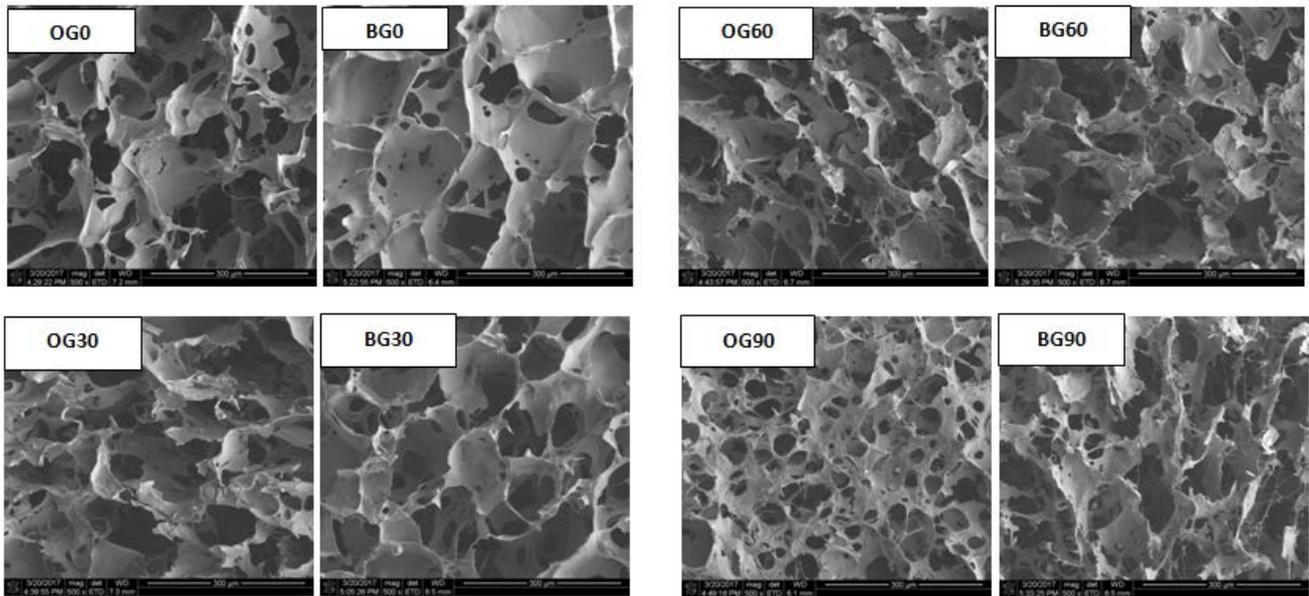
Figure 7. SEM images of 4% OG and BG with different molecular weights after eight freeze-thaw cycles

4. Conclusions

In this study, the imperative effects of the network structure of β-glucan polymers and the presence of water has been found on the properties of β-glucan gels after specific freeze-thaw cycles. What has also been found is that three groups of relaxation components with transverse relaxation times T_{21}, T_{22} and T_{23}; the corresponding signal peak proportions were A_{21}, A_{22} and A_{23}. With the increase of freezing and thawing cycles, T_{21}, T_{22} and T_{23} of different β-glucan mass fractions and molecular weights generally increased at first and then did not change noticeably. However, T_{22} and A_{22} of Highland barley β-glucan increased after either three or five cycles of freezing and thawing. A_{21} and A_{22} of different β-glucan mass fractions first increased and then did not change noticeably, and A_{23} first decreased and then did not change noticeably. The variation of gels with different molecular weight β-glucan and different numbers of freeze-thaw cycles was distinguished by principal component analysis of the relaxation distribution curve. Rheological studies showed that the larger the mass fraction and the less the molecular weight, and that the larger the elastic modulus were and the more viscosity modulus it shows, indicating that more cross-linked structures were formed in the gel. The result of scanning electron microscopy indicated that the gel structure was being denser with the molecular weight becoming smaller, and the gel network was being looser with the molecular weight becoming larger.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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