Freeze-thaw Properties of β-glucan Gels with Different Concentrations

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Abstract: The β-glucan gel was prepared in freeze-thawing method. The formation of freeze-thawed gel was studied by low-field nuclear magnetic resonance (NMR). Three relaxing components with different transverse relaxation time (T21, T22 and T23) were founded in the β-glucan system. With the increase of the number of freezing and thawing, the T22 of barley β-glucan increased rapidly after 5th freezing and thawing and A22 increased significantly after 3th freezing and thawing. The relaxation time T21, T22 and T23 rose first and then did not change. A21 and A22 first increased and then changed little while A23 first declined and then changed little. By performing the principal component analysis on the relaxation distribution curve, β-glucan gel of different concentrations. The rheological study showed that β-glucan gel with higher mass fraction has a larger elastic viscous modulus. This indicated that it formed more more physical ross-linked structure in gel. The results of scanning electron microscopy showed that β-glucan solut ion with higher mass fraction formed structure compact gel while β-glucan solution with lower mass fraction formed structure loose gel.

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

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1. Introduction

β-glucan is a linear polysaccharide located in the endosperm and aleurone layer of cereal grains [1,2]. Oat β-glucan is a linear polysaccharide formed by β-(1→3) and β-(1→4) glycosidic linkages connected to glucopyranose units [3,4], with more than 85% of β-glucan chains. Among the 2 to 3 β-(1→4) glycosidic bonds, there is a β-(1→3) glycosidic bond, and about 15% of the segments are composed of β-(1→3) glycosidic bonds are alternately connected. The structure of barley beta-glucan is similar to that of oat beta-glucan [5,6].

A large number of studies have confirmed that β-glucan has important physiological functions, promoting the proliferation of probiotics, preventing colon cancer [7], reducing blood lipids and cholesterol [8], regulating blood sugar [9,10], improving immunity [11], anti-aging and moisturizing the skin [12,13].

After research, β-glucan solutions (3 to 5%, w/v) with different mass fractions and different molecular weights can form frozen gels after repeated freeze-thaw cycles, mainly due to the effective inter-molecular chain in the freeze-thaw process. Collisions resulted in the rearrangement of β-glucan molecules to form a cross-linked structure and then a gel [14]. In a high-quality fraction of the β-glucan solution system, the probability of β-glucan molecules colliding with each other will increase, there are many cross-linked regions, and β-glucan molecules are easy to aggregate, so it will form a gel faster. Lakli [15] and others found that the formation of β-glucan gel is related to substances such as polyhydroxy compounds. When a certain mass fraction of glucose, fructose, maltose, xylose, etc. are added to the β-glucan solution, they can extend the gel formation time and have a certain effect on the strength of the gel.

Both the polymer network structure and the presence of water in the gel have a great influence on the performance of the hydrogel. The properties of the gel are affected by the fine structure, molecular size, and presence of β-glucan [16]. Gel properties can be studied by low-field nuclear magnetic resonance (LF-NMR) technology, rheology, and scanning electron microscopy (SEM). Low-field NMR can obtain measurement results efficiently and accurately without damaging the sample. Rheometers can determine the viscoelastic properties of gels. Scanning electron microscope can observe the micro-morphology of biological macromolecules including polysaccharides.

Compared with other detection methods, NMR detection technology has the advantages of less sampling and maintaining the integrity of the sample. It can non-destructively study the mobility and distribution of water in the sample. It is a non-destructive detection method and can be used to detect gels. Mobility and
distribution of water in food. Studied the effect of salt solution rinsing on gel quality of minced fish with low-field NMR. Vaikousi [17] and others took barley β-glucan as the research object, and studied the performance of rheological characteristics at different temperatures and shear rates. It was found that when the barley β-glucan solution had a mass fraction of 1%, it exhibited a shear-thinning pseudoplasticity, so the high-viscosity barley β-glucan gel had good fat substitution. Burkus [18], Lazarido [19], and Temelli [20] also discussed the mass fraction and conditions of β-glucan to form a gel. The gel strength was found to be related to the β-glucan mass fraction and molecular weight. When the mass fraction is higher and the molecular weight is lower, the strength of β-glucan to form a gel is stronger. At the same time, the higher the ratio of celiotriose to cellobiose, the shorter the gelation time of the β-glucan gel had good fat substitution.

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 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 method described by Lazaridou et al with minor modification. Briefly, the bran was used for β-glucan extraction with 52°C water, involving a thermostable amylase and a pan creatin digestion. After obtaining the principal component score data, the principal components with eigenvalues greater than 1 are retained, and the score map is drawn using Origin9.0 and analyzed accordingly [21].

2.2. Preparation of β-glucan Frozen Gel

Prepare oat β-glucan solutions and barley β-glucan solutions of various molecular weights (mass fractions: 3%, 4%, 5%), magnetically stir for 3 h in a 85 °C water bath. 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.

2.3. Nuclear Magnetic Resonance Measurements

A 0.010 g sample of dry β-glucan was weighed and dissolved in 1.0 mL of D₂O. The 1H NMR and 13C NMR spectra were measured on a Bruker AVANCE III-500 nuclear magnetic resonance instrument, and the temperature was 27 °C.

2.4. 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, T2, 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.

2.5. Principal Component Analysis of LF-NMR

Use the principal component analysis module in SPSS18.0 to analyze the LF-NMR relaxation time distribution data of oat and barley β-glucan gel systems with different mass fractions of 8 freeze-thaw cycles. After obtaining the principal component score data, the principal components with eigenvalues greater than 1 are retained, and the score map is drawn using Origin9.0 and analyzed accordingly.

2.6. 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.

2.7. Scanning Electron Microscopy

Freeze-thaw cycles were repeated 8 times to obtain oat β-glucan and barley β-glucan frozen gel. After the frozen gel is treated with liquid nitrogen and vacuum freeze-dried, a section is cut along the longitudinal surface with a blade, and a small sample is adhered to the conductive adhesive.
on the sample stage. In the process, the surface morphology of the sample is not damaged as much as possible. (Gold spraying conditions are vacuum, 30 mA, 90 s) Observe the surface morphology of the sample in high vacuum mode, and the acceleration voltage of the electron gun is 3 KV.

3. Results and Discussion

3.1. Nuclear Magnetic Resonance Analysis

It can be seen from Figure 1 that the carbon spectrum can confirm the existence of β-D-(1→3) and (1→4) bonds in the β-glucan molecule. From Figure 1 (a), it can be seen that the residue of 4G3 C1 has a resonance peak at 102.8 ppm due to the effect of β-(1→3) bond, and C1 has a resonance peak at 102.6 ppm due to the effect of β-(1→4) bond, and C3 at 3G4 residue A single peak at 84.1 ppm indicates the existence of a single β-(1→3) glycosidic bond in the sugar chain. The resonance peak of C4 in the 4-O-substituted residue and the 3-O-substituted residue moves to the low field direction. The C6 of 3-O-substituted residues and 4-O-substituted residues have resonances at 60.8 ppm and 60.2 ppm, respectively. These results are consistent with those in previous studies [22,23].

![Figure 1](Image 58x133 to 294x467)

**Figure 1.** $^{13}$C NMR spectra of OG0 (a) and BG0 (b) samples (in D$_2$O, 500 MHz, 27°C)

3.2. LF-NMR Analysis

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 is the transverse relaxation time of OG0 and BG0 with different mass fractions after different freezing and thawing times. From Figure 2 (a and b), it can be seen that $T_{21}$ of OG0 and BG0 showed an upward trend several times before freezing and thawing, indicating that the physical cross-linking between polysaccharide molecules increased, but the newly formed physical cross-linking structure was relatively loose as the number of freeze-thaw cycles continues to increase, $T_{21}$ changes little. In general, the smaller the mass fraction of β-glucan is, the larger the $T_{21}$ is, to a certain extent, it is proved that the larger the mass fraction, the more physical crosslinking.

From Figure 2 (c and d), it can be seen that during the first 4 freeze-thaw cycles, as the number of freeze-thaw cycles increased, $T_{22}$ increased accordingly, and the smaller the mass fraction, the greater the increase. After 4 freeze-thaw cycles, $T_{22}$ as the number of freeze-thaw cycles continues to increase, the change is not large. The larger the mass fraction, the smaller the lateral relaxation time $T_{22}$. During the first 5 freeze-thaw cycles of BG0, $T_{22}$ was almost unchanged. After 5 freeze-thaw cycles, $T_{22}$ increased sharply, probably because the molecular weight of barley β-glucan was larger, which resulted in a faster freeze-thaw gel formation rate slow.

It can be seen from Figure 2 (e and f) that the lateral relaxation time $T_{23}$ decreases as the mass fraction increases. This is because the greater the mass fraction of β-glucan, the more active hydrogen that can be chemically exchanged with water, resulting in a decrease in the lateral relaxation time of the water protons exchanged with it. At the same time, the degree of interaction between polysaccharide molecules in the β-glucan solution with a higher mass fraction is higher, which reduces the mobility of the polysaccharide molecules, and the active hydrogen relaxation time on the polysaccharide molecules is shorter, resulting in the chemically exchanged water proton $T_{23}$ is smaller. From Figure 2 (e), it can be seen that the lateral relaxation time $T_{23}$ of water in the OG0 system with various mass fractions shows a certain upward trend during the first 4 freeze-thaw cycles, and $T_{23}$ remains almost unchanged when the number of freeze-thaw cycles is increased. This is because during the first 4 freeze-thaw processes, β-glucan interactions reduced the amount of active hydrogen for chemical exchange with water molecules, and the $T_{23}$ of water in the system had a certain upward trend. As the number of freeze-thaw cycles increased, a part of the β-glucan solution began to form a gel, and the mobility of the β-glucan molecules weakened, resulting in a decrease in the $T_{23}$ of water. The combined effect of the above two effects made the measured freeze the change in $T_{23}$ after 4 fusions was not significant. In the BG0 system, the mass fractions of $T_{23}$ of 3%, 4%, and 5% of BG0 increased during the first three
freeze-thaw freeze-thaw cycles, and the $T_{22}$ of 3 to 11 freeze-thaw cycles remained almost unchanged. To a degree, the larger the mass fraction, the easier it is to form a gel.

Figure 3 is the ratio of the relaxation signal peaks of OG0 and BG0 with different mass fractions after different freezing and thawing times. It can be seen from Figure 3, (a and b) that during the freeze-thaw process, except for the $T_{23}$ component of 3% BG0, the other mass fractions of OG0 and BG0 $T_{21}$ components increase with the number of freeze-thaw cycles. And increase, on the whole, the mass fraction has no regular effect on the $T_{21}$ component of OG0 and BG0, to a certain extent, the larger the mass fraction, the easier it is to form a gel.

In Figure 3(c), after a freeze-thaw cycle, OG0 with different mass fractions appears as a $T_{22}$ component due to the increase in gel structure. In Figure 4(d), BG0 undergoes 3 passes. After freezing and thawing, the obvious $T_{22}$ component appeared. From Figure 3 (c and d), it can be seen that the larger the mass fraction of the dextran system, the more $T_{23}$ increases. The main reason for the smaller proportion of $T_{22}$ peaks of BG0 than OG0 is that the molecular weight of BG0 is large, which limits the movement and diffusion of β-glucan molecules, and the speed of gel formation is slower.

The peak proportion $A_{23}$ of $T_{23}$ in OG0 frozen gels with different mass fractions decreases with the increase of the number of freeze-thaw cycles, indicating that during the freeze-thaw process, a corresponding proportion of gels are formed, and $T_{23}$ in BG0 frozen gels with different mass fractions The reduction ratio of the peak ratio $A_{23}$ is smaller than the peak ratio $A_{23}$ of OG0, and the change of $A_{23}$ of BG0 is not obvious when frozen and thawed 0 to 3 times, and most of them are close to 100%, which indicates that the larger molecular weight β-glucan is formed The speed of the gel is slower.

**Figure 2.** Effects of freeze-thaw cycles on $T_2$ of OG0 (a, c, e) and BG0 (b, d, f) with different concentrations
3.3. Principal Component Analysis

As shown in Figure 4, samples can be compared and classified according to their position on the scoring map. Those in the map that are distributed in the same area can be classified into one category. From the figure, we find that the positions of gel samples with different mass fractions on the score chart are clearly separated, indicating that the relaxation characteristics of gel samples with different mass fractions are significantly different. Figure 4 (a) shows the main component scores of OG0 with different quality scores. The score contributions of PC1 and PC2 are 50.24% and 35.73%, respectively, and the cumulative contribution rate is 85.97%. And 5% OG0 is at the right end of the first principal component. OG0 with different mass fractions can be well distinguished along the PC1 axis. Figure 4 (b) shows the main component scores of BG0 with different quality scores. The score contribution rates of PC1 and PC2 are 75.61% and 24.39%, respectively, and the cumulative contribution rate is 100%. 3% of BG0 is at the lower end of the second principal component, and 5% of BG0 is at the upper end of the second principal component. On the PC2 axis, BG0 with different mass fractions can be well distinguished. It is shown that about 4% has a key point for the mass fraction of β-glucan gel formation. When it is less than 4%, gel formation is slower; when it is more than 4%, the speed of gel formation is faster due to the large degree of cross-linking of β-glucan molecules [24]. Therefore, the combined use of low-field nuclear magnetic resonance technology and PCA can distinguish different β-glucan gels with different mass fractions.
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 $\delta = G''/G'$ ($\delta$ is the loss angle) is an index of the viscoelastic properties of the system. Generally, tan $\delta = 1$ is the limit. The larger the tan $\delta$, the more dominant the viscous component, showing more liquid solid properties [25].

The behavior of the fluid against the applied stress can be expressed in two aspects, namely elasticity and viscosity. In this paper, $G'$ and $G''$ are used to represent elasticity and viscosity, respectively. $G'$ is the storage modulus or elastic modulus, which reflects the ability of the polymer to deform with the change of external force, and $G''$ is the loss modulus or the viscosity modulus (Pa) reflects the energy loss caused by the internal or intermolecular stretching of the molecular chain when the polymer changes its external force [26]. $\tan\delta=G''/G'$ ($\delta$ is the loss angle) is an indicator of the viscoelastic properties of the system. Generally, $\tan\delta=1$ is the limit. The larger the $\tan\delta$, the more viscous the component will be dominant, showing more liquid characteristics. More solid characteristics. Figure 6. is a viscoelastic chart of OG0 and BG0 with different mass fractions of 8 freeze-thaw cycles. From the figure, it can be seen that $G'$ is larger than $G''$, indicating the hydrogel formed. It has a certain elasticity, and with the increase of OG0 and BG0 mass fractions, both $G'$ and $G''$ increase. The reason may be that as the mass fraction increases, the molecular chain density increases, and the gel network structure can form with more cross-linking points and cross-linking regions, the gel stability is stronger. The $G'$ of the three mass fractions of OG0 gels is almost unchanged with the increase of the oscillation frequency; $G''$ increases with the increase of the oscillations, and BG0 the $\tan\delta$ of the gel is larger than the $\tan\delta$ of the OG0 gel, indicating that the gel performance of OG0 is better than that of BG0, and probably because the molecular weight of OG0 is smaller than that of BG0.

Figure 5. Viscoelasticity of OG0 (a) and BG0 (b) gel with different concentrations after 8 freeze-thaw cycles

3.5. Scanning Electron Microscopy

From the scanning electron microscope results of oat and barley $\beta$-glucan gel, we can see that oat and barley dextran gels with different mass fractions have porous structures (as shown in Figure 6). The pore size has a certain effect. The $\beta$-glucan gel structure with a low mass fraction is relatively loose, and the gel structure with a high mass fraction is relatively homogenous and compact. The possible reason is that the higher the mass fraction of $\beta$-glucan, the stronger the interaction between $\beta$-glucan molecular chains.
4. Conclusions

This chapter uses low-field nuclear magnetic resonance (LF-NMR) technology, rheology, and scanning electron microscopy (SEM) to study the gel properties. The main conclusions are as follows:

(1) The formation of freeze-thaw gel was studied by low field nuclear magnetic resonance. It was found that there are mainly three groups of relaxation components in the β-glucan system, which have lateral relaxation times $T_{21}$, $T_{22}$, and $T_{23}$, corresponding to the signal fraction ratio. They are $A_{21}$, $A_{22}$, and $A_{23}$. As the number of freeze-thaw cycles increases, the transverse relaxation times $T_{21}$, $T_{22}$, and $T_{23}$ of different mass fractions OG0 rise first and then change little, $A_{21}$ and $A_{22}$ rise first and then change little, and $A_{23}$ decrease first and then change little.

(2) β-glucan gels with different mass fractions can be better distinguished by principal component analysis. With the increase of the number of freeze-thaw cycles, the score interval of the samples became smaller and smaller. The OG0 and BG0 samples with a mass fraction of 4% at the 8th and 11th freeze-thaw cycles were almost 0, indicating the freeze-thaw cycles. After 8 times, the gel state basically stabilized.

(3) The rheological studies show that the larger the mass fraction of the β-glucan gel, the greater its elastic modulus and viscosity modulus, indicating that the larger the mass fraction of the β-glucan solution is, the greater the number of freeze-thaw cycles. Under the formation of more cross-linked structure.

(4) The results of scanning electron microscopy showed that the larger the mass fraction of the β-glucan solution, the denser the gel structure, and the smaller the mass fraction of the β-glucan solution, the looser the gel network.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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