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Research Paper

Effects of L.*plantarum* dy-1 fermentation time on the characteristic structure and antioxidant activity of barley β-glucan in *vitro*

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ABSTRACT

This article explored the effect of *Lactobacillus plantarum dy-1 (L. plantarum dy-1)* fermentation on the basic physicochemical properties and associated *in vitro* antioxidant activity of barley β-glucan, including its molecular weight, monosaccharide composition, characteristic structure and rheology. Its DPPH, ABTS, hydroxyl radical scavenging capacity, and ferric reducing antioxidant potential (FRAP) were measured at different fermentation times. The results showed that the molecular weight of barley β-glucan was decreased from 1.052×10^5 Da to 4.965×10^4 Da within 0–24 h by L. *plantarum dy-1* fermentation, but there was no effect on its characteristic structure. The water- and oil-holding properties of barley β-glucan were significantly enhanced with increased fermentation time, and the fluid viscous behavior of barley β-glucan was enhanced at 6% concentration, while elastic characteristics were weakened. The fermentation had no significant effect on the scavenging effect of DPPH and ABTS radicals of barley β-glucan, but the hydroxyl radical scavenging activity and total antioxidant capacity of FRAP were enhanced with increased fermentation time. Fermentation time may change the physicochemical properties and enhance antioxidant activity of barley β-glucan by reducing its molecular weight.

1. Introduction

β-Glucan is an important functional component in barley. It plays important roles in health foods and pharmaceutical products due to its widely-known beneficial effects, including immunomodulation, antitumor activity, serum cholesterol and glucose reduction, and obesity prevention (Bai et al., 2019).

Some polysaccharide or dietary fiber can be used in foods as an antioxidant agent to improve the bioactivity of final food products (Alakhrash et al., 2016). Studies have shown that β-glucan is potentially a good antioxidant (Johansson et al., 2004; Kofuji et al., 2012; Song, 2006). In recent years there has been more and more research on the oxidative stress of cereals or barley β-glucan. Oxidative stress is considered one of the primary causal factors for various diseases and aging (Błaszczyk et al., 2015; Comert et al., 2020; Paiva et al., 2020). Barley β-glucan has been found to have antioxidant activity *in vitro*, such as DPPH radical scavenging activity, ABTS radical scavenging activity, metal chelating capacity, capacity reduction, ferric reducing antioxidant potential (FRAP), and so on (Kofuji et al., 2012; Shah et al., 2015; Sinthusamran and Benjakul, 2018).

The antioxidant activity of β-glucan is influenced by different physical and physiologic properties, such as structure (Ahmad et al., 2016) and molecular size (Kofuji et al., 2012)), which can vary depending on the source and extraction method used.

Moreover, some studies have shown that physical processing such as microwaving (Papageorgiou et al., 2005) and radiation treatment (Hussain et al., 2018)) can change the structure or reduce the molecular weight of β-glucan, which can significantly improve its antioxidant activity. However, there has been limited information about effect of lactic acid bacteria fermentation on the antioxidant activity of barley β-glucan. Although our group's previous study found that the structure and function of barley β-glucan changed after barley was fermented by *L. plantarum dy-1* (Xiao et al., 2020), the effect on its antioxidant activity was unclear. Therefore, to further explore effect of *L. plantarum dy-1*

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fermentation on the basic physicochemical properties and *in vitro* antioxidant activity of barley β-glucan, the following characteristics were measured at different fermentation times: its molecular weight, monosaccharide composition, characteristic structure and rheology, its DPPH, ABTS, and hydroxyl radical scavenging capacity, and its ferric reducing antioxidant potential (FRAP).

2. Materials and methods

2.1. Materials and chemicals

The barley used was hulled barley (*Yang si mai* 3) purchased from Yan Cheng of China. The lactobacillus strain *Lactobacillus plantarum dy-1* (*L. plantarum dy-1*) had been previously isolated and assigned a preservation number CGMCC NO. 6016 in the database of the Chinese Common Microbe Bacterial Preservation Administration Center. 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) TCI Shanghai (Shanghai, China). 2,2″-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Shanghai Yien Chemical Technology Co., Ltd (Shanghai, China). FRAP kits were purchased from Beyotime Biotechnology Co., Ltd (Shanghai, China). All of the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and were of either analytical or chromatographic grade.

2.2. Preparation of barley β-glucan with different fermentation times

After sieving and removing impurities, the raw barley powder (RB) was obtained by crushing with the High Speed Universal Milling Machine (Zhejiang, China) and filtering through a 100 mesh sieve. We added 2.5% *L. plantarum dy-1* (4×10^8 cfu/mL) to the barley flour with seven-times deionized water to ferment at 30 °C for 8 h, 16 h, 24 h, and 32 h in a microbiological incubator, respectively. After fermentation, the solution was centrifuged at 12,000 g for 15 min at 4 °C using a refrigerated centrifuge (Jouan, France), then the supernatant was freeze-dried into a powder (FBE) using a vacuum freeze dryer (Marin Christ, Germany). The extract was centrifuged and the supernatant collected and then freeze-dried into a powder (RBE) under the same conditions as the fermented barley. For barley b-glucan extraction, the method described by Limberger-Bayer (Limberger-Bayer et al., 2014) and adapted by Xiao (Xiao et al., 2020) was used with a few modifications. Soluble β-glucans from *L. plantarum dy-1* fermented barley (FBG) and raw barley (RBG) were obtained by twice-repeated water extraction of FBE and RBE at 80 °C for 1 h, separately followed by starch removal by enzymatic treatment, deproteination using the Sevag reagent, and precipitation with three volumes of anhydrous ethanol addition. The pellet obtained was resuspended in distilled water and precipitated with 0.6 vol of ammonium sulfate, followed by centrifugation (10 min at 6000 g), resuspension in distilled water, dialysis (MWCO 8000D to 10000D) and freeze drying. The freeze-dried product containing β-glucan was then collected as a powder and stored at −20 °C.

2.3. Determination of molecular weight of barley β-glucan

Determination of the molecular weight of barley β-glucan was performed according to the method previously reported by Xiao (Xiao et al., 2020). Briefly, the concentration of soluble β-glucan was 1 mg/mL, which was filtered through 0.22 μM aqueous membrane filtration. Then, the weight-average molecular weight (Mw), number-average molecular weight (Mn), molecular weight distribution (Mw/Mn), z-average radius of gyration (Rg), and some conformational properties of β-glucan were determined by using high-performance size exclusion chromatography (HPSEC) connected to a refractive index detector (RID,Agilent) and multiangle laser light scatterer (MALLS, DAWN HELLOS II λ = 658 nm; Wyatt Technologies Corporation, USA). The HPSEC-MALLS was performed based on the Agilent 1100 HPLC system (Agilent, USA) equipped with two SEC columns (OHpak SB-806 M HQ and SB-805 HQ, 8 mm Φ × 30 cm, Shodex, Japan) at 25 °C. The flow rate of the mobile phase (0.1 M NaCl solution) was 0.5 mL/min; the differential refractive index increment (dn/dc) of the solution was 0.138 mL/g and injection volume was 20 μ L. Data were analyzed and processed using Astra.

2.4. Fourier transform infrared spectra analysis

The Fourier transform infrared (FT-IR) spectra of β-glucan with different fermentation times were measured using the KBr disk method on a Nicolet IS50 FT-IR spectrometer (Thermo Nicolet Co., USA) in the frequency range of 450–4000 cm^{-1} with KBr pellets and referenced against air.

2.5. Determination of water- and oil-holding capacity

The water-holding capacity (WHC) and oil-holding capacity (OHC) were determined in triplicate according to the method described by Liu et al. (2015). and Wang et al. (2015) with slight modification. Briefly, either distilled water (4 mL) or olive oil (1 mL) was transferred into 50 mL centrifuge tubes containing 100 mg of FBG. For WHC, the mixture was stirred and left at room temperature for 24 h. For OHC, the mixture was stirred and left at 4 °C for 1 h. After centrifugation at 8000 r/min for 10 min in a J-26 XP high-speed freezing centrifuge (Beckman Coulter Diagnostics, USA), the pellet was weighed. WHC and OHC were determined as gram weight of water or oil adsorbed per gram of FBG or RBG.

2.6. Determination of rheological properties

The rheological properties (static and dynamic) of 6% RBG and FBG were determined using an oscillatory rheometer (MCR 102, Anton Paar, Germany) equipped with plate geometry (40 mm diameter, and 29 μm gap) at 25 °C.The test conditions of static viscoelasticity were as follows: the shear rate was 0.01–1000 s^{-1}. The test conditions of dynamic viscoelasticity were as follows: the frequency range was 0.1–100 rad/s, and the strain was 0.1%. Note that, because concentration plays an important role in determining the rheological properties of β-glucans, a concentration of 6% was selected.

2.7. Determination of antioxidant capacity in vitro

2.7.1. DPPH radical scavenging activity

The DPPH scavenging activity of the samples at a concentration ranging from 0.25 to 4 mg/mL was measured by the method with some modifications (Fu et al., 2010). The DPPH ethanol solution (2.0 mL, 0.1 mmol/L) was mixed with 2.0 mL of samples at varying concentrations, and incubated in darkness (30 min). The absorbance of the solution was read at 517 nm against a blank. The DPPH scavenging activity was calculated as follows: scavenging effect (%) = (1- A_s/A_0) x 100%, where A_s is the absorbance of the sample, and A_0 is the absorbance of the blank control. The percentage of inhibition was plotted against the concentration of the FBG, and Vitamin c (Vc) was used as a comparison with the same concentration range of FBG.

2.7.2. ABTS radical scavenging activity

ABTS radical scavenging activity was determined by ABTS assay, as described by Sinthusamran et al. (Sinthusamran and Benjakul, 2018) with some modifications. Briefly, the stock solutions included a 7.0 mM ABTS solution and a 140 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions (5 mL+88 μL) and allowing them to react for 12 h at room temperature in the dark, and then diluting with absolute ethanol to the absorbance at 734 nm of 0.70 ± 0.020. The sample (10 μL) was mixed with 0.2 mL of freshly prepared ABTS assay solution. Then the mixture was left at room temperature for 20min in the dark. The absorbance at 734 nm was read using a double beam spectrophotometer (Model UV-1900, Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that

distilled water was used instead of the sample. The ABTS radical scavenging activity was calculated as follows: scavenging effect (%) = (1-A_s/A_0) x 100%, where A_s is the absorbance of the sample, and A_0 is the absorbance of the blank control.

2.7.3. Hydroxyl radical scavenging capacity

The hydroxyl radical assay was evaluated by the Fenton reaction method. The reaction mixture contained 2 mL 1.8 mM $FeSO_4$, 1.5 mL 1.8 mM salicylic acid and 0.1 mL 0.3% H_2O_2 and 1 mL samples of varying concentrations (0.25–4 mg/mL). After incubation at room temperature for 30 min, the absorbance of the mixture was measured at 510 nm. Distilled water was used as a control group. Hydroxyl radicals gave a crimson colour, so the absorbance change of the reaction mixture indicated the scavenging ability for hydroxyl radicals. The hydroxyl radical-scavenging activity was expressed as: scavenging effect (%) = (1-A_s/A_0) x 100%. Where A_s is the absorbance of the sample, and A_0 is the absorbance of the blank control.

2.7.4. FRAP assay

The total antioxidant capacity test was performed according to the instructions of the FRAP kit. 180 μL of FRAP working solution to the 96-well plate, and then 5 μL of FeSO4 standard solution or 5 μL sample solution of each concentration were added at room temperature at 37 °C and incubated for 5 min; the absorbance of the resulting solution was measured at 595 nm against a blank. The concentration of the FeSO4 standard curve is 150, 300, 600, 900, 1200, 1500 μmol/mL FeSO4 aqueous solution, and the measurement result uses FeSO4 as mmol FeSO4/g dry weight (mmol Fe/g DW). The FRAP activity was expressed as: scavenging effect (%) = (1- A_s/A_0) x 100%, where A_s is the absorbance of the sample, and A_0 is the absorbance of the blank control.

2.8. Statistical analysis

Significant differences were measured using SPSS Statistics software version 17.0 (SPSS, Chicago, IL, USA) at $P < 0.05$. GraphPad prism 7.0 (GraphPad Software Inc., USA) and OriginPro 9.0 (OriginLab Inc., USA) were also used for statistical analysis and plotting. The data are expressed as the arithmetic mean ± standard deviation (SD). Data were analyzed by analysis of variance (ANOVA) with different letters indicating a significant difference ($P < 0.05$).

3. Results and discussion

3.1. Effect of fermentation time on the molecular weight of β-glucan

Physical properties of β-glucan such as solubility, viscosity and gel properties depend upon molecular properties (like molecular weight and molecular structure). In addition, its physiological activities are also related to molecular weight and molecular structure (Bai et al., 2019).

In order to clarify the effect of fermentation time on barley β-glucan, the molecular weight of β-glucan was studied by HPSEC-RI-MALLS. The weight average molecular weight (Mw), number average molecular weight (Mn), and molecular weight distribution coefficient (Mw/Mn) of β-glucan are shown in Table 1 and Fig. 1. The HPSEC-RI-MALLS chromatogram of RBG showed a single symmetrical peak (Mw: 1.052×10^5), which indicated that the molecular weight distribution of RBG was uniform. With the increase of fermentation time, the molecular weight of FBG decreased gradually, and a second peak appeared in the HPSEC-RI-MALLS chromatogram. The data collection of a light scattering detector depends on the fluctuation of scattered light intensity. The diffusion rate of smaller particles in a solvent is faster, while the fluctuation of scattered light intensity is faster and the peak time is shorter. The results showed that the molecular weight of barley β-glucan was degraded from 1.052×10^5 Da to 3.056×10^4 Da within 0–32 h by *L. plantarum dy-1* fermentation. In addition, increased fermentation time led to a gradual increase in small-molecular-weight barley β-glucan (Ahmad et al., 2016). This may be due to the enzymatic hydrolysis of lactic acid bacteria during the fermentation process (Liu et al., 2015) followed by the chain breaking of the β-glucan polymer (Xiao et al., 2019). Therefore, enzymatic hydrolysis of lactic acid bacteria observably decreased the average Mw of barley β-glucans via chain breaking of the β-glucan polymer.

Table 1
Molecular conformation parameters of β-glucan in barley at different fermentation times.

Samples	M_w (g/mol)	M_n (g/mol)	M_w/M_n
RBG	1.052×10^5	1.017×10^5	1.03
FBG 8 h	5.158×10^4	5.069×10^4	1.01
FBG 16 h	5.274×10^4	4.825×10^4	1.09
FBG 24 h	4.965×10^4	4.861×10^4	1.02
FBG 32 h	3.056×10^4	2.625×10^4	1.16

Mw, weight average molecular weight; Mn, number average molecular weight; Mw/Mn, molecular weight distribution coefficient; FBG, soluble β-glucans from *L. plantarum* dy-1 fermented barley; RBG, soluble β-glucans from raw barley.

3.2. Effect of fermentation time on the characteristic structure of β-glucan

FT-IR spectroscopy was used to understand the possible structural variations in the β-glucan concentrate after different fermentation times. The FT-IR spectra of RBG at different fermentation times of FBG concentrate are shown in Fig. 2. The RBG and different fermentation times of FBG showed an absorption band around 3200 cm^{-1} to 3700 cm^{-1}, which is assigned to the symmetric and asymmetric stretching of the OH groups. The peaks at 2800 cm^{-1} to 3000 cm^{-1} correspond to the vibrational symmetric and asymmetric stretching of C–H groups. The bands appearing at 1400 and 1500 cm^{-1} were mainly caused by the bending vibration of C–H. In addition, the characteristic absorption peaks in the range from 1200 to 1000 cm^{-1}, as the typical infrared spectral signals of glucan, can probably be ascribed to the combination of the asymmetric stretching vibration of the C–O–C acetal linkage, the ring vibration, as well as the vibration of the pyranose glycosidic bond and the hydroxyl group. Moreover, the absorption peak at 896 cm^{-1} indicated the presence of β-glycosidic anomeric bonds in the samples (Dangi and Yadav, 2020; Shah et al., 2015; Xiao et al., 2020). Similar FT-IR spectra have been observed for barley and oat β-glucans in the previous studies (Fan et al., 2019; Limberger-Bayer et al., 2014; Mikkelsen et al., 2010).

It can be seen that no transformation of existing groups or addition of groups occurred on account of fermentation. Overall, these results showed that the fermentation time did not change the barley β-glucan within 32 h of fermentation, and that a small-molecular-weight barley β-glucan was produced with a gradual increase in fermentation time, with no effect on its characteristic structure.

3.3. Effect of fermentation time on the water- and oil-holding capacity of β-glucan

WHC and OHC are basic physical and chemical properties of dietary fiber. The WHC and OHC of barley β-glucan resulting from different fermentation times are shown in Table 2. During 8 h fermentation, the WHC and OHC had no significant change, but the WHC and OHC of barley β-glucan increased significantly after 16 h fermentation. When the fermentation time was 32 h, the WHC and OHC of β-glucan were significantly lower than after 24 h. The reason may be that unfermented barley β-glucan has a tighter molecular gap. The increase of fermentation time led to β-D-G polymer chain breaks (Ahmad et al., 2016), which changed the network structure of dietary fiber and improved the WHC and OHC of barley β-glucan. However, cleavage of more glycosidic bonds destroyed the fiber network structure of β-glucan, which reduced its ability to lock in water and oil. Therefore, an appropriate extension of fermentation time could improve the WHC and OHC of barley β-glucan.

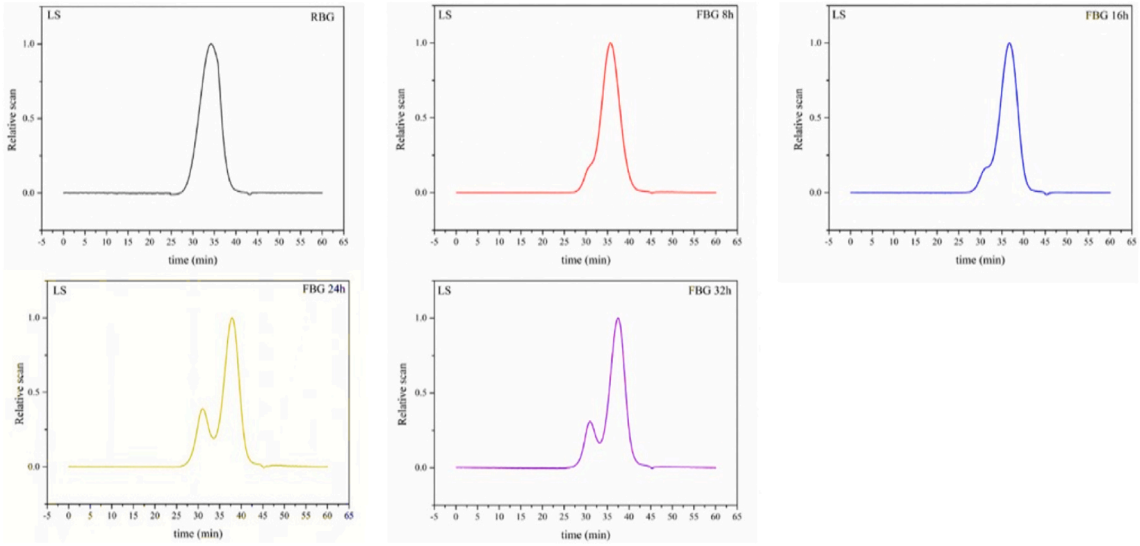

Fig. 1. HPSEC-RI-MALLS chromatograms of barley β-glucan under different fermentation times. FBG, soluble β-glucans from *L. plantarum* dy-1 fermented barley ; RBG, soluble β-glucans from raw barley.

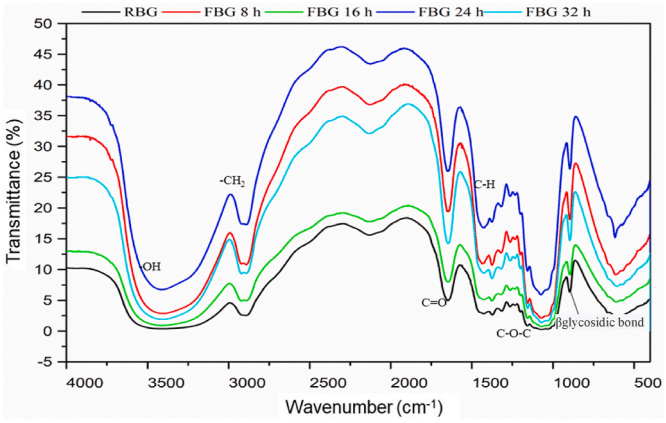

Fig. 2. Infrared spectra of barley β-glucan at different fermentation times.

Table 2
The water and oil holding capacity of barley β-glucan at different fermentation time.

	RBG	FBG 8 h	FBG 16 h	FBG 24 h	FBG 32 h
WHC (g/ g)	10.57 ± 0.74^{a}	10.53 ± 1.09^{a}	11.40 ± 0.37^{a}	18.57 ± 0.97^{b}	11.33 ± 0.36^{a}
OHC (g/ g)	5.53 ± 0.42^{a}	6.40 ± 0.39^{ab}	6.58 ± 0.45^{ab}	7.35 ± 0.28^{b}	5.47 ± 0.38^{a}

WHC, water-holding capacity OHC, oil-holding capacity.
Different letters mean different significance $P < 0.05$, n = 3.

In addition, WHC has been reported to be influenced by strongly-bonded micellar networks and amylopectin molecular structure (Kratz et al., 2013). Liu et al. (2015) found that WHC showed significant correlation with molecular weight distribution.

3.4. Effect of fermentation time on the rheological properties of β-glucan

As mentioned above, because apparent viscosities of dispersion are dependent on β-glucan concentration (Xu et al., 2013), the 6% β-glucan concentration was investigated. The static rheological properties of 6% barley β-glucan at different fermentation times are shown in Fig. 3A. The initial apparent viscosity of β-glucan increased with fermentation time. In addition, the apparent viscosity of β-glucan decreased with an increase in shear rate, and finally tended to be stable. This may be due to the high initial viscosity of the system, and to the greater resistance present just under the action of external force. With the increase in shear rate, the hydration structure between polysaccharide molecules and water molecules was destroyed, resulting in a decrease in viscosity. However, many studies have shown that at higher molecular weight β-glucan has a lower DP3/DP4 ratio, higher degree of molecular interaction, stronger flow resistance, and enhanced apparent viscosity (Xu et al., 2013). These are not consistent with our results, which may be due to the promotion of fermentation by the interaction of water molecules with water β-glucan binding, or to the different instruments and molds used in the test process. There is some slope between the lamina and the contact surface, and the frictional force between the plate and the contact surface is greater. Therefore, under the extrusion effect of external force, intermolecular combination is closer, so as to improve its apparent viscosity.

The dynamic viscoelasticity of barley β-glucan with different fermentation times are shown in Fig. 3 B. As seen in Fig. 3 B, in the increased-malt-content-β-glucan solution, Tan δ decreased with fermentation time. However, with increased oscillation frequency, the storage modulus G′ and loss modulus G″ of β-glucan increased under different fermentation times, while Tan δ decreased. Because extraction conditions can effect the yield, composition, and viscosity stability of barley beta-glucan gum (Burkus Z., 1998), the fluid viscosity of RBG was less than its elasticity. The elasticity of RBG was stronger than that of FBG. These results indicate that the apparent viscosity of β-glucan gradually increased, and its elastic properties gradually decreased with different fermentation times at a concentration of 6%. Previous studies related to the structural and molecular characteristics of β-glucan revealed that molecular weight is the key factor that determines its functional properties, such as solubility and rheological properties (Hu et al., 2015; Wolever et al., 2010).

3.5. Effect of fermentation time on the DPPH, ABTS, hydroxyl radical scavenging capacity, FRAP of β-glucan

As a stable and good qualitative solid free radical, DPPH free radical measurement is a typical *in vitro* antioxidant evaluation method. *In vitro* DPPH free radical scavenging test results of barley β-glucan under different fermentation times are shown in Fig. 4 A. It can be seen from Fig. 4 A that barley β-glucan with a concentration of 0.25–4 mg/mL

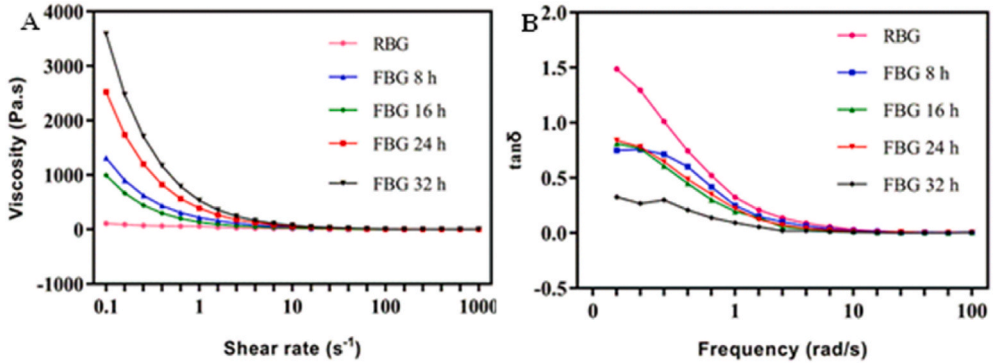

Fig. 3. Apparent viscosity curve (A) and dynamic viscoelastic change curve (B) of barley β-glucan at different fermentation times.

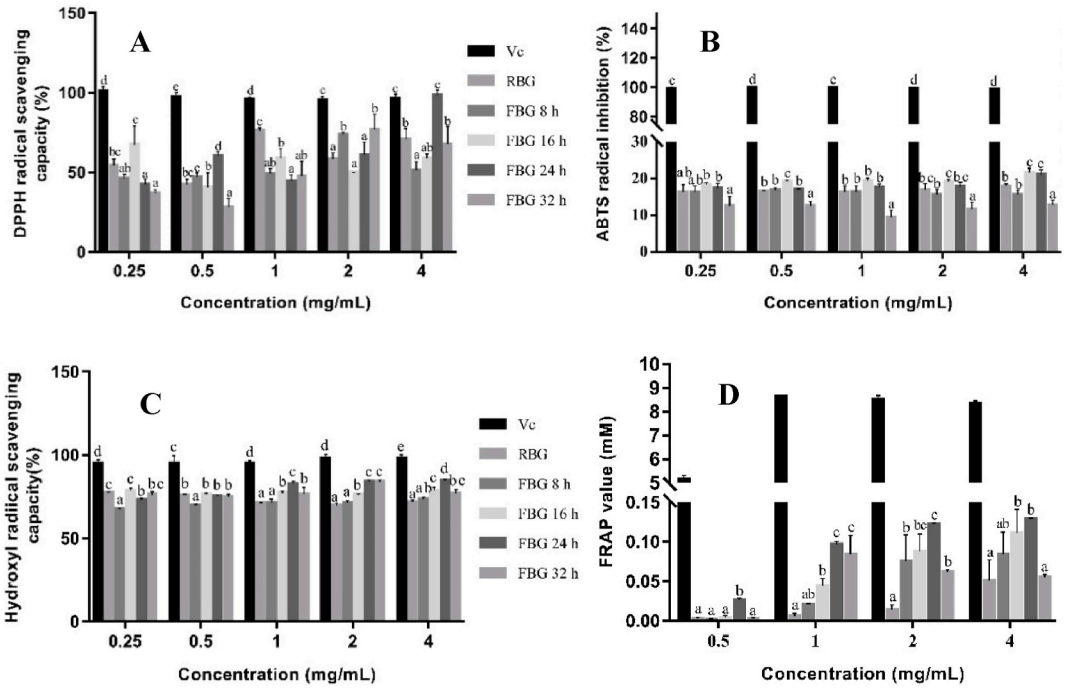

Fig. 4. DPPH free radical scavenging (A), ABTS free radical scavenging (B), hydroxyl radical scavenging (C) and FRAP (D) effect of barley β-glucan at different fermentation times. Vc stands for vitamin C. Different letters mean different significance $P < 0.05$, n = 3.

under different fermentation times had DPPH free radical scavenging ability. The DPPH free radical scavenging effect of barley β-glucan was close to the positive control group concentration. However, the fermentation time had no tendency to influence the DPPH free radical scavenging effect of barley β-glucan. The enhanced DPPH free radical scavenging activity of fermented barley β-glucan instead may be related to the low molecular weight subunits in fermented polysaccharides (Hussain et al., 2018).

The ABTS free radical scavenging method can be used to evaluate the antioxidant capacity of plants and pure compounds. After a substance reacts with the ABTS free radical solution, if the absorbance at 734 nm decreases, it indicates that the substance has free radical scavenging activity and belongs to an antioxidant. Barley β-glucans with different fermentation times had some ABTS free radical scavenging activity (Fig. 4 B), but in the concentration range of 0.25–4 mg/mL, there was no concentration dependence on the scavenging effect of ABTS free radicals. After 32 h of fermentation, the ABTS scavenging activity of FBG was significantly lower than that of barley β-glucan during the other fermentation times, which may be due to excessive β-D-G chain bond breakage and the loss of some groups that can bind to ABTS free radicals.

Hydroxyl radical (•OH) is an important reactive oxygen species formed from the molecular formula by the loss of one electron from hydroxide (OH-). Hydroxyl radicals can cause oxidative damage to carbohydrates, amino acids, proteins, nucleic acids, and other substances in tissues due to their strong oxidizing ability. Therefore, hydroxyl free radicals have strong biological toxicity in the metabolism of animals. The results of the determination of hydroxyl radical scavenging activity of barley β-glucan at different fermentation times are shown in Fig. 4 C. Barley β-glucan with different fermentation times had some hydroxyl radical scavenging effect. Studies have shown that the dissociation energy of the CH bond in β-glucan is higher than that of the OH bond. Therefore, the hydroxyl group can easily react with carbohydrates by extracting the hydrogen bonded to the carbon, thereby forming a carbon-centered free radical, HC–OH + •OH •C–OH + H2O (Hussain et al., 2018). With the extension of fermentation time, the hydroxyl radical scavenging effect of FBG gradually increased, but the hydroxyl radical of barley β-glucan fermented to 32 h was significantly lower than that of FBG to 24 h. In addition, the hydroxyl free radical scavenging activity of barley β-glucan before and after fermentation was stronger than that of the positive control. It may be that the hydroxyl free radical scavenging activity of barley β-glucan mainly comes from the dissociation of C–H bond.

FRAP has been widely used in the analysis of the antioxidant capacity of food and health products. Through the determination of the *in vitro* FRAP of barley β-glucan at different fermentation times (Fig. 4 D), the total antioxidant capacity of barley β-glucan also increased with the increase in concentration. The FRAP of barley β-glucan increased significantly with the extension of fermentation time at 1–4 mg/mL barley β-glucan concentration. However, the FRAP of barley β-glucan fermented for 32 h was significantly lower than that of barley β-glucan fermented for 24 h. The principle underlying the FRAP method to determine total antioxidant capacity is that antioxidants can reduce Fe^{3+} to Fe2+ under acidic conditions, and the breakage of the glycosidic bond leads to an increase in the number of reducing sugars, which in turn helps to enhance the reducibility of degraded polysaccharides (Hussain et al., 2018). The reducing ability is usually related to the presence of reducing sugars, and may also be related to the hydrogen supply capacity. The hydrogen supply capacity of barley β-glucan after 32 h of fermentation may not be strong, resulting in a decrease in the total antioxidant capacity.

4. Conclusion

From the present investigation, it can be concluded that *L. plantarum dy-1* fermentation had a positive effect on barley β-glucans, while enzymatic hydrolysis of lactic acid bacteria observably decreased the average Mw of barley β-glucans via chain breaking of the β-glucan polymer. In addition, a gradual increase in small molecular weight barley β-glucan was produced with an increase in fermentation time, but there was no effect on its characteristic structure. The water- and oil-holding properties of barley β-glucan were significantly enhanced with the increase of fermentation time, and the fluid viscous behavior of barley β-glucan was enhanced at 6% concentration, while elastic characteristics were weakened. With the increase of fermentation time there was no significant effect on the scavenging effect of the DPPH and ABTS radicals of barley β-glucan, but the hydroxyl radical scavenging activity and total antioxidant capacity of the FRAP were enhanced. Fermentation time may change the physicochemical properties and enhance antioxidant activity of barley β-glucan by reducing its molecular weight.

CRediT authorship contribution statement

Jiayan Zhang: Conceptualization, Methodology. **Ping Wang:** Data curation, Writing – original draft. **Cui Tan:** Visualization, Investigation. **Yansheng Zhao:** Supervision. **Ying Zhu:** Software, Validation. **Juan Bai:** Writing – review & editing. **Xiang Xiao:** Supervision. **Lili Zhang:** Resources. **Donghai Teng:** Investigation. **Jing Tian:** Resources. **Liangcheng Liu:** Resources. **Haibo Zhang:** Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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