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Processing and Prebiotics Characteristics of β -Glucan Extract from Highland Barley

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Abstract: β -glucan extract (GE) was obtained from highland barley bran using alkaline–acid–alcohol extraction method. The stability, solubility, foaming ability, and prebiotics characteristics of GE were assessed consecutively. GE demonstrated excellent heat stability (hardly degraded at 220 °C) and pH stability, especially at neutral or alkaline condition, and its solubility was significantly influenced by temperature instead of pH or NaCl, achieving 0.91 g/100 g at 100 °C. Good foaming ability and foam stability of GE were observed during low temperatures (≤ 40 °C), neutral or alkaline condition. GE indicated a strong anti-digestibility capacity of resisting the hydrolysis of α -amylase and simulated human gastric acid. Interestingly, GE could effectively promote the growth of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis*, which was close to fructooligosaccharide. The results of this study could offer valuable information for the application of β -glucan from highland barley as prebiotics in promoting human intestinal health metabolism.

Keywords: β -glucan; highland barley; processing characteristics; prebiotics

1. Introduction

Highland barley, one of the superb cereal crops, is endowed with high content of fiber, vitamin, protein, and low content of fat, and is distributed mainly in highland sections of Tibet, Qinghai, Gansu, Sichuan, and Yunnan provinces in China [1]. It is regarded as staple meal everyday by Tibetan residents [2]. Although Tibetan people are fond of beef, mutton and milk instead of vegetable, demonstrating a diet with high energy, high fat, and high protein, they seldom suffered from diseases—such as hypertension, diabetes, and coronary heart diseases—which presumably benefits from the nutrition and bioactive factors from highland barley [3].

β -glucan is the main active ingredient in highland barley, and its content is about three to nine times higher than in oat and barley [4]. It has three significant functions, namely regulating blood sugar, lowering blood cholesterol, and improving immunity, and is a potential raw functional material for food industry [5–7]. The β -glucan molecule, with a specific helical structure, consists of some cellotrioses or cellotetraoses by β -1,4-glycosidic bonds, and these fiber glycosyls are connected each other by β -1,3-glycosidic bonds. The molecular structure of β -glucan is shown in Figure 1.

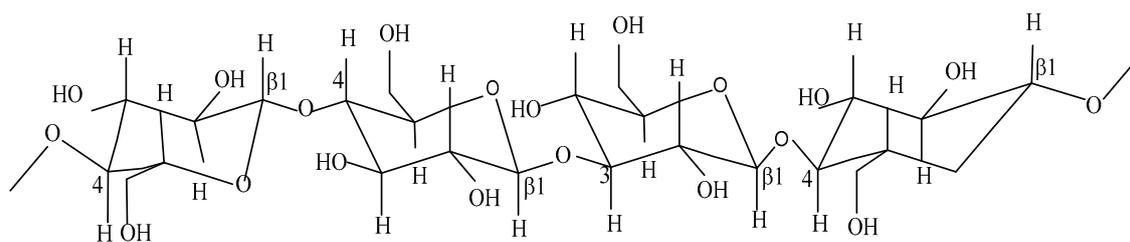


Figure 1. Molecular structure of β -glucan.

Highland barley is usually cooked as Zanba which is a traditional food for Tibetan people. Apart from this, it is mainly applied in animal feed making, grain processing, and beer production [8]. Nonetheless, a mass of highland barley is wasted every year. In addition, β -glucan mainly lies in the surface layer of the bran, and represents 25% of the weight of the whole highland barley, while a large amount of bran is wasted in highland barley processing [9]. Economically exploiting the β -glucan would therefore contribute to easing a significant waste disposal issue.

In the present research, β -glucan extract (GE) was prepared from highland barley. Some significant processing characteristics including stability and solubility were determined against temperature, pH, and NaCl concentration, and then its prebiotics characteristics consisting of anti-digestibility and effect on probiotics growth were evaluated, which were helpful for the high value-added utilization of β -glucan from highland barley bran.

2. Materials and Methods

2.1. Materials and Reagents

Highland barley was purchased in Ganzi county, Ganzi Tibetan autonomous prefecture, Sichuan province, China. *Lactobacillus delbrueckii* subsp. *Bulgaricus* ATCC 11842 and *Bifidobacterium adolescentis* ATCC 15703 were purchased from China Center of Industrial Culture Collection, Beijing, China. Man, Rogosa and Sharp (MRS) medium was bought from Qingdao Rishui Biological Technology Co., Ltd., Qingdao, China, and trypticase-phytone-yeast extract (TPY) medium was bought from Qingdao Haibo Biological Technology Co., Ltd., Qingdao, China. Fructooligosaccharide was purchased from Shandong Baolingbao Biological Technology Co., Ltd., Dezhou, China. Both α -amylase (8000 U/mL) and Congo Red were purchased from Aladdin Reagent Co., Ltd., Shanghai, China. All other chemicals and reagents were of analytical grade commercially available.

2.2. Preparation of β -Glucan Extract (GE) from Highland Barley

Highland barley bran was processed by smashing and then sieving through 60-mesh sieve. A 15 g dose of the processed bran was dispersed in 285 mL of distilled water, followed by the pH adjustment to 8 using 0.05 M NaOH and 2.5-h incubation at 80 °C with a stirring speed of 150 r/min in a water-bath shaker (Suzhou Bing Lab Equipment Co., Suzhou, China). Then the mixture was centrifuged at 7000 r/min for 10 min using a 5804R high speed refrigerated centrifuge (Eppendorf Co., Hamburg, Germany) at room temperature. The pH of the isolated supernatant was adjusted to 4.5 with 1 M HCl, and then centrifuged at 7000 r/min for another 10 min. The supernatant was reduced to 100 mL by vacuum concentration, and then followed by the addition of 300 mL of ethanol. After 10-h incubation at 4 °C, it was centrifuged at 7000 r/min for further 10 min. The precipitate (GE) was collected by freeze drying using a Christ Alpha 1-2 freeze dryer (Christ Co., Osterode, Germany). The content of β -glucan was determined using the following method.

2.3. Determination of β -Glucan

A 0.2 mL dose of 0.1 g/mL prepared β -glucan solution was added into 4.0 mL of 0.1 g/L Congo red dissolved in 0.1 mol/L PBS (pH 8.0), following with a 10-min incubation at 25 °C. The maximum absorption wavelength of the incubation solution was found out using a spectrophotometer (722, grating spectrophotometer, Shanghai Inesa Analytical Instrument Co., Ltd., Shanghai, China). 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mL of 0.1 g/mL β -glucan solution were diluted with distilled water to 2.0 mL and then added into the same Congo Red solution, respectively. After 10-min incubation at 25 °C, the solution absorbance was measured at the maximum absorption wavelength. The standard curve was drawn and regression equation was given. The sample was determined as the same steps above and the β -glucan content was calculated according to the standard curve.

2.4. Processing Characteristics of GE

The processing characteristics of GE were investigated from the three aspects, including stability, solubility, and foamability.

2.4.1. Stability Assay

Heat stability—Six portions of 0.2 g of GE were ready for the analysis of heat stability. One of them was dissolved into 100 mL of distilled water and then 6-h incubation at 100 °C, the other five portions were put into the oven set at 140, 160, 180, 200, and 220 °C for 6 h, respectively. After being cooled, they were respectively dissolved into 100 mL of distilled water to measure the content of β -glucan. Another GE sample (1 g) without heat treatment was served as a negative control. The retention rates of GE of these six samples were measured to assess their heat stability, which were calculated as

$$\text{Retention rate (\%)} = \frac{C_1}{C_0} \times 100 \quad (1)$$

where C_1 is the content of GE of the heat-treated samples; C_0 is the content of GE of negative control.

pH stability—Five portions of 0.2 g of GE were respectively dissolved into 100 mL of distilled water. The pHs were separately adjusted to 3, 5, 7, 9, and 11 using 1 M HCl or 1 M NaOH and then 2-h incubation at 100 °C. The retention rates of GE of the five samples were measured to assess their pH stability, which were calculated as

$$\text{Retention rate (\%)} = \frac{C_2}{C_0} \times 100 \quad (2)$$

where C_2 is the content of GE of the acid or alkali-treated samples; C_0 is the content of GE of negative control, which is the same to that in Equation (1).

2.4.2. Solubility Assay

Various effects of temperature, pH and NaCl concentration on GE solubility were investigated.

Temperature effect—Five portions of 2.0 g of GE were dissolved into 100 mL of distilled water (pH 7.0) and then 1-h incubation at 20, 40, 60, 80, and 100 °C in a water-bath shaker with a stirring speed of 800 r/min, respectively, and then centrifuged at 3000 r/min for 20 min. The supernatant was concentrated and dried to a constant weight. The solubility (g/100 mL) of GE at different temperature was measured as the mass value of the dried product.

pH effect—Five portions of 0.5 g of GE were dissolved into 100 mL of distilled water. Their pHs were respectively adjusted to 3, 5, 7, 9, and 11, and followed by a 1-h incubation at 100 °C with the stirring speed of 800 r/min. The solubility of GE at different pHs was measured according to the same method as the temperature effect.

NaCl effect—The solubility of GE in the presence of 0, 1, 2, 3, and 4% NaCl (pH 7.0) was also respectively measured according to the same method as the pH effect.

2.4.3. Foam Assay

Various effects of temperature, pH and NaCl concentration on foaming ability and foam stability of GE were investigated by reference to the methods [10].

Five portions of 0.2 g of GE were respectively dissolved into 100 mL of distilled water (pH 7.0) and then stirred at 20, 40, 60, 80, and 100 °C with the speed of 10,000 r/min for 90 s using a high-speed organization broken machine (DS-1, Shanghai specimen model factory, Shanghai, China). The foam volumes were measured and recorded. They were respectively incubated at 20 °C–100 °C for 30 min, and then the volumes were also recorded. Foaming ability (%) and foam stability (%) of GE at different temperatures were calculated as

$$\text{Foaming ability (\%)} = \frac{V_1}{V_0} \times 100 \quad (3)$$

$$\text{Foam stability (\%)} = \frac{V_2}{V_1} \times 100 \quad (4)$$

where V_1 is the initial foam volume of GE; V_2 is the final foam volume of GE after 30-min incubation, V_0 is the initial volume of GE solution.

The temperature was set as 20 °C, foaming ability and foam stability of GE at the pH of 3, 5, 7, 9, 11, or the NaCl concentration of 0, 1, 2, 3, 4% were conducted at pH 7.0 as the method above.

2.5. Prebiotics Characteristics of GE

2.5.1. Anti-Digestibility Analysis

α -amylase assay—Five portions of 0.5 g of GE were respectively dissolved into 100 mL of 20 mmol PBS at the pH of 4, 5, 6, 7, and 8. After a 0.1 g dose of α -amylase was added, the mixture was incubated at 37 °C for 6 h. During this process, 1 mL of the sample solution was taken out at 0, 1, 2, 4, and 6 h for determining the contents of reducing sugar and total sugar by dinitrosalicylic acid method and phenol sulfuric acid, respectively [11,12]. The degree of hydrolysis (DH) of GE was employed to assess the anti-digestibility of GE to α -amylase, which was calculated as

$$\text{DH (\%)} = \frac{M_t - M_0}{M_1 - M_0} \times 100 \quad (5)$$

where M_t is the reducing sugar content of GE sample at the different incubation time; M_1 is the total sugar content of GE; M_0 is the initial reducing sugar content of GE. The DH of polysaccharide is usually represented using the ratio of the reducing sugar content of sample generated by hydrolysis and the total sugar content except initial reducing sugar. The lower the DH of GE is, the stronger the anti-digestibility is.

Simulated human gastric acid assay—Five portions of the buffer solution were adjusted to pH 1, 2, 3, 4, and 5 using 1 M HCl, following by the addition of 0.2 g of GE. The mixture was then incubated at 37 °C for 6 h, and the DH of GE was determined as the method above.

2.5.2. Effect of GE on Probiotics Growth

The strain of *Lactobacillus bulgaricus* was inoculated on MRS medium plate for a 48 h-anaerobic culture at 37 °C. Some of the activated strains were dispersed in sterile saline (0.9% NaCl solution) to form the suspension with bacterial concentration of 4×10^7 cfu/mL. 0.2, 0.4, 0.6, 0.8, and 1.0 mL of 20 mg/mL GE solution were respectively added into a tube with 8.5 mL of sterile MRS liquid medium, and then their volumes were fixed to 9.5 mL with the liquid medium. After 0.5 mL of the suspension was added, a 28 h-anaerobic culture was carried out at 37 °C, and the OD₆₀₀ (the absorbance of culture solution at 600 nm) detected every 2 h was used to characterize the strains biomass. The culture at the same condition without the addition of any GE was considered as a negative control, while an addition

of fructooligosaccharide (2.0 mg/mL) was positive control. Each test was conducted in triplicate. *Bifidobacterium adolescentis* was inoculated on TPY medium plate for 48-h anaerobic culture at 37 °C. The strain biomass was characterized as the above-mentioned method [13].

2.6. Statistical Analysis

Statistical calculation was performed by one-way analysis of variance (ANOVA) using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation (SD) of triplicate determinations. Differences were considered to be significant at $p < 0.05$ or 0.01 .

3. Results and Discussion

3.1. Heat Stability and pH Stability of GE

GE is usually applied as material in food processing, so adjusting the temperature and pH of the surrounding environment is common. On the other hand, high temperature and strong acid or alkaline conditions may cause degradation of β-glucan. Therefore, it is necessary to investigate the stability of GE to heat and pH [14]. Firstly, the yield of GE was measured as $6.99 \pm 0.23\%$, and the β-glucan content of GE was $62.63 \pm 1.54\%$ according to the regression equation, which was

$$Y = 3.279X + 0.002 \tag{6}$$

where Y was the OD₅₅₀, and X was the β-glucan content (mg/mL). Heat stability of the GE was systematically evaluated by wet heat in the water at 100 °C and dry heat in the oven between 140 °C and 220 °C. As shown in Table 1, β-glucan content of GE almost kept constant from 100 °C (wet heat) to 200 °C (dry heat) for 6 h. When the temperature climbed to 220 °C, the content reduced to $98.21 \pm 1.83\%$ but even this decline was not significant ($p > 0.05$). Thus, GE exhibited excellent heat stability under boiling and high-bake.

Table 1. Stability, solubility, and foaming ability of β-glucan extract (GE) from highland barley.

Stability		Solubility		Foaming Ability		
Treatment	β-Glucan Retain Ratio (%)	Treatment	Solubility (g/100 g)	Treatment	Foam Capacity (%)	Foam Stability (%)
Heat (°C)	-	Temperature (°C)		Temperature (°C)		-
Boiling	-	20	0.28 ± 0.04a	20	45.65 ± 1.24b	55.55 ± 1.23e
100	100 ± 0.14a	40	0.39 ± 0.02b	40	45.54 ± 1.41b	50.15 ± 0.35d
Dry heat (°C)	-	60	0.66 ± 0.02c	60	44.12 ± 1.83b	25.14 ± 1.02c
140	100 ± 0.21a	80	0.79 ± 0.02d	80	41.67 ± 2.42ab	13.98 ± 1.32b
160	99.96 ± 0.55a	100	0.91 ± 0.06e	100	38.21 ± 2.68a	7.21 ± 0.80a
180	99.36 ± 1.01a	pH	-	pH	-	-
200	99.01 ± 0.88a	3	0.41 ± 0.03a	3	20.35 ± 1.24a	25.87 ± 2.12a
220	98.21 ± 1.83a	5	0.66 ± 0.03b	5	31.08 ± 1.92b	32.26 ± 1.82b
-	-	7	0.91 ± 0.05c	7	45.76 ± 1.23d	55.55 ± 1.23e
-	-	9	0.87 ± 0.02c	9	39.55 ± 2.06c	51.28 ± 1.37d
-	-	11	0.86 ± 0.05c	11	32.67 ± 1.34b	46.87 ± 1.95c
pH	-	NaCl concentration (% w/v)		NaCl concentration (% w/v)		-
3	70.45 ± 3.78a	0	0.91 ± 0.01c	0	45.58 ± 1.34a	55.61 ± 1.08a
5	92.96 ± 2.83b	1	0.87 ± 0.02b	1	48.21 ± 1.02b	56.25 ± 1.84a
7	100 ± 0.85c	2	0.84 ± 0.02b	2	54.67 ± 2.25c	64.81 ± 1.23b
9	100 ± 1.04c	3	0.81 ± 0.02ab	3	61.27 ± 1.32d	68.85 ± 2.18c
11	99.27 ± 0.57c	4	0.78 ± 0.01a	4	65.73 ± 1.86e	69.23 ± 1.56c

Means with different letters (a, b, c, d, e) are significantly different ($p < 0.05$).

The pH of natural GE solution was measured as 6.6. The results of pH stability of the GE are also shown in Table 1. The content of β-glucan was basically unchanged at neutral and alkaline conditions of pH 7–11, but it decreased dramatically to $92.96 \pm 2.83\%$ as the pH was lowered to 5 ($p < 0.05$). Furthermore, the strong acid condition of pH 3 generated a lower β-glucan content of $70.45 \pm 3.78\%$. Therefore, the GE had better pH stability at neutral or alkaline condition than acid one, which demonstrated the GE is more suitable to be applied to weak acidic, neutral, or alkaline food.

In summary, highland barley or bran which is rich in β -glucan can be smoothly processed with no loss of β -glucan under high temperature and weak acidic, neutral or alkaline condition in food industry.

3.2. Solubility of GE

Solubility of GE was investigated in the presence of different temperature, pH and NaCl, which is important to assess its application in food processing. As shown in Table 1, temperature had a significant effect on the solubility of GE. The solubility increased along with the temperature. It was observed that the solubility was only 0.28 ± 0.04 g/100 g at 20 °C, while 0.91 ± 0.06 g/100 g at 100 °C. Likely, higher temperature led to more active thermal motion of β -glucan and other molecules in GE, thereby weakening the intermolecular interaction. On the other hand, higher temperature increased the collision frequency among β -glucan molecules in GE and water molecules thereby causing a stronger interaction. As a result, wet heating is an effective way for improving solubility of GE in food production.

The effect of pH on the solubility of GE was also shown in Table 1. The solubility was observably lower in acidic medium than in alkaline or neutral ($p < 0.05$). The acid in medium could degrade some of β -glucan in GE, but also H^+ could combine naturally with the OH^- in β -glucan, which would weaken the electrostatic repulsion among β -glucan molecules. Thus, the molecular aggregation emerged, and a lower solubility of GE was observed under acidic condition.

Effect of NaCl concentration on the solubility of GE was also shown in Table 1. The solubility decreased as the NaCl concentration increased. NaCl solution with a certain concentration could break surface hydration layer around β -glucan molecules and lead to a decline of GE solubility. However, high-concentration NaCl is not usually adopted in food processing. It was reported that only 0.5% NaCl had been employed to make oat bran bread by using sour dough containing β -glucan [15]. Therefore, the addition of NaCl in conventional food industry could not practically affect the solubility of GE.

3.3. Foaming Ability and Foam Stability of GE

Foaming ability and foam stability of the β -glucan from highland barley are very important for its further application in food industry. Thus, these properties at different temperatures and pHs as well as NaCl concentrations were investigated.

As shown in Table 1, the foaming ability of GE displayed slight decline and foam stability decreased substantially with the temperature ($p < 0.05$). High temperature could reduce the viscosity of solution, thus accelerating the evaporation of liquid film of the foam. When the liquid film of the foam was thin enough, a burst occurred [16]. Foaming ability and foam stability were better under neutral condition than alkaline, and worst in acidic environments. It is likely that a strong base could make β -glucan particles on the solution interface dissolve into solution with changing the interface property of solution, and under acidic condition there were more undissolved β -glucan particles that broke the interface property of solution. The effect of NaCl concentration on foaming ability and foam stability of the GE was shown in Table 1. The addition of NaCl could enhance simultaneously the foaming ability and foam stability of GE. Likely, Na^+ could weaken the electrostatic repulsion between and within β -glucan molecules, and promote stronger intermolecular interaction thereby enlarging their steric configuration, which was very helpful for the formation and stability of foam.

Most of carbohydrates cannot perform great foaming ability due to strong hydrophilic property. Nevertheless, some polysaccharide can achieve it because of high molecular cross-linking degree and molecular weight. It was reported that oat containing blistery polysaccharide added into coffee could successfully improve the fine and smooth taste by promoting the formation of foam [10]. Additionally, in the production of beer, hydrophilic polysaccharide such as β -glucan from barley could be employed to improve the foam stability [17]. Meanwhile, β -glucanase exogenous enzymes are of common use for solving filterability issues caused by the addition of β -glucans in brewing [18].

Based on the results of the processing characteristics study above, GE was considered to have a good processing performance, and it is promising to be applied in baked foods, dairy products, health-care products, beers, and so on.

3.4. Anti-Digestibility of GE

Prebiotics intake is inevitable of going through human mouth and stomach. Therefore, it needs to resist the hydrolysis of salivary amylase and gastric acid, and then arrive at intestinal tract to participate in metabolism of probiotics groups.

The normal pH of human mouth is 6–7, but it changes temporarily when intaking different kinds of food [19]. Therefore, the anti-digestibility assay of GE to α -amylase was implemented at pH 4–8 (37 °C), and the result was shown in Table 2. The DHs of GE were all low during these pHs. When the pH was 4 and 5, almost no hydrolysis of GE was observed. When the pH was 6, 7, and 8, there was only a slight increase of DH within initial 2 h ($p < 0.05$), and then a balance occurred. The DH was the highest at pH 7, but it was only 2.72%, indicating an excellent anti-digestibility of GE to α -amylase.

Table 2. Hydrolysis of GE under the addition of α -amylase and simulated gastric acid (SGA), respectively.

pH	DH of GE Acted with α -Amylase (%)				pH	DH of GE Acted with SGA (%)			
	t = 0 h	t = 2 h	t = 4 h	t = 6 h		t = 0 h	t = 2 h	t = 4 h	t = 6 h
4	0	0	0	0	1	0	4.05 ± 0.24	4.95 ± 0.26	4.95 ± 0.34
5	0	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.01	2	0	2.21 ± 0.17	2.72 ± 0.07	2.72 ± 0.15
6	0	0.74 ± 0.04	0.82 ± 0.06	0.82 ± 0.07	3	0	0.74 ± 0.05	0.82 ± 0.05	0.82 ± 0.09
7	0	2.21 ± 0.12	2.72 ± 0.12	2.72 ± 0.14	4	0	0.31 ± 0.02	0.31 ± 0.03	0.31 ± 0.04
8	0	1.51 ± 0.08	1.75 ± 0.14	1.75 ± 0.09	5	0	0	0	0

The normal pH of human gastric acid is about 2, but it rises to 3–5 in 2 h after meal for the dilution of gastric acid. The food remains in stomach for 4–6 h [20]. Thus, the anti-digestibility of GE to simulated gastric acid at pH 1–5 (37 °C) was investigated, and the result was shown in Table 2. The DHs of GE at different pHs were diverse. It was the highest at pH 1, but only 4.95%, showing a strong resistance of GE to acid. As a result, it was indicated that GE has a good anti-digestibility to gastric acid.

Based on the results above, GE can resist the hydrolysis of salivary amylase and gastric acid, and go through mouth, stomach, and then enter successfully into intestinal tract, meeting various probiotics in these digestive organs.

3.5. Improving Growth of Probiotics

Lactobacillus bulgaricus and *Bifidobacterium adolescentis* are two classical kinds of probiotics in human intestinal tract, so effect of GE on the growth of the two probiotics was studied. Results showed that the OD₆₀₀ (X) of the culture medium was linearly correlated with the strain biomass (Y), and the regression equation was

$$Y = 17.24X - 0.04 \tag{7}$$

The correlation coefficient R² was 0.9947 for *Lactobacillus bulgaricus*. The strain biomass (N, OD₆₀₀) and culture time (t, h) were nonlinear fitted by Logistic Model, whose equation was

$$Y = A / \{1 + \exp[4\mu_{\max}(\lambda - t) / A + 2]\} \tag{8}$$

Y was log N_t/N₀, in which N_t was real-time strain biomass (OD₆₀₀), and N₀ was initial strain biomass (OD₆₀₀). A, the relative maximum concentration of strain, was log N_{max}/N₀, in which N_{max}

was the maximum strain biomass (OD_{600}). μ_{max} (h^{-1}) is the specific growth rate of strain, and λ is the hysteresis period. Meanwhile, the equation was

$$Y = 35.73X - 3.47 \quad (9)$$

R^2 was 0.9944 for *Bifidobacterium adolescentis*. Specifically, the effects of the addition of GE with different concentrations of 0–2.0 mg/mL on the growth of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis* are shown in Figure 2A,B. The strains biomass and growth rate of the two probiotics increased with GE concentration. When the culture time reached 22 h, the OD_{600} with adding 1.6 mg/mL GE to *Lactobacillus bulgaricus* was 1.370 ± 0.017 , while the OD_{600} without adding any GE was only 1.141 ± 0.026 ($p < 0.05$). Similarly, the OD_{600} with adding 1.6 mg/mL GE to *Bifidobacterium adolescentis* was 1.718 ± 0.028 , while the OD_{600} without the addition of GE was only 1.506 ± 0.019 ($p < 0.05$). Hence, the addition of GE could effectively boost the strains biomass of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis* at the same time.

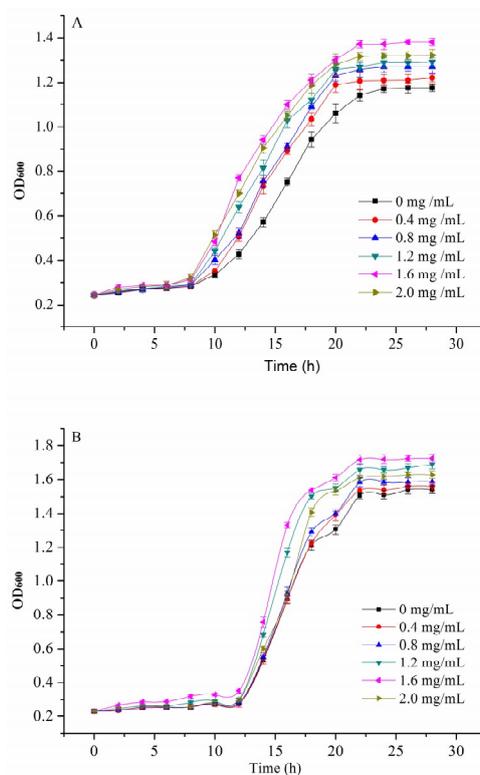


Figure 2. Effect of the addition of GE on the growth of *Lactobacillus bulgaricus* (A) and *Bifidobacterium adolescentis* (B).

Through non-linear fitting of a logistic equation, the initial strain biomass (N_0), the maximum strain biomass (N_{max}), the maximum specific growth rate (μ_{max}) and the arrival time (t_{max}) under different GE concentrations were calculated and listed in Table 3. Both the N_{max} and μ_{max} of *Lactobacillus bulgaricus* grew overall but t_{max} declined with the increase of GE concentration. As GE concentration increased from 0 mg/mL to 1.6 mg/mL, the N_{max} and μ_{max} climbed up from 1.196 ± 0.059 (OD_{600}) to 1.391 ± 0.029 (OD_{600}), and $0.098 \pm 0.005 h^{-1}$ to $0.118 \pm 0.007 h^{-1}$, respectively. A further increase of GE concentration made the N_{max} and μ_{max} keep steady. Similarly, as for *Bifidobacterium adolescentis*, the N_{max} and μ_{max} were changed from 1.537 ± 0.036 (OD_{600}) to 1.710 ± 0.017 (OD_{600}), and $0.183 \pm 0.006 h^{-1}$ to $0.283 \pm 0.013 h^{-1}$, respectively. As the concentration further increased, the N_{max} and μ_{max} decreased a little. Therefore, GE was able to improve the growth of the two probiotics. Zhao indicated that the addition of different β -glucans—from barley, seaweed, mushroom, etc.—could increase observably the

populations of *Bifidobacterium adolescentis* during 24 h of in vitro fermentation [21]. It was reported that β -glucan from oat bran also made a positive contribution to the growth of *Bifidobacterium adolescentis* and *Lactobacillus bulgaricus* under different fermentation conditions, respectively [22,23].

Table 3. Growth dynamic parameters of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis*.

β -Glucan (mg/mL)	<i>Lactobacillus bulgaricus</i>			
	N_0 (OD ₆₀₀)	N_{max} (OD ₆₀₀)	t_{max} (h)	μ_{max} (h ⁻¹)
0	0.264 ± 0.011	1.196 ± 0.059	14.98 ± 0.247	0.098 ± 0.005
0.4	0.260 ± 0.014	1.235 ± 0.024	13.58 ± 0.356	0.111 ± 0.006
0.8	0.263 ± 0.013	1.295 ± 0.013	13.58 ± 0.236	0.111 ± 0.008
1.2	0.259 ± 0.008	1.310 ± 0.018	12.73 ± 0.121	0.112 ± 0.009
1.6	0.261 ± 0.006	1.391 ± 0.029	12.16 ± 0.147	0.118 ± 0.007
2.0	0.261 ± 0.015	1.340 ± 0.023	12.16 ± 0.054	0.112 ± 0.005
β -Glucan (mg/mL)	<i>Bifidobacterium adolescentis</i>			
	N_0 (OD ₆₀₀)	N_{max} (OD ₆₀₀)	t_{max} (h)	μ_{max} (h ⁻¹)
0	0.240 ± 0.008	1.537 ± 0.036	15.83 ± 0.214	0.183 ± 0.006
0.4	0.242 ± 0.006	1.566 ± 0.047	15.83 ± 0.175	0.195 ± 0.012
0.8	0.245 ± 0.011	1.594 ± 0.032	15.55 ± 0.236	0.206 ± 0.014
1.2	0.251 ± 0.010	1.666 ± 0.025	15.55 ± 0.147	0.234 ± 0.008
1.6	0.259 ± 0.008	1.710 ± 0.017	14.70 ± 0.089	0.283 ± 0.013
2.0	0.253 ± 0.014	1.640 ± 0.028	14.98 ± 0.072	0.263 ± 0.007

N_0 , initial strain biomass. N_{max} , maximum strain biomass. t_{max} , arrival time. μ_{max} , maximum specific growth rate.

Fructooligosaccharide is commonly regarded as an excellent prebiotic factor, so it was chosen in our study to compare with GE on the ability of improving the growth of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis* [24], and the results were shown in Figure 3A,C.

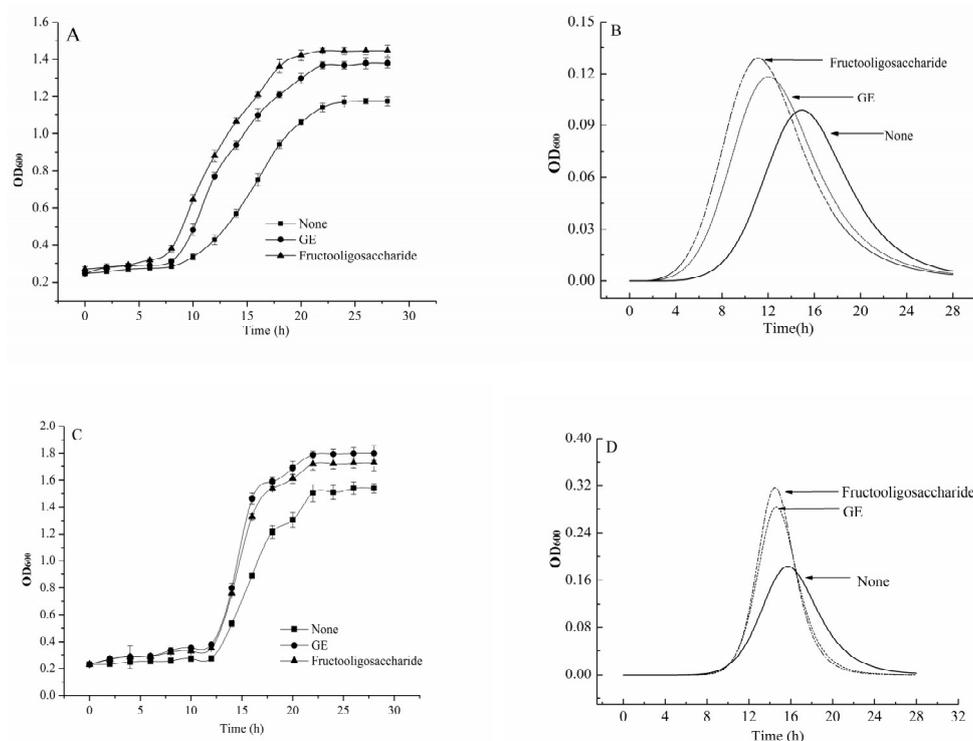


Figure 3. Evolution profiles of strains biomass (A,C) and specific growth rate (B,D) of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis* involving in the addition of GE (20 mg/mL) and fructooligosaccharide (20 mg/mL) with culture time.

The strains biomass of the two probiotics increased with culture time. When the time reached 22 h, the biomass peaked. Noteworthy, the addition of fructooligosaccharide could promote the growth of *Lactobacillus bulgaricus* more than GE, while GE could improve the growth of *Bifidobacterium adolescentis* more than fructooligosaccharide ($p < 0.05$). Maximum specific growth rate was shown in Figure 3B,D, when GE, and more so fructooligosaccharide was added to *Lactobacillus bulgaricus*, its maximum specific growth rate appeared earlier than negative control ($p < 0.05$). As for *Bifidobacterium adolescentis*, although the addition of GE or fructooligosaccharide could enhance maximum specific growth rate, the times to peak were close one another. Therefore, the addition of GE or fructooligosaccharide could significantly improve the growth of both *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis*. The improving abilities of GE were close to that of fructooligosaccharide.

4. Conclusions

GE was proved to have excellent processing and prebiotics characteristics. Its heat stability is remarkable, as almost no degradation was observed at 220 °C, while pH stability was also great, especially at neutral or alkaline condition. Meanwhile, its solubility increased with temperature, and a good solubility of 0.91 g/100 g was observed at high temperature (100 °C). Moreover, it also demonstrated excellent foaming ability and foam stability at low temperature (≤ 40 °C) and at neutral or alkaline condition. GE exhibited strong anti-digestibility to α -amylase and simulated gastric acid, and it could improve the growth of *Lactobacillus bulgaricus* and *Bifidobacterium adolescentis*, close to the level observed with fructooligosaccharide. Therefore, the GE from highland barley could be applied potentially as a prebiotic factor into the processing of baked food, beer, and so on. Meanwhile, this work provided a reference for the β -glucan extraction processes from highland barley. Furthermore, scalability of the processing methods and sensitivity analysis of the GE in industrial manufacture should be explored in future.

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