Effects of ultrasound-assisted H\textsubscript{2}O\textsubscript{2} on the solubilization and antioxidant activity of yeast β-glucan

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\textbf{A B S T R A C T}

Yeast β-glucan (YG) possess an extensive range of biological activities, such as the inhibition of oxidation, but the poor water solubility of macromolecular YG limits its application. In this study, through the combined degradation of ultrasonic waves and H\textsubscript{2}O\textsubscript{2}, and the optimization of the main process parameters for solubilizing YG by response surface methodology (RSM), a new product of YG\textsubscript{UH} was generated. The molecular weight, structural characteristics and degradation kinetics before and after solubilization were evaluated. The results showed that under these conditions, ultrasound-assisted H\textsubscript{2}O\textsubscript{2} increased the solubility (from 13.60 % to 70.00 %) and reduced molecular weight (from 6.73 × 10\textsuperscript{6} Da to 1.22 × 10\textsuperscript{6} Da). Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), Congo red (CR), scanning electron microscopy (SEM) revealed that ultrasound-assisted H\textsubscript{2}O\textsubscript{2} increased the conformation’s flexibility greatly, without changing the main structure of YG. More importantly, solubilization of YG improved free radical scavenging activity with YG\textsubscript{UH} exhibiting the highest levels of DPPH and ABTS\textsuperscript{+} free radical scavenging activity. These results revealed that ultrasound-assisted H\textsubscript{2}O\textsubscript{2} degradation could be a suitable way to increase the solubility of YG for producing value-added YG.

1. Introduction

Yeast β-glucan is a natural polysaccharide extracted from yeast cell wall [1], which is composed of a linear backbone of α-glucose connected by β-1,3 bonds and branches of β-1,6 bonds [2], and has higher biological activity than other β-glucans [3]. More and more studies have proved that yeast β-glucan has good biological activities, such as antivirus [4], enhancing immunity [5], anti-cancer [6], lowering cholesterol [7], anti-tumor [8], improve the intestinal flora [9] and other physiological functions. In recent years, yeast β-glucan has been widely used in the functional food, cosmetics and pharmaceutical industries [10,11]. However, the tight triple helix structure and high molecular weight of yeast β-glucan leads to its insolubility, which affects its biological activities and limits its application range. Therefore, it is of great significance to improve the biological activity by using appropriate methods to degrade polysaccharides in order to increase their solubility.

The biological activity of polysaccharides is affected by its molecular weight, structure and conformation [12]. Currently, the commonly used degradation methods of polysaccharides include chemical degradation, physical degradation and biological degradation [13]. Chemically degrading is easy to destroy the original structure and groups of polysaccharides and causes great environmental pollution [14]. The requirement of physical degradation equipment is relatively high [15,16]. The enzymatic degradation method is limited for the enzyme’s sensitivity to the surrounding environment and poor stability, furthermore various factors in the solution and the environment can significantly affect the physiology of the enzyme, activity and catalytic reaction rate [17].

Oxidative degradation by H\textsubscript{2}O\textsubscript{2} has attracted much attention because of its non-toxicity and low cost. The free radical catalyzed H\textsubscript{2}O\textsubscript{2} method is often used to degrade Tremella fuciformis polysaccharide, and the oxidative degradation does not change the main structure of Tremella fuciformis polysaccharide with lower molecular weight possessing higher antioxidant activity [18,19]. Wang et al [20] used free radicals to degrade a new glycosaminoglycan in Holothuria mexicana (HmG), and found that the degradation rate had a positive correlation with peroxide concentration without destroying the primary structure. Yet, the H\textsubscript{2}O\textsubscript{2} concentration used in the process determines the rate of degradation to a lower molecular weight. In late research, modified polysaccharides with higher bioactivity could be obtained by combining different degradation
2. Materials and methods

2.1. Materials and reagents

The yeast β-glucan content extracted from Candida utilis in our laboratory (Shanghai, China) exceeded 82.65%. Hydrogen peroxide (H₂O₂) was purchased from Aladdin Biochemical Technology Co., Ltd., China. All other chemicals and reagents were of analytical reagent grade and used without further purification.

2.2. Preparation of YG

The polysaccharides were extracted from Candida utilis using high pressure steam synergistic enzymatic method according to our previous report [24]. The extract was prepared with a 0.02 mol/L pH 7.5 sodium phosphate buffer to prepare a yeast cell suspension with solid–liquid ratio of 10%, 50 g of glass beads with a diameter of about 0.3 and 0.4 cm were added, and high-pressure steam treatment was performed at 121 °C for 2 h, cooled to room temperature, centrifuged at 8000 rpm for 5 min, and the precipitate was washed twice. Add 2% neutral protease weight, dried at 105 °C to constant weight, and weighed repeatedly until the mass difference between the two weights did not exceed 2 mg, then the supernatant was put into an aluminum box dried to constant weight. In this study, we used ultrasound-assisted H₂O₂ to solubilize YG, optimized its solubilization conditions by response surface methodology, characterized the structures, and evaluated their antioxidant activity. The purpose of this study was to investigate the effect of ultrasound-assisted H₂O₂ degradation on the structure of YG, as well as the degradation kinetic model and its possible mechanism of solubilization of YG.

2.3. Polysaccharide degradation experiment

2.3.1. Solubility of YG from Candida utilis

The solubility was determined according to the previous method [25]. Briefly, the YG solution was centrifuged at 8000 rpm for 5 min, then the supernatant was put into an aluminum box dried to constant weight, dried at 105 °C to constant weight, and weighed repeatedly until the mass difference between the two weights did not exceed 2 mg, keeping the final constant weight value. Calculate the solubility according to the following formula.

\[ \text{Solubility} = \frac{W}{(C \times V)} \]  

Where W (g) is the weight of the dried product of the supernatant, C (g/mL) is the initial solution concentration, and V (mL) is the volume of the supernatant.

2.3.2. Response surface optimization experiment

On the basis of single factor experiment, the reaction conditions were optimized by Box-Behnken design (BBD) with three factors and three levels. The Box-Behnken Design (BBD) principle of Design Expert 8.0.5 software was used to design and fit the response surface methodology.

2.4. Evaluation of intrinsic viscosity and viscosity-average molecular weight

The intrinsic viscosity \( [\eta] \) of polysaccharides was measured according to the previously reported method [26]. Measured by Ubbelohde viscometer at 25 ± 0.5 °C. The difference in each measurement is <0.2 s, and the average value was taken as the outflow time. From the outflow time, the relative viscosity \( \eta_r \) and the specific viscosity \( \eta_sp \) were obtained from the Eqs. (2) and (3). The intrinsic viscosity value \( [\eta] \) can be calculated according to Eqs. (4).

\[ \eta_r = t/t_0 \]  
\[ \eta_sp = \eta_0 - 1 \]  
\[ [\eta] = \sqrt{2\eta_{sp} - \ln\eta_r/c} \]  

Where \( t \) and \( t_0 \) are respectively the flowing times or relative viscosity of the polysaccharide solution and the solvent, \( \eta_r \) is the relative viscosity, \( \eta_{sp} \) is the incremental viscosity, and \( c \) is the concentration of polysaccharide (g/mL).

2.5. Fitting of degradation kinetics model

According to the first-order and second-order reaction kinetics, the kinetic model of YG degradation with ultrasound-assisted H₂O₂ treatment was evaluated [27]. It is expressed as Eqs. (5) and (6), which are:

\[ \ln(M_t/M_0) = -kt \]  
\[ 1/M_t - 1/M_0 = kt \]  

where \( M_0 \) and \( M_t \) represent the viscosity average molecular weight at the beginning of the reaction and at reaction time, respectively; \( k \) is the degradation rate constant (mol·g⁻¹·min⁻¹); \( t \) is the degradation time (min).

2.6. Structure characterization

2.6.1. Molecular weight measurement

The \( M_w \) of β-glucan content before and after solubilization was determined by the coupling technique of High performance size exclusion chromatography (HPSEC), and multiangle light scattering (MALLS) connected with differential refractive index detector (HPSEC-MALLS-RID, Wyatt, USA). Chromatographic conditions: ShodexOHpak SB HQ 804 and 806 were connected in series, the mobile phase was 0.05 M NaNO₃ solution, the flow rate was 0.5 mL/min, the column temperature was 35 °C, and the injection volume was 100 μL.

2.6.2. Fourier transform infrared spectroscopy (FTIR) analysis

The dried polysaccharide sample was added into agate mortar containing KBr, evenly ground, compressed and scanned by FTIR (Bruker, Germany) at the wavelength range of 4000–500 cm⁻¹.

2.6.3. Nuclear magnetic resonance spectroscopy (NMR) analysis

Dissolve 30 mg of β-glucan in 0.6 mL of d₆-DMSO at 80 °C. Each β-glucan sample dissolved in d₆-DMSO was subjected to ¹H and ¹³C NMR analyses.

2.6.4. Congo red test

Congo red solution (80 μmol/L, 1 mL) was mixed with 2 mL of
3.1. Optimization of ultrasound-assisted H₂O₂ solubilization of YG

Generally, time, reaction temperature, H₂O₂ concentration, and ultrasonic power significantly affect the solubility of YG. In this study, reaction temperature of 60 °C was set as a fixed factor to determine the optimal conditions for solubilization of YG, as this condition provided the highest solubility by determining the solubility of YG according to a preliminary study (data not shown). Thus, three independent variables, namely time, H₂O₂ concentration and ultrasonic power, were established in response surface methodology.

The reaction time (A), ultrasonic power (B) and hydrogen peroxide concentration (C) were selected as three independent variables. An additional 17 BBD experiments were performed to optimize the three independent variables. An analysis of variance (ANOVA) was used to determine the statistical significance, and the value of P < 0.05 was considered to be statistically significant.

3. Results and discussions

3.1. Optimization of ultrasound-assisted H₂O₂ solubilization of YG

All experiments were conducted three times, and the data were expressed as means ± standard deviation. Data analysis was performed using Origin 9.0 software (Origin Lab Corporation, Northampton, Mass., USA). One-way analysis of variance (ANOVA) was used to determine the statistical significance, and the value of P < 0.05 was considered to be statistically significant.

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the two factors, and the circular shape is the opposite. The interaction was also significant because the response surface curve is steeper [32]. Only the interaction term of response time and ultrasonic power (AB) was significant, as shown in Fig. 1, which was consistent with Table 2’s findings. The effects of reaction time (A) and ultrasonic power (B), as well as their interactions, on the solubility of the polysaccharide are shown in Fig. 1A. The solubility of the YG increased rapidly with increasing reaction time (A) until 3 h, after which it began to decline. This might be related to the acceleration of molecular motion and response as time passes from 1 h to 3 h. However, after a certain time of reaction, the free radical content no longer increases and the polysaccharide degradation reaction was not significant [33]. The solubility
of YG rose fast with an increase in hydrogen peroxide concentration until 24 %, then increased marginally, according to Fig. 1 B and C. From 15 to 24 %, the hydrogen peroxide concentration exhibited a strong reaction. The solubility then gradually reduced over time. As a consequence, the ANOVA findings indicated that the hydrogen peroxide concentration had the greatest impact on the response value.

According to the solubility measurement, the main effect of ultrasound-assisted H\textsubscript{2}O\textsubscript{2} on YG was that ultrasonic excitation of H\textsubscript{2}O\textsubscript{2} produced active radicals. More active radicals such as OH\textsuperscript{⋅} promoted the degradation of YG. High concentration of H\textsubscript{2}O\textsubscript{2} was not required for the effective solubilization of YG, because the newly formed hydroxyl radicals might be damaged by excessive H\textsubscript{2}O\textsubscript{2}.

### 3.2. Optimization of degradation condition and its validation

According to the results of the optimization and fitting of the test, the optimal conditions can be obtained: the reaction time was 4 h, the ultrasonic power was 2.80 W/mL, and the hydrogen peroxide concentration was 23.61 %. Adjust according to the actual situation: the reaction time was 4 h, the ultrasonic power was 3 W/mL, and the hydrogen peroxide concentration was 24 %. After model optimization, the solubility of YG reached 70.00 %, indicating that the obtained model has certain practical guiding significance.

Ultrasound-assisted H\textsubscript{2}O\textsubscript{2} treatment was used to improve the solubility of YG. Ultrasonic wave could promote the oxidative degradation of H\textsubscript{2}O\textsubscript{2} due to the following two aspects: first, ultrasonic wave could promote the further dissolution of YG and the shearing action caused by the relative motion of solvent molecules and YG molecules would reduce the viscosity of the solution and increase the solubility [34,35]; Second, ultrasonic “cavitation” caused YG to rupture or generate micropores, which promoted the decomposition of H\textsubscript{2}O\textsubscript{2}. The generated -OH could destroy glycosidic bonds and reduce their molecular weight, thus increasing the solubility [36].

#### 3.3. Degradation kinetics model

The kinetic curves and related kinetic parameters of the first- and second-order reactions are shown in Fig. 2 and Table 3 to evaluate the degradation effect of YG by ultrasound-assisted H\textsubscript{2}O\textsubscript{2} treatment. It was shown that the first-order kinetic model had a higher correlation coefficient (R\textsuperscript{2}) than the second-order kinetic model, which was more suitable for fitting the degradation of YG. The intrinsic viscosity[\eta] of YG decreased continuously during 2 h of ultrasound-assisted H\textsubscript{2}O\textsubscript{2} treatment (from 176.54 to 83.59 cm\textsuperscript{3}/g) (Supplementary Table 1).

### Table 3
Degradation kinetic equation and parameters.

| Kinetics Equation | Kinetics Equation | k | R\textsuperscript{2} |
|------------------|------------------|---|-----------------|
| ln (M\textsubscript{t}/3567595.19) − 8.72 t | 1/M\textsubscript{t}/3567595.19 − 0.7478 t | 8.72 | 8.72 | 0.945 | 0.74 | 0.862 |

### Table 4
\(\beta\)-glucans M\textsubscript{w}, M\textsubscript{n}, M\textsubscript{z}, and M\textsubscript{w}/M\textsubscript{n} of YG and YG\textsubscript{UH} solubilization.

| \(\beta\)-glucans | M\textsubscript{w} (×10\textsuperscript{6} Da) | M\textsubscript{n} (×10\textsuperscript{6} Da) | M\textsubscript{w}/M\textsubscript{n} | M\textsubscript{z} (×10\textsuperscript{6} Da) | M\textsubscript{w}/M\textsubscript{n} |
|------------------|------------------|------------------|------------------|------------------|------------------|
| YG               | 6.73 ± 1.02 %    | 6.55 ± 1.01 %    | 1.03 ± 1.43 %    | 9.60 ± 1.17 %    | 6.93 ± 2.29 %    |
| YG\textsubscript{UH}1 | 3.38 ± 1.27 %    | 3.23 ± 1.26 %    | 1.03 ± 1.43 %    | 5.33 ± 1.13 %    | 3.56 ± 2.84 %    |
| YG\textsubscript{UH}2 | 3.19 ± 1.42 %    | 2.90 ± 1.45 %    | 1.03 ± 1.43 %    | 4.05 ± 1.28 %    | 3.545 ± 3.15 %   |
| YG\textsubscript{UH}3 | 2.04 ± 1.68 %    | 1.83 ± 1.80 %    | 1.12 ± 2.46 %    | 2.95 ± 1.58 %    | 2.29 ± 3.67 %    |
| YG\textsubscript{UH}4 | 1.91 ± 1.68 %    | 1.72 ± 1.79 %    | 1.11 ± 2.45 %    | 2.19 ± 1.60 %    | 2.14 ± 3.66 %    |
| YG\textsubscript{UH}5 | 1.22 ± 2.56 %    | 1.09 ± 3.13 %    | 1.12 ± 4.04 %    | 1.64 ± 1.89 %    | 1.37 ± 5.30 %    |
early stage of treatment, the intrinsic viscosity dropped sharply to a very low level, and the decrease in intrinsic viscosity was equivalent to the decrease in the average relative molecular mass. Therefore, it can be considered that the ultrasound-assisted $H_2O_2$ method led to the degradation of YG. The reduction in viscosity is not only related to polymer degradation (polymer chain scission or glycosidic bond scission), but also to polymer breakdown. The above results indicated that ultrasound-assisted $H_2O_2$ treatment of polysaccharides was helpful to break the glycosidic bonds, resulting in the decrease of $M_\eta$ and the formation of some small molecular polysaccharides [37]. The production of OH· was related to the content of $H_2O_2$ [38], ultrasonic could promote $H_2O_2$ to generate free radicals (OH·), and then OH· attacked the H atom of the C bond, leading to the formation of carbon-centered free radicals in polysaccharides. A series of free radical reactions occurred in polysaccharides, leading to the breakage of glycosidic bonds and increasing the solubility of YG. Free radicals were slowly produced and gradually disappeared with the decrease of $H_2O_2$ content in the late stage, so $M_\eta$ almost remained unchanged.

3.4. Molecular weight

As the degradation time increased, the molecular weight of YG decreased from $6.73 \times 10^6$ Da to $1.22 \times 10^6$ Da. The molecular weights of a series of degraded polysaccharides are shown in Table 4. The molecular weight of YG decreases with time in all $\beta$-glucan samples. By controlling the reaction conditions, YG with molecular weights ranging from $1.22 \times 10^6$ Da to $3.38 \times 10^6$ Da can be prepared. Therefore, by selecting reaction conditions, yeast $\beta$-glucans of a specific molecular weight range can be prepared [39]. The decrease of molecular weight confirmed that ultrasound-assisted $H_2O_2$ could degrade YG. The bubble collapse caused by ultrasonic induced cavitation generated shear force and formed shock wave, which promoted the decomposition of -OH by $H_2O_2$ and led to the destruction of intramolecular and intermolecular bonds in YG, cutting the glycosidic bond of YG, leading to the reduction of its molecular weight.

3.5. Fourier transform infrared spectroscopy

The spectra of YG (a), YG$_{UH}$ (b) are shown in Fig. 3 in the range of
The FT-IR spectra of YG and YG\textsubscript{UH} were not significantly different. 3430 cm\(^{-1}\) was the stretching vibration peak of OH in sugar compound C-OH [40], the absorption peak near 1650 cm\(^{-1}\) was the C=O asymmetric tensile vibration in –CHO, and the absorption peak in the range of 1400–1200 cm\(^{-1}\) further indicated that the sample was a carbohydrate [41]. The characteristic peak near 2930 cm\(^{-1}\) was the characteristic absorption peak of C–H on saturated carbon [42]. The absorption peak at 1380 cm\(^{-1}\) can indicate that the YG was still connected by β-(1–3) bonds after degradation [43]. The two polysaccharides had similar characteristic absorption peaks in the range of 4000–500 cm\(^{-1}\), indicating that ultrasound-assisted H\textsubscript{2}O\textsubscript{2} degradation of YG did not affect the main functional groups of polysaccharides.

3.6. Nuclear magnetic resonance spectroscopy

The chemical structures of YG before and after solubilization were preliminarily identified by \(^1\)H- and \(^{13}\)C NMR. The \(^{13}\)C NMR spectrum of YG and YG\textsubscript{UH} are shown in Fig. 4. As shown in the Fig. 4a, YG had six strong signal peaks at \(\delta\) 103.55, 73.31, 76.83, 86.76, 70.30 and 68.87 ppm, respectively, which were attributed to the C-1, C-2, C-3, C-4, C-5 and C-6 formants on the β-glucan ring, consistent with the structure of YG in the previously reported [44], indicating that YG was a macro-molecular polysaccharide connected by β-(1–3) glycosidic bond [45]. Several weak signal peaks at \(\delta\) 69–70 ppm, \(\delta\) 73–76 ppm, indicating that there were β-(1–6) glycosidic-linked branches in the YG molecule, but due to the weaker signal peaks, the number of branches was low [46]. In Fig. 4b, the six strong signal peaks at \(\delta\) 103.52, 73.32, 76.81, 86.70, 70.26 and 68.89 ppm of the YG\textsubscript{UH} can be assigned to C-1, C-2, C-3, C-4, C-5 and C-6 [47], indicating that the solubilization of YG by ultrasound-assisted H\textsubscript{2}O\textsubscript{2} method did not damage its main chain structure, and it was still a glucan molecule linked by β-(1–3) glycosidic bonds [48]. Different from the infrared spectrum of YG, YG\textsubscript{UH} showed new signal peaks at \(\delta\) 74.33 ppm, 76.99 ppm and 103.73 ppm, indicating that a carbon position in the sugar molecule has been replaced. Therefore, the basic skeleton of the modified and solubilized YG was not changed, and the ultrasound-assisted H\textsubscript{2}O\textsubscript{2} solubilization of YG might act on the 1,6-glycosidic bond in the side chain.

In the \(^1\)H NMR spectrum, the α- or β-configuration of the glycosidic bond is mainly determined by the proton peak on the anomeric C-1. If the chemical shift of the anomeric proton is \(\delta\) 4.00–5.00 ppm, it is generally considered to be the β-type. Conversely, if the chemical shift \(\delta\) 5.00–6.00 ppm, it is considered to be α type [49]. According to Fig. 5a,
2.50 ppm was the signal peak of DMSO, the H-1 chemical shift of YG was $\delta$ 4.51 ppm, and it can be seen that most of the chemical shifts of all the anomeric protons were in the range of 3.00–5.00 ppm, which was a typical polysaccharide absorption peak signal [50]. The chemical shift at $\delta$ 3.34 ppm represents H-2, 4, and 5 of the 1,3-linked $\beta$-D-Glcp. In addition, the chemical shift of yeast $\beta$-glucan at $\delta$ 3.70 ppm was the proton signal of H-6. As shown in Fig. 5b, H-4 and H-5 protons of YG show slight high-field shift, and it was possible that the combination of hydroxyl radical and hydrogen atom destroys the pre-existing intramolecular and intermolecular hydrogen bonds of $\beta$-glucan and thus increases its solubility.

3.7. Congo red

Congo red is an acid dye which can combine with triple helix polysaccharide in a certain concentration of NaOH solution, and the maximum absorption wavelength ($\lambda_{\text{max}}$) of Congo red-polysaccharide complex was red-shifted [51]. In Fig. 6, the red shift of the Congo red-polysaccharide complex formed by the polysaccharide sample and Congo red solution in 0.05–0.50 mol/L NaOH was observed. With the increase of NaOH concentration, the maximum absorption wavelength of Congo red-polysaccharide complex was gradually decreased, indicating that YG and YG$_{UH}$ had triple helix structure. The stability of the triple helical structure of polysaccharides in solution mainly depends on intramolecular and intermolecular hydrogen bonds. The breaking of hydrogen bonds will destroy the triple helix structure [52]. The triple helix structure of YG was not damaged by ultrasound-assisted H$_2$O$_2$ degradation.

3.8. Scanning electron microscope observation

As shown in Fig. 7, the morphology and structure of YG were significantly different before and after solubilization. Before treatment, YG existed in the form of compact aggregated clusters with rough surface and honeycomb-like structure. Fig. 7b was a SEM image of YG after degradation, which was looser and has smaller fragments than YG before treatment. The results suggested that ultrasound-assisted H$_2$O$_2$ degradation could hinder the aggregation of YG macromolecules and lead to microstructural changes. This might be due to the generation of more hydroxyl radicals by ultrasound-assisted H$_2$O$_2$, which led to the breakage of YG glycosidic bond, and the decrease of molecular weight also confirmed this.

3.9. In vitro antioxidant activities of YG

The in vitro antioxidant activity of YG before and after solubilization was studied by determining the DPPH radical and ABTS$^+$ radical scavenging activity (Fig. 8). As shown in Fig. 8a, with the increase of polysaccharide concentration, the scavenging rate of YG and YG$_{UH}$ on DPPH free radical showed an upward trend. At the polysaccharide concentration of 3.0 mg/mL, the DPPH radical scavenging activities of YG and YG$_{UH}$ were 58.50 % and 80.10 %, respectively. At 3.0 mg/mL, the DPPH radical scavenging rate of V$_C$ reached 95.67 %, and the scavenging rate remained basically unchanged with the increase of concentration. Within the concentration range of 0.4–2.0 mg/mL, the DPPH scavenging activity of YG$_{UH}$ solubilized by ultrasound-assisted H$_2$O$_2$ method was significantly higher than that of YG. This might be due to the degradation of YG by ultrasound-assisted H$_2$O$_2$, resulting in the decrease of the molecular weight of YG, and more hydroxyl groups exposed to the solution, thus providing more hydrogen or electrons [53].

As shown in Fig. 8b, the removal efficiency of ABTS$^+$ by the solubilized yeast $\beta$-glucan was significantly better than that of the original YG. The results showed that the removal efficiency was enhanced with the increase of polysaccharide concentration. At a polysaccharide concentration of 2.0 mg/mL, the clearance of ABTS$^+$ by YG and YG$_{UH}$ was 34.40 % and 44.00 %, respectively. At 1.2 mg/mL, the ABTS$^+$ radical...
scavenging activity of VC was 95.50 %, and there was no significant change after that. It was possible that low molecular weight polysaccharides contain more free hydroxyl groups, which might be beneficial to improve their antioxidant activity. The antioxidant activity of YG in vitro was higher than that of YG, which may be due to the better water solubility of degraded YG, so it was easier to contact free radicals.

4. Conclusion

In this study, it was discovered that ultrasound-assisted H$_2$O$_2$ treatment was a simple and effective method to increase YG solubility and reduce molecular weight. When the ultrasonic intensity was 3 W/mL, the reaction time was 4 h, and the hydrogen peroxide concentration was 24 %, the solubility of YG reached a maximum value. The effect of ultrasound-assisted H$_2$O$_2$ degradation of YG conformed to the first-order kinetic model, which can believe that ultrasound-assisted H$_2$O$_2$ can cause degradation of YG. During the degradation of YG, ultrasonic accelerated the decomposition of H$_2$O$_2$, which resulted in the breakage of YG glycosidic bonds, hindered the aggregation of macromolecules, reduced the molecular weight and improved the solubility, but the primary structure did not change obviously. The antioxidant activity of YG was significantly increased after solubilization. In summary, this study provided an effective method to increase the solubility of yeast β-glucan which would further contribute to the development and utilization of the biological function of yeast β-glucan.

CRediT authorship contribution statement

Xia Ma: Conceptualization, Formal analysis, Supervision, Writing – review & editing. Lin Dong: Conceptualization, Data curation, Formal analysis, Writing – review & editing. Yan He: Investigation, Methodology, Software, Visualization, Writing – original draft. Shiwen Chen: Investigation, Methodology, Software, Visualization, Writing – original draft.

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.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

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