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Ultrasonic effects on the degradation kinetics, preliminary characterization and antioxidant activities of polysaccharides from *Phellinus linteus* mycelia

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ABSTRACT

In this study, a high-molecular-weight polysaccharide PL-N isolated from the alkaline extract of *Phellinus linteus* mycelia was degraded by ultrasound. Results showed that ultrasound treatment at different ultrasonic intensities decreased the intrinsic viscosity and molecular weight of PL-N, as well as narrowed the molecular weight distribution. A larger reduction in intrinsic viscosity and molecular weight was caused by a higher ultrasonic intensity. The degradation kinetics model was fitted to $(1/M_t - 1/M_0) = k \cdot t$, and the reaction rate constant (k) increased with increasing ultrasonic intensity. Ultrasound degradation did not change the primary structure of PL-N, and scanning electron microscopy analysis indicated that the morphology of the original PL-N was different from that of degraded PL-N fractions. Antioxidant activity assays *in vitro* indicated that the degraded PL-N fraction with low molecular weight had stronger hydroxyl radical scavenging capacity and higher TEAC and FRAP values.

1. Introduction

Phellinus linteus (Berkeley & M.A. Curtis) Teng, generally called Sanghuang, is a species of medicinal mushrooms belonging to *Hymenochaetaceae Basidiomycetes* that is valuable in traditional Chinese medicine. This species is widely used in East Asia, particularly China, Japan, and Korea, for preventing ailments as diverse as gastroenteric dysfunction, diarrhea, hemorrhage, and cancers [1,2]. Polysaccharides represent one of the most abundant components in this mushroom. A major group of bioactive constituents have been extracted and isolated from the fruiting bodies, cultured mycelium, and fermentation broth of *P. linteus*, which are structurally diverse biomacromolecules with notable and excellent bioactivities, such as antitumor, antioxidative, anti-inflammatory, and immunoregulatory properties [1,2]. As an important antioxidant, polysaccharides isolated from *P. linteus* can be explored as novel and natural functional or healthy foods to prevent oxidative damage in living organisms. Recently, our group reported a novel polysaccharide named PL-N, which was isolated from an alkaline extract of *P. linteus* mycelia. Antioxidant activity assays *in vitro* showed that PL-N exhibits strong free radical scavenging capacity and cytoprotective activity [3]. However, PL-N has a high molecular weight (MW: ~311,000 kDa) and intrinsic viscosity (127.47 dL/g), which greatly limit the absorption and utilization of polysaccharides in the body and its pharmaceutical applications. Thus, considering the potential applications of PL-N in functional foods and medicine, the degradation of PL-N must be carried out in a controlled manner with low-MW fractions for specific uses.

The depolymerization of polysaccharides using various methods, including physical, chemical, and biological approaches [4], has gained increasing attention in recent years. Chemical degradation, such as hydrogen peroxide degradation and acid or alkaline hydrolysis, is often time and energy consuming. Moreover, chemical degradation involves a large consumption of organic reagents, thereby leading to unwanted monomers and oligomers [5]. The enzymatic method is a relatively complex approach, and the high cost of different enzymes has greatly restricted their industrial-scale applications. Notably, ultrasound degradation, unlike chemical or biological decomposition, is a non-random process, with cleavage occurring probably at the midpoint of the molecule and larger molecules degrading faster than others [6]. In particular, the ultrasound irradiation method has the advantage of being rapid, mild, and environment-friendly. Over the last several decades, numerous studies have focused on the use of ultrasound irradiation for the degradation of water-soluble polysaccharides, such as dextran [7], chitosan [8], carboxymethylcellulose [9], pullulan [10], pectin [11], and schizophyllan [12]. The mechanism involved in the degradation of these polysaccharides is generally accepted to

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be due to acoustic cavitation (mechanical effect). Acoustic cavitation is well explained by the rapid formation and collapse of cavitation bubbles within the irradiated liquid media, generating intense stress and resulting in irreversible chain scission [7]. Interestingly, a desirable feature of ultrasonic polysaccharide degradation is the high degradation rate for large molecules, resulting in a narrow or more uniform MW distribution. Nevertheless, reports on the ultrasound degradation of the high-MW polysaccharide PL-N with enhanced physicochemical properties and bioactivities are rare or lacking.

Therefore, this study aimed to investigate the depolymerization of PL-N solution isolated from the alkaline extract of *P. linteus* mycelia using ultrasound irradiation. Effects of ultrasound treatment on the MW and intrinsic viscosity of PL-N solution at various ultrasonic intensities as a function of time were investigated. The preliminary characterizations and antioxidant activities *in vitro* of PL-N before and after ultrasound treatment were evaluated. In addition, the degradation kinetics of PL-N under ultrasound was also investigated.

2. Materials and methods

2.1. Materials and chemicals

PL-N with higher weight-average molecular weight (M_w) of $\sim 311 \times 10^6$ g/mol was isolated from the alkaline extract of *P. linteus* (Strain No. KCTC 6190) mycelia [3]. 2,6-Hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS), and 2,4,6-tris(2-pyridyl)-s-triazine were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Ultrasonic degradation

Ultrasonic degradation experiments were performed on a Model VCX 750 horn-type ultrasonic processor (Sonics & Materials Inc., Newton, USA) at a frequency of 20 kHz and 750 W maximum output power. The probe horn with a 13 mm tip diameter was used in all experiments. A total of 20 mL of the PL-N solution (5.0%, w/v) was placed into a 50 mL plastic centrifuge tube, and the ultrasonic probe was dipped into the solution to a depth of about 20 mm. The PL-N solution was treated with ultrasound at different powers (200, 400, and 600 W) in pulse mode (2 s on, 2 s off) for 30, 60, 90, 120, 150, and 180 min. The solution was maintained at 40 ± 0.5 °C in an ice-cold bath. After ultrasonication, the solutions were centrifuged and lyophilized for further analysis. The ultrasonic intensity was predicted according to the following equation: $I = P/(\pi r^2)$, where I is the ultrasound intensity, P is the input power, and r is the radius of the ultrasound probe [13]. The ultrasonic powers were set at 200, 400 and 600 W, which corresponded to ultrasonic intensities of around 151, 302, and 453 W/cm^2, respectively.

2.3. Intrinsic viscosity determination

Intrinsic viscosity $[\eta]$ was determined by the serial dilution method [14], and the intrinsic viscosities of PL-N before and after ultrasound treatment were measured at 30 ± 0.1 °C with an Ubbelohde viscometer (0.5–0.6 mm capillary diameter). The kinetic energy correlation was always negligible. The $[\eta]$ value of each sample was estimated by the Huggins and Kraemer equations:

$$\eta_{sp}/c = [\eta] + k' \cdot [\eta]^2 \cdot c; \quad \ln \eta_r/c = [\eta] - \beta \cdot [\eta]^2 \cdot c \tag{1}$$

where k' and β are constants for a given polymer at a given temperature in a given solvent, c is the polymer concentration, η_{sp}/c is the reduced specific viscosity, and $(\ln \eta_r)/c$ is the inherent viscosity.

2.4. High-performance gel permeation chromatography (HPGPC) analysis

The MW and the polydispersity index (PDI) of PL-N before and after ultrasound treatment were determined by HPGPC, which was performed on a Waters 1515 isocratic pump and a Waters 2414 refractive index detector with two ultrahydrogel columns 250 and 2000 (7.8 mm × 300 mm, Waters Corp., Milford, MA, USA) in series at 50 °C. The detailed experiment conditions were reported previously [3]. Dextran MW standards ranging from 5.2 to 1482 kDa (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) were used for calibration. The online Breeze software package (Waters Corp., Milford, MA, USA) was used for data collection and analysis.

2.5. Degradation kinetics model

The degradation behavior of PL-N under ultrasound was expressed according to the first- and second-order reaction kinetics model [12,15], and the rate constant (k) was derived from the following formulas:

$$\ln(M_t/M_0) = k \cdot t \tag{2}$$

$$1/M_t - 1/M_0 = k \cdot t \tag{3}$$

where k is the rate constant (mol/g min) of MW degradation during ultrasonic treatment; t is the treatment time (min); M_t and M_0 are the weight-average MW at time t and at time 0 (g/mol), respectively.

2.6. Monosaccharide composition analysis

The monosaccharide compositions of PL-N before and after ultrasound treatment were investigated by a combination of acid hydrolysis and gas chromatography (GC) according to our previous study [16]. In brief, the samples were hydrolyzed by 2 M H_2SO_4 at 100 °C for 8 h and then neutralized by $BaCO_3$. The hydrolysate was converted into nitrile acetate and subjected to GC analysis on an Agilent 7890A instrument (HP-5 fused-silica capillary column, 30 m × 0.25 mm × 1 μm). D-Arabinose (D-Ara), D-glucose (D-Glc), D-galactose (D-Gal), D-mannose (D-Man), L-rhamnose (L-Rha), and D-xylose (D-Xyl) (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) were used as monosaccharide standards.

2.7. Fourier transform infrared (FTIR) spectroscopic analysis

The FTIR spectra of the PL-N before and after ultrasound treatment were performed with a Nexus 670 FTIR spectrometer (Thermo Nicolet Co., USA) in the wavenumber ranging from 500 to 4000 cm^{-1} with KBr pellets and then referenced against air.

2.8. Scanning electron microscopy (SEM) observation

The freeze-dried samples were fixed onto a copper stub. After sputtering with a layer of gold, the SEM images were observed and recorded using a JSM-6460LV SEM (JEOL Ltd., Japan) under high vacuum condition at an accelerating voltage of 20 kV and image magnifications of 600× and 800×.

2.9. Antioxidant activity assays in vitro

In this study, three assays, namely, hydroxyl (·OH) radical scavenging, Trolox equivalent antioxidant capacity (TEAC), and ferric