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# Pulsed counter-current ultrasound-assisted extraction and characterization of polysaccharides from *Boletus edulis*

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ABSTRACT

Four methods for extracting polysaccharides from *Boletus edulis*, namely, hot-water extraction, ultrasonic clearer extraction, static probe ultrasonic extraction, and pulsed counter-current probe ultrasonic extraction (CCPUE), were studied. Results showed that CCPUE has the highest extraction efficiency among the methods studied. Under optimal CCPUE conditions, a *B. edulis* polysaccharide (BEP) yield of 8.21% was obtained. Three purified fractions, BEP-I, BEP-II, and BEP-III, were obtained through sequential purification by DEAE-52 and Sephadex G-75 chromatography. The average molecular weights of BEP-I, BEP-II, and BEP-III were 10,278, 23,761, and 42,736 Da, respectively. The polysaccharides were mainly composed of xylose, mannose, galactose, and glucose; of these, mannose contents were the highest. The antioxidant activities of the BEPs were further investigated by measurement of their ability to scavenge DPPH and hydroxyl radicals as well as their reducing power. The results indicated that the BEPs have good antioxidant activity.



## 1. Introduction

In recent years, polysaccharides from various mushrooms have sparked great scientific interest because of their potent ability to prevent oxidative damage to living organisms by enhancing the activities of antioxidant enzymes or increasing the efficiency of free radical scavenging (Tian, Zeng, Xu, Zheng, & Lin, 2012; Yin, You, & Jiang, 2011). Mushrooms have been used as food for centuries because of their high nutrient content, low lipid content, and excellent flavor and texture (Chiang, Yen, & Mau, 2006; Emilia, Grazyna, & Zofia, 2006). *Boletus edulis*, also known as king bolete, is an edible mushroom that is popular in Europe, North America, and Asia (Tsai, Tsai, & Mau, 2007). *B. edulis* grows in many districts of China, such as Henan, Heilongjiang, Zhejiang, Sichuan, Yunnan, and Tibet. However, while modern pharmacological studies demonstrate that *B. edulis* has anti-diabetic and antitumor activities (Zhang, Xiao, He, & Sun, 2011), little attention has been devoted to the extraction of *B. edulis* polysaccharides (BEPs).

Polysaccharide extraction methods have been widely studied all over the world. Conventional hot-water extraction (HWE), the most commonly used method for polysaccharide extraction, is simple and safe; however, the high temperature and long extraction time used in HWE may lead to polysaccharide degradation and a decrease in the pharmacological activity of extracted polysaccharides (Y. J. Wang, Cheng, Mao, Fan, & Wu, 2009). Therefore, the development of a new effective and economical method for polysaccharide extraction is desirable. Various novel techniques for extracting bioactive polysaccharides from mushrooms have recently been developed, including microwave-assisted extraction (J. Wang et al., 2010), supercritical fluid extraction (Turner, King, & Mathiasson, 2001), and ultrasonic-assisted extraction (X. Li, Wang, Wang, Walid, & Zhang, 2012; X. L. Li et al., 2012). Among these methods, ultrasonic-assisted extraction has received a significant amount of attention. Ultrasound-assisted extraction has been employed for preparing bioactive compounds from different plant materials and has proven to be fairly effective (Wen et al., 2011). Ultrasonic-assisted extraction has many advantages over traditional extraction methods, including shorter extraction times, use of less solvent, and higher extraction rates (Chen et al., 2010). The high extraction efficiency of ultrasonic treatment is mainly attributed to breakage of the cell wall and enhancement of mass transfer through broken cell walls as a result of cavitation effects (Entezari, Hagh Nazary, & Haddad Khodaparast, 2004). Today, ultrasonic-assisted extraction is widely employed for the extraction of polysaccharides from different materials with high extraction efficiency.

Ultrasonic cleaners (Tian et al., 2012) or ultrasonic cell disruptors are instruments commonly used for ultrasonic-assisted

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extraction. The power of these instruments is usually low and the state of material is static which makes the materials cannot touch the ultrasound sufficiently. In this study, pulsed counter-current ultrasound was applied to extract polysaccharides from *B. edulis*. "Pulsed" means that the ultrasound waves emanate at given intervals, and the operation and pause times may be set in the instrument. "Counter-current" means that the flow direction of the material solution is from low to high whereas the direction of the ultrasound wave is from high to low. This working mode is more efficient for the material touching the ultrasound.

In this study, four extraction methods, including HWE, ultrasonic clearer extraction (UCE), static probe ultrasonic extraction (SPUE), and counter-current probe ultrasonic extraction (CCPUE), were studied to determine the best extraction method featuring a low extraction temperature, low energy consumption, and minimal time to achieve high polysaccharide yields. The purification and preliminary characterization of the polysaccharides obtained were studied, and the antioxidant activities of the polysaccharides, including their ability to scavenge DPPH and hydroxyl radicals as well as their reducing power, were assayed in vitro.

## 2. Materials and methods

### 2.1. Materials and chemicals

Dried *B. edulis* samples were purchased from the local market. DEAE-52 cellulose, Sephadex G-75, 1,1-diphenyl-2-picryldydrazyl (DPPH), standards of the monosaccharides, and glucuronic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of analytical grade.

### 2.2. Extraction of polysaccharides by HWE, UCE, SPUE, and CCPUE

Using polysaccharide yield as a target, four extraction methods to obtain BEP were employed. A water bath was used to keep the temperature of the materials at 90 °C and mechanical stirring (MS) was needed to keep the materials moving. A three-blade turbine with a diameter of 1.5 cm was used as the stirrer (mechanical stirring). The operating speed used in the HWE method was 500 rpm. An ultrasonic clearer (BL6-180, Shanghai Bilang Instrument Co., Ltd.) with a power of 200 W and a frequency of 20 kHz was used in the UCE method. The instruments used for SUPE and CCPUE were nearly identical except for two peristaltic pumps used to keep the material solutions in a counter-current flow state in the CCPUE method. The SUPE and CCPUE instruments (Wuxi Finebao Bio-engineering Co., Ltd.) were equipped with an ultrasonic probe with a frequency of 20 kHz, and the temperature was controlled using a water bath.

*B. edulis* was dried at 60 °C for 12 h, ground using a grinder, and sieved through a 60-mesh sieve. The dried *B. edulis* powder was first defatted with petroleum ether (60–90 °C) and pretreated with 95% ethanol to remove oligosaccharides, small-molecule materials, and some colored substances. The pretreated samples were separated from the organic solvent by centrifugation (5000 × *g* for 10 min) and dried at 60 °C for 12 h. The dried material was extracted with distilled water using the methods described above. After extraction, the solutions were filtered to remove debris fragments, and the filtrate was concentrated using a vacuum concentrator (BUCHI 409, Buchi Corp., New Castle, DE, USA). After cooling, proteins and pigments were removed by the addition of trichloroacetic acid and activated carbon (H. Wang, Jiang, Wang, & Li, 2013). Alcohol was then added to a final concentration of 80% (v/v), and the precipitates were collected by centrifugation for 15 min at 5000 rpm in a high-speed centrifuge and subsequently lyophilized. The percentage of polysaccharide yield (%) was calculated using the following formula:

$$\text{Yield}(\%,\ \text{w/w}) = \frac{\text{Weight of dried crude extraction}}{\text{Weight of } B.\ \ edulis \text{ material}} \times 100 \qquad (1)$$

### 2.3. Purification of BEPs

The crude polysaccharides were redissolved in distilled water and loaded on a DEAE-52 cellulose column (3.0 cm × 5 cm). The polysaccharide was first eluted with distilled water followed by NaCl solutions of different concentrations (0.1, 0.3, 0.5, and 1.0 M). An automatic fraction collector was used to collect 5 mL fractions. The carbohydrates were determined by the phenol–sulfuric acid method using glucose as a standard (Masuko et al., 2005). The eluates were concentrated to obtain the main fractions and then fractionated using a Sephadex G-75 column (3.0 cm × 50 cm). Elutions were performed with distilled water at a flow rate of 1.0 mL $min^{-1}$. The obtained fractions were combined according to the total carbohydrate content as quantified by the phenol–sulfuric acid colorimetric method.

### 2.4. Preliminary characterization of BEPs

The polysaccharides were dissolved in distilled water, and their contents were measured using the phenol–sulfuric acid colorimetric method with glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein and uronic acid contents of the BEPs were determined according to the literature (Bradford, 1976; Dubois et al., 1956) using bovine serum albumin and glucuronic acid as the standards, respectively. The weight–average molecular weights of the polysaccharides were determined by gel permeation chromatography (GPC) in combination with high-performance liquid chromatography (HPLC) (Agilent 1100, USA). Samples (2.0 mg) were dissolved in distilled water (2 mL), passed through a 0.45 μm filter, and applied to a gel-permeation chromatographic system of TSK-GEL G3000SWxl columns (5 μm, 7.8 mm × 300 mm) maintained at 40 °C. Samples were eluted with 0.05 mol $L^{-1}$ $Na_2SO_4$ at a flow rate of 0.5 mL $min^{-1}$ and detected using a refractive index detector (RID-10A) (X. Li et al., 2012; X. L. Li et al., 2012). The molecular weight of the BEPs was estimated by referencing the calibration curve made from Pullulan standards of known molecular weight (P-100, P-50, P-20, P-10, and P-5). Monosaccharide compositions of the crude BEPs and their corresponding purified fractions were determined by gas chromatography (GC, Agilent 6890, USA) according to the literature (J. E. Li, Nie, Yang, Qiu, & Xie, 2011). Monosaccharide standards, including glucose, galactose, xylose, mannose, ribose, fucose, arabinose, and rhamnose, were applied as previously reported (Tian et al., 2012).

### 2.5. Antioxidation of BEPs

#### 2.5.1. Scavenging activity for DPPH radicals

The DPPH radical scavenging activity of the polysaccharides was measured according to a previously described method (Chen, Ma, Liu, & Liao, 2012). Briefly, 2.0 mL of the polysaccharide samples of different concentrations were added to the following freshly prepared reagents: 2.0 mL of phosphate buffer (0.02 M, pH 6.0) and 2.0 mL of 0.2 mM DPPH in 95% ethanol. The mixtures were shaken and allowed to stand for 30 min at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Shanhai Metash Instruments Co., Ltd.).

Ascorbic acid was used as the positive control. Scavenging activity was calculated using the following equation:

$$\text{Scavenging activity}\ (\%) = \frac{1-(A_2-A_1)}{A_0} \times 100 \quad (2)$$

where $A_0$ is the absorbance of the DPPH solution without the addition of the positive control or sample, $A_1$ is the absorbance of the sample without the DPPH solution, and $A_2$ is the absorbance of the mixture containing both the DPPH solution and the sample.

*2.5.2. Scavenging activity for hydroxyl radicals*

The hydroxyl radical scavenging activity of the BEPs was measured using a previously reported method with slight modifications (Wu, Zhu, Zhang, Yang, & Zhou, 2012). The polysaccharides were dissolved in distilled water to various concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL). The reaction mixture contained 1 mL of water, 1 mL of 1,10-phenanthroline ethanol solution (0.75 mM), 1 mL of $FeSO_4$ (0.75 mM), 1 mL of $H_2O_2$ (0.01%), and different volumes of the BEP solutions (1 mg/mL). The final reaction volume was made up to 4 mL using distilled water. After incubation at 30 °C for 20 min, the absorbance of the mixtures was measured at 624 nm against a blank (distilled water instead of the ABPS solution) using ascorbic acid as a positive control. The hydroxyl radical-scavenging activity of the BEPs was calculated according to the following formula:

$$\text{Scavenging activity}\ (\%) = \frac{A_0-A_1}{A_0} \times 100 \quad (3)$$

where $A_0$ is the absorbance of the blank and $A_1$ is the absorbance of the sample.

*2.5.3. Reducing power assay*

The reducing ability of the samples was measured according to the method of Oyaizu (1986). Mixtures containing 2.5 mL of samples of different concentrations, 2.5 mL of pH 6.6 phosphate buffer (0.2 mol $L^{-1}$), and 2.5 mL of 1% (w/v) potassium ferricyanide were incubated at 50 °C for 20 min. Afterwards, 2.5 mL of trichloroacetic acid (10%, w/v) was added to terminate the reaction, and the mixture was centrifuged at 2000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of water and 1 mL of 0.1% (w/v) $FeCl_3$. After reacting for 10 min, the absorbance of the mixture was measured at 700 nm against a blank. An increase in the absorbance of a reaction mixture indicates an increase in its reduction capability.

*2.6. Statistical analysis*

All experiments were performed in triplicate, and data are expressed as mean ± SD.

## 3. Results and discussion

*3.1. Extraction of BEPs*

*3.1.1. Effect of liquid–solid ratio on the BEP yield*

The liquid–solid ratio significantly affects the extraction yield. The polysaccharides cannot be completely extracted if the liquid–solid ratio is too low. In this study, extraction was carried out under different liquid–solid ratios; other extraction variables were set as follows: the temperature for HWE was 90 °C with an extraction time of 60 min; the extraction time for UCE, SPUE, and CCPUE was 40 min; the pulse duration and interval times for SPUE and CCPUE were 4 and 2 s, respectively; the frequency used for UCE, SPUE, and CCPUE was 20 kHz; and the extraction time was 40 min. The effect of solid–liquid ratios on the BEP yields was studied and the results are shown in Fig. 1.

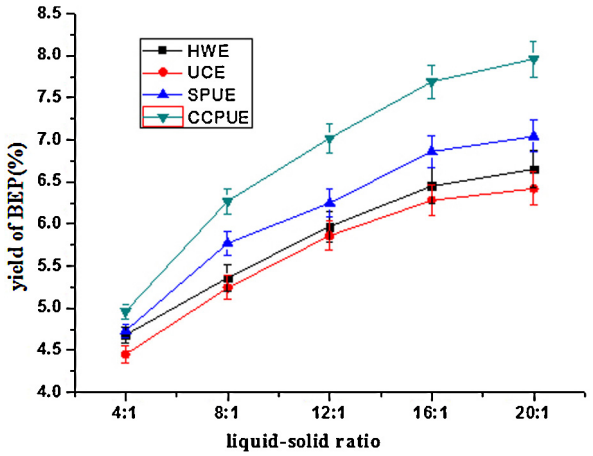


**Fig. 1.** Effect of liquid–solid ratios on the yield of BEP.

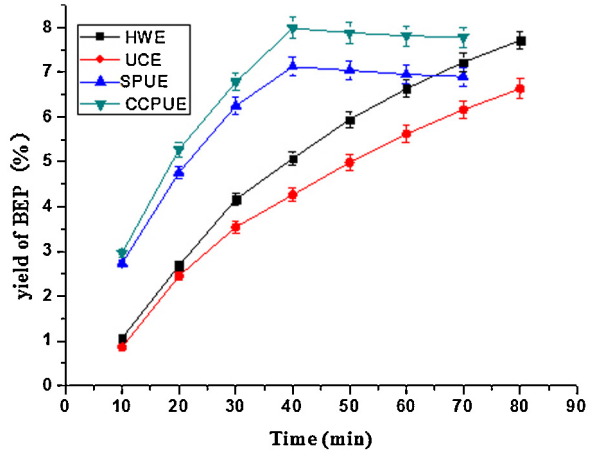


**Fig. 2.** Effect of extraction time on the yield of BEP.

The BEP yield increased rapidly with increasing liquid–solid ratios from 4:1 to 16:1 (mL/g). No obvious changes in BEP yield were observed as the ratio increased beyond 16:1. A larger liquid–solid ratio indicates a larger concentration difference between the exterior solvent and the interior plant cells and allows faster diffusion of the polysaccharides from the material to the solvent. The results also show various BEP yields from different methods. At the same liquid–solid ratio, the BEP yield of UCE was the lowest, followed by those of HWE and SPUE; the BEP yield of CCPUE was the highest among the methods studied. These results indicate that ultrasound enhances extraction efficacy. UCE has a lower extraction efficiency than SUPE or CCPUE. As a kind of flat plate, the solvents in it is almost static which makes the mass transfer rate is low. Compared with SUPE, CCPUE has higher extraction efficiency, possibly because its counter-current flow state allows better distribution of the solutions to touch the ultrasound.

*3.1.2. Effect of extraction time on the BEP yield*

The extraction time is another factor that can affect the extraction yield and selectivity of the extracting fluid. Penetration of the liquid into dried materials requires time to release BEP, and this time depends on the enhancing abilities of different extraction methods. The effect of extraction time on the BEP yield was studied using a liquid–solid ratio of 16:1; all other conditions were set as described above.

The effect of extraction time on the BEP yield is shown in Fig. 2. The BEP yield increased with increasing extraction time. Using

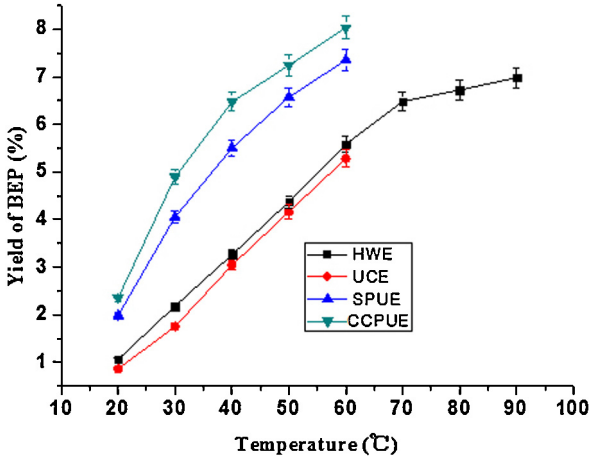


**Fig. 3.** Effect of extraction temperature on the yield of BEP.

CCPUE, SPUE, HWE, and UCE, the highest yields were got at 40, 45, 70, and 80 min, respectively. These results indicate that CCPUE and SPUE require considerably less time to obtain maximum yields compared with HWE and UCE; yields of the former were higher than those of the latter. Using CCPUE and SPUE, the BEP yields decreased slightly at extraction times exceeding 40 and 45 min, respectively. Longer extraction times may induce the degradation of polysaccharides under ultrasound conditions.

### 3.1.3. Effect of extraction temperature on the BEP yield

The effect of extraction temperature on the BEP yield was studied at a liquid–solid ratio of 16:1, while all other conditions were kept identical to those described above. The results are shown in Fig. 3. Generally, high temperatures can increase diffusion coefficients and increase extraction rates. Fig. 3 shows increases in yield with increasing extraction temperature. Under HWE, the BEP yield increased from 20 °C to 100 °C. Unfortunately, the equipment employed by the three other methods using ultrasound effects did not work optimally at temperatures exceeding 60 °C. The final BEP yield by UCE was lower than that by HWE but the final BEP yields by SPUE and CCPUE were higher than that of HWE. Such a finding indicates that similar BEP yields can be obtained using SPUE and CCPUE at lower temperatures than HWE. Therefore, ultrasound could enhance diffusion coefficients.

### 3.1.4. Energy consumption of the four extraction methods

The energy consumptions of the four extraction methods were analyzed using a DDS666-electronic single-phase power meter (Chint Instrument & Meter Co., Ltd., Zhejiang, China). Energy consumption data were read when the BEP yield was about 5.00%. Table 1 shows that the HWE method requires the highest energy consumption (0.47 kW h) because the temperature of the water bath must be kept at 90 °C. For CCPUE, less time is needed to obtain the same yield but the method has the second highest energy consumption (0.36 kW h) because the system includes two components: the ultrasound instrument and the two peristaltic pumps. The power consumed by the instruments for UCE and SPUE was not higher than that consumed by CCPUE; however, their longer extraction times compared with CCPUE rendered their energy consumptions only slightly lower than that of CCPUE.

Combining all of the results obtained thus far, CCPUE appears to be the best extraction method because of its high extraction efficiency and relatively low energy consumption. The optimal working conditions for CCPUE were further studied using single-factor experiments.

### 3.1.5. Effect of the pulse duration of CCPUE on the BEP yield

The effect of pulse duration of CCPUE on the BEP yield was studied at a constant liquid–solid ratio of 16:1, interval time of 2 s, frequency of 20 kHz, extraction temperature of 60 °C, and total extraction time of 40 min. Results are shown in Fig. 4a. The BEP yield increased rapidly when pulse durations ranged from 2 s to 6 s; no obvious changes in the yield were observed as the pulse duration increased. Given that total extraction time was fixed, the time during which ultrasound waves could affect the solution increased with increasing pulse duration. As a result, BEP yield increased with increasing pulse duration. When the pulse duration exceeded 6 s, the BEP yield increased only slightly. Given that a longer pulse duration could damage the ultrasonic probe, 4 s was considered the optimal pulse duration.

### 3.1.6. Effect of the interval time of CCPUE on the BEP yield

The effect of the interval time of CCPUE on the BEP yield was studied at a liquid–solid ratio of 16:1, pulse duration of 4 s, frequency of 20 kHz, extraction temperature of 60 °C, and total extraction time of 40 min. The results shown in Fig. 4b demonstrate that the BEP yield decreases with increasing interval time. Given that the total extraction time was fixed, the time during which ultrasound waves could affect the solution decreases with increasing interval time. Thus, the BEP yield decreased as the interval time increased. At an interval time of 1 s, the BEP yield was 8.44%. Considering that a very short interval time could damage the ultrasonic probe, 2 s was chosen as the optimal interval; under this condition, the BEP yield was 8.21%.

## 3.2. Purification and preliminary characterization of BEP

### 3.2.1. Purification of crude BEP

Samples were prepared by CCPUE under the following conditions: liquid–solid ratio of 16:1, pulse duration of 4 s, interval time of 2 s, frequency of 20 kHz, total extraction time of 40 min, and extraction temperature of 50 °C. The crude BEP was fractioned by chromatography on a DEAE-52 column, and three independent elution peaks (BEP-I, BEP-II, and BEP-III) were detected by phenol–sulfuric acid assay (Fig. 5a). Each fraction was collected, concentrated, dialyzed, and loaded onto a Sephadex G-75 column. The column was eluted with deionized water, and the resulting eluate was collected. A single elution peak was obtained from each fraction, which indicates that BEP-I, BEP-II, and BEP-III are single polysaccharides. The linearity of the proposed method was determined using Pullulan standards of different molecular weight. The calibration curve of Pullulan was plotted as the molecular weights on a log scale versus the retention time. The molecular weights of BEP-I, BEP-II, and BEP-III were approximately 10,278, 23,761, and 42,736 Da, respectively.

### 3.2.2. Carbohydrate, protein, uronic acid and monosaccharide compositions of BEP

The carbohydrate, protein and uronic acid contents of in the crude BEPs as well as their corresponding purified fractions were detected. As seen in Table 2, the carbohydrate content of crude BEP was 68.32%, and the carbohydrate contents in purified fractions of BEP-I, BEP-II, and BEP-III were 86.63%, 85.41%, and 86.34%, respectively. The uronic acid and protein contents of the BEPs were low. The monosaccharide composition was determined by GC, and GC chromatograms of the monosaccharide standards and BEP-III are shown in Fig. 6. The polysaccharides were mainly composed of xylose, mannose, galactose, and glucose. The mannose contents were the highest among the polysaccharide components detected, showing ratios of 34.32%, 32.62%, 33.56%, and 36.60% for crude BEP, purified BEP-I, BEP-II, and BEP-III, respectively.

**Table 1**
The energy consumptions of the four enhancing methods.

| Energy consumption | HWE | | UCE | CCPUE | | SPUE |
|---|---|---|---|---|---|---|
| | Water bath | MS | | CCPUE | Peristaltic pumps | |
| Energy consumption (kW h) | 0.42 | 0.05 | 0.33 | 0.24 | 0.12 | 0.31 |
| Total energy consumption (kW h) | 0.47 ± 0.02 | 0.33 ± 0.01 | 0.36 ± 0.02 | 0.31 ± 0.01[a] | | |

[a] Mean ± standard deviation ($n$ = 3).

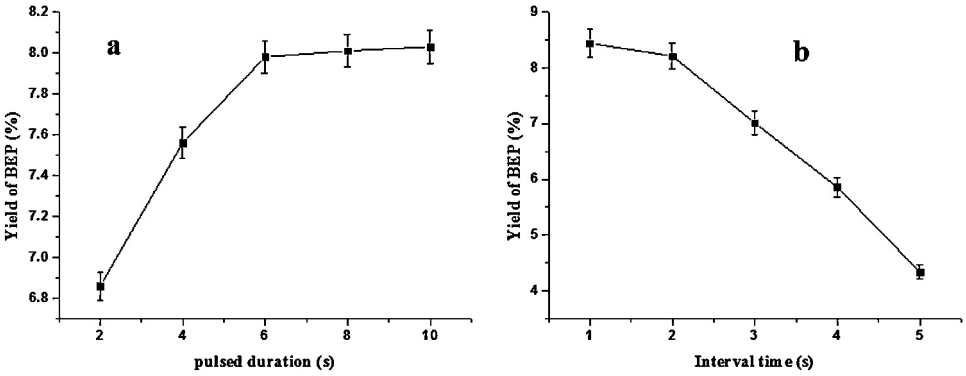


**Fig. 4.** Effect of pulsed duration for CCPUE on the yield of BEP (a) and effect of interval times for CCPUE on the yield of BEP (b).

### 3.3. Antioxidation of BEP

#### 3.3.1. Scavenging activity for DPPH radicals

DPPH, a compound that possesses a free proton radical with a characteristic absorption, decreases significantly upon exposure to proton radical scavengers (Yamaguchi, Takamura, Matoba, & Terao, 1998). The effect of antioxidants on DPPH radical scavenging is believed to be due to the hydrogen donating ability of these compounds. When DPPH encounters a hydrogen-donating substance, the radical is scavenged and the absorbance is reduced (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The scavenging effects of purified BEPs on DPPH radicals were measured, and the results are shown in Fig. 5b. The polysaccharides showed good antioxidant capacities. The scavenging activity of polysaccharides and ascorbic acid on DPPH radical inhibition was related to the concentration of the samples. Scavenging effects increased with increasing concentration from 0.2 mg/mL to 1.2 mg/mL, and the scavenging activities of the polysaccharides on DPPH radicals ranged from 4% to 79%.

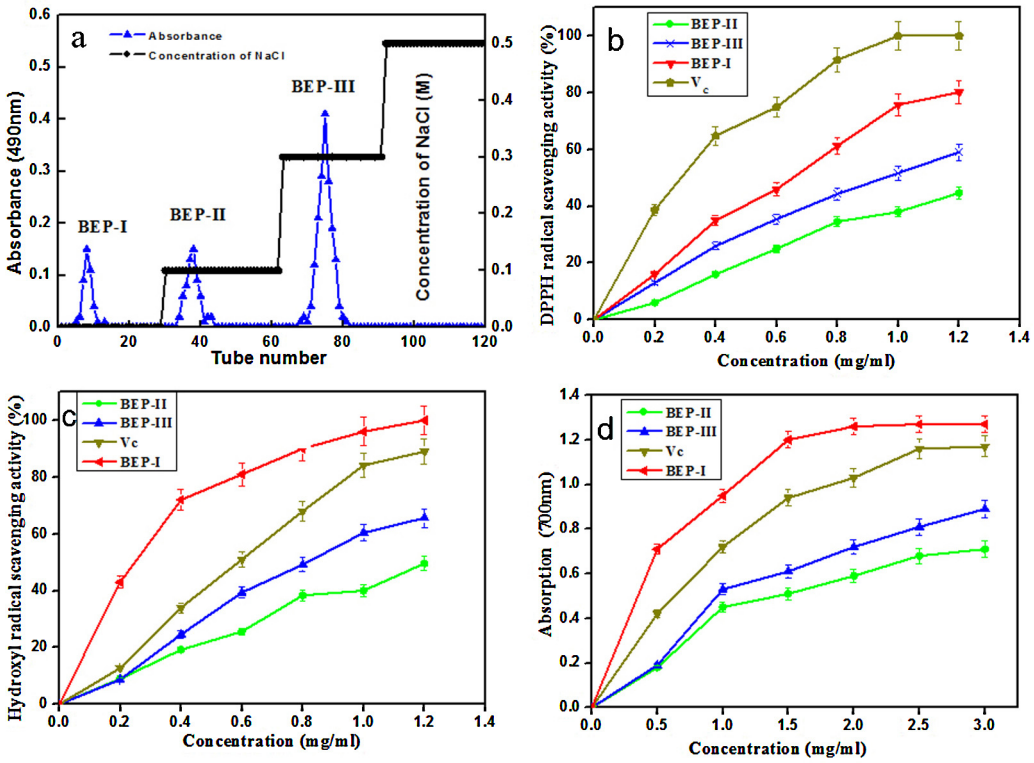


**Fig. 5.** Elution profiles of crude BEP on DEAE-52 cellulose anion-exchange chromatography column (a) and scavenging effects on DPPH radical (b), hydroxyl radical and (c) and reducing power (d) of purified BEP-I, BEP-II and BEP-III.

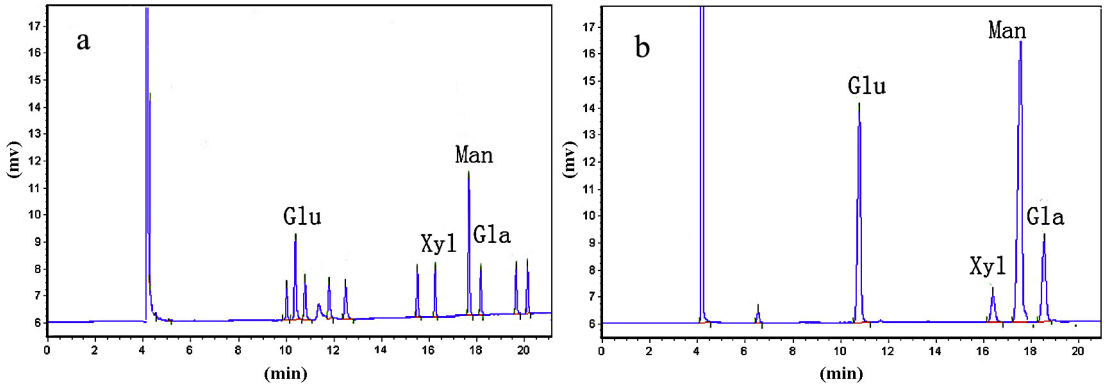


**Fig. 6.** GC spectra of monosaccharides composition of standards (a) and BEP-III (b). Man, mannose; xyl, xylose; gal, galactose; glu, glucose.

**Table 2**
The chemical compositions and the contents of carbohydrate, protein and uronic acid for Crude BEP, BEP-I, BEP-II, and BEP-III.

| Samples | Crude BEP | BEP-I | BEP-II | BEP-III |
|---|---|---|---|---|
| Carbohydrate (%) | 68.32 | 86.63 | 85.41 | 86.34 |
| Protein (%) | 1.59 | 0.14 | 0.13 | 0.37 |
| Uronic acid (%) | 2.92 | 0.08 | 1.72 | 2.36 |
| Sugar components (%) | | | | |
| Xylose | 10.23 | 11.52 | 12.62 | 9.65 |
| Mannose | 34.32 | 32.62 | 33.56 | 36.60 |
| Galactose | 11.13 | 14.16 | 9.13 | 13.35 |
| Glucose | 25.03 | 23.65 | 27.37 | 22.83 |

At a concentration of 1.2 mg/mL, the scavenging activities of BEP-I, BEP-II, and BEP-III were 78.7%, 59.3%, and 43.2%, respectively. The scavenging effects of BEP-I were higher than those of BEP-II and BEP-III but lower than that of ascorbic acid. These results imply that the purified fractions of BEP contain substances that are hydrogen donors and could react with free radicals to convert them into more stable products and terminate the radical chain reaction. Therefore, the purified polysaccharides may act as electron or hydrogen donors to scavenge DPPH.

### 3.3.2. *Scavenging activity for hydroxyl radicals*

Hydroxyl radicals are very harmful reactive free radicals. Therefore, hydroxyl radicals must be eliminated by the body to protect itself from oxidative damage. The hydroxyl radical scavenging activities of the polysaccharides are shown in Fig. 5c. The hydroxyl radical scavenging activities of the polysaccharides were concentration-dependent and increased in the order of BEP-II < BEP-III < ascorbic acid < BEP-I. The hydroxyl radical scavenging activity of BEP-I was higher than that of ascorbic acid. At a concentration of 1.2 mg/mL, the hydroxyl radical scavenging activities of BEP-I, BEP-II, and BEP-III were 99.3%, 61.6%, and 48.6%, respectively, which indicates that the three purified fractions, especially BEP-I, are effective hydroxyl radical scavengers.

### 3.3.3. *Reducing power assay*

The reducing power of a compound serves as a good indicator of potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). Results of the reducing power of the polysaccharides are shown in Fig. 5d; the reducing power of all of the samples increased gradually with concentration increased. BEP-I showed considerably higher reducing ability than BEP-II and BEP-II and significantly higher reducing power than ascorbic acid, which suggests that the polysaccharides can act as electron donors and react with free radicals to convert them into more stable products.

## 4. Conclusion

Four methods for extracting polysaccharides from *B. edulis*, namely HWE, UCE, SPUE, and CCPUE, were studied. Under the same experimental conditions, CCPUE and SPUE showed higher extraction efficacy and less energy consumption than HWE. Despite the energy consumption of CCPUE being slightly higher than that of SPUE, the former was regarded as the best extraction method because of its high extraction efficacy. The results indicated that ultrasound waves could enhance the extraction procedure and increase the extraction efficacy. The best conditions for CCPUE were as follows: liquid–solid ratio of 16:1, total extraction time of 40 min, extraction temperature of 60 °C, pulse duration of 4 s, and interval time of 2 s. Under these conditions, the BEP yield by CCPUE was 8.21%. Three purified fractions, BEP-I, BEP-II, and BEP-III, were obtained from the crude BEP through sequential purification using DEAE-52 and Sephadex G-75 chromatography. The average molecular weights of the polysaccharides BEP-I, BEP-II, and BEP-III were 10,278, 23,761, and 42,736 Da, respectively.

The chemical compositions and antioxidant activities of the polysaccharides were also studied. The polysaccharides were mainly composed of xylose, mannose, galactose, and glucose. Mannose contents were highest at ratios of 34.32%, 32.62%, 33.56%, and 36.60% in crude BEP, BEP-I, BEP-II, and BEP-III, respectively. The antioxidant activities of BEP-I, BEP-II, and BEP-III were further investigated by measuring their corresponding scavenging abilities for DPPH and hydroxyl radicals as well as reducing power in vitro. The results indicated that the BEPs exhibit good antioxidant activities.

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## References

Bradford, M. M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Chen, G. T., Ma, X. M., Liu, S. T., & Liao, Y. L. (2012). Isolation, purification and antioxidant activities of polysaccharides from *Grifola frondosa*. *Carbohydrate Polymers, 89*, 61–66.

Chen, X. P., Wang, W. X., Li, S. B., Xue, J. L., Fan, L. J., Sheng, Z. J., & Chen, Y. G. (2010). Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. *Carbohydrate Polymers*, *80*, 944–948.

Chiang, P. D., Yen, C. T., & Mau, J. L. (2006). Non-volatile taste components of canned mushrooms. *Food Chemistry*, *97*(3), 431–437.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Emilia, B., Grazyna, J., & Zofia, L. (2006). Edible mushroom as a source of valuable nutritive constituents. *Acta Scientarum Polonorum: Technologia Alimentaria*, *5*(1), 5–20.

Entezari, M. H., Hagh Nazary, S., & Haddad Khodaparast, M. H. (2004). The direct effect of ultrasound on the extraction of date syrup and its micro-organisms. *Ultrasonics Sonochemistry*, *11*, 379–384.

Li, J. E., Nie, S. P., Yang, C., Qiu, Z. H., & Xie, M. Y. (2011). Extraction optimization, characterization and bioactivity of crude polysaccharides from *Herba moslae*. *Carbohydrate Polymers*, *83*, 1201–1206.

Li, X., Wang, Z., Wang, Lu., Walid, E., & Zhang, H. (2012). Ultrasonic-assisted extraction of polysaccharides from *Hohenbuehelia serotina* by response surface methodology. *International Journal of Biological Macromolecules*, *51*, 523–530.

Li, X. L., Wang, Z. H., Zhao, Y. X., Luo, S. J., Zhang, D. W., Xiao, S. X., & Peng, Z. H. (2012). Purification of a polysaccharide from *Gynostemma pentaphyllum* Makino and its therapeutic advantages for psoriasis. *Carbohydrate Polymers*, *89*, 1232–1237.

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in a microplate format. *Analytical Biochemistry*, *339*, 69–72.

Meir, S., Kanner, J., Akiri, B., & Hadas, S. P. (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of Agricultural Food and Chemistry*, *43*, 1813–1819.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidant activities of products browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidant properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural Food and Chemistry*, *40*, 945–948.

Tian, Y., Zeng, H., Xu, Z., Zheng, B., & Lin, Y. (2012). Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). *Carbohydrate Polymers*, *88*, 522–529.

Tsai, S. Y., Tsai, H. L., & Mau, J. L. (2007). Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea*, and *Boletus edulis*. *LWT*, *40*, 1392–1402.

Turner, C., King, J. W., & Mathiasson, L. (2001). Supercritical fluid extraction and chromatography for fat-soluble vitamin analysis. *Journal of Chromatography A*, *936*, 215–237.

Wang, Y. J., Cheng, Z., Mao, J. W., Fan, M. G., & Wu, X. Q. (2009). Optimization of ultrasonic-assisted extraction process of *Poria cocos* polysaccharides by response surface methodology. *Carbohydrate Polymers*, *77*(4), 713–717.

Wang, H., Jiang, H., Wang, S., & Li, X. (2013). Extraction, purification and preliminary characterization of polysaccharides from *Kadsura marmorata* fruits. *Carbohydrate Polymers*, *92*, 1901–1907.

Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y., & Yao, J. (2010). A comparison study on microwave-assisted extraction of *Potentilla anserina* L. polysaccharides with conventional method: Molecule weight and antioxidant activities evaluation. *Carbohydrate Polymers*, *80*(1), 84–93.

Wen, L., Lin, L., You, L., Yang, B., Jiang, G., & Zhao, M. (2011). Ultrasound-assisted extraction and structural identification of polysaccharides from *Isodon lophanthoides* var. gerardianus (Bentham) H. Hara. *Carbohydrate Polymers*, *85*, 541–547.

Wu, W. L., Zhu, Y. T., Zhang, L., Yang, R. W., & Zhou, Y. H. (2012). Extraction, preliminary structural characterization, and antioxidant activities of polysaccharides from *Salvia miltiorrhiza* Bunge. *Carbohydrate Polymers*, *87*, 1348–1353.

Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry*, *62*, 1201–1204.

Yin, X. L., You, Q. H., & Jiang, Z. H. (2011). Optimization of enzyme assisted extraction of polysaccharides from *Tricholoma matsutake* by response surface methodology. *Carbohydrate Polymers*, *86*, 1358–1364.

Zhang, A., Xiao, N., He, P., & Sun, P. (2011). Chemical analysis and antioxidant activity in vitro of polysaccharides extracted from *Boletus edulis*. *International Journal of Biological Macromolecules*, *49*, 1092–1095.