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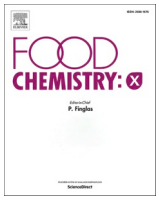

Impact of high pressure pre-treatment and hot water extraction on chemical properties of crude polysaccharide extract obtained from mushroom (*Volvariella volvacea*)

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ABSTRACT

An examination of the process of extracting crude polysaccharides from *Volvariella volvacea* solely through hot water treatment (HWE) at 60, 80, and 100 °C and through an approach involving high pressure processing (HPP) at 200, 400, and 600 MPa followed by HWE. The physiological properties of the polysaccharides could be explained by the structural analysis performed via FT-IR spectroscopy and NMR spectroscopy, which revealed the extract composition of the protein-bound polysaccharides connected by β-glycosidic bonds. Under the extraction conditions investigated in this current study, the recommended extraction condition was a combination of HPP (600 MPa, 10 min) and HWE (60 °C, 2 h). This condition gave high crude polysaccharide yields (with a 2–12% increase), and β-glucan content (with a 15–20% increase) without disrupting the β-glycosidic bond, as compared to using HWE alone. High pressure extraction could be an alternative technique for reduced extraction temperatures of active compounds from mushrooms.

Introduction

In East and Southeast Asia, straw mushrooms (*Volvariella volvacea* (Bull. ex Fr.) Sing.) are commonly consumed due to their continuous accessibility, convenience of purchase, and distinctive flavor and consistency. They are a rich source of protein and various bioactive compounds, including non-digestible carbohydrates, polyphenols, and flavonoids that have antioxidant activities (Cheung, 2008). Additionally, they contain bioactive polysaccharides that are of particular interest to researchers. One particular primary active ingredient derived from straw mushrooms is β-glucan (Ghosh, 2020), and several researchers have conducted studies of its effective extraction and its potential usefulness for therapeutic purposes. Straw mushroom glucans are known to provide antioxidative and anticancer effects, as well as stimulating the immune system. These qualities are due to the backbone, comprising glucose residues linked by β-(1–3)-glycosidic bonds and coupled β-(1–6) branch points (Cheung, 2008). Both the polysaccharide and total phenolic components of mushroom extracts were shown to be linked with their antioxidant properties (Cheung, Cheung, & Ooi, 2003). Therefore, it can be regarded as a highly prospective component. Additionally, it is important to note that the extraction method plays an extremely important role as it can affect yield, physicochemical characterization, and functional qualities.

The typical conventional technique for isolating edible mushroom crude polysaccharides uses hot water extraction (HWE) in conjunction with the alcohol precipitation approach. In many studies, HWE methods involve heating the sample for one to six hours at a temperature between 65 and 100 °C (Kim and Iwahashi, 2015; Chen et al., 2020; Sangthong, Pintathong, Pongsua, Jirarat, & Chaiwut, 2022). Low operational costs, safety, environmental friendliness, and straightforward equipment needs are among the benefits of HWE (Gong et al., 2020). Unfortunately, HWE also has a number of drawbacks, including a high extraction temperature that might degrade the most important chemicals and diminish polysaccharide activity, as well as a lengthy extraction procedure and a lengthy treatment process that restricts productivity during industrial processing (Ren et al., 2017). Due to their reduced time and

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energy requirements, recent research on the harvesting of mushroom polysaccharides using ultrasonic, microwave, enzyme, pulsed-electric-field, and high-pressure extraction has assisted in the creation of experimental designs for the recovery of bioactive ingredients from mushroom polysaccharides (Huang et al., 2022). Additionally, the use of non-thermal pre-treatment can enhance mass transfer as a substitute for heating, which may enable greater preservation of the end product's nutritional qualities (Luo et al., 2019; Fu, Belwal, Cravotto, & Luo, 2020).

High-pressure processing (HPP) is a non-thermal technique that employs high pressure delivered via a liquid medium (usually water) at pressures between 100 and 600 MPa (Gomathy, Pandiselvam, Kothakota, & Ramesh, 2021). Since HPP may shatter cell walls, increasing cell permeability, which in turn encourages the infiltration of solvents into cells, increasing mass transfer rates and chemical solubility, HPP can be utilized to enhance the extraction of bioactive components from animal, plant, and fungal sources. These features make HPP a high-efficiency and eco-friendly alternative method for polysaccharide extraction. Compared with traditional extraction technologies, HPP could ensure the biological activity of compounds because of the mild treatment temperature involved (<45 °C), can enhance the extraction efficiency of target components, and can reduce the extraction time and energy consumption (Li et al., 2021). Moreover, it could be used to modify the structure of extracted polysaccharides, such as by reducing the molecular weight, changing the ratio of monosaccharides and promoting the chemical reaction (Li et al., 2021). High-pressure processing was previously assessed for extracting polysaccharides from *Phellinus linteus* (Kim and Iwahashi, 2015), *Schizophyllum commune*, *Phellinus linteus* (Chen et al., 2020) and *Volvariella volvacea* (Sangthong, Pintathong, Pongsua, Jirarat, & Chaiwut, 2022) in order to enhance the separation of natural components with additional value in terms of biological characteristics. However, there are no well-known reports on polysaccharide contents of *Volvariella volvacea.* The objective of this study was to evaluate the application of high-pressure processing as a pre-treatment, followed by hot water extraction, for extracting polysaccharides from *Valvariella volvacea* and compare this approach with the traditional method of using only heated water. The chemical properties, antioxidant activity, β-glucans content, and structure analysis of the extracts were evaluated.

Materials and methods

Materials and chemicals

The fruiting bodies of straw mushrooms (*Volvariella volvacea*, *V. volvacea*) were purchased from a mushroom farm in Chachoengsao province, Thailand. Ethanol and methanol were purchased from Merck (Darmstadt, Germany), while 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich Co., Ltd. (Steinheim, Germany). Folin-Ciocalteu's phenol reagent was purchased from Loba Chemie (Mumbai, India). The standards (gallic acid and d-glucose) were purchased from Sigma-Aldrich Co., Ltd. (Steinheim, Germany). All other chemicals used were of analytical grade. The analytical mushroom β-glucan kit was obtained from Megazyme Int. (Wicklow, Ireland). Distilled and deionized water was used for the preparation of all solutions.

Preparation of mushroom powder

The fruiting bodies of the mushrooms were dried using a tray dryer (CD-1, PML tray dryer, Thailand) at 50 °C for 18 h to remove all moisture. Then, the dried mushroom was ground into fine particles (through a 20-mesh sieve) and kept in air-tight plastic bags at room temperature for further analysis.

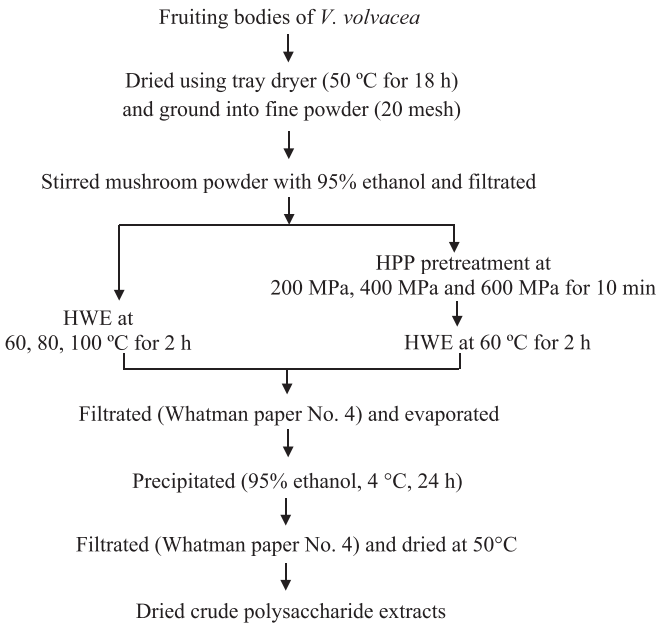

Fig. 1. Preparation of dried *Volvariella volvacea* crude polysaccharide extracts by using hot-water extraction (HWE) and high-pressure extraction (HPP) followed by HWE.

Crude polysaccharide extraction

Water-soluble substances were extracted from *V. volvacea* according to the method described by Kim, & Iwahashi, (2015) with additional modifications as shown in Fig. 1. Crude polysaccharide extracts of *V. volvacea* were prepared by hot-water treatment, alone and in combination with-high pressure treatment. *V. volvacea* powdered samples were stirred with 95% ethanol (ratio 1:10 *w/v*) for 1 h at 60 °C to eliminate low-molecular-weight components such as mono-and disaccharides, oligosaccharides, amino acids, lipids, and some phenols.

For hot-water extraction (HWE), 20 g of dried *V. volvacea* powder was extracted twice with hot water (ratio 1:25 *w/v*) at various temperatures (sample names: HWE 60 at 60 °C, HWE 80 at 80 °C and HWE 100 at 100 °C) at 200 rpm for 2 h. Then, the extracted solutions were centrifuged (8,000 rpm for 10 min) to collect the supernatant and concentrated with a rotary evaporator at 50 °C under vacuum. The concentrate was precipitated using 95% ethanol (ratio 1:4 *v/v*) at 4 °C for 24 h. After the centrifugation process, the precipitate was collected and dried at 50 °C to obtain the crude polysaccharide extract.

For high-pressure extraction (HPP) followed by HWE (HPPE), 20 g of dried *V. volvacea* powder in distilled water (ratio 1:25 *w/v*) was packed in a small plastic pouch and placed in the vessel of a laboratory-scale high-pressure processor with a 5-L capacity (HPP600MPa, KFHPP, China). The vessel was then filled with distilled water. The water was used as a pressure-transmitting medium and the internal temperature was set at room temperature. HPP was performed at 3 levels of 200–600 MPa for 10 min. After that, the hydrated samples following HPP were extracted two times with hot water at 60 °C (200 rpm for 2 h), and then filtered (sample names: HPPE 200; HPP 200 followed by HWE 60, HPPE 400; HPP 400 followed by HWE 60 and HPPE 600; HPP 600 followed by HWE 60). The extracted solution was centrifuged (8,000 rpm for 10 min) to collect the supernatant and concentrated with a rotary evaporator at 50 °C under a vacuum. The concentrate was precipitated using 95% ethanol (ratio 1:4 *v/v*) at 4 °C for 24 h. After the centrifugation process, the precipitate was collected and dried at 50 °C to obtain the crude polysaccharide extract.

Determination of proximate composition

The proximate composition (moisture, ash, fiber, protein, fat, and carbohydrate (by difference) of the mushrooms was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2000).

Determination of crude polysaccharide yields

The weight of crude polysaccharide extracts was recorded, and the crude polysaccharide yield (%) was calculated using the following formula:

$$\text{Yield (\%)} = (\text{Weight of dried extract/Weight of raw material}) \times 100$$

Determination of polysaccharide content

The polysaccharide content of crude extracts was determined by phenol–sulfuric acid assay as described by Sangthong, Pintathong, Pongsua, Jirarat, & Chaiwut, (2022), with d-glucose as the standard. The crude polysaccharide solutions (0.5 mL) were mixed with 0.50 mL of 5% phenol solution followed by 2.50 mL of sulfuric acid. The mixture was vortexed and incubated at 50 °C for 20 min and then stored at room temperature to cool. After cooling to room temperature, the absorbance of the mixture was measured at 490 nm. All assays were conducted in triplicate, and standard curves were obtained using d-glucose. The result was expressed as grams of d-glucose equivalent per gram of dry weight (g/g dw).

Determination of protein content

The protein content of the crude extracts was determined according to the method developed by Bradford, (1976), using bovine serum albumin (BSA) as a standard. The crude extracts (100 μL) were mixed with 3,000 μL of Coomassie reagent from Thermo Scientific (MN, USA) and then incubated at room temperature for 5 min. Protein concentration was determined by measuring the absorption at 595 nm.

Determination of total phenolic content

The total phenolic content (TPC) was measured according to the method of Tepsongkroh, Jangchud, & Trakoontivakorn, (2019). One milliliter of the crude polysaccharide extract was added to 4 mL of Folin-Ciocalteu reagent, which was diluted with distilled water (1:10). After 3 min, 5 mL of 7.5% Na_2CO_3 was added. The mixture was agitated and allowed to stand for a further 30 min in the dark. The absorbance of the extract and a prepared blank was measured at 765 nm using a spectrophotometer (X-ma 1200, Human Corporation, Korea). The TPC was standardized against gallic acid, and the data were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g dw).

Determination of antioxidant activity

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of crude polysaccharide extracts was determined according to the method of Liu, Jia, Kan, & Jin, (2013); Tepsongkroh, Jangchud, & Trakoontivakorn, (2019). Briefly, 2 mL of DPPH radical solution (0.2 mM DPPH in methanol) was mixed with 2 mL of each crude polysaccharide extract. The mixture was agitated and allowed to stand for a further 30 min in the dark. Then, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. The percentage inhibition was calculated according to the formula: $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control solution (without mushroom extracts) and A_1 is the absorbance of the DPPH radical solution containing mushroom extracts. The DPPH radical-scavenging activity was standardized against gallic acid, and the data were expressed as mg GAE/g dw.

Table 1
Proximate composition of *Volvariella volvacea* mushroom.

Composition	%, dry basis
Moisture[1]	7.46 ± 0.11
Protein[2]	24.60 ± 0.29
Fat	0.75 ± 0.36
Fiber	13.68 ± 0.25
Ash	10.01 ± 0.12
Carbohydrate[3]	50.96 ± 0.33

Values expressed as mean ± standard deviation of triplicate measurements.
[1] Percentage on a wet basis.
[2] Calculated using the conversion factor of N × 4.38.
[3] Calculated by difference.

Ferric ion reducing antioxidant power

The ability of polysaccharides to reduce ferric ions was analyzed according to the method described by Sudha, Vadivukkarasi, Shree, & Lakshmanan, (2012). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed in a water bath at 37 °C for 30 min. A 3 mL FRAP reagent was mixed with 300 μL water and 100 μL of the crude polysaccharide extracts for 30 min in the dark at room temperature, and the absorbance was measured at 593 nm. The $FeSO_4 \cdot 7H_2O$ solution was used to generate the standard curve. The FRAP values were expressed as milligrams of $FeSO_4$ equivalents per gram of dry weight (mg $FeSO_4$/g dw).

Determination of β-glucan

The glucan crude polysaccharide contents of *V. volvacea* were determined using a mushroom and yeast β-glucan assay kit from Megazyme (Wicklow, Ireland), according to methods reported by Kim, & Iwahashi, (2015); Sangthong, Pintathong, Pongsua, Jirarat, & Chaiwut, (2022), The β-glucan content was calculated by subtracting the α-glucan from the total glucan content. All values of glucan contents were expressed as grams per 100 g of dry weight.

Fourier transform infrared (FT-IR) spectroscopy

Infrared spectroscopy of crude polysaccharide extracts was performed by the KBr pellet approach with slight modifications according to the methods of Chen et al. (2020). The operation was as follows: 2 mg of dried polysaccharide extract was mixed with 100 mg of KBr powder and pressed. The FT-IR spectrum was obtained by Fourier-transform infrared spectroscopy (Nicolet-IS50, Thermo Scientific, USA) in the range of 4000–400 cm^{-1}.

Nuclear magnetic resonance (NMR) spectroscopy

Dried crude polysaccharide extracts were dissolved in D_2O for the detection of ^1H NMR and ^{13}C NMR spectra using an NMR spectrometer (Ascend TM 600/Avance III HD, Bruker, Switzerland). The NMR spectra were recorded at room temperature by standard.

Bruker software.

Statistical analysis

All data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed to determine whether differences

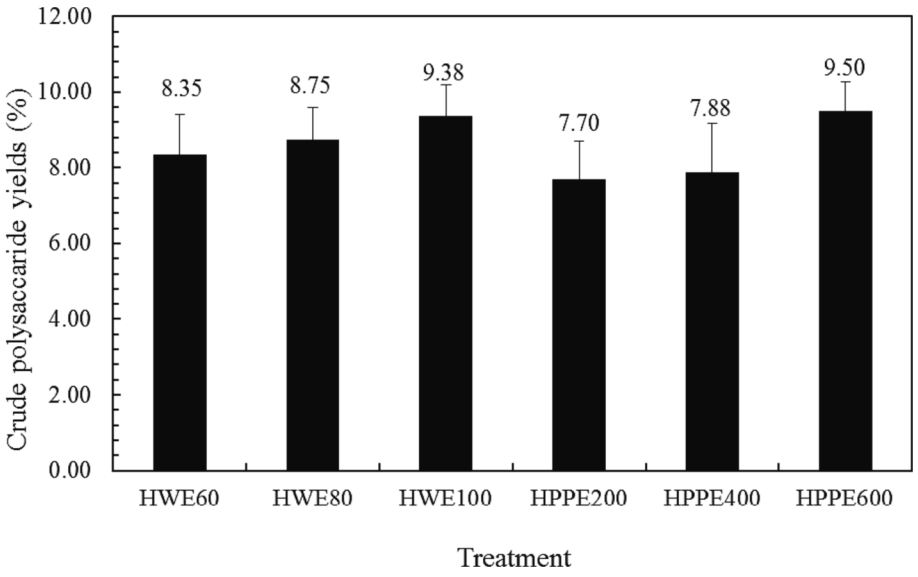

Fig. 2. Extraction yields of *Volvariella volvacea* crude polysaccharide extracts. HWE60; hot water extraction (60 °C), HWE80; hot water extraction (80 °C), HWE100; hot water extraction (100 °C), HPPE200; HPP pre-treatment (200 MPa) followed by HWE60, HPPE400; HPP pre-treatment (400 MPa) followed by HWE60, HPPE600; HPP pre-treatment (600 MPa) followed by HWE60.

existed among treatments in terms of chemical and antioxidant properties. Duncan's new multiple-range test was used to determine the differences between means established at $p < 0.05$. Data analysis was performed using SPSS software (Version 16, SPSS Inc., USA) and Microsoft Excel software (Version 16, Microsoft Corporation, USA).

Results and discussion

Table 1 presents the composition of dried *V. volvacea*, suggesting that *V. volvacea* contained the largest amounts of carbohydrate, at 50.96%, while protein comprised 24.60% and fiber comprised 13.68%. The overall carbohydrate content for these mushrooms, encompassing both digestible and non-digestible carbohydrate types ranged from 35% to 70% on a dry weight basis (Chen et al., 2011). The carbohydrates of *V. volvacea* typically exist as polysaccharides such as glycogen, hemicelluloses including mannans, xylans, and galactans, as well as other indigestible fibers such as chitin, cellulose, glucans, and dietary fibers (Singdevsachan et al., 2016). The results agreed with those reported previously by Tepsongkroh, Jangchud, & Trakoontivakorn, (2019); Sangthong, Pintathong, Pongsua, Jirarat, & Chaiwut, (2022), who found the prominence of carbohydrate, protein, and fiber in *V. volvacea*. Meanwhile, moisture, ash and fat comprised, respectively, 7.46%, 10.01%, and 0.75%. Differences in these values can arise in different climates and locations as well as between species.

Crude polysaccharide extraction yields and the contents of polysaccharide and protein

The yields of crude polysaccharide are significantly influenced by the conditions pertaining to the extraction process. One common approach

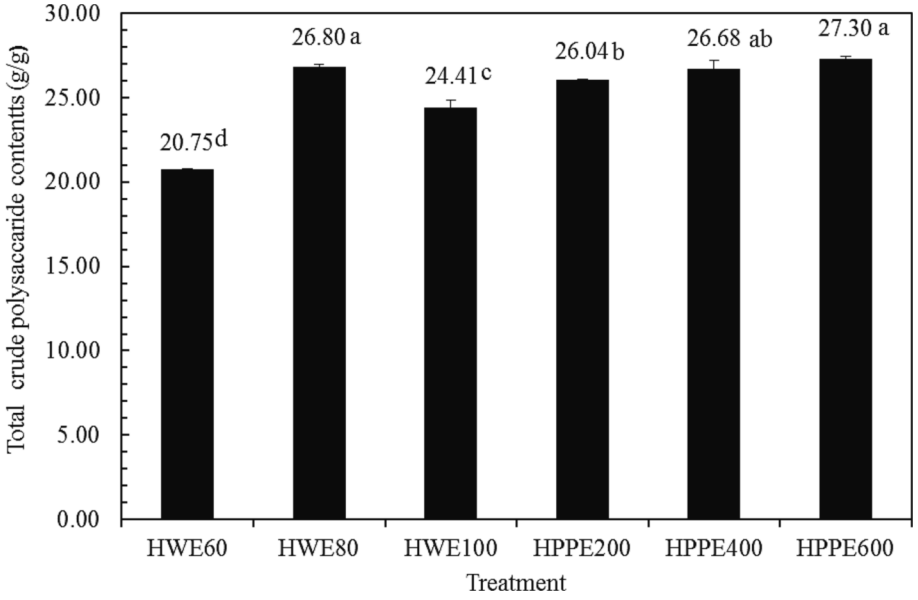

Fig. 3. Total crude polysaccharide contents of *Volvariella volvacea* crude polysaccharide extracts. HWE60; hot water extraction (60 °C), HWE80; hot water extraction (80 °C), HWE100; hot water extraction (100 °C), HPPE200; HPP pre-treatment (200 MPa) followed by HWE60, HPPE400; HPP pre-treatment (400 MPa) followed by HWE60, HPPE600; HPP pre-treatment (600 MPa) followed by HWE60.

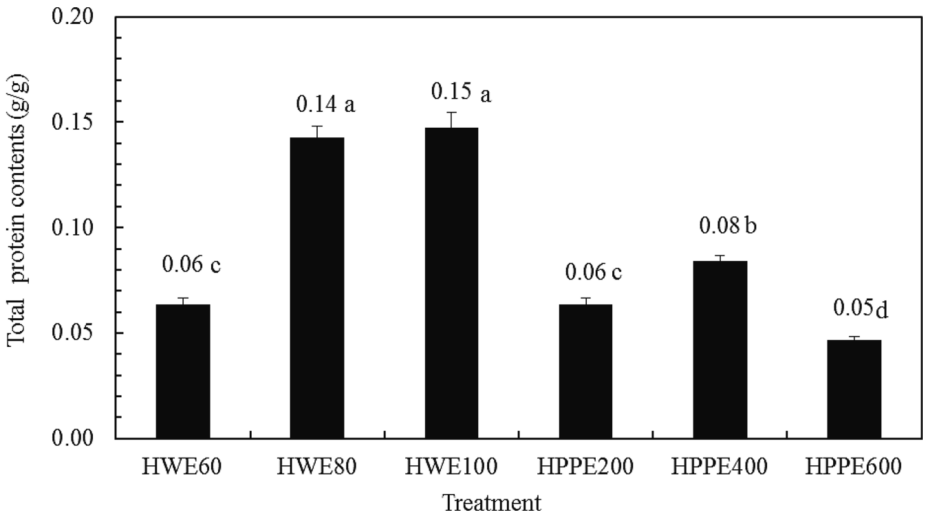

Fig. 4. Total protein contents of crude polysaccharide extracts of *Volvariella volvacea*. HWE60; hot water extraction (60 °C), HWE80; hot water extraction (80 °C), HWE100; hot water extraction (100 °C), HPPE200; HPP pre-treatment (200 MPa) followed by HWE60, HPPE400; HPP pre-treatment (400 MPa) followed by HWE60, HPPE600; HPP pre-treatment (600 MPa) followed by HWE60.

is to extract polysaccharides via HWE followed by ethanol precipitation. Fig. 2 presents the yield of crude polysaccharide extracts, revealing no significant differences in yields ($p > 0.05$). The various extraction conditions resulted in a range of crude polysaccharide extract yields from 7.70% to 9.50%, while the highest yields were observed for the HWE 100 and HPPE 600 treatments. The combination of HPP at 600 MPa followed by HWE at 60 °C increased crude polysaccharide yields about 2–12% compared to the use of HWE alone. It expressed that high pressure combined with hot water treatment can be effectively used to improve the extraction rate by increasing the mass transfer rate and possible rupture of the cell wall, membrane and organelles leading to higher product yields with reduced processing time and solvent consumption (Huang, Hsu, Yang, & Wang, 2013). Increasing the extraction temperature from 60 °C to 90 °C tended to increase crude polysaccharide yields, since the higher temperature increased the potential for the cell walls to rupture and release polysaccharides in greater quantities (Yuan, & Macquarrie, 2015). In the case of increased crude polysaccharide yields when applying high pressure pre-treatment, this can be attributed to the deprotonation of charged groups while salt bridges and hydrophobic interaction can be disrupted in the membranes of the cells, thus allowing heightened levels of permeability (Prasad et al., 2010).

Similar results were reported by Zhang, Xiao, He, & Sun, (2011); Chen et al. (2020), who used boiling water to extract polysaccharides from *Schizophyllum commune* and *Boletus edulis* and obtained respective yields of 8.26% and 8.83%. Meanwhile, according to Kim, & Iwahashi, (2015), the use of high pressure pre-treatment maximized the yields obtained from *Phellinus linteus*, although other changes in the extraction conditions might cause the results to vary. Moreover, the HPP and HPP followed by HWE treatment in this study provided higher extraction yields (7.70–9.50 %) compared to other extraction methods. Treatment with ultrasound-assisted under condition of 150 W powers, 50 min extraction time at 53 °C gave 2.46% of polysaccharide yields from *Acanthus ilicifolius* (Mtetwa et al., 2020). Microwave-assisted treatment under 550 W for 5 min condition gave 4.98% of polysaccharide yields from *Schizophyllum commune* (Chen et al., 2020).

It has also been found that the relative abundance of total crude polysaccharide content can be significantly altered by HWE alone and by high-pressure pre-treatment followed by hot water. Both temperature and pressure are factors that cause variation in polysaccharide content as soluble proteins are also extracted and sample compression occurs, so that water molecules cannot be internally diffused among the solid particles (Barbosa et al., 2020). Total crude polysaccharides content

Table 2
Total phenolic content, antioxidant activity and β-glucan content of *Volvariella volvacea* crude polysaccharide extracts.

Treatment	TPC (mg GAE/g dw)	DPPH (mg GAE/g dw)	FRAP (mg GAE/g dw)	β-glucans (% w/w)
Hot water Extraction (HWE)				
HWE 60	3.51 ± 0.13^{bc}	0.23 ± 0.07^{b}	0.05 ± 0.00^{c}	7.62 ± 0.10^{bc}
HWE 80	3.52 ± 0.12^{bc}	0.47 ± 0.02^{a}	0.05 ± 0.00^{bc}	8.41 ± 0.36^{b}
HWE 100	4.42 ± 0.14^{a}	0.46 ± 0.11^{a}	0.06 ± 0.00^{a}	8.05 ± 0.13^{b}
High pressure followed by HWE 60 (HPPE)				
HPPE 200	3.30 ± 0.37^{c}	0.26 ± 0.10^{b}	0.05 ± 0.01^{d}	6.96 ± 0.19^{c}
HPPE 400	3.70 ± 0.06^{b}	0.39 ± 0.05^{a}	0.06 ± 0.00^{b}	7.78 ± 0.89^{bc}
HPPE 600	3.63 ± 0.01^{b}	0.41 ± 0.01^{a}	0.07 ± 0.00^{a}	9.43 ± 0.01^{a}

Values expressed as mean ± standard deviation of triplicate measurements. Means with different lowercase superscript letters in the same column are significantly ($p < 0.05$) different.

ranged between 22.44 and 29.51 g/g (Fig. 3), with the lowest and highest content observed from HWE 60 and HPPE 600, respectively. Clearly, using a higher temperature over 80 °C resulted in decreased total polysaccharide content, as these conditions would damage the structure of the polysaccharides and lead to negative reactions (Zhang et al., 2015). However, the total polysaccharides content will be affected by the temperature and pressure applied for the extraction process as well as by the choice of equipment used.

The protein content of the crude polysaccharides is shown in Fig. 4. Among all the treatments, the protein content were in the range of 0.05–0.15 g/g. Considering HWE treatment, the protein content increased when the extraction temperature increased. The increase in protein content was probably because protein was partly denatured and the polymeric components (protein-starch complexes) are degraded at high temperatures, which resulted in an increased amount of free protein in the water (Imjongjairak et al., 2016). Considering HPPE treatments, the protein content increased when the HPP increased from 200 to 400 MPa but decreased slightly when the HPP increased from 400 to 600 MPa. The decrease in the protein content was probably caused by partial protein denaturation and condensation. The surface exposure of hydrophobic bonding and –SH groups will be connected with –SH binding in new hydrophobic interactions, which leads to the aggregation of denatured proteins (Yin, Tang, Wen, Yang, & Li, 2008). These results agreed with those reported by Zhao, Huo, Qian, Ren, & Lu, (2017), who worked on the extraction of bioactive peptides from mushroom and found that increasing HPP from 400 to 500 MPa decreased the protein

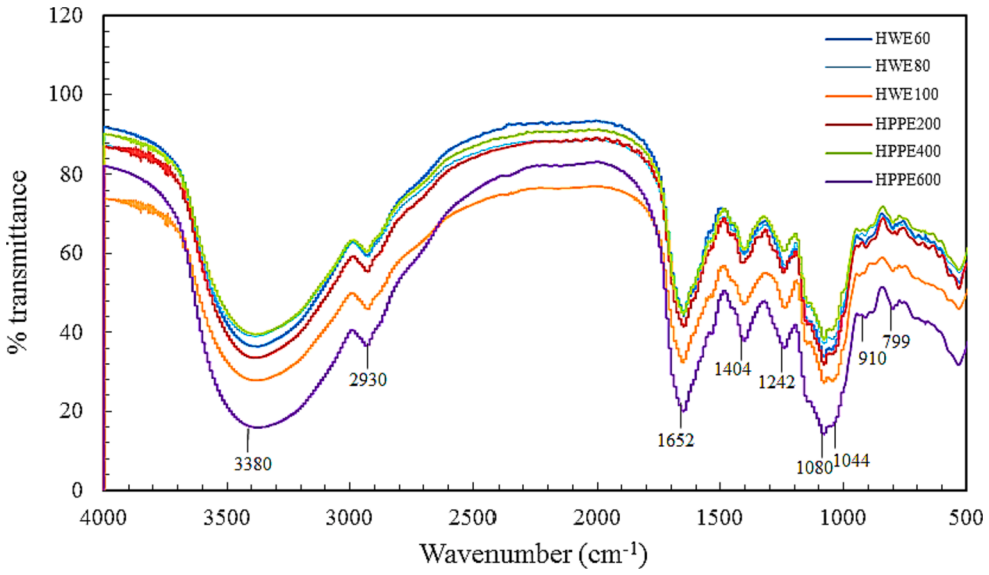

Fig. 5. Fourier-transform infrared spectrum of crude polysaccharide extracts from *Volvariella volvacea*.

content.

Total phenolic content

Among the extraction conditions, the TPC of *V. volvacea* polysaccharide extracts was found to be in the range of 3.30–4.42 mg GAE/g dw (Table 2) and showed a slight increase with increasing the extraction temperature from 60 to 100 °C. The maximum value of 4.42 mg GAE/g dw occurred in the case of HWE 100. Extraction at high temperatures allowed the phenolic–matrix bonds within the cellular matrix to be broken, thus changing the chemical structure and releasing significant quantities of polyphenolic compounds (Liu et al., 2020; Fu et al., 2021). Considering HPPE treatments, an increase in TPC could be seen as the pressure increased from 200 MPa to 400 MPa, although no significant difference ($p > 0.05$) could be achieved as the pressure increased further from 400 MPa to 600 MPa. The increase in TPC might be a consequence of temperature- and pressure-induced cell disruption, ensuring that as the cell walls break down, certain antioxidant complexes see an increase in their bioavailability (Barba, Esteve, & Frigola, 2013). Therefore, high-pressure pre-treatment along with hot water can achieve similar TPC levels when employed with lower temperatures than are needed when hot water is used alone at high temperature.

Antioxidant activity

Table 2 shows the results for antioxidant activity of crude polysaccharide extracts measured via DPPH and the FRAP assay. The differences might be adjusted if the conditions of temperature and pressure were altered. Mushroom polysaccharide extracts have the ability to scavenge free radicals due to the existence of hydrogen in some of the monosaccharide units, which are able to bind to the side branches connecting to the main chain (Lo et al., 2011).

Free-radical scavenging in polysaccharides is usually evaluated via DPPH assay. The greater extraction efficiency at high temperature and pressure could account for the high antioxidant activity and TPC. Increasing the pressure and temperature led to a significant increase ($p < 0.05$) in the scavenging ability related to DPPH radicals. Considering HWE treatments, the highest DPPH radical-scavenging abilities were found in HWE 80 and HWE 100. This increase in scavenging activity could be due to the applied heat causing the degradation of polysaccharides. In such degraded polysaccharides, it would be more likely that the chemical groups they contain would come into contact with radicals due to the greater surface area involved and enhanced water solubility (Chen et al., 2019). As the pressure increased, the ability to scavenge DPPH radicals also increased, with the best performance exhibited in HPPE 400 and HPPE 600. The effect of high pressure is to expand the extracellular matrix, allowing the solvent to enter the sample matrix in greater quantities, ensuring an increased likelihood of contact between the target compounds and the solvent (Huang, Cheng, Chen, & Wang, 2019). This situation would occur because the pressure of 400–600 MPa upon *V. volvacea* would be able to break certain non-covalent bonds, thus enhancing the saccharide solubility in water at high pressures (Li et al., 2021). Another important consideration is ferric reducing power, which can be assessed by determining the capacity to perform Fe^{3+} to Fe^{2+} reduction, thus indicating antioxidant activity. Polysaccharides are known to serve as electron donors capable of reacting with free radicals to create stable products (You, Yin, & Ji, 2014). The results showed that all the treatments exhibited reducing capacity. The *V. volvacea* polysaccharide extracts acted as electron donors capable of creating stable products by reacting with free radicals, thus causing the termination of radical chain reactions. The highest reduction ability was found in HWE 100 and HPPE 600. The application of high pressure or extraction at higher temperatures may increase the solvent density and power, as well as boosting the polar compound solubility (Prasad et al., 2010).

On the basis of the DPPH and FRAP assay results, it can be seen that *V. volvacea* polysaccharide extracts offer antioxidant activity. This heightened level of antioxidant activity may result from the presence of an impure fraction, as is the case of those phenolic compounds that display high levels of antioxidant activity (Wang, Hu, Nie, Yu, & Xie, 2016). Furthermore, some authors have stated that the antioxidant properties of polysaccharides are a consequence of their underlying chemical structure, including factors such as carbohydrate content, and the presence of proteins and phenolics. In addition, polysaccharides contain the –C—O, –OH, and –COOH functional groups, which are commonly linked to antioxidant activity (Deveci et al., 2019).

β-glucans content

The β-glucans in polysaccharide extracts from *V. volvacea* mushrooms were affected by different extraction conditions and are shown in Table 2. Determination of the β-glucan contents in the *V. volvacea* polysaccharide extracts was carried out due to the importance of this particular mushroom polysaccharide. The method used was to subtract

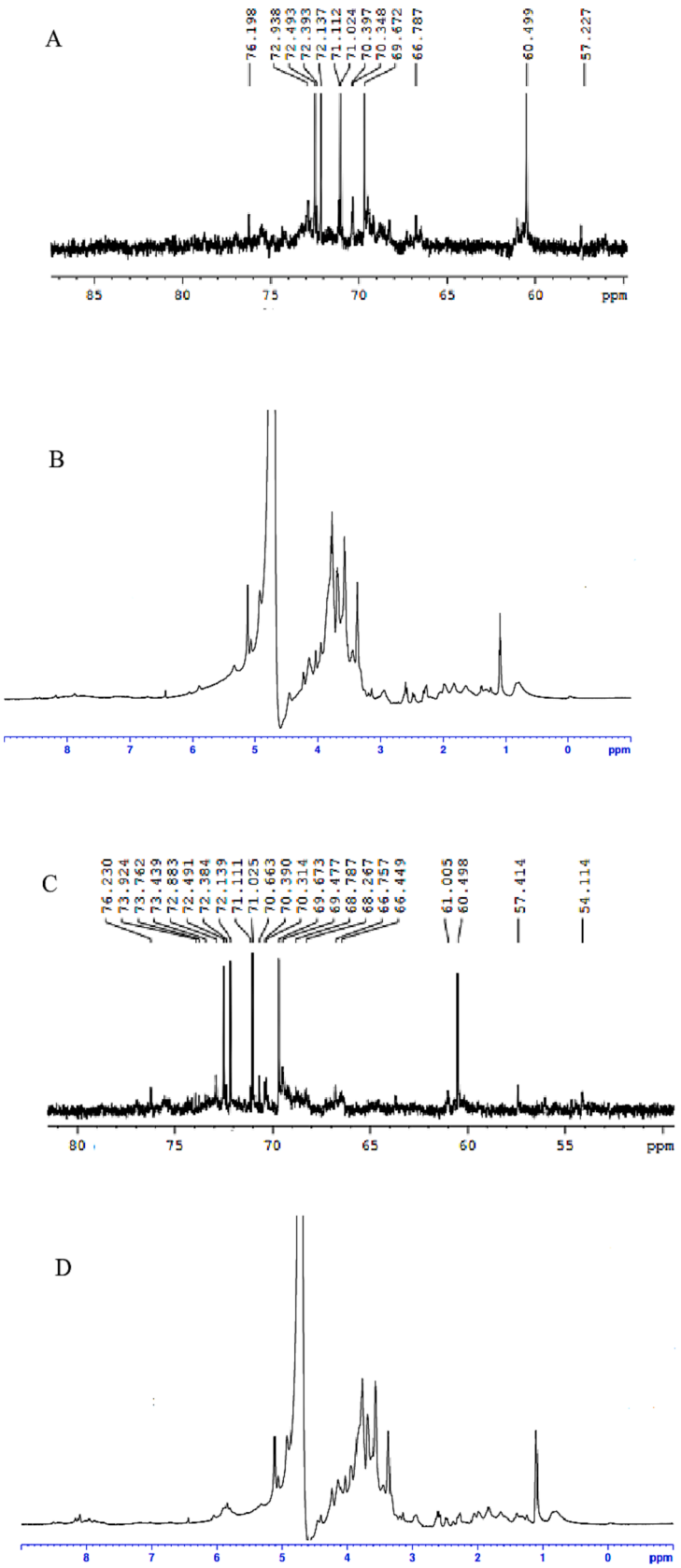

Fig. 6. The ^{13}C NMR spectrum of HWE100 (A), ^{1}H NMR spectrum of HWE100 (B) and ^{13}C NMR spectrum of HPPE600 (C), ^{1}H NMR spectrum of HPPE600 (D).

the amount of α-glucan from the total glucan content, thus arriving at a figure for the β-glucan content. The highest β-glucan contents were observed in HPPE 600 (9.43% w/w). The combination of HPP at 600 MPa followed by HWE at 60 °C increased β-glucan contents about 15–20% compared to the use of HWE alone. Increasing the pressure and water temperature serves to destroy interactions and break bonds linking the polysaccharides within the matrix, thus allowing β-glucans to be released into the solvent (Benito-Román, Alonso, & Cocero, 2013). A similar result was reported by Kim, & Iwahashi, (2015) who found that β-glucan content of HPP at 300 MPa combined with 65 °C hot water treatments was higher than that of 95 °C hot water treatment alone.

Fourier transform infrared (FT-IR) spectroscopy analysis

The characteristic functional groups of polysaccharides can be identified via FT-IR. Fig. 5 presents the FT-IR spectra for *V. volvacea* polysaccharides, with all treatments returning similar spectra, from which it can be inferred that the chemical structures of the polysaccharides extracted under different conditions might be rather similar. There was a strong and broad peak around 3,300–3,400 cm^{-1} indicative of the stretching vibration of the carbohydrate O—H bonds (Kim, & Iwahashi, 2015). Meanwhile, an absorption peak around 2,900–2,935 cm^{-1} revealed C—H stretching, demonstrating that polysaccharides were present (Deveci et al., 2019). Additional peaks in the range of 1075–1082 cm^{-1} confirmed that a pyranose ring was present (Yan et al., 2019), while peaks occurring around 1,600–1,650 cm^{-1} showed the stretching vibration of the carbohydrate C—O along with a clear protein absorption peak in the N—H stretching region (peak around 3300–3400 cm^{-1}), thereby indicating that the polysaccharides were comprised of protein (Kim, & Iwahashi, 2015). The presence of α-linked glycosyl groups within the main chain was revealed by an absorption peak around 900–930 cm^{-1}, while a further strong peak around 990–1,050 cm^{-1} represented the β-linked glycosyl groups of the main chain (Deveci et al., 2019). Considering these criteria, the HWE and HPPE samples produced spectra showing that all of the samples comprised protein-bound polysaccharides connected by α-glycosidic and β-glycosidic bonds. Kim, & Iwahashi, (2015) reported similar polysaccharide spectra for extracts prepared from *Phellinus linteus* via HPP pre-treatment where the pressure was 300 MPa and the temperature was 65 °C. They compared this to hot water treatment alone at 95 °C, finding protein-bound polysaccharides connected by β-glycosidic bonds. Moreover, similar FT-IR results were found by Deveci et al. (2019) who reported that polysaccharide extracts from *Ganoderma applanatum* revealed absorption peaks around 3,000 –3,500 cm^{-1}, indicative of –OH stretching; around 2,924 cm^{-1}, revealing $–CH_2$ stretching; around 2,856 cm^{-1}, showing C—H stretching; in the range of 1690–1,750 cm^{-1}, for C═O bonds; at 1,639 cm^{-1}, for proteins; then at 1,155 and 890 cm^{-1}, confirming β-glycosidic linkage; and finally at 920 cm^{-1}, showing α-linkage.

Nuclear magnetic resonance (NMR) spectroscopy analysis

In order to further verify the structural of crude polysaccharide extracts from *Volvariella volvacea*, ^{1}H NMR and ^{13}C NMR spectra were characterized. The ^{1}H NMR spectrum of all treatments showed signals in the range of 3.5–5.0 ppm, whereas the ^{13}C NMR spectrum showed signals in the range of 60–80 ppm (data not shown). The presence of these NMR signals indicates the presence of α and β-configurations of the glycosidic bond substituted in the crude polysaccharide extracts (Zhao et al., 2020). Fig. 6 presents the representative treatments as the ^{1}H NMR and ^{13}C NMR spectra of HWE 100 and HPPE 600. For both treatments, the ^{1}H NMR spectra exhibited a proton signal peak predominantly ranging from 4.4 to 4.8 ppm and from 3.5 to 3.7 ppm, thus confirming the presence of both β-linkages and α-linkages (Zhao et al., 2020), in accordance with similar findings from the FT-IR spectrum shown in Fig. 5. In the ^{13}C NMR spectrum of both treatments, most of the signals appearing at 70 to 75 ppm were assigned to unsubstituted *C*2, C3, C4, and C5, and the peaks appearing at 60 ppm were attributed to unsubstituted C6 (Gong et al., 2020). The results were similar to those of Wang et al., (2021), who reported that the signals appearing at 75.52, 72.99, 69.43, 68.20, and 60.61 ppm indicated the identity of the primary chain in *Stropharia rugosoannulata* polysaccharides as (1 → 3)-β-D-Glcp linkage. Accordingly, our findings from NMR demonstrate that the β-glucan (1, 3/1, 6) structure was predominant in the extracts of crude polysaccharide obtained from *Volvariella volvacea*, consistent with the results of FT-IR.

Conclusion

The extracts of crude polysaccharides from *Volvariella volvacea* prepared using HWE treatment and also using HPP followed by HWE at 60 °C provided differing yields, polysaccharide content, and protein content. All extracts seem to consist of a mixture/complex of polysaccharides, proteins, and polyphenols that could be bound or even covalently and α and β-glycosidically linked. Along with the changes in chemical properties caused by the HWE treatments and by HPP followed by HWE at 60 °C, increasing temperature or pressure tended to increase the TPC, antioxidant activities and β-glucan content. The recommend condition for polysaccharide extraction was combining the HPP at 600 MPa followed by HWE at 60 °C which gave the highest yields, antioxidant activities and β-glucan content. Therefore, HPP technology may have an important future role to play in the extraction of crude polysaccharides, as it enables extraction at lower temperature and can be considered as a form of green chemistry.

CRediT authorship contribution statement

Benjarat Tepsongkroh: Conceptualization, Funding acquisition, Methodology, Data curation, Supervision, Writing – original draft, Writing – review & editing. **Chuttida Thaihuttakij:** Methodology, Data curation. **Supattra Supawong:** Review & editing. **Kamolwan Jangchud:** Visualization, Writing – review & editing.

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

Data will be made available on request.

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