

Physicochemical properties and antimicrobial activity of glucan from mushroom by-products by ultrasonic extraction compared to the conventional hot water method

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Abstract

Mushroom by-products are viewed as a source of bioactive polysaccharides known as glucans. The physicochemical properties and antimicrobial activity of glucan extracted from mushroom (*Pleurotus sajor-caju* (Fr.) singer) by-products using an ultrasonic method were compared with the conventional hot water method. Ultrasonic extraction was conducted using an ultrasonic water bath with frequency 40 kHz and 200 W ultrasonic power at 40°C for 1 h, while the conventional extraction was carried out using hot water at 100°C for 4 h. The chemical composition, Fourier transform infrared spectroscopy, rheological property, functional properties and antimicrobial activity were investigated. The results showed that the ultrasonic extraction delivered lower extract yield and glucan content ($p < 0.05$). The FTIR spectra showed similar primary structure and functional groups of the two extracts. The ultrasonic extract had lower viscosity, swelling power, fat binding capacity, and foaming capacity ($p < 0.05$). Both extracts exhibited inhibitory action against *Bacillus cereus*, with the ultrasonic extract demonstrating a significantly wider inhibition zone ($p < 0.05$). The ultrasonic extract showed significant inhibition of *Staphylococcus aureus* ($p < 0.05$), while neither extract showed inhibition of *Escherichia coli*.

1. Introduction

Mushrooms are recognized as a functional food containing bioactive compounds that promote human health. Recently, the global mushroom industry has been expanded, resulting high amount of by-products including stripes, discarded space bags and broken fruiting bodies, mushrooms with irregular shape or dimensions, accounting for 5 to 25% of production volume.

Mushroom by-products are viewed as sources of bioactive polysaccharides known as glucans. These homopolysaccharides are composed of D-glucopyranose that can be linked by α - or β -glycosidic bonds called α -glucan or β -glucan. Glucans performed biological functions such as immunomodulation, antitumor, anticancer, antioxidative, antidiabetic, antihypertensive effects, and antimicrobial activity, and they have attracted increased attention over the past decades (Zhu *et al.*, 2015; Zhu, Du and Xu, 2016). However, their efficacy as nutraceutical agents differs due to disparities in their structure and physicochemical properties (Khan

et al., 2017; Ma *et al.*, 2022; Yuan *et al.*, 2022).

The variety in glucan characteristics has been attributed to diverse sources and extraction methods. Common methods used for extracting glucan are hot water, acid/alkali and enzyme extractions. New extraction methods such as ultrasonic, pulsed electric fields, microwave and high pressure were also used to improve the efficiency of glucan recovery. Among these, the ultrasonic-assisted method attracted greater attention due to its simpler and safer, less time and energy, increased production and quality, more convenient and environmentally friendly features. Ultrasound refers to sound waves beyond the audible frequency range (> 20 kHz). When the ultrasound extraction process is performed under the action of ultrasound, cavitation induced by ultrasonic waves generates high shear force, leading to disruption of cell walls, thereby enhancing mass transfer and extraction yield.

Several studies have reported the efficiency of the ultrasonic method in the extraction of mushroom glucans, with most focusing on extraction rate and yield.

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Extraction yield was improved using ultrasonic extraction at optimal temperature, frequency, and duration (Cheung *et al.*, 2013; Alzorqi *et al.*, 2017; Morales *et al.*, 2019; Wang, 2020; Liu *et al.*, 2021; Cao *et al.*, 2023). Some studies noted the effect of ultrasound on glucan structure and antioxidant activity. Ultrasound application induces ultrasonic cavitation, which causes fragmentation of the native molecular chains and reduces the molecular weight, thereby increasing the activity of scavenging free radicals and antioxidant activity (Alzorqi *et al.*, 2017; Li *et al.*, 2017; Wang, 2020; Cao *et al.*, 2023). The biological activities of the glucan were similarly influenced by its molecular weight. Reducing the molecular weight altered the anti-inflammatory activity (Liu *et al.*, 2023) and inhibitory effects on lipid peroxidation, tyrosinase, and elastase (Sangthong *et al.*, 2022). However, no data are available regarding the influence of ultrasound on antimicrobial activity of mushroom glucan, while studies on viscosity, solubility, water absorption capacity, swelling power, oil absorption capacity, and emulsifying and foaming properties describing the behavior of glucan during processing and their impacts on the finished products in terms of look, feel, and taste are limited. Previous reports indicated that these characteristics were correlated with the structure of glucan and its biological activity (Zhu *et al.*, 2016; Bai *et al.*, 2019; Sun *et al.*, 2020; Cui and Zhu, 2021; Yang and Huang, 2021).

Therefore, this study investigated the physicochemical properties of glucan ultrasonically extracted from mushroom by-product, mainly focusing on rheological, functional and antibacterial properties in compared to conventional hot water extraction.

2. Materials and methods

2.1 Sample preparation

Mushroom (*Pleurotus sajor-caju* (Fr.) singer) by-product, obtained from a mushroom farm in Sakon Nakhon province, Thailand, was cleaned with tap water, cut into small pieces and dried using a tray dryer at 55°C. The dried sample was ground and sieved to get mushroom powder with particle size smaller than 250 micron (MP), then packed in a sealed aluminium foil bag and stored at 4°C.

2.2 Glucan extraction

Two different methods, that is, hot water and ultrasonic extractions, were performed to prepare glucan extracts. Commercial yeast glucan was employed as a control for glucan characteristics.

The hot water extraction method followed Alzorqi *et al.* (2017). A 10 g of mushroom powder was mixed with

250 mL of distilled water and heated in a water bath at 100°C for 4 h. The solution was centrifuged at 5000×g for 10 min, and the supernatant was collected and evaporated at 50°C (Büchi Rotavapor R-200/205, Switzerland). The concentrate was precipitated by mixing with 95% ethanol (1:4 v/v) at room temperature and kept at 4°C for 24 h. The precipitate was separated by 5000×g centrifugation for 10 min, then freeze-dried and ground to get a hot water extract sample (HW).

The ultrasonic extraction was conducted following the methodology outlined by Alzorqi *et al.* (2017) and Morales *et al.* (2019), with slight modifications. First, 10 g of mushroom powder was mixed with 250 mL of distilled water and placed in the ultrasonic bath (Ultrasonic bath GT Sonic, model:VGT-1990QTD, Frequency: 40KHz, Ultrasonic Power: 200W, Heating Power: 300W, China) controlled at 40°C for 1 h. Then, the solution was subjected to the same procedure used for hot water extraction to obtain the ultrasonic extract sample (US). The duration of the ultrasonic extraction differed from the hot water extraction and was determined based on the maximum yield according to Alzorqi *et al.* (2017).

The yield of extracts (g/100 g dried mushroom) was calculated as follows:

$$\text{Yield} = \frac{\text{weight of extract}}{\text{weight of mushroom powder}} \times 100$$

2.3 Proximate analysis

Proximate composition, i.e., moisture, protein, fat, and ash contents of mushroom powder and glucan extracts, was analyzed by the method of AOAC INTERNATIONAL (2000). The moisture content was determined by drying each sample in a hot air oven to a constant weight (AOAC Method 930.15). The crude protein was determined by the Kjeldahl method (AOAC Method 954.01). Crude fat was extracted using a Soxhlet apparatus with petroleum ether (AOAC Method 920.39). The total ash content was determined by dry mineralization in a muffle furnace (AOAC Method 942.05).

2.4 Glucan content quantification

The glucan content was determined using an assay kit (Megazyme, Bray Business Park, Co. Wicklow, Ireland). Total glucan, β-glucan, and α-glucan were quantified.

For total glucan measurement, the mushroom powder (90 mg) was weighed in a culture tube and 2.0 mL of 12 M sulfuric acid was added and stirred vigorously on a vortex mixer. The tube was then placed in an ice water bath for 2 h. Distilled water (10 mL) was

then added and kept at 100°C in a water bath for 2 h. After cooling to room temperature, the mixture was transferred to a 100 mL volumetric flask, 6 mL of 10 M KOH was added, and the volume was adjusted to 100 mL with 200 mM sodium acetate buffer (pH 5.0). After mixing well, the solution was centrifuged at 1500×g for 10 min and collected the aliquot. An aliquot of 0.1 mL was mixed with 0.1 mL of a mixture of exo-1,3-β-glucanase (20 U/mL) plus β-glucosidase (4 U/mL) in 200 mM sodium acetate buffer (pH 5.0), and incubated at 40°C for 60 min. Approximately 3 mL of GOPOD reagent was then added, incubated at 40°C for 20 min, and the absorbance of the solution was measured at 510 nm against the reagent blank.

For α-glucan measurement, 100 mg of mushroom powder was weighed in a culture tube, and 2 mL of 2 M KOH was added. The mixture was stirred in an ice water bath using a magnetic stirrer for 20 min. Then, 8 mL of sodium acetate buffer (1.2 M, pH 3.8) and 0.2 mL of amyloglucosidase plus invertase were added, mixed well, and placed in a 40°C water bath. After 30 min incubation with intermittent vortex stirring, the mixture was centrifuged at 1500×g for 10 min. A 0.1 mL supernatant volume was collected and mixed with 0.1 mL of sodium acetate buffer (0.2 M, pH 5.0) plus 3.0 mL of GOPOD reagent and incubated at 40°C for 20 min. The absorbance was measured at 510 nm against the reagent blank.

Total glucan, α-glucan, and β-glucan contents (g/100 g) were calculated as follows:

$$\text{Total glucan} = \Delta E \times \frac{F}{W} \times 90$$

$$\alpha - \text{glucan} = \Delta E \times \frac{F}{W} \times 9.27$$

$$\beta - \text{glucan} = (\text{Total glucan}) - (\alpha - \text{glucan})$$

where ΔE = Reaction absorbance - Blank absorbance, F = 100/Absorbance of D-glucose standard and W = Weight of sample

The blank consisted of 0.2 mL of sodium acetate buffer (0.2 M, pH 5.0) plus 3.0 mL of GOPOD reagent. The D-glucose standard consisted of a mixture of 0.1 mL of D-glucose (1 mg/mL) and 0.1 mL of sodium acetate buffer (0.2 M, pH 5.0) plus 3.0 mL of GOPOD reagent.

2.5 Fourier transform infrared spectroscopy

The chemical structure analysis was performed using a Fourier transform infrared spectrophotometer, FTIR (Thermo Scientific, Nicolet 6700, USA) with 32 scans in the wavelength range between 4000 and 400 cm⁻¹.

2.6 Rheological property measurement

The glucan solutions were prepared at 1, 2, and 3% (w/v) concentrations by dissolving the required amount of glucan extracts in distilled water at room temperature. Rheological property was measured at 25°C using a Rheometer (Physica MCR 51, Anton-Paar, Germany) fitted with a cone and plate geometry (50 mm diameter and 4° angle) with a gap of 0.046 mm (Bao *et al.*, 2016) and shear rate from 0.01 to 1000 1/s. The experimental flow curve at different concentrations was fitted with the Power law model:

$$\eta = K \times \dot{\gamma}^{n-1}$$

Where K is the apparent viscosity (cP), K is the consistency index, γ is the shear rate, and n is the flow behavior index.

2.7 Functional property measurement

2.7.1 Swelling power

Swelling power was determined following Khan *et al.* (2017). A 0.1 g was dissolved in 10 mL of distilled water and heated in a shaking water bath at 70°C for 10 min, followed by heating at 100°C for 5 min. The mixture was then cooled with tap water and centrifuged at 1700×g for 4 min. The wet sediment was weighed, and the swelling power was calculated as:

$$\text{Swelling power (g/g sample)} = \frac{\text{weight of sediment}}{\text{weight of dry sample}}$$

2.7.2 Fat binding capacity

Fat binding capacity was determined following the method of Khan *et al.* (2017). A 0.2 g sample was dissolved in 10 mL soybean oil and agitated using a vortex mixer every 15 min for 1 h. The mixture was centrifuged at 1600×g for 20 min, then the supernatant was removed and the sediment was weighed. The fat absorption was calculated by the amount of oil bound to 1 g of dry sample (g/g sample) as:

$$\text{Fat binding capacity (g/g sample)} = \frac{\text{weight of sediment} - \text{weight of dry sample}}{\text{weight of dry sample}}$$

2.7.3 Emulsifying property

The emulsion was prepared by mixing 0.05 g sample with 5 mL of soybean oil using homogenizer. The emulsion was then centrifuged at 1100×g for 5 min (Sridaran *et al.*, 2012). The emulsion capacity was evaluated as:

$$\text{Emulsion capacity (\%)} = \frac{\text{height of the emulsified layer}}{\text{total height of mixture}} \times 100$$

The emulsion stability was determined by heating the emulsion at 80°C for 30 min, followed by centrifuging at 1100×g for 5 min. The emulsion stability was calculated as:

$$\text{Emulsion stability (\%)} = \frac{\text{height of the emulsified layer after heating}}{\text{height of the emulsified layer before heating}} \times 100$$

2.7.4 Foaming property

The foaming property was determined according to the method of Khan *et al.* (2017) with slight modification. A 0.5 g sample was dissolved in 25 mL of distilled water, and homogenized with homogenizer at 10,000 rpm for 1 min. The foaming capacity was calculated as:

$$\text{Foaming capacity (\%)} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{volume before whipping}} \times 100$$

The foam stability was measured at 60 min standing time as:

$$\text{Foaming stability (\%)} = \frac{\text{volume of foam after standing time}}{\text{volume of foam at initial time}} \times 100$$

2.8 Antibacterial activity

Antibacterial activity of mushroom extract was assessed by the agar-well diffusion method (Standard Protocol of Institute of Biological Sciences, 2004). The bacteria used in this study, obtained from the Thailand Institute of Scientific and Technology Research, were gram-negative *Escherichia coli* TISTR 074 and gram-positive *Staphylococcus aureus* TISTR 2329 and *Bacillus cereus* TISTR 1527. The bacteria were cultured in nutrient agar (NA) at 37°C for 24 h. Before experimental use, cultures from solid medium were subcultivated in nutrient broth, incubated at 37°C for 24 h until the initial cell cultures reached 10⁸ CFU/mL. The turbidity of microbial suspension was adjusted to 0.5 McFarland standard before use.

Then, 100 mL of bacterial cell suspensions was spread on NA surfaces, and the inoculated plates were left for 15-20 min at room temperature. An 8 mm hold was made in each inoculated plate by a cork borer. For antibacterial activity test, the extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 500 mg/mL and filter-sterilization through a 0.22 mm membrane filter, and the 100 µL (equivalent to 50 mg) of the extract was loaded in each hold. As a reference, a paper disc of 10 mg streptomycin was used as a positive control, and DMSO was used as negative control. The plates were incubated at 37°C for 24 h. After incubation,

the zone of inhibition was measured, with data recorded as the diameter of the inhibition zone minus the hole diameter.

2.9 Experimental design and statistical analysis

This study used analysis of variance (ANOVA) with a complete randomized design, with differences between treatments compared using Duncan's multiple range test at a 95% confidence interval ($p < 0.05$).

3. Results and discussion

3.1 Yield and chemical composition

The glucan extract yields are presented in Table 1. Ultrasonic extraction delivered lower extract yields compared with hot water extraction. Sangthong *et al.* (2022) also found that ultrasonic extraction provided a lower extraction yield of glucan extract compared to hot water extraction, while Marales *et al.* (2019) found that ultrasonic extraction gave a higher yield when compared with conventional hot water extraction. They also revealed that the yield of extracts increased with extraction time when using ultrasonic extraction, but decreased with extraction time when hot water extraction was used. Alzorqi *et al.* (2017) reported that the extraction yield increased with extraction time when using hot water extraction, while Yang *et al.* (2011) reported that the yield of mushroom glucan ultrasonically extracted decreased with increasing of temperature but increased with increasing extraction time. These diverse results highlight the different ranges of temperature, time, and ultrasonic power used in the extraction. The higher yield of hot water was probably due to longer extraction time.

Except for ash levels, the proximate composition analysis revealed no significant differences in compositions between the ultrasonic and hot water extracts, as indicated in Table 2. The two extracts exhibited lower fiber content but higher carbohydrate content compared to mushroom powder. When compared to commercial glucan, the extracts exhibited lower carbohydrate content but higher protein content. Results indicated that most extracts consisted of water-soluble polysaccharides and the extraction techniques did

Table 1. Yield and glucan content of glucan extracts.

Sample	Yield (g/100 g dried mushroom)	Glucan content (g /100 g sample)		
		Total glucan	α-glucan	β-glucan
Not extracted	-	55.19±5.01 ^a	1.54±1.58 ^a	53.65±6.59 ^a
Hot water	7.04±1.35	49.36±0.85 ^a	3.13±1.97 ^a	46.23±2.68 ^a
Ultrasonic	5.88±0.54	37.37±3.16 ^b	1.65±1.25 ^a	35.72±3.54 ^b
Control (49% yeast β-glucan)	-	51.35±3.16 ^a	0.54±0.03 ^a	50.51±4.42 ^a

Values are presented as mean±standard error of triplicates. Values with different superscripts in the same row are statistically significantly different compared using Duncan's multiple range test ($p < 0.05$).

Table 2. Proximate composition of mushroom powder and glucan extracts.

Sample	Proximate composition (g/100 g sample)					
	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Not extracted	8.78±0.69 ^a	11.77±0.21 ^a	0.46±0.19 ^a	18.01±0.26 ^a	7.30±0.07 ^c	53.67±0.63 ^c
Hot water	7.89±0.42 ^b	11.61±0.15 ^a	0.31±0.19 ^b	0.09±0.02 ^c	9.71±0.16 ^b	70.39±0.19 ^b
Ultrasonic	8.71±0.06 ^a	11.42±0.12 ^a	0.36±0.01 ^b	0.05±0.01 ^c	10.50±0.24 ^a	68.96±0.06 ^b
Commercial glucan	5.60±0.05 ^c	1.72±0.01 ^b	0.13±0.03 ^c	5.09±0.35 ^b	0.61±0.07 ^d	86.85±0.41 ^a

Values are presented as mean±standard error of triplicates. Values with different superscripts in the same row are statistically significantly different compared using Duncan's multiple range test ($p < 0.05$).

not eliminate the presence of protein.

The glucan content quantification (Table 1) showed no significant difference in total glucan and β -glucan levels between the hot water extract and mushroom powder, while the ultrasonic extraction approach gave significantly reduced total glucan and β -glucan concentrations compared to the hot water extraction method. In addition, there was no significant difference of α -glucan among the extract samples. Variations in glucan level were ascribed to the influence of ultrasonic cavitation, temperature, and time span, which resulted in fragmentation and reaggregation of the extracted water-soluble polysaccharides (Sangthong *et al.*, 2022).

3.2 Fourier transformed infrared

FTIR spectroscopy is a technique used to determine the chemical structure and identify the functional groups present in a given compound. The result showed that FTIR spectra of glucan extracted by ultrasonic and hot water methods were not different and very similar to non-extracted mushroom powder (Figure 1). The spectra of all samples showed peak region of 3200-3400 cm^{-1} indicating the presence of -OH group. Whilst the band around 2930 cm^{-1} and 1370 cm^{-1} indicated the C-H bond and the peak around 1640 cm^{-1} was attributed to C=O bond. The peak region of 1310-1320 cm^{-1} was assigned to C-O bond stretching. The peaks around 1020 cm^{-1} , 1080 cm^{-1} and 1040 cm^{-1} indicated the presence of the glucosidic linkage, which is mainly ascribed to glucan (Khan *et al.*, 2017; Huang *et al.*, 2015; Shaheen *et al.*, 2022; P'erez-Bassart *et al.*, 2023). The FTIR result suggested that the mushroom polysaccharide has typical molecular characteristics. The molecular structure of extracts could be altered by the extraction procedure, as observed by different glucan contents, but the two extraction methods did not modify the primary structure and functional groups of the extracts (Alzorqi *et al.*, 2017; Huang *et al.*, 2015; Cao *et al.*, 2023).

3.3 Rheological property

Viscosities of the glucan solutions are shown in Figure 2, and the parameters fitting with the Power Law model are given in Table 3. Results showed that the

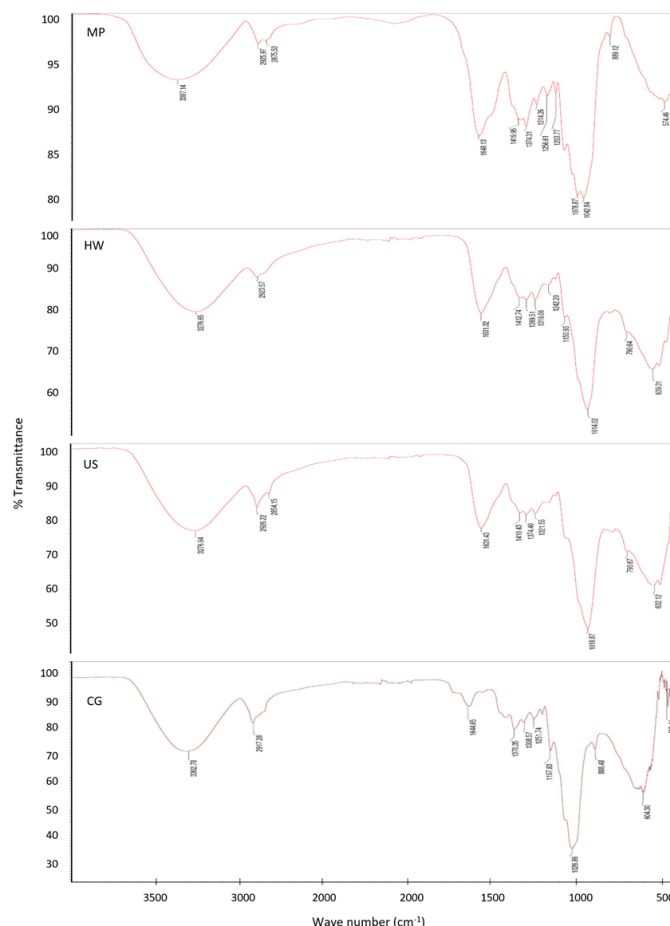


Figure 1. FTIR spectra of mushroom powder (MP), glucan extracts (HW and US) and commercial glucan (CG).

Table 3. Rheological characteristics of glucan extract solutions at various concentrations.

Extraction method	Concentration	K	n	R ²
Hot water	1%	0.547±0.156	0.428±0.019	0.980
	2%	7.846±1.433	0.174±0.031	0.997
	3%	25.483±9.960	0.125±0.028	0.999
Ultrasonic	1%	0.687±0.161	0.352±0.077	0.933
	2%	2.183±1.178	0.252±0.076	0.979
	3%	5.093±2.936	0.228±0.079	0.991

Values are presented as mean±standard error of triplicates.

viscosity of all glucan solutions decreased with increasing shear rate, exhibiting shear-thinning behavior or pseudoplastic fluid. The n values, representing shear-thinning capacity, decreased with the increase of concentration; while the K values, referring to the

magnitude of viscosity or consistency index, increased with increasing concentration. Sun *et al.* (2020) reported that the glucan solution exhibited pseudoplastic behavior at high concentration and molecular weight, with Newtonian behavior observed at low concentration and molecular weight.

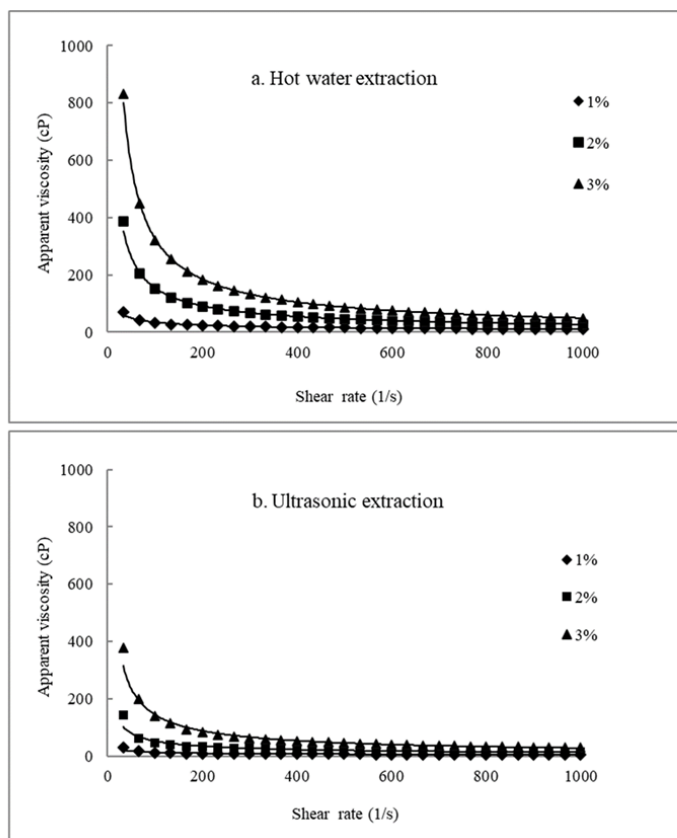


Figure 2. Flow behavior of glucan extracts solutions at various concentrations; symbols stand for measured values, lines stand for fitted model values.

The viscosity of the ultrasonic extract was lower than the hot water extraction due to the lower glucan content in the ultrasonic extract. Glucan extract viscosity was reported to be related to its molecular structure, molecular weight and solubility, resulting from different extraction methods (Liu *et al.*, 2021; Mäkelä *et al.*, 2021). β -glucans with higher molecular weights have higher viscosities because of stronger entanglements of molecular chains and flow resistance of high-molecular polymers (Sun *et al.*, 2020; Liu *et al.*, 2021). Huang *et al.* (2015) reported that ultrasonic treatment modified the structure of glucans, with molecular weight decreasing in the first period of ultrasonication. Liu *et al.* (2021) compared the extraction yield and physicochemical properties of glucan from hull-less barley. They found that different extraction methods affected the molecular weight distribution of β -glucan, while M_n and M_w values of the ultrasonic extract were lowest compared with hot water extraction, microwave extraction, and microwave-assisted ultrasonic extraction. Studies conducted by Hu *et al.* (2021) and Cao *et al.* (2023) revealed that alteration in the glucan structure during ultrasonic

extraction was time-dependent. Application of ultrasonic treatment for a short time caused major disruption in the glucan structure and led to a decrease in both M_n and M_w values, eventually resulting in reduced viscosity. However, prolonged ultrasonication led to the reaggregation of glucan structures into a dense network structure, resulting in an increase in molecular size and weight, and subsequently, an increase in viscosity. Findings indicated that the lower viscosity of the ultrasonic extract in this study was related to its lower glucan content and lower molecular weight, as a result of the short duration of the ultrasonic extraction process.

The viscosity of β -glucan has been reported to significantly affect the biological activity of glucan since it relates to the ability to form viscous solutions in the gastrointestinal (GI) tract, leading to slowing down the intestinal transit and absorption of glucose and sterol (Bai *et al.*, 2019; Sun *et al.*, 2020; Mäkelä *et al.*, 2021). Ellegård and Andersson (2007) also reported that bile acid excretion was mostly linked with the viscosity of β -glucan in the small intestine, while Cai *et al.* (2018) and Cao *et al.* (2023) reported that ultrasonic treatment could enhance the antioxidant activity of glucans due reduction in particle size and increased specific surface area, thereby providing more active sites to react with free radicals. These findings demonstrated that the biological functionality of the ultrasonic extract might differ from the hot water extract due to decreased molecular weight and lower viscosity.

3.4 Functional properties

3.4.1 Swelling power

Swelling power was also found to be related to the ability of glucan to reduce cholesterol; the higher swelling power, the higher efficacy of cholesterol reduction or hypolipidemic effect (Sarteshnizi *et al.*, 2015; Kim *et al.*, 2016; Sun *et al.*, 2020). The swelling power of glucan also increased with molecular weight and insoluble fraction (Khan *et al.*, 2017; Sun *et al.*, 2020). The swelling power of the glucan extracts was greater than both mushroom powder and commercially available glucan. The swelling power of the ultrasonic extract (16.42 g/g sample) was lower than the hot water extract (20.99 g/g sample) (Table 4). This suggested that the glucan extracted by ultrasonication had lower cholesterol-lowering efficacy due to lower swelling power, resulting from lower molecular weight and insoluble fraction.

3.4.2 Fat binding capacity

Fat binding capacity is attributed to proper mouthfeel and is an important indicator for predicting hypolipidemic effect. Polysaccharides like glucan bind to

fat in the digestive tract and become larger, reducing fat absorption by the body (Sun *et al.*, 2020; Quin *et al.*, 2021). Fat binding capacity was 8.55, 6.03, 2.98, and 2.80 g/g for hot water extract, ultrasonic extract, mushroom powder, and commercial glucan, respectively (Table 4), concurring with a study by Khan *et al.* (2017) who reported that fat binding capacity of mushroom glucan ranged from 5.38 to 6.65 g/g sample. Sun *et al.* (2020) reported fat-binding capacity of Oat β -glucan as 1.97 g/g sample, and this increased after acid degradation to β -glucan oligosaccharide from 1.83 to 6.30 g/g sample due to the release of active hydroxyl group. The glucan extracts showed greater fat binding capacity compared to both mushroom powder and commercial glucan, with the ultrasonic extract demonstrating lower fat binding capacity compared to the hot water extract (Table 4). This suggested that extraction techniques potentially improved the hypolipidemic impact, with hot water extracts demonstrating greater hypolipidemic effects compared to ultrasonic extracts.

3.4.3 Emulsifying property

Both mushroom extracts exhibited superior emulsifying properties compared to mushroom powder and commercial glucan, with no apparent differences in the emulsifying characteristics between the ultrasonic and hot water extracts. The emulsifying capacity and stability of the two extraction methods ranged from 34.16 to 42.13% and from 85.33 to 92.52%, respectively (Table 4). The emulsifying capacity and stability of glucan from different mushroom varieties were reported by Khan *et al.* (2017), ranging from 64.16 to 65.47% and 94.64 to 97.68%, respectively.

3.4.4 Foaming property

Foaming capacities were 28.46, 44.00, 16.00, and 4.80% for mushroom powder, hot water extract, ultrasonic extract, and commercial glucan, respectively (Table 4). Greater foaming capacity and stability of the extracts and mushroom powder compared to commercial glucan were attributed to their higher protein content, as protein is an effective foaming agent. The ultrasonic extract exhibited lower foaming capability compared to

the hot water extract. Differences in foaming properties were attributed to variations in molecular weight. The foaming capacity of mushroom glucan, extracted using hot water and dialysis for low molecular weight, was examined by Khan *et al.* (2017). They reported low foaming capacity ranging from 9.80 to 10.20%. The reduced foaming ability of the ultrasonic extract in this study was attributed to its low molecular weight.

3.5 Antimicrobial activity

Antibacterial activity was investigated by agar-well diffusion method, with results summarized in Table 5. The mushroom powder showed no antimicrobial effect against all bacteria tests. The ultrasonic extract had higher antibacterial activity than the hot water extract, with a significant inhibitory effect between the ultrasonic extract and the commercial glucan. The hot water extract had an inhibition zone of 9.00 ± 0.00 mm against *B. cereus* growth, but no inhibitory effect against *S. aureus*. The inhibition zone against *S. aureus* was 9.00 ± 0.00 mm for both the ultrasonic and commercial glucan, while the inhibition zone against *B. cereus* was 13.00 ± 2.12 mm and 12.00 ± 0.70 mm for the ultrasonic and commercial glucan, respectively. Variations in bacterial inhibition abilities were attributed to differences in glucan structure and solubility. However, in this study, none of the samples showed inhibitory effects against the growth of *E. coli*.

Sun *et al.* (2020) and Qin *et al.* (2021) reported the influence of acid degradation and molecular weight on antimicrobial activity of oat β -glucan, which showed no antimicrobial activity due to its poor water solubility; however, its oligosaccharides obtained by acid degradation exhibited concentration-dependent antibacterial activities against *S. aureus* and *E. coli*. The authors concluded that the degradation of β -glucan led to the exposure of active hydroxyl groups and decreased intermolecular hydrogen bonding. Polysaccharides possessing hydroxyl or carboxyl groups have good chelating ability, and the chelating of metal ions influences the nutrient uptake of bacteria. Amer *et al.* (2021) compared the antibacterial activity of yeast glucan extracted using a modified acid-base extraction

Table 4. Functional properties of mushroom powder and glucan extracts.

Sample	Swelling power (g/g sample)	Fat binding capacity (g/g sample)	Emulsifying property		Foaming property	
			Emulsifying capacity (%)	Emulsifying stability (%)	Foaming capacity (%)	Foaming stability (%)
Not extracted	9.13 \pm 0.68 ^d	2.98 \pm 0.18 ^c	14.43 \pm 18.50 ^b	86.56 \pm 17.55 ^a	28.46 \pm 5.56 ^b	93.63 \pm 6.46 ^a
Hot water	20.99 \pm 0.96 ^a	8.55 \pm 0.59 ^a	42.13 \pm 1.48 ^a	85.33 \pm 6.66 ^a	44.00 \pm 3.46 ^a	94.06 \pm 2.15 ^a
Ultrasonic	16.42 \pm 0.71 ^b	6.03 \pm 0.32 ^b	34.16 \pm 2.57 ^{ab}	92.52 \pm 2.73 ^a	16.00 \pm 2.00 ^c	96.00 \pm 1.99 ^a
Commercial glucan	13.48 \pm 0.23 ^c	2.80 \pm 0.66 ^c	8.50 \pm 1.32 ^b	55.00 \pm 7.07 ^b	4.80 \pm 0.80 ^d	24.29 \pm 4.29 ^b

Values are presented as mean \pm standard error of triplicates. Values with different superscripts in the same row are statistically significantly different compared using Duncan's multiple range test ($p < 0.05$).

Table 5. Inhibition zone diameter of mushroom powder and glucan extracts.

Sample	Inhibition zone diameter (mm)		
	Gram-positive bacteria		Gram-negative bacteria
	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>
Not extracted	0.00±0.00 ^c	0.00±0.00 ^b	0.00±0.00 ^a
Hot water	9.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^a
Ultrasonic	13.00±2.12 ^a	9.00±0.00 ^a	0.00±0.00 ^a
Commercial glucan	12.00±0.70 ^a	9.00±0.00 ^a	0.00±0.00 ^a
DMSO (negative control)	0.00±0.00	0.00±0.00	0.00±0.00
Streptomycin 10 µg (positive control)	25.00±0.00	20.00±0.00	19.00±0.70

Values are presented as mean±standard error of triplicates. Values with different superscripts in the same row are statistically significantly different compared using Duncan's multiple range test ($p < 0.05$).

method and a water extraction method. Results indicated that the acid-base sample exhibited higher antimicrobial activity, while Ahmad *et al.* (2021) reported antibacterial activities of β -glucan extracted from cultured termite mushrooms. The antibacterial activities of different crude extracts from fruiting bodies, mycelium and fermentation broth were investigated against two Gram-positive bacteria (*S. aureus* and *Streptococcus sp.*) and three Gram-negative bacteria (*Ralstonia sp.*, *E. coli* and *Salmonella sp.*). All crude extracts exhibited antibacterial activities, where the hot water extract being the most effective crude extract, possibly due to higher extract concentrations. Srikrum *et al.* (2018) reported that the methanolic extract of *P. sajor-caju* (Fr.) singer showed no antimicrobial effect against gram-negative bacteria *S. Typhimurium* and *E. coli* and gram-positive bacteria *S. aureus* and *B. cereus*, possibly because of a different extraction method, as the major fraction of the methanolic extract was phenolics and flavonoids, not β -glucan.

4. Conclusion

Ultrasonic extraction at 40°C for 1 h was compared with conventional hot water extraction at 100°C for 4 h. The ultrasonic extraction was found to deliver lower extract yield with lower glucan content, with no difference in proximate composition. The FTIR spectra showed similar primary structure and functional groups of the two extracts. The ultrasonic extract had lower viscosity and functional properties, i.e., swelling power, fat binding capacity, emulsifying and foaming capacity. Neither of the extracts exhibited inhibition of *E. coli*, but the ultrasonic extraction sample exhibited superior inhibitory effects against *B. cereus* and *S. aureus* that were similar to commercial glucan. Glucan content and also glucan structure impacted the functional properties of mushroom glucan. However, further studies on extraction conditions are required for a more profound understanding of the relationship between glucan structure and physicochemical properties.

Conflict of interest

The authors declare no conflict of interest.

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