

# Ultrasound-assisted extraction of polysaccharides from edible and medicinal fungi: major factors and process kinetics

## Abstract

Polysaccharides (PS) from medicinal fungi (mushrooms) provide an important source of nutraceutical and pharmaceutical compounds with notable antitumor, immunomodulatory and other health benefits. Extraction (solid-liquid) is the first key step for isolating PS from the fungal materials. Power ultrasound (US) is a special and favorable means for processing food and medicinal products because of several merits such as simple equipment, lower temperature and less use of chemicals. The various effects of power US can be mostly attributed to the hydrodynamic activities of acoustic cavitation, leading to cell wall disruption and more effective mass transfer. The major factors affecting the rate of US-assisted extraction (UAE) include the solid particle size, solid-liquid ratio; US power intensity and temperature, plus the physical properties of solid materials being extracted. Besides the extraction process (rate and yield), US may also affect the properties and bioactivities of extract products. This article is to give a brief review of UAE for extraction of PS from edible and medicinal mushrooms.

**Keywords:** extraction, ultrasound, polysaccharide, mushroom, process factors, kinetic model

Volume 4 Issue 2 - 2017

**Jian-Yong Wu**

Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong

**Correspondence:** Jian-Yong Wu, Department of Applied Biology and Chemical Technology, State Key Laboratory of Chinese Medicine and Molecular Pharmacology in Shenzhen, The Hong Kong Polytechnic University, Kowloon, Hong Kong, Tel +852 3400 8671, Fax +852 2364 9932, Email jian-yong.wu@polyu.edu.hk

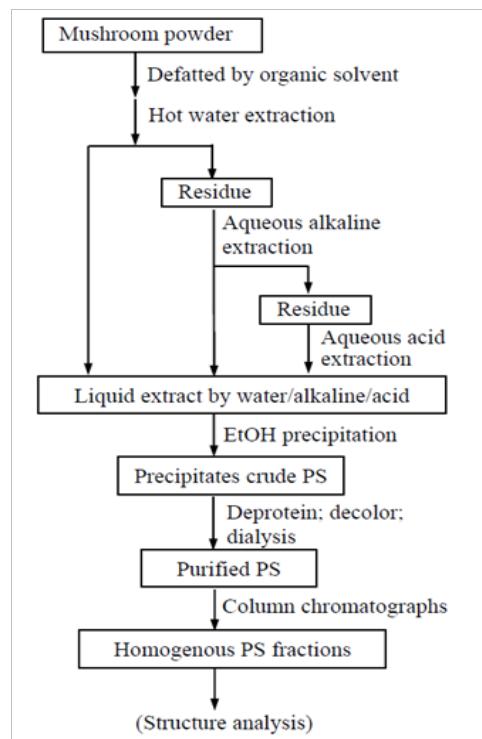
**Received:** January 28, 2017 | **Published:** April 03, 2017

## Introduction

Mushrooms are favorite foods in our daily diet, many having tonic effects and medicinal properties. Polysaccharides (PS) are major functional constituents of edible and medicinal mushrooms (fungi), which are well known for their antitumor and immunomodulatory activities and other bioactivities such as anti-inflammation, hypoglycemic, hypolipidemic, antioxidant, and antiviral.<sup>1-3</sup>

Some mushroom PS have been used commercially as immunotherapeutic agents and adjuvant drugs for cancer therapy and PS-rich extracts of mushroom fruit bodies or fungal mycelia are widely used in functional foods.<sup>4</sup> Unlike the small antitumor molecules with strong cytotoxicity, most of the mushroom PS have low toxicity and produce antitumor effects by acting as biological response modifiers or immunomodulators on the host immune responses.<sup>5</sup>

Extraction is the first key step for separation of PS from mushrooms as well as other material sources. The specific extraction procedure and conditions depend on the PS structure and water-solubility and (Figure 1) shows a general procedure for extraction, isolation and purification of polysaccharides from mushrooms. In brief, the dry mushroom or fungal mycelium in the form of dry powder is first defatted with an organic solvent such as acetone and ethanol to remove water-insoluble organic components. The defatted mushroom is extracted successively with boiling water, hot aqueous alkaline solution, and hot aqueous acid solution.<sup>6,7</sup> The liquid extract from each extraction step is precipitated with ethanol to attain the crude PS. The crude PS is purified through a series of steps for removal of proteins, pigments and other low molecular weight components.



**Figure 1** A general procedure for extraction, isolation and purification of polysaccharides from mushrooms.

The purified PS can be further purified by ion-exchange and gel permeation chromatography to retain homogenous PS fractions for structure analysis or other uses. The molecular structure of purified PS fractions can be determined through specialized protocols involving extensive degradation reactions and analytical measurements.

Power ultrasound (US) is reagreded as a special and favorable means for processing food and medicinal products because of several merits such as simple equipment, direct and quick effect (fast start-up), clean and safe operation (milder conditions, e.g. lower temperature and less use of chemicals).<sup>8-10</sup> Ultrasound-assisted extraction (UAE) has been widely exploited to enhance or replace the hot-water and solvent extractions for the separation of bioactive components from food and medicinal products.<sup>11-13</sup> The potential advantages of UAE over the conventional methods for separation of bioactive natural products include higher extract yield and extraction rate, and lower extraction temperature. The low-temperature operation of UAE is most favorable for preserving the properties and activities of heat-sensitive food and medicinal products which could be deteriorated in hot water or solvent extraction processes. UAE is also more favourable than other non-conventional techniques for laboratory analysis and large-scale separation of food and medicinal products in the simplicity, energy efficiency, scalability, versatility, safety and environmental compatibility of equipment and operation.<sup>9,10</sup> While most early studies on UAE of food and natural products were aimed at the small organic molecules, many recent studies have applied power US to improve the extraction of natural PS from plants, mushrooms and other sources.

## UAE of PS from plants and fungi

Power ultrasound (US) is a versatile tool in the laboratory and industry for various processes such as cleaning, extraction, homogenization and chemical reactions.<sup>14</sup> Ultrasound-assisted extraction (UAE) is the application of high-intensity power US to enhance the extraction of solid constituents in a liquid solvent. Practically UAE has been applied in the laboratory for a long time for extraction and homogenization of chemical, food and biological samples with ultrasonic cleaning baths or probe horns, often as a routine sample preparation step prior to analysis and measurement.<sup>15</sup> However, the application of UAE in large-scale commercial processes of food and natural products is still classified as a non-conventional process.<sup>8,16,17</sup>

Most previous studies on UAE of natural products have been aimed at the low-MW organic molecules such as phenolic and aromatic compounds, alkaloids and essential oils from plants and various other sources.<sup>12,18</sup> With increasing interest in the bioactivities and other useful properties of natural PS, more recent studies have devoted to UAE of PS from plant materials such as herbs and agriculture residues<sup>19,20</sup> and from edible/medicinal mushrooms such as *Lentinus edodes* (Shiitake),<sup>21,22</sup> *Ganoderma lucidum* (Lingzhi),<sup>23</sup> *Agaricus bisporus* (button)<sup>24</sup> and *Cordyceps sinensis* (caterpillar fungus) mycelium.<sup>25</sup> These studies suggest that UAE is a viable alternative to classical extraction methods such as hot water extraction (HWE) for isolation of PS from various sources. A specific concern for UAE of PS and related biopolymers is depolymerization with high intensity US and long exposure period.

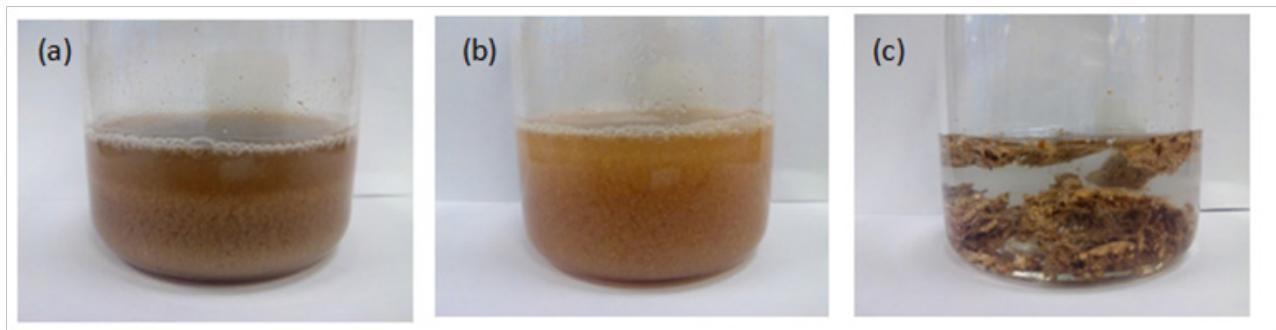
We have accomplished several studies on UAE of PS and PS-protein complexes (PSPs) from selected species of the most important and valuable edible and medicinal fungi in both fruiting body and

mycelia form.<sup>26-28</sup> In these studies, power US was applied to the powdered samples dispersed in water with an ultrasonic probe horn at a fixed power (Figure 2). During UAE, the sample bottle was immersed in ice to avoid overheating and maintain a low temperature (<50°C). In one study with three species of mushrooms, the yield and extraction rate of PSP by UAE was notably higher with *G. frondosa* and only slightly higher with *L. edodes*, but even lower with *C. versicolor*, than by HWE (Table 1). The different effects of UAE on the efficiency of PSP extraction from the three mushroom species was mainly attributed to the different physical properties of mushroom tissue and the aggregation or dispersion of mushroom powder in the extraction liquid. In particular, the powder *G. frondosa* and *L. edodes* samples subjected to UAE were in the form of small, well-dispersed particles in water but the *C. versicolor* powder formed large, irregular aggregates (Figure 3). Under scanning electron microscopy (SEM), the *G. frondosa* and *L. edodes* mushroom powder appeared as irregular particles before UAE and became looser and more porous after UAE, while the *C. versicolor* powder was in fibrous form before and after UAE (Figure 4).

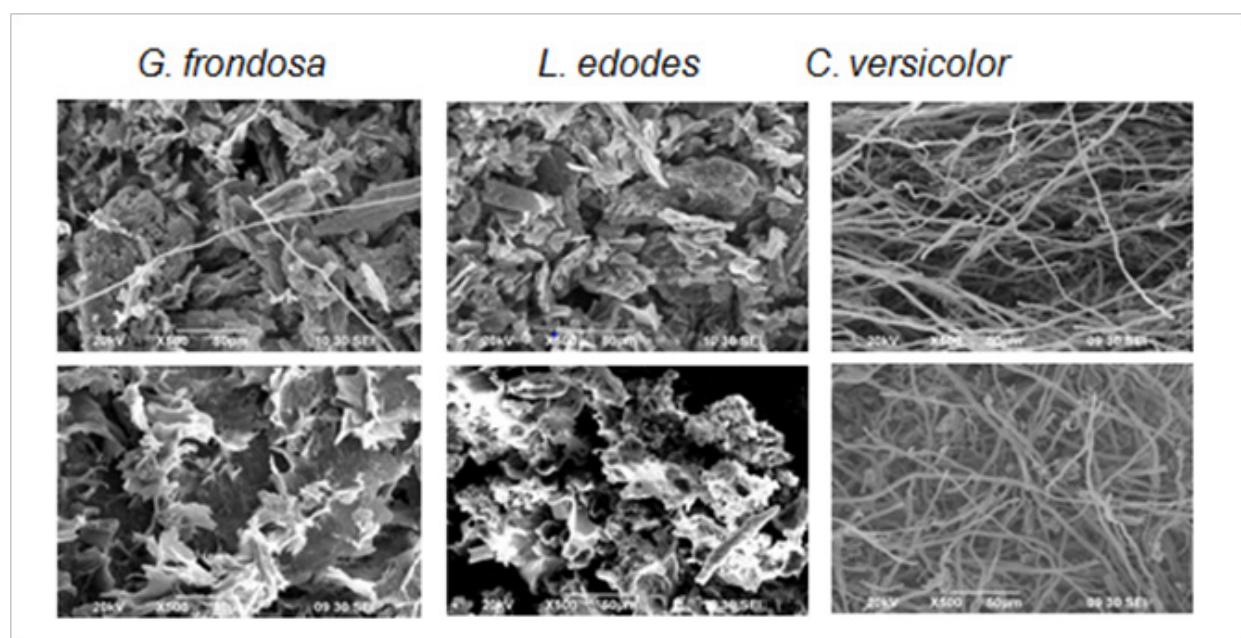
Our studies have also shown significant differences in the composition and molecular properties between the PSPs attained by UAE and those by HWE.<sup>26</sup> Compared with those extracted by HWE, the PSPs from all the three mushrooms extracted by UAE had much higher protein contents and lower carbohydrate contents (Table 1). The molecular weight distribution spectra of PSPs from UAE showed an overall shift of the peaks to lower molecular weight range, which was due probably to the lower extraction temperature and/or partial degradation of the PSPs by the US power.<sup>26</sup> In addition, only the PSPs extracted by UAE but not by HWE exhibited distinct protein bands on SDS-PAGE, suggesting that UAE at low temperature retained the protein constituents which could be denatured during HWE. The PSPs by UAE from three mushrooms also exhibited higher antioxidant activities.



Figure 2 Ultrasonic processor with a probe horn for UAE.



**Figure 3** Mushroom particles dispersed in water: (a) *G. frondosa*, (b) *L. edodes* and (c) *C. versicolor* (all mushrooms ground into powder and screened through 250 µm mesh) [26].



**Figure 4** Scanning electron micrographs of three mushrooms before (top row) and after UAE (bottom row). (All mushrooms ground into powder and screened through 250µm mesh before UAE; Scanning electron microscopy (SEM) images taken on a JEOL JSM-6490F

**Table 1** Yield and composition of PS extracted from three mushrooms (fruit body) by UAE and HWE (UAE at <50°C; all values in wt%). UAE was performed with a probe horn ultrasonic processor of 20kHz frequency and 130W power (Sonics & Materials Inc., Newton, USA).<sup>26</sup>

Mushroom	PS Yield		Carbohydrate		Protein	
	UAE	HWE	UAE	HWE	UAE	HWE
<i>G. frondosa</i>	6.30± 0.07	5.71± 0.07	28±0.4	60±4.8	33±2.9	21±0.3
<i>C. versicolor</i>	1.61± 0.07	6.58± 0.04	21±0.5	66±3.0	25±0.3	15±0.7
<i>L. edodes</i>	12.4± 0.70	6.57± 0.05	36±1.4	46±1.9	34±0.3	29±0.1

## UAE process kinetics

Ultrasound-assisted solid-liquid extraction is a complex physical process depending on several classes of factors, namely ultrasonic (equipment structure and operation), solid material (physical properties, particle size and morphology), liquid solvent (property and volume ratio), and environmental (temperature, agitation). Ultrasonic enhancement of extraction is attributed mostly to disruption of cell walls, reduction of particle size and enhanced mass transfer through the solid particle by the hydrodynamic activities of cavitations' bubbles.<sup>29</sup> The kinetic models for UAE of natural products are usually adapted from those for conventional solid-liquid extraction or leaching, a well-known mass transfer process in chemical engineering. Several

**Table 2** The linear regression coefficients ( $R^2$  values) for the UAE data (total extract yields versus time) of various mushroom materials fitting to different kinetic models.<sup>27</sup>

Kinetic model	Fruit body		Mycelium	
	<i>G. frondosa</i>	<i>L. edodes</i>	<i>C. versicolor</i>	Cs-HK1
Parabolic diffusion: $y = y_0 + y_1 t^{1/2}$	0.907	0.871	0.778	0.995
Power Law: $y = Bt^n$	0.984	0.964	0.559	0.926
Weibull: $y = 1 - e^{(-t_m/D)}$	0.984	0.964	0.559	0.913
Elovich: $y = E_0 + E_1 \ln t$	0.984	0.963	0.564	0.913
Unsteady diffusion: $y = (1 - b)e^{-kt}$	0.743	0.717	0.823	0.906

## Conclusion

Power US is a convenient and effective means for improving the extraction of bioactive natural products in both laboratory and industry applications. For extraction of PS from mushrooms, high-intensity US affects not only the extraction rate and yield but also the molecular composition and properties of PS. The US effect on the extraction process is dependent on the microstructure of mushroom particles and their dispersion in the exacting solvent, in addition to the process factors such as the solid particle size, solid-liquid ratio, US power intensity and temperature. The UAE process kinetics of mushrooms can be well represented by simple empirical kinetic models for solid-liquid extraction. These models are useful for prediction and optimization of the UAE process factors and also for understanding the mechanisms of ultrasonic effects on the solid-liquid mass transfer process.

simplified empirical models have been developed for describing the extraction kinetics of natural products from plant materials in various mathematical forms, parabolic, hyperbolic, power-law and exponential.<sup>30</sup> The experimental data (extract yields versus time) of UAE of mushrooms and fungal mycelia in our previous studies were fitted to several kinetic models for solid-liquid extraction.<sup>27,28</sup> Based on the  $R^2$  values from linear regression (Table 2), three models including the power law, Weibull exponential model and Elovich logarithmic model, fitted closely to the data for fruit body of *G. frondosa* and *L. edodes*, and the parabolic diffusion model showed a close fit for all mycelia, Cs-4, Cs-HK1 and *G. lucidum*, and no model fitted well for *C. versicolor* fruit body.

**Table 2** The linear regression coefficients ( $R^2$  values) for the UAE data (total extract yields versus time) of various mushroom materials fitting to different kinetic models.<sup>27</sup>

## Acknowledgements

None.

## Conflict of interest

The author declares no conflict of interest.

## References

1. Moradali MF, Mostafavi H, Ghods S, et al. Immunomodulating and anticancer agents in the realm of macromycetes fungi. *Int Immunopharmacol*. 2007;7(6):701–724.
2. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Appl Microbiol Biotechnol*. 2011;89(5):1323–1332.
3. Stachowiak B, Regula J. Health-promoting potential of edible macromycetes under special consideration of polysaccharides: a review. *European Food Research and Technology*. 2012;234(3):369–380.

4. Giavasis I. bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology*. 2014;26:162–173.
5. El Enshasy HA, Hatti-Kaul R. Mushroom immunomodulators: unique molecules with unlimited applications. *Trends Biotechnol*. 2013;31(12):668–677.
6. Mizuno T. Development of antitumor polysaccharides from mushroom fungi. *Foods & Food Ingredients Journal of Japan*. 1996;167:69–87.
7. Zhang M, Cui SWW, Cheung PCK, et al. Antitumor polysaccharides from mushrooms: A review on isolation process, structural characteristics and antitumor activity. *Trends in Food Science and Technology*. 2007;18(1):4–19.
8. Patist A, Bates D. Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science and Emerging Technologies*. 2008;9(2):147–154.
9. Kentish S, Feng H. Applications of power ultrasound in food processing. In: Doyle MP, et al. editors. *Annual Review of Food Science and Technology*. 2014;5:263–284.
10. Tao Y, Sun DW. Enhancement of food processes by ultrasound: a review. *Crit Rev Food Sci Nutr*. 2015;155(4):570–594.
11. Vinatoru M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*. 2001;8(3):303–313.
12. Vilkhu K, Mawson R, Simons L, et al. Applications and opportunities for ultrasound assisted extraction in the food industry - A review. *Innovative Food Science and Emerging Technologies*. 2008;9(2):161–169.
13. Chemat F, Rombaut N, Sicaire AG, et al. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*. 2017;34:540–560.
14. Mason TJ, Lorimer JP. *Applied sonochemistry: the uses of power ultrasound in chemistry and processing*. Weinheim: Wiley-VCH; 2002.
15. Chemat F, Tomao V, Virot M. Ultrasound-assisted extraction in food analysis. In: Ötles S editor. *Handbook of food analysis instruments*. (Chapter 5), Boca Raton, USA: CRC Press; 2009.
16. Soria AC, Villamiel M. Effect of ultrasound on the technological properties and bioactivity of food: a review. *Trends in Food Science and Technology*. 2010;21(7):323–331.
17. Chandrapala J, Oliver C, Kentish S, et al. Ultrasound in food processing. *Ultrasonics Sonochemistry*. 2012;19(5):975–983.
18. Toma M, Vinatoru M, Paniwnyk L, et al. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics Sonochemistry*. 2001;8(2):137–142.
19. Ebringerová A, Hromádková Z. An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides. *Central European Journal of Chemistry*. 2010;8(2):243–257.
20. Rosello-Soto E, Parniakov O, Deng Q, et al. Application of non-conventional extraction methods: toward a sustainable and green production of valuable compounds from mushrooms. *Food Engineering Reviews*. 2016;8(2):214–234.
21. Nian BY, Wang ZM, Huang HN. Study on methodology of lentinan by ultrasonic extraction and ultrafiltration. *Chemistry & Bioeng (in Chinese)*. 2004;4:16–18.
22. Ke LQ. Optimization of ultrasonic extraction of polysaccharides from *Lentinus edodes* based on enzymatic treatment. *Journal of Food Processing and Preservation*. 2015;39(3):254–259.
23. Chen XP, Wang WX, Li SB, et al. Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. *Carbohydrate Polymers*. 2010;80(3):944–948.
24. Tian YT, Zeng HL, Xu ZB, et al. Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). *Carbohydrate Polymers*. 2012;88(2):522–529.
25. Yan JK, Wang YY. Random-centroid optimization of ultrasound-assisted extraction of polysaccharides from *Cordyceps sinensis* mycelium. *Current Topics in Nutraceutical Research*. 2015;13(4):167–172.
26. Cheung YC, Siu KC, Wu JY. Molecular properties and antioxidant activities of polysaccharide-protein complexes from selected mushrooms by ultrasound-assisted extraction. *Process Biochemistry*. 2012;47(5):892–895.
27. Cheung YC, Siu KC, Wu JY. Kinetic models for ultrasound-assisted extraction of water-soluble components and polysaccharides from medicinal fungi. *Food and Bioprocess Technology*. 2013;6(10):2659–2665.
28. Cheung YC, Wu JY. Kinetic models and process parameters for ultrasound-assisted extraction of water-soluble components and polysaccharides from a medicinal fungus. *Biochemical Engineering Journal*. 2013;79:214–220.
29. Veličković DT, Milenović DM, Ristić MS, et al. Kinetics of ultrasonic extraction of extractive substances from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. *Ultrasonics Sonochemistry*. 2006;13(2):150–156.
30. Kitanovic S, Milenovic D, Veljkovic VB. Empirical kinetic models for the resinoid extraction from aerial parts of St. John's wort (*Hypericum perforatum* L.). *Biochemical Engineering Journal*. 2008;41(1):1–11.