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Effects of Axial Circulation and Dispersion Geometry on the Scale-Up of Ultrasonic Extraction of Polysaccharides

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The ultrasonic-assisted extraction of polysaccharides (PS) from Ganoderma lucidum, was subjected to a scale-up study. 0.25 L extractor was used to optimize the extraction conditions toward maximum yield of PS. The extracted PS was observed to be reduced by increasing the scale from 1 to 6 L. To intensify the extraction, axial circulation at different stirring rates was induced and optimized in a 3 L U-tube extractor. Although circulation at 300 rpm improved the yield of PS for 3 L, introducing dispersion geometry (conical funnel) and adjusting the radiation distance in a 6 L U-tube extractor further intensified the extraction efficiency. A radiation distance of 4 cm and circulation induced using 600 rpm enhanced the PS as compared to the conventional 6 L extractor. Overall, the scale-up from 0.25 to 6 L was successful and introducing circulation and dispersion geometry intensified the extraction efficiency under similar dissipation of ultrasonic power. *AIChE J*, 61: 1483–1491, 2015

Keywords: ultrasound, cavitation, extraction, scale-up, circulation, polysaccharides

Introduction

Ganoderma lucidum (*G. lucidum*) has been reported to be one of the most important species in the traditional Chinese medicine, due to its ability to treat a number of diseases and hence used for wider medicinal applications. The fruiting body, mycelia, and spores of *G. lucidum* contain approximately 400 bioactive compounds. Diverse groups of these bioactive compounds such as triterpenes, polysaccharides (PS), proteins, nucleotides, metals, and so forth. with different pharmaceutical activities have been isolated from this species.[1–3] Among these bioactive compounds, PS, a group of carbohydrate sugar has attracted the attention of many researchers due to their potential ability to act as antiaging[4] and antitumor,[5] antidiabetic,[6] and as an antioxidant to reduce the damage caused by the free radicals generated by the oxidation reaction.[7]

Many studies have reported the advantages of using power ultrasound in improving the efficiency of extraction.[8–10] Remarkably ultrasound has been found to enhance the water uptake and hydration of vegetable materials; provides greater penetration of solvent into the cellular materials and also enlarges the pores of cells and increases the contact surface area owing to the size-reduction of cellular structure of the plant.[11] Particularly, the bioactivity of PS depends on the extraction conditions such as extraction temperature, intensity of output power and time of exposure. Ultrasound assisted extraction (UAE) has been reported to have advantages over many other conventional methods for extracting the PS from different plants. This is mainly due to its ability to effectively separate and extract the PS while maintaining its bioactivity as well as increasing the extraction yield at shorter extraction time and less power consumption.[12–14]

The scale-up of UAE has gained considerable global interest as a result of increased awareness toward applying energy-saving, cost-effective, and green technologies in diverse industrial applications. Besides increasing the demands for a unit operation of prospective commercial and industrial opportunities, the positive outcomes achieved in the laboratory and pilot scales could make UAE as a viable alternative to the traditional extraction processes.[12] Many studies have presented different concepts for the scale-up of ultrasonic assisted processes,[15,16] however, the scale-up of a horn-type ultrasound system is still restricted considering the volume and the operational conditions. It is believed that the ultrasonic horn-type systems are generally preferable for laboratory scale investigations due to their poor ability to transmit the acoustic energy into larger process volumes, erosion and particle shedding at the tip surface, and the formation of cavitational blocking. Moreover, there is a probability of equipment failure due to the large transducer displacement.[17]

Gogate discussed two aspects of cavity dynamics which are of prime importance to be considered in the design of cavitational reactors. First, the maximum size reached by the cavity before its violent collapse which determines the cavitational intensity of the system by affecting the magnitude of pressure on bubble collapse. Second, the life of cavity determines the distance travelled by the cavity from the point of generation to the moment of collapse and hence the boundary of active volume of a cavitational reactor.[18] Therefore to maximize the life and the size of a cavity before its collapse, it is of great importance to optimize the parameters, that is,

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Table 1. The Chemical Composition of *G. lucidum*

Content		Analysis Method Code and Reference
Moisture	11.4%	D23A/M1M-1Malaysian standard (MS 1191:1991), UDC 642.2:641.13, SIRIM, 1991, pg 8.
Ash	0.8%	D23A/M2M-1AOAC Official method of analysis (2000), 17th edition, chapter 32, page 2, 32.1.05.
Crude protein	8.6%	D23A/M5P-1TECATOR manual- Kjeltec Auto 2300 Analyzer, 10007729, Rev. 1.2 yr 2002.
Fat	2%	D23A/M3CFTFAO Food and nutrition paper 14/7, Manuals of food quality control, 7. Food Analysis: General techniques, additives, contaminants, and composition (1986), p 210 – 213.
Carbohydrates	77.2%	Calculation base Department of chemistry Malaysia, Jabatan Kimia proficiency testing program. Year 2005.
Energy	511 kcal/100 g	Calculation base Food regulations 1985.
Dietary fiber	74.8 g	Dietary fiber determination using the fibertec e system. Tecator application note (AN 302) 97-09-18.

ultrasonic power, time, and temperature toward optimum equipment geometry taking into the consideration of concentration (ratio of solid/liquid) and the rigidity of extracted raw materials. Gogate reported that the cavity dynamics using ultrasonic horn systems could be effectively improved by the geometry of the reactor where most of the working liquid will be constrained within the longitudinal high intensity region or where the liquid is stirred vigorously. In another approach by Horst et al., they used a high intensity ultrasound concentrator horn and a dispersion conical funnel to improve the radiation effectiveness and intensify the Grignard reaction. According to Horst et al., the tip of an ultrasound horn acts as a point source and radiates spherical waves as well as the plane waves into the liquid.[16,19] A decrease in the sound pressure was observed by increasing the radiation distance which has been attributed to the geometric dispersion of the conical funnel, the strong absorption of sound waves and the change in the speed of sound in the bubbly liquid associated with cavitation.[20] The sound pressure is responsible for the cavitation, whereby a lower sound pressure generates less cavitation intensity and smaller sound absorption. Therefore, when the sound pressure approaches the so called threshold pressure (the last level of sound pressure where cavitation believed to occur), the cavitation intensity diminishes and finally vanishes beyond this pressure.[16] According to Horst et al., the cavitation zone in a reactor could be classified into three sections; zone-1, a heavily cavitating region above the sonotrode that occurs at a distance of 1 cm. Zone-2 is a transition region of cavitation where a quick increase in wave resistance and sound speed takes place. Zone-3 is a noncavitation zone.

The concept of classifying the cavitation zone into three sections has been adopted in this study. A U-tube extractor was designed to enable modifying the dispersion geometry of the cavitational intensity in a longitudinal pipe. A dispersion conical funnel was used to induce different dispersion geometries inside the pipe at different radiation distances from the horn tip. The effects of combining optimal ultrasonic parameters achieved through a preliminary stage with different stirring rates (to induce axial circular motion) and different radiation distance for the ultrasonic U-tube extractor were investigated to improve the efficiency of extraction of PS from *G. lucidum*.

Materials and Methods

Materials

The fruiting bodies of *G. lucidum* were supplied by the local company, *Ganofarm Sdn. Bhd*. (Tanjung Sepat, Selangor, Malaysia). The chemical composition of this mushroom was analyzed by Malaysian agriculture research and development institution and has been reported in Table 1 (Test certificate No. E-14-009). All the chemicals (ethanol, butanol, and chloroform) used were of analytical grade.

Isolation of PS from G. lucidum

The fruiting bodies of *G. lucidum* were first subjected to open air-drying and further drying was followed using an oven at 60°C to reduce its moisture content up to 11.4%. The fruiting bodies were then crushed using a heavy miller (Retch, SM-100, GmbH Co., Germany) and then passed through a 4 mm screen. To exclude some of the constituents of this mushroom such as reducing sugars, fatty acids, amino acids, phenols, and endogenous enzymes, pretreatment was performed using aqueous ethanol (80%) for 24 h using a shaker (Unimax 1010, Heidolph Instrument, Germany) at room temperature.[21,22] The residual fiber of the defatted mushroom was filtered and dried to evaporate the remaining solvent using an oven at 60°C. The defatted dried fiber was then subjected to ultrasonic extraction. An ultrasonic processor (HD-3400, SONOPLUS, Germany) with a tip diameter of 25 mm, frequency of 20 kHz and with the power in the range of 0–1000 W was used for the extraction. For each gram of the fiber, 25 mL of distilled water was used. Once the extraction was completed, the extract was left to cool to room temperature and filtered. The filtered solution of crude extract was stirred to ensure a homogeneous solution. 100 mL of this solution was concentrated using a rotary vacuum evaporator (Eyela N1100, Eyela Co., Shanghai, China). The concentrated sample (50 mL) was subjected to Sevag method (5:1 of $CHCl_3$/n-BuOH)[23,24] to exclude the protein components. After the denaturation of proteins, the samples were dialyzed using dialysis tubes (40 × 25 mm, Sigma-Aldrich) with the molecular weight cut-off, 10,000 Da to exclude smaller molecules. Fourfold volumes of solvent (95% ethanol) were added to the samples to precipitate the PS under 4°C for 24 h.[25,26] The precipitates were then centrifuged at 2935 g (rcf) for 15 min (Centrifuge 5430, Eppendorf, Germany), washed with ethanol, acetone, and then dried at 60°C (Oven, D-91126, Memmert, Gmbh Co., Germany) to obtain PS with the moisture content below 4%.

Scale-up of UAE

The extraction efficiency of ultrasound using different scales of operation has been examined by monitoring the concentration of extracted PS (mg PS/ 100 mL extract). The study was conducted in 3 stages using the same ratio of solid/liquid (1 g/25 mL) under the same optimal conditions of ultrasonic time (1 h), power (600 W) and temperature (80°C). In a preliminary study, the ultrasonic extraction

Table 2. Calorimetric Study: The Power Supplied and Their Corresponding Actual Power Dissipated Into the System

Power supplied (*W*)	400	500	600	700	800
Actual power output (*W*)	67.64	77.58	85.07	90.48	98.85

conditions were optimized to maximize the yield of PS using extraction volume of 0.25 L. An experimental design namely Box Behnken Design, one of the statistical design of response surface methodology has been used to study the interactive effects of these parameter on the yield of PS. The optimal conditions under which maximum yield achieved were 600 W, 80°C and 60 min.

To determine the amount of actual power dissipated by ultrasonic horn into the extraction system, a calorimetric study was conducted in the power range, that is, 400–800 W. The actual power dissipated was calculated by measuring the total heat generated in 250 mL of distilled water using a thermo-flask unit. As shown in Table 2, the optimal ultrasonic power of 600 W could be approximately equivalent to 85.07 W.

In the 1st stage, the extraction efficiency of UAE at different scales of operation, that is, 0.25, 1, 2, 3, 4, and 6 L was investigated using conventional jacketed extractors (borosilicate) with a thermostated bath. In the 2nd stage, the ultrasonic horn was coupled with an agitating system in a U-tube extractor of 3 L. This extractor has an internal and outer diameter of 70 mm and 80 mm, respectively and provided with a coil that acts as a heat exchanger to control the temperature as shown in Figure 1A. Axial circular motion was induced at different stirring rates, that is, 300, 600, 800, and 1000 rpm to increase the exposure time of the mushroom to high intensity power ultrasound. A 4-bladed propeller stirrer of 50 mm diameter was used as part of the stirring system.

In the 3rd stage, the U-tube of 3 L was scaled-up to 6 L along with incorporating a dispersion conical funnel (L=50 mm) to reduce the cross sectional area of the flow-path by reducing the internal diameter of the U-pipe from 90 to 30 mm as shown in Figure 1B. Moreover the addition of a dispersion conical funnel acts as a constriction to congest and focus the mushroom within the intensive radiating zone (zone-1) under the horn tip. The dispersion conical funnel was made of stainless steel to ensure better sound reflection and hence intensifies the cavitation intensity.[16] The distance between the horn tip and the bottom tip of a conical funnel is the radiation distance (radiation zone) which is adjustable (up to a distance of 8 cm) and studies were made on the effects of different radiation distances (2, 4, 6, and 8 cm) on the distribution of cavitational intensity in intensifying the extraction of PS (Figure 2). Overall, optimization was carried out at different radiation distances, different stirring rates and hence at different circulation rates to obtain a higher concentration of PS.

Results and Discussion

Effect of increasing the process scales on the concentration of PS

The main parameters that affect the performance of an ultrasonic liquid processing are the amplitude, external pressure, temperature, viscosity of solvent and concentration of solid materials subjected to extraction.[27] The optimal interactions of these parameters can be materialized into an improvement in the yield of extracted materials as a function of power density and power intensity. Power intensity is the amount of power dissipated into the extractor through the available surface area (W/cm^2) of horn tip[27,28] which is fixed in this study to a power of 600 W through an area of 4.9 cm^2. While the power density represents the total power input in a given volume of materials subjected to ultrasound[29] during 1 h of extraction (kWh/L). Therefore the power density for a batch process with fixed power intensity tends to decrease by increasing the scale of operation which may decrease the overall extraction efficiency of PS ingredients from the mushroom.

Using the inverse relationship between the power density and the scale of operation we can explain the variation in the concentration of extracted PS from different scale of operations as shown in Table 3. By using a conventional extractor, the concentration of extracted PS has been observed to be reduced from 20.3 to 17.23 mg/100 mL of

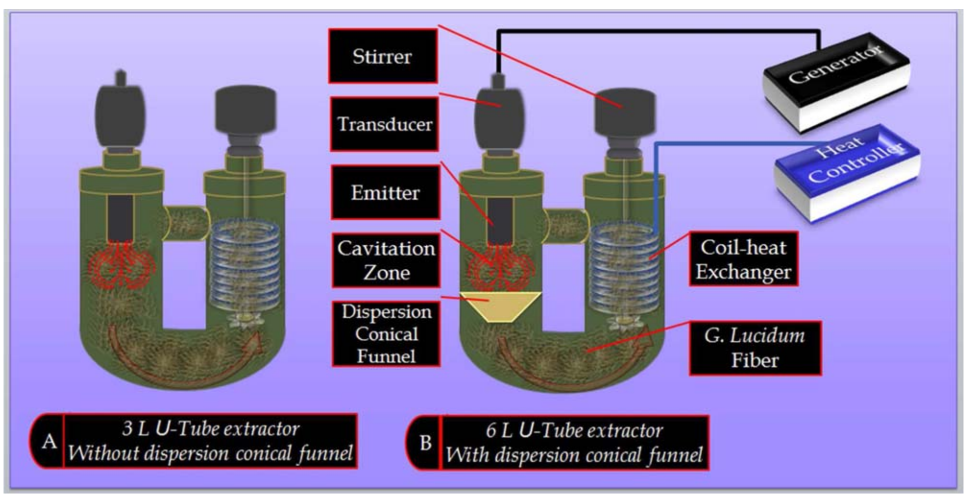

Figure 1. Design of U-tube extractors (A) 3 L and (B) 6 L.

Probe diameter is 25 mm. For 6 L U-tube extractor; the external and internal diameter is 100 mm and 90 mm, respectively. The funnel diameter is 30–89 mm and funnel length 50 mm. For 3 L U-tube extractor; the external and internal diameter is 80 mm and 70 mm, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

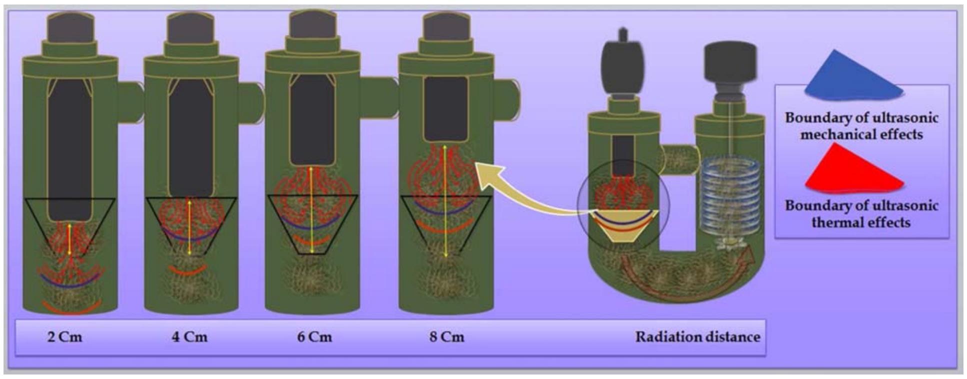

Figure 2. Representation of different radiation distance between the horn tip and the dispersion conical funnel.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

extracting solution as a result of increasing the volume from 0.25 to 1 L under similar ultrasonic conditions. Also, further increasing the volume to 2 or 6 L did not improve the concentration of PS per 100 mL of extracting solution due to the continuous reduction in the power densities up to 0.1 kWh/L in the extraction systems which is responsible for limited boundary of active cavitation zone (Figure 3).

The mechanical effects of transient cavitation of UAE could be intensified with smaller scale of operation such as 0.25 L due to the limited boundaries of the active volume of the extractor, the congestion of mushroom under the ultrasonic radiating tip and the higher power densities in the radiation distance within 1–4 cm away from the ultrasonic radiating tip. However, the cavitational intensity decreases exponentially by moving away from the surface of the horn-tip and vanishes at a distance as near as 2–5 cm, depending on the energy supplied to the equipment and on the operating frequency of used horn.[18,30] These mechanical effects are mainly attributed to the asymmetrical collapse of short-lived oscillation bubbles at solid surfaces. These effects could be represented by the generation of microjets,[31] on collapse of bubbles which causes local microturbulence and liquid microcirculation,[20,32] high velocity interparticle collisions; perturbation in the microporous of particles[8,33] and break down of larger aggregates into smaller particles.[34,35] These effects enhance the diffusion of PS solutes and thus the overall mass-transfer rate due to the local eddy diffusion caused by the implosion of cavitational bubbles. Conversely, the physical effects of inertial cavitation contribute significantly in the intensification of the extraction process. On violent bubble collapse, the highly localized energy generated affects the surface morphology of adjacent cell-wall of mushroom, induces penetration of the liquid solvent into the mushroom cell-wall and thereby increasing the mass-transfer rate.[27,36,] For the scale of 1 L, the internal circulation induced by the local turbulence and acoustic streaming of ultrasound cavitation[20] was sufficient to maintain a proper exposure of mushroom to the ultrasonic radiating tip which in turn resulted into extracting a relatively higher concentration of PS. While for the scales, that is, 2–6 L, introducing an external stirring source is recommended to improve the axial circular motion which increases the exposure time of mushroom particles to the radiating source in the active cavitational zone.

Table 3. Concentration of Extracted PS by Using Different Scales of Extraction

Scale of operation (L)	Power Density in 1 h treatment time (kWh/L)	Power Intensity (W/cm^2)	Concentration of PS (mg/100 mL)	Yield of PS[a] (mg)
0.25	2.4	122.22	20.3	50.75
1	0.6		17.23	172.3
2	0.3		12.4	248
3	0.2		12.12	363.6
4	0.15		11.1	444
6	0.1		12.21	732.6

[a]Yield was calculated based on the total volume used during the extraction.

Effect of circulation rate on the concentration of extracted PS using an ultrasonic U-tube extractor with the scale of 3 L

To improve the concentration of PS, a 3 L ultrasonic U-tube extractor was coupled with a stirring system (Figure 1A) to maintain a continuous axial circulation at different rates of stirring. It is believed that any ultrasonic process will be scalable using parameters such as energy and intensity.[37] In this study, the power intensity remained constant as there is no change in the power supplied to the system and the diameter of the tip. However, the energy input into the solid particles of the mushroom might be affected indirectly by the time of exposure due to its direct relation with the flow rate of mushroom solution. For example at high stirring

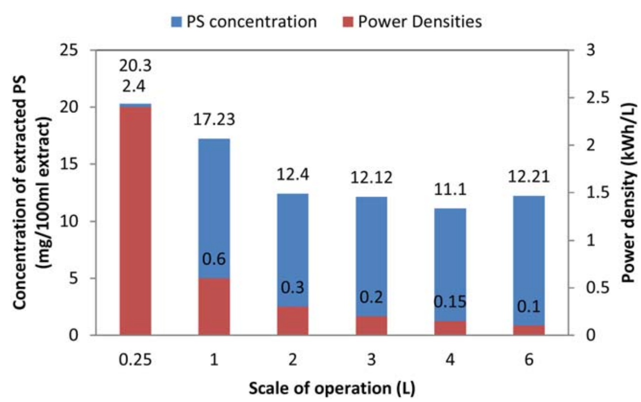

Figure 3. Effect of different scales of operation and power densities on the concentration of extracted PS.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

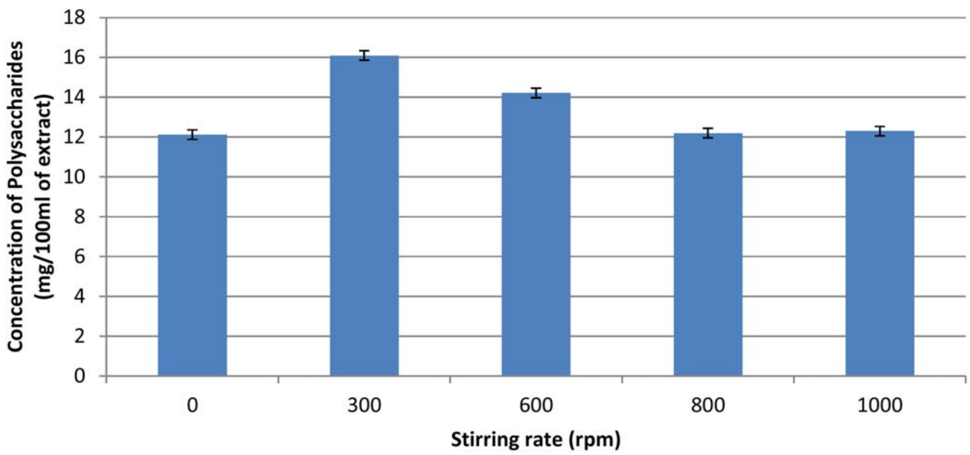

Figure 4. Concentration of extracted PS at different stirring rates using a 3 L ultrasonic U-tube extractor.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

rates (beyond 600 rpm) increasing the circulation rate leads to an increase in the flow rate of mushroom solution under the ultrasonic tip and thus reduces the residence time. This is in agreement with the findings of Patist and Bates. They reported that the energy input is a function of power and the flow rate. On the other hand, they considered it as the product of power output and time of exposure.[27] Therefore the relationship between the power input and flow rate in general could affect the overall rate of ultrasonic extraction and could be described by Eq. 1 (for continuous process) and Eq. 2 (for batch process)

$$W_{\text{input}}(\text{kWh/L}) \text{ for continuous process} = \text{Power of sonotrode (kW)}/\ Q\ (\text{L/h}) \tag{1}$$

$$W_{\text{input}}(\text{kWh/L}) \text{ for batch process} = [\text{Power of sonotrode (W)} \times \text{treatment time (s)}]/[\ 3.6E6\ (\text{J/kWh}) \times \text{volume of treated materials } (L)] \tag{2}$$

As shown in Figure 4, inducing an axial circular motion for the mushroom solution in a 3 L U-tube extractor had positive effects in improving the concentration of extracted PS as compared to the conventional 3 L extractor. In comparison with the PS concentration as obtained by the conventional extractor of 3 L (12.12 mg/100 mL), the concentration of PS increased by 32.75 and 17.32% when the circulation was induced at the stirring rates of 300 and 600 rpm, respectively. The circular motion increased the exposure time of mushroom solution to ultrasonic radiating tip as a result of increasing the flow rate of mushroom solution. An increase in the concentration of PS could also be attributed to the gas bubbles induced into the fluid by the effects of agitation. Interestingly when ultrasonic waves encounter bubbles in liquids, these gas bubbles are set into the motion of the acoustic wave, dissipate heat due to the collision between the acoustic waves and the gas bubbles as a result of thermal and viscous damping mechnisms.[38] Nevertheless a further increase in the stirring rate (beyond 600 rpm) increases the overall circulation, reduces the residence time of mushroom solution under the ultrasonic radiating tip in the active cavitation zone and thus affected the efficiency of extraction by reducing the concentration of PS to 12.3 mg/100 mL at 1000 rpm. At stirring rates beyond 600 rpm, the flow rate of the mushroom solution will be high enough to reduce its

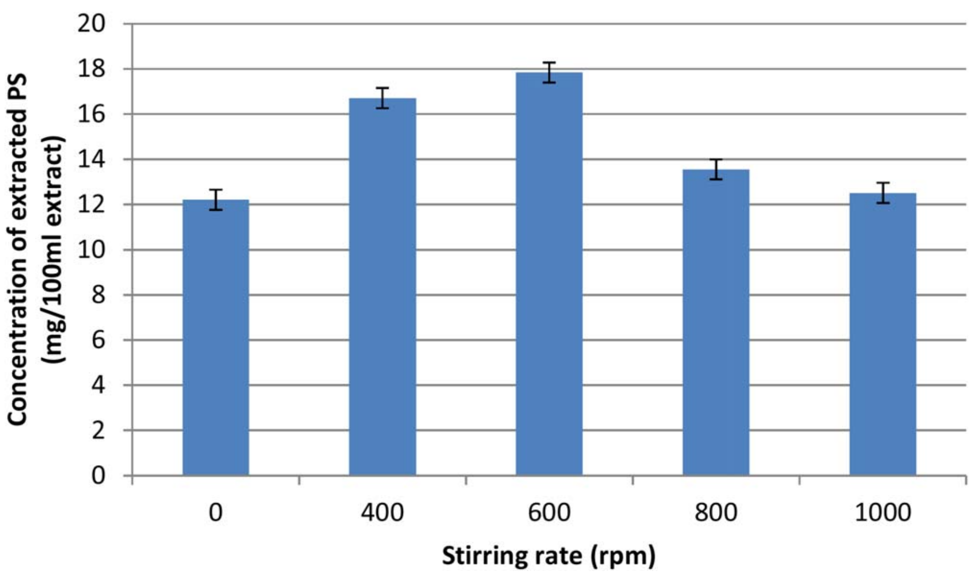

Figure 5. Concentration of extracted PS at different stirring rates using a 6 L ultrasonic U-tube extractor.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

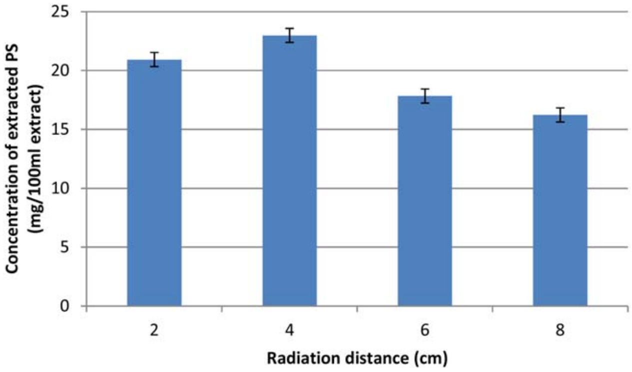

Figure 6. Effect of radiation distance (using dispersion conical funnel) on the concentration of extracted PS.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

active exposure to the ultrasonic irradiation tip and also interrupt the acoustic cycles that cause incomplete growth of cavitational bubbles and hence less intense cavitational conditions.

Effect of circulation and the dispersion geometry on the concentration of extracted PS in an ultrasonic U-tube extractor of 6 L

To optimize the impact of different radiation distances on the concentration of extracted PS, a preliminary investigation was conducted to select the optimal circulation rate using the scale of 6 L. Different stirring rates (400, 600, 800, and 1000 rpm) were subjected to study the effects of changing the circulation rate on the exposure time of mushroom solution to the ultrasonic radiating tip. The distance between the horn tip and the dispersion conical funnel was fixed initially at 6 cm. As shown in Figure 5, it was found that 400 rpm was the minimum stirring rate to maintain a circular flow motion in the U-tube with the volume of 6 L. Below 400 rpm, the circulation rate was not sufficient to avoid the sedimentation of mushroom powder at the bottom of the extractor. While 600 rpm was the optimum stirring rate to induce a good circulation and hence the exposure of mushroom solution which achieved the highest concentration of extracted PS (17.84 mg/100 mL). At 600 rpm the circular motion of the liquid successfully improved the concentration of extracted PS by 46.1% compared to using the same extractor of 6 L but without any circulation. However, a further increase in the circulation rate led to a reduction in the concentration of PS as a result of an increase in the flow rate and hence a decrease in the residence time. The latter could lead to incomplete growth of cavitational bubbles owing to the obstruction of ultrasonic waves which tend to move in spherical or plane motions.[16]

Effect of dispersion Geometry (dispersion conical funnel) and its radiation distance on the extraction of PS

The optimized circulation achieved at a stirring rate of 600 rpm from the preliminary study was then adopted to study the influence of change in the radiation distance using a dispersion conical funnel on the concentration of extracted PS. As shown in Figure 6 the concentration of extracted PS varied from 16.23 to 22.97 mg/100 mL by reducing the radiation distance from 8 to 4 cm. Thus the efficiency of ultrasonic extraction was significantly improved by reducing the radiation distance between the radiation source of horn tip and the dispersion conical funnel. This could be attributed to several factors that have contributed individually and interactively to improve the ultrasonic extraction efficiency. First, the mechanical effects of short-life oscillation bubbles in the transient cavitation were intensified at a radiation distance of 2 and 4 cm within the boundary of active cavitation zone-1 under the sonotrode. On the asymmetrical bubble collapse near the solid surfaces, millions of microjets of liquid occur at the solid surfaces of the congested mushroom fiber in the high intensity region under the tip. The flow of mushroom solution will be restricted due to the constriction in the diameter of the longitudinal pipe (90–30 mm) at a distance of 2–4 cm (between the irradiation tip and the bottom of the conical funnel). Besides the reflection of sound waves at the surface of conical funnel leads to extend the acoustic cycles which in turn enhance the mechanical effects on bubble collapse. However, the reduction in the concentration of extracted PS (20.93 mg/100 mL) observed at a radiation distance of 2 cm could be attributed to the higher attenuation of sound that occurs when the power densities close to the delivery point at the solid surfaces (congested mushroom) are very high and hence a probability of incomplete bubble growth resulted from shortening the number of acoustic cycles. Second, the effects of circular motion induced at a stirring rate of 600 rpm and the microturbulence of the transient cavitation contributed interactively in inducing steady eddy diffusion of PS solutes at the surface of mushroom particles which resulted in an enhancement in the local mass-transfer rate. While the overall mass-transfer rate was improved due to the local eddy diffusion as well as the highly localized energy resulted from the generated hot spots on violent collapse of the cavities. The inertial cavitation results into high amplitude pulsations in which extreme

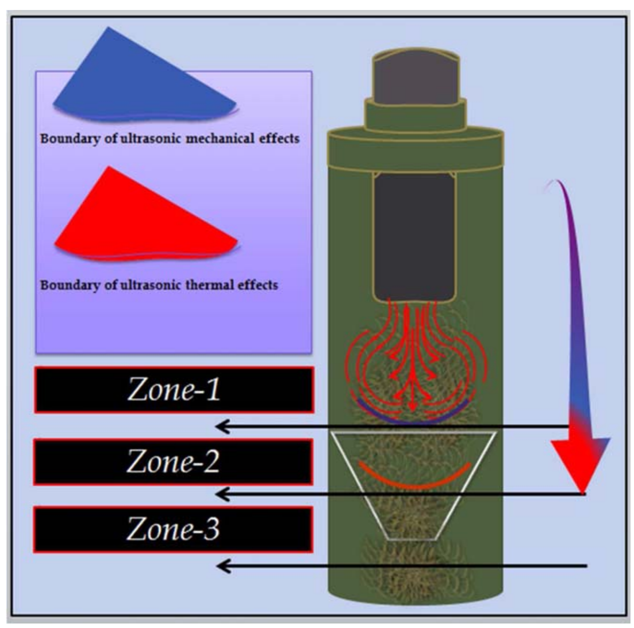

Figure 7. Distribution of cavitational intensity in the three zones using a dispersion conical funnel at a radiation distance of 8 cm.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

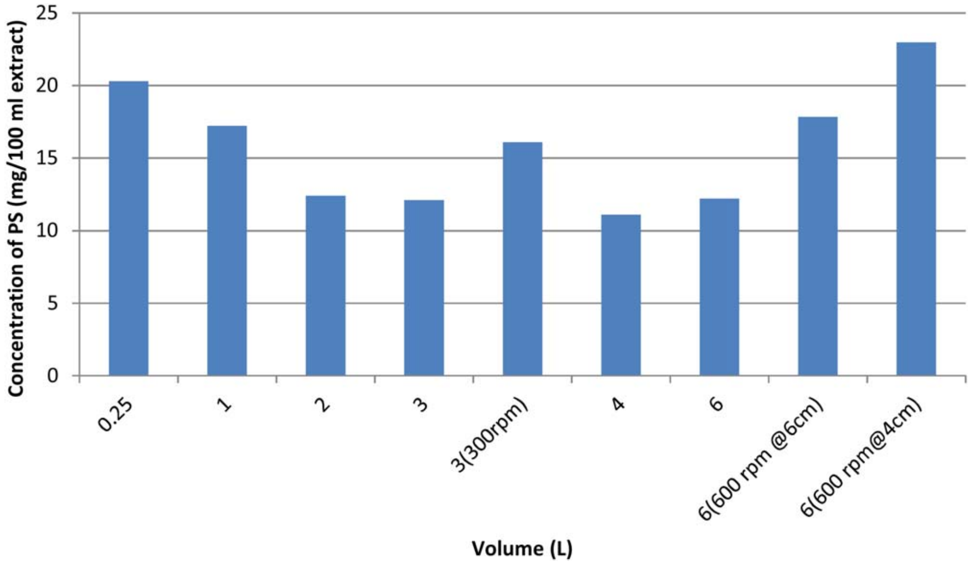

Figure 8. Comparison of PS concentration extracted by different scales of operation, stirring rates and at different radiation distances using dispersion conical funnel.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

expansion, shrinkage and eventual collapse of bubbles occur to produce large number of smaller bubbles associated with high localized energy which is believed to enhance the chemical and biological processes.[18,38–42] It is expected that inside a cavity (bubble), there is always a gas (such as air) and vapor (such as water vapor). Thus, during the bubble collapse, the temperature and pressure of the gas and vapor inside a bubble increase due to quasiadiabatic process where "quasi" referring to appreciable thermal conduction taking place between the heated interior of the bubble and the surrounding liquid.

The inertial cavitation is apparently has the major role in intensifying the cavitational intensity at distances of 6 and 8 cm as compared to the transient cavitation. At these distances extending the number of acoustic cycles (many hundreds) resulted in expanding the cavitational zone (zone-1, zone-2, and zone-3). The expansion of cavitational zone could be attributed to two reasons. First is the existence of nanobubbles (cavities of gas) at the interfaces between water and mushroom fiber (hydrophobic materials). These nanobubbles were believed to act as nuclei to assist in the formation of microbubbles,[43] and thereby increasing the bubbles population. Second is the bubble expansion phase of inertial cavitation that occurs at certain bubble size and acoustic pressure (Figure 7). For instance, on bubble expansion phase the initial bubble radius of 0.5 μm could be expanded up to 30 times of its initial radius and is followed by a violent collapse back to a smaller size. This mode of bubble oscillation can persist for many hundreds of acoustic cycles.[40,44]

Looking into the impact and results of different radiation distances, it could be speculated that the three regions of cavitation zone[16] were most likely extended in the radiation path of 6 and 8 cm. However, the lower concentration of extracted PS obtained at the radiation distances of 6 and 8 cm might be resulted from smaller sound absorption and the reduction in sound pressure to a level below threshold pressure where no longer cavitational activity can exist. A radiation distance of 4 cm may involve a heavily cavitating region (zone-1) and a cavitation transition region (zone-2) which positively intensified the extraction of PS. While a radiation distance of 2 cm involves the effects of zone-1 as well as the negative effects of attenuation of sound. Overall, using the U-tube extractor of 6 L, the scale-up has been successfully achieved whereby the concentration of extracted PS was enhanced, more importantly exceeding the concentration of PS obtained using extraction volume of 0.25 L (Figure 8). Table 4 shows the economic value of this scale-up by indicating the total energy consumption for each scale after incorporating the axial motion.

These outcomes are in agreement with the findings reported using an ultrasonic energy meter (PB-500, Megasonics, US) to observe the distribution of cavitational energy

Table 4. Energy Consumption for Different Scales of Extraction

Scale (L)	Energy (J/h)[a]	PS Concentration (mg/100 extract)	Yield of PS (mg)	Yield/Energy (mg/J)
0.25	**306252**	**20.3**	**50.75**	**1.657×10^{-4}**
1	306252	17.23	172.3	5.626×10^{-4}
2	306252	12.4	248	8.097×10^{-4}
3	**306252**	**12.12**	**363.6**	**1.187×10^{-3}**
3(300 rpm)	**325152**	**16.09**	**482.7**	**1.484×10^{-3}**
4	306252	11.1	444	1.449×10^{-3}
6	**306252**	**12.21**	**732.6**	**2.392×10^{-3}**
6(600rpm@6cm)	**344052**	**17.84**	**1070.4**	**3.111×10^{-3}**
6(600rpm@4cm)	**344052**	**22.97**	**1378.2**	**4.005×10^{-3}**

[a]Total energy consumed calculated based on the actual power dissipated into the system from a calorimetric study. Bold text represent the major points of comparison.

Table 5. Analysis of Variance by Genstat-16

Source of Variation	DF	S.S.	M.S.	*F*-Ratio	*P*-Value	e.s.e.	s.e.d.
Scale of operation (0.25–6 L)	5	202.9165	40.5833	77.73	<.001	0.417	0.590
3 L (Circulation)	4	36.5964	9.1491	53.11	<.001	0.240	0.339
6 L (Circulation)	4	78.2106	19.5526	33.18	<.001	0.443	0.627
6 L (Circulation with dispersion conical funnel)	3	82.529	27.510	25.72	<.001	0.597	0.844

DF, degree of freedom; S.S, Sum of squares; M.S, Mean squares; e.s.e, Standard errors of means; s.e.d, Standard errors of differences of means.

in an ultrasonic U-tube system of 2 L.[45] When the test was performed in pure water a sharp decline in the power intensity from 16.49 to 4.22 W/cm^2 was observed by moving the meter away from the ultrasonic radiating source (a horn-tip of 15 mm diameter) from 1 to 6 cm. While after the addition of the extraction biomaterials into water the power intensity significantly reduced to 2.82 W/cm^2 at a distance of 1 cm. A reduction in power intensity could be due to the attenuation of ultrasonic waves and due to an increase in the resistance to the diffusion of ultrasonic energy caused by higher concentration of solid particles in the extracting solution (solid/liquid ratio of 1 g per 30 mL) and the integrity of the solid matrix (cellular structure of extracted materials). However, when they coupled the ultrasonic system with an agitator the power intensity was enhanced markedly to 9.42 W/cm^2 at a distance of 1 cm and the cavitational intensity extended to reach a distance up to 4 cm.

Statistical analysis

Statistical Analyses section: All single factor experiments were analyzed statistically. The data were represented as the mean of three replications. GenStat-16 (Version 16.1.0.10916, VSN International, UK) was used to perform the computational analysis. The analysis of variance was performed using one way ANOVA. Tukey test was used to determine the degrees of freedom, mean square, sum of squares, standard errors of means, and standard errors of differences of means as well as *P*-values and *F*-values. A *P*-value of < 0.05 was considered to be the level of significance and the resulted data were statistically significant as shown in Table 5.

Conclusion

The extraction of PS from *Ganoderma lucidum* with the aid of ultrasound was subjected to a scale-up study to enhance the concentration and hence the extracted yield under constant ultrasonic conditions (power, 600 W; time, 1 h; and temperature, 80°C). The concentration of PS was observed to be reduced by 39.85% by increasing the scale of extraction from 0.25 to 6 L due to the reduction in the ultrasonic power density. An ultrasonic U-tube extractor was designed to enhance the extraction efficiency of 3 and 6 L, where axial circular motion was induced at different stirring rates (300, 400, 600, 800, and 1000 rpm). The axial circular motion was positively found to be influential in improving the concentration of PS in the following conditions, that is, 3 L, 300 rpm and 6 L, 600 rpm due to increasing the exposure of mushroom powder to the ultrasonic radiation tip. The ultrasonic U-tube extractor was modified by introducing a dispersion geometry (conical funnel) to reduce the cross-sectional area of the circulation flow path and intensify the mushroom powder in the active cavitation zone at different radiation distances (2, 4, 6, and 8 cm). At a stirring rate of 600 rpm and at a radiation distance of 4 cm the concentration of extracted PS increased by 88.21% as compared to the conventional extractor without circulation and dispersion conical funnel. Besides, adjusting the radiation distance between the ultrasonic tip and the tip of conical funnel to 4 and 2 cm has improved the concentration of extracted PS by 28.75 and 17.32% compared to 6 cm under same circulation rate. Overall the scale-up from a volume of 0.25–6 L was successful and the concentration of extracted PS increased by 13.15% as compared to using an extractor of 0.25 L under the same ultrasonic power dissipation.

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