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Research paper

Optimization of ultrasound assisted extraction (UAE) of β-D-glucan polysaccharides from *Ganoderma lucidum* for prospective scale-up

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Abstract

Three levels of three ultrasonic independent variables were optimized to obtain the maximum yield of water-soluble polysaccharides (PS) extracted from *Ganoderma lucidum* using response surface methodology (RSM). Box–Behnken design (BBD) was employed to evaluate the effects of ultrasonic variables on the yield of PS. The parameters that were considered for the optimization are ultrasound power (500–700 W), ultrasonic irradiation time (45–65 min) and temperature (70–90 °C). The analysis of variance suggested that the response dependent variable of yield of PS could be expressed by a quadratic polynomial model. The optimal theoretical extraction conditions were found to be an ultrasonic power of 590 W, an irradiation time of 58 min and a temperature of 81 °C. Under these conditions the predicted optimal yield was 52.33 mg. Whereas by following the optimized conditions, the yield of PS by experiments was found to be 52.28 mg which is in a very good agreement with the theoretically predicted one. These outcomes indicate the adequacy of quadratic polynomial model to represent the ultrasonic extraction variables within the ranges of investigation for a volume of 0.25 L; and any prospective scale-up may require modifications in the geometry of the extracting vessel due to the non-linear effects of power ultrasound.

Keywords: Ultrasound; Extraction; Response surface methodology (RSM); Optimization; *Ganoderma lucidum*; Polysaccharide

1. Introduction

The fruiting body, mycelia and spores of *Ganoderma lucidum* (GL) contain more than 400 types of bioactive ingredients which have been reported historically to cure many diseases, especially in the traditional Chinese medicine (TCM). These bioactive ingredients were classified in groups such as triterpenes, polysaccharides, proteins, nucleotides, metals etc., and have different pharmaceutical activities in the human body [1–7]. Particularly polysaccharides (PS) have been found to be a good antioxidant to reduce the damage caused by the free radicals produced during the oxidation reaction. These free radicals have been considered to be harmful to the human body due to the potential damages they cause such as losing tissue, promoting diseases and skin ageing [8–13]. PS function as antidiabetic agents as a result of lowering the concentration of blood glucose and due to its potential hypoglycaemic activities [14,15]. PS also possess inhibitive effects on the tumour growth, especially against the growth of S-180 Sarcoma and Lewis lung carcinoma in mice [2,16]. Moreover PS have been reported as effective antitumour agents against human breast cancer cells due to their ability to increase the proliferation of macrophage cells in the immune system [17].

In essence, preserving the structure of PS during their isolation is crucial to maintain their bioactivity for the intended application. Using conventional methods such as heating or boiling water to extract the water-soluble PS might have some negative effects on the structure of PS owing to hydrolysis, ionization or oxidation as a result of longer time of extraction followed to achieve higher efficiency [18]. Alternatively newer techniques such as microwave assisted extraction (MAE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and

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ultrasound assisted extraction (UAE) have been developed to improve the extraction in terms of reducing the energy and time along with obtaining a higher bioactivity [19,20]. Among these methods, UAE has been found to be a cost-effective technique due to shortening the extraction time and reducing the power consumption attributed to the physiochemical effects of cavitational phenomenon induced with ultrasound waves into the extraction medium [21,22]. However, the successful application of UAE relatively depends upon the optimum operating conditions and the matrix of the plants subjected to ultrasound [23] which should be investigated carefully towards obtaining a higher yield of targeted components and their bioactivity.

Significantly the operational parameters such as power intensity, irradiation time and temperature could be optimized to obtain the best combination of their interacted levels for better influence on the concentration, stiffness and matrix of the extracted raw materials. Actually, one of the different approaches that illustrate the effects of power intensity on the ultrasonic assisted process was reported by Patist and Bates [21]. According to Patist and Bates, the ultrasonic liquid processing can be affected by the parameters of amplitude, pressure, temperature, viscosity and concentration of solids. If the pressure, viscosity of the solvent and the concentration of the solid materials were considered constant, the optimal effects of the remaining parameters could be a function of (1) Energy (the total of energy input per volume of treated materials, kWh/L) and (2) Intensity (the actual output power per surface area of the sonotrode, W/cm^2) [21]. For a fixed tip diameter, the total energy input into the extraction medium is actually the result of power output (kW) dissipated across the cross-sectional area of tip and time of exposure of the materials subjected to ultrasound. Therefore, the optimal power intensity could be best functioning for larger scales depends upon the capacity of the extraction, i.e. the size of the extraction vessel, as well as the geometry of the extractor.

Response surface methodology (RSM) is a statistical analysis technique and has been employed to systematically optimize the ultrasonic parameters involved in the extraction and achieve their best possible combination. RSM has been widely used in optimizing the ultrasonic parameters for the extraction of PS from different herbs such as *Zizyphus jujube* cv. *jinsixiaozao* [24], *Isodon lophanthoides* var. *gerardianus H. Hara* [11] and *Agaricus bisporus* [25]. RSM involves a number of statistical designs such as Central Composite Design (CCD), Box–Behnken Design (BBD), Optimal Design and other statistical procedures. Among these designs, the BBD has been distinguished as a simplified design to cover three levels of experimental factors with less number of experiments [26–28]. It was also widely applied for optimizing the extraction of PS from *Litchi* [29], *Boletus edulis* mycelia [18], *Asparagus officinalis* [26], *Longan* fruit pericarp [10] and *Opuntia milpa alta* [30]. Specifically for *Ganoderma lucidum*, RSM-BBD has been used to optimize the UAE at low frequencies (4, 6 and 8 kHz) to investigate the inhibitory effects of PS on cervical cancer cells [31]; RSM-BBD has also been used to optimize the conditions of hot water extraction to increase the yield for higher antioxidant activity [32], while RSM-CCD was used to optimize UAE for antioxidant and antiproliferative activities [33].

In the present investigation, RSM-BBD has been employed to optimize the effects of high power ultrasound (500–700 W) at an ultrasonic frequency of 20 kHz, ultrasonic irradiation time (45–65 min) and temperature (70–90 °C) on the yield of PS extracted from *G. lucidum* cultivated locally in Malaysia for commercial scale-up. The optimal conditions achieved in this study have been applied to scale-up the UAE of polysaccharides [34]. The results indicated that the optimal conditions could be better applicable after modifying the geometry of the extracting vessel with the aid of axial circulation.

2. Materials and methods

2.1. Materials

The fruiting bodies of *G. lucidum* were supplied by the local company, *Ganofarm Sdn. Bhd.* (Tanjung Sepat, Selangor, Malaysia). All the chemicals used, i.e. ethanol, butanol and chloroform, were of analytical grade.

2.2. Methods: isolation of polysaccharides and characterization

The isolation steps followed for the extraction of PS by employing the power ultrasound have been reported in our previous work [35]. In the same study the sugar profile, molecular weight and degree of branching of isolated polysaccharides have been characterized to having physiochemical properties of (1–3; 1–6)-β-D-glucan configuration. The ratio of solid/liquid was fixed at 10 mg fibre/250 ml water in all the experiments.

2.3. Description of the ultrasonic system employed in the study

An ultrasonic horn of 25 mm tip diameter, 20 kHz frequency, with the power range of 0–1000 W (HD-3400, SONOPLUS, Germany) has been used. The amount of actual power dissipated by the ultrasonic system into the extraction medium was determined by conducting a calorimetric study for the power amplitude of 400–800 W and measuring the total heat generated in 250 ml of distilled water. The power range of 400–800 W has been reported to be equivalent to 67–98 W, and is in accordance with our previous work [34], as shown in Table 1. The temperature was controlled by utilizing a jacketed system to avoid the probable degradation of PS which could be induced by the thermal effects of high intensity power ultrasound. The jacketed reactor of 300 ml was designed to control and maintain the temperature with the aid of a chilling system (Model DRC4, CPT Co. Ltd., Korea). The system was covered during the experiments to maintain solvent condensation and recovery.

2.4. Optimization of ultrasonic parameters by RSM and statistical analysis

An optimization study for the parameters involved in the UAE was carried out in two stages. In the first stage, a

Table 1
Calorimetric study: the theoretical power levels (amplitudes) and their corresponding actual power dissipated into the system.

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

Table 2
Independent variables and their investigated coded levels for Box–Behnken design matrix.

Independent variables	Codes	Levels		
		−1	0	1
Power (W)	(X1)	500	600	700
Time (h)	(X2)	45	55	65
Temperature (°C)	(X3)	70	80	90

single-factor experiment was conducted to determine the preliminary effective range of each of the extraction factors; power, time and temperature towards achieving the highest level of PS yield. In this stage, the analysis of variance and regression analysis for each factor were performed using the statistical software, Genstat 16th (Version 16.1.0.10916, VSN International Ltd., UK). The ranges followed were ultrasonic power, 200–700 W; temperature, 40–90 °C; and ultrasonic irradiation time, 20–60 min.

In the second stage, three-level-three-factor Box–Behnken Design [27] was employed to optimize the parameters achieved in the first stage in the best ranges, and their optimal combinations were established. The Design expert (Version 8.0, Stat-Ease Inc., Minneapolis, MN, USA) software was used for the experimental design, data analysis and model building. The independent variables (ultrasonic power, irradiation time and temperature) and their levels followed in the second stage have been shown in Table 2. The whole BBD design consists of 15 factorial points (Table 3). The design involves 12 randomized points of the independent variables with their responses of dependent variable (yield of the extracted PS) and three replicates at the centre point (13–15) to evaluate the pure error. The response variable could be fitted into the general form of a quadratic polynomial model as shown in the following Eq. (1),

$$Y = \beta_0 + \sum \beta_j X_j + \sum \beta_{jj} X_j^2 + \sum \sum \beta_{ij} X_i X_j \quad (1)$$

Table 3
Levels of independent factors and the actual and predicted values of dependent variable for Box–Behnken Design (BBD).

Runs	Power (W)	Time (min)	Temperature (°C)	Yield (mg)	
	X1	X2	X3	Actual	Predicted
1	500	45	80	38.4	38.26
2	700	45	80	40.2	41.84
3	500	65	80	48.7	47.06
4	700	65	80	40.7	40.84
5	500	55	70	39.2	39.88
6	700	55	70	38.8	37.70
7	500	55	90	41.5	42.60
8	700	55	90	42.8	42.13
9	600	45	70	35.8	35.26
10	600	65	70	43.7	44.66
11	600	45	90	45.3	44.34
12	600	65	90	42.2	42.74
13	600	55	80	52.4	52.00
14	600	55	80	52.1	52.00
15	600	55	80	51.5	52.00

where Y is the response variable measured for each combination of factorial level; β_0, β_j, β_{jj} and β_{ij} are the regression coefficients for intercept, linearity, square and interaction respectively; X_i and X_j are the codes of the independent variables. The coefficients of second order polynomial model were determined by a multiple regression analysis for the values of responses obtained from the experiments. The coefficient of determination (R^2) represents the quality of fit of the polynomial model. F-test and t-test were used to check the statistical significance of R^2 and the regression coefficients respectively.

3. Results and discussion

3.1. Preliminary screening of ultrasonic irradiation time on the yield of extracted polysaccharides

Preliminary experiments were conducted to examine the effects of ultrasonic irradiation time on the yield of PS. Many factors influence in determining the required extraction time such as the structure of cell-wall, mass transfer resistance for the diffusion of solvent and the penetration rate of solvent into the porous microstructure of the plant [23]. The woody structure of *G. lucidum* probably requires extended time to ensure effective isolation of PS. In order to fairly represent the factor of time in the optimization study, the ultrasonic extraction was conducted at different irradiation times (20, 30, 40, 50 and 60 min), while other two parameters were kept constant at a power of 200 W and at a temperature of 40 °C. As shown in Fig. 1, the yield of PS was found to increase from 25.8 mg to 30.13 mg by increasing the extraction time from 20 to 50 min. Following this, a marginal reduction in the yield (30 mg) was observed by increasing the ultrasonic irradiation further to 60 min. These results indicate that an extraction time between 40 and 60 min would effectively increase the concentration of extracted PS due to the higher ultrasonic energy introduced during 40–60 min. However, extended exposure to power ultrasound may lead to reduction in the yield, as shown at 60 min, due to the degradation of PS.

The ultrasonic energy enhances the hydration of medicinal herbs and water uptake; provides greater penetration of the solvent into the cellular matrix; enlarges the pores of cells and increases the surface area of solid particles owing to the size-reduction of the cellular structure [36,37].

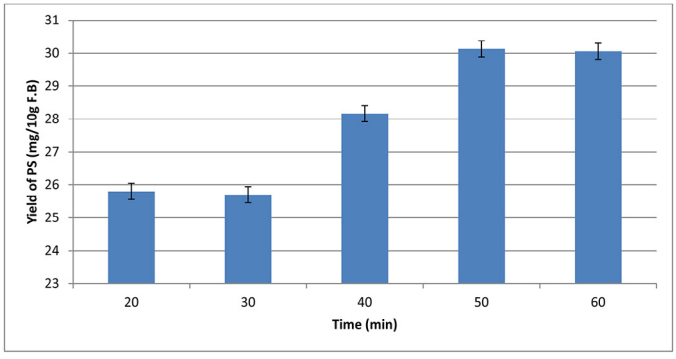

Fig. 1. Effect of ultrasonic irradiation time on the yield of extracted polysaccharides.

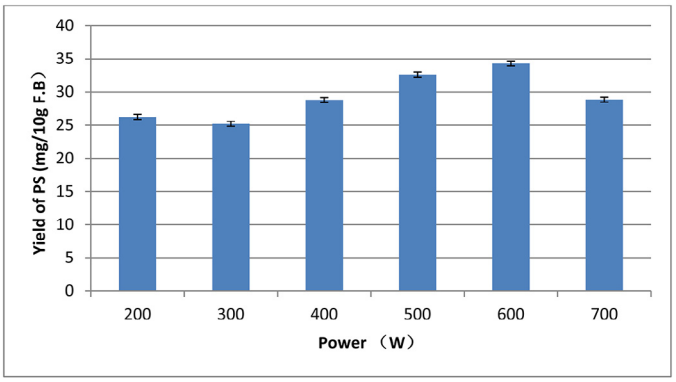

Fig. 2. Effect of ultrasonic power on the yield of extracted polysaccharides.

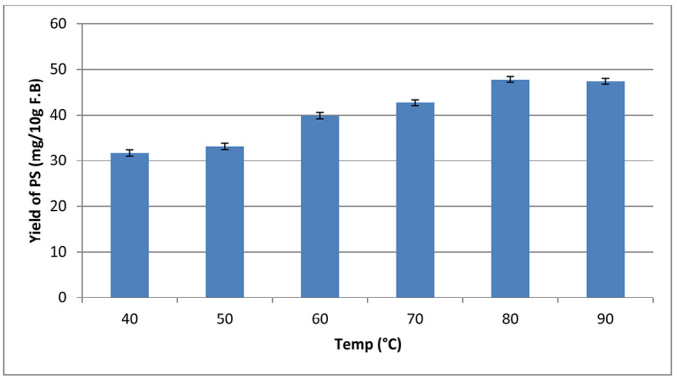

Fig. 3. Effect of temperature on the yield of extracted polysaccharides.

3.2. Preliminary screening of ultrasonic power on the yield of extracted polysaccharides

The ultrasonic power is another essential parameter that influences the extraction. To determine the effective range of this parameter, a number of preliminary experiments covering the ultrasonic power levels of 200, 300, 400, 500, 600 and 700 W were subjected to check the efficiency of extraction. The other two parameters, i.e. temperature and irradiation time, were kept constant at 30 min and at 40 °C respectively. At 200 W, the extraction yield was 26.66 mg which increased to 32.63 and 34.3 mg by increasing the ultrasonic power to 500 and 600 W respectively (Fig. 2). An increase in the yield of PS at higher ultrasonic power is attributed to an increase in the power intensity and thus the cavitational intensity [21].

Cavitational intensity depends upon the ultrasonic energy dissipated into the extraction system, the irradiation distance away from the ultrasonic tip and the operating frequency [38,39]. Cavitational intensity acts essentially to increase the diffusion of PS solutes due to the violent disruption of solvent into the cellular structure of *G. lucidum* and hence an increase in the overall mass transfer rate [23,40,41]. Accordingly, the power range of 500–600 W was chosen as an optimum for the next stage of RSM design.

3.3. Preliminary screening of temperature on the yield of extracted polysaccharides

The ambient temperature might affect the cavitational activity of an ultrasonic process. It was reported that increasing the temperature positively leads to an increase in the number of cavitational bubbles due to reducing the surface tension and viscosity of the solvent [42]. However, any excessive temperatures result into increasing the vapour pressure which in turn cushions the collapsed bubbles [21] and thus suppresses the cavitational activity. Therefore optimizing the temperature of extraction is important to exploit its positive effects and mitigate the negative ones.

By considering these facts, different levels of extraction temperatures (40, 50, 60, 70, 80 and 90 °C) were examined to study their effects on the extraction and yield of PS under the influence of ultrasound. The temperature was measured using a digital thermometer. The sensor was put inside the extraction vessel under the irradiation tip directly within 2–5 cm. As shown in Fig. 3, the highest yield of extracted PS was found to be within a temperature range of 70–90 °C which is attributed to improving the mass transfer rate due to an increase in the number of cavitational bubbles with an increase in the temperature of ultrasonic extraction. However, excessive exposure of PS to high temperatures may lead to degradation of its molecular structure [22,43]. Eventually the range of 70–90 °C was considered for further investigations in the RSM study.

3.4. Optimization of extraction conditions by RSM-BBD (ANOVA, regression analysis and model fitting)

In Table 2 the relationship of ultrasonic parameters and their levels has been presented. The levels of coded variables (X_1: power, X_2: time, X_3: temperature) estimated for each of the experiments in the design matrix and the actual experimental and predicted results have been shown in Table 3. The response variable of different experimental combinations has been represented by the yield of PS (Y).

The results of multiple regression analysis for the values of responses obtained from the experiments indicated that the response variable of PS yield (Y) could be predicted by a quadratic polynomial model (1) within the levels of investigation as represented in the following Eq. (2),

$$Y = 52 - 0.66X_1 + 1.95X_2 + 1.79X_3 - 2.45X_1X_2 + 0.43X_1X_3 - 2.75X_2X_3 - 5.59X_1^2 - 4.41X_2^2 - 5.84X_3^2 \quad (2)$$

where Y is the yield of PS, and X_1, X_2 and X_3 represent the coded variables of ultrasonic power, irradiation time and temperature respectively.

The fitness of mathematical model to adequately represent the relationships between the independent and dependent variables is essential and is of high importance to be tested [10,44]. The degree of fitness of the model could be indicated by the values; coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), *p*-value and lack-of-fit. First, the coefficient of determination was defined by the ratio of explained variation to the total variation [45]. The *P*-value is an indicator of the significance of each coefficient to represent the variables and its interactions, while the lack-of-fit test was designed to measure the lack of the model to represent the factorial points

Table 4
ANOVA for response surface of the quadratic polynomial model.

Source	Sum of squares	Degree of freedom	Mean square	F value	p-value Prob > F	Significance
Model	386.65	9	42.96	18.55	0.0025	Significant
Residual	11.58	5	2.32			
Lack of fit	11.16	3	3.72	17.72	0.0539	Not significant
Pure error	0.42	2	0.21			
Cor total	398.24	14				
R-squared	0.97					
R^2_{Adj}-squared	0.92					

that exist in the domain but not involved in the regression. The R^2_{adj} is designed to measure the correlation and the degree of goodness-of-fit of the regression equation [25]. When the coefficient of determination (R^2) approaches the integrity (≈1), the *p*-value equals or below 0.05 and lack-of-fit test has a small value, and the model is expressed to have a good fitness. The relationship between R^2 and R^2_{adj} is important, i.e. as much as R^2 and R^2_{adj} in higher agreement is the better correlation between the actual and predicted experimental values of a design matrix.

The fitness of the model is summarized in Table 4. The F-test and analysis of variance (ANOVA) for the response surface quadratic model showed that the *p*-value of the model is 0.0025. This suggests that the model is significant and adequate for predicting the yield of PS within the range of investigation. The value of R^2 calculated from the quadratic regression model was 0.97, which implied a high degree of fitness of the model and closer agreement between the experimental and predicted values [46], i.e. 97% of the variation could be explained by the fitted model. The F-value and *p*-value of the lack-of-fit test were 17.72 and 0.0539 respectively for the regression model. The lack-of-fit test is considered significant if the F-value is very high and *p*-value is very low. Therefore the lack-of-fit test is not significant for the model relative to the pure error.

The results of multiple regression analysis have been shown in Table 5. It involves the significant and non-significant coefficients of the polynomial model as well as their terms of interaction. It could be seen that the linear coefficients of X_2 and X_3, the quadratic coefficients of x_1^2, x_2^2, x_3^2 and the coefficients of interaction for x_1x_2 and x_2x_3 are significant with *p*-values below 0.05. These terms significantly affected the yield of PS.

Table 5
Estimated regression model between the response and independent variables.

Source	SS	DF	MS	F-value	P-value	Significance
X_1	3.51	1.00	3.51	1.52	0.2730	Not significant
X_2	30.42	1.00	30.42	13.13	0.0152	Significant
X_3	25.56	1.00	25.56	11.03	0.0210	Significant
X_1X_2	24.01	1.00	24.01	10.36	0.0235	Significant
X_1 X_3	0.72	1.00	0.72	0.31	0.6006	Not significant
X_2 X_3	30.25	1.00	30.25	13.06	0.0153	Significant
X_1^2	115.27	1.00	115.27	49.76	0.0009	Significant
X_2^2	71.89	1.00	71.89	31.03	0.0026	Significant
X_3^2	125.82	1.00	125.82	54.31	0.0007	Significant

The model terms of *p*-values less than 0.05 were considered significant. X_1 – Power (W), X_2 – Time (min) and X_3 – Temperature (°C).

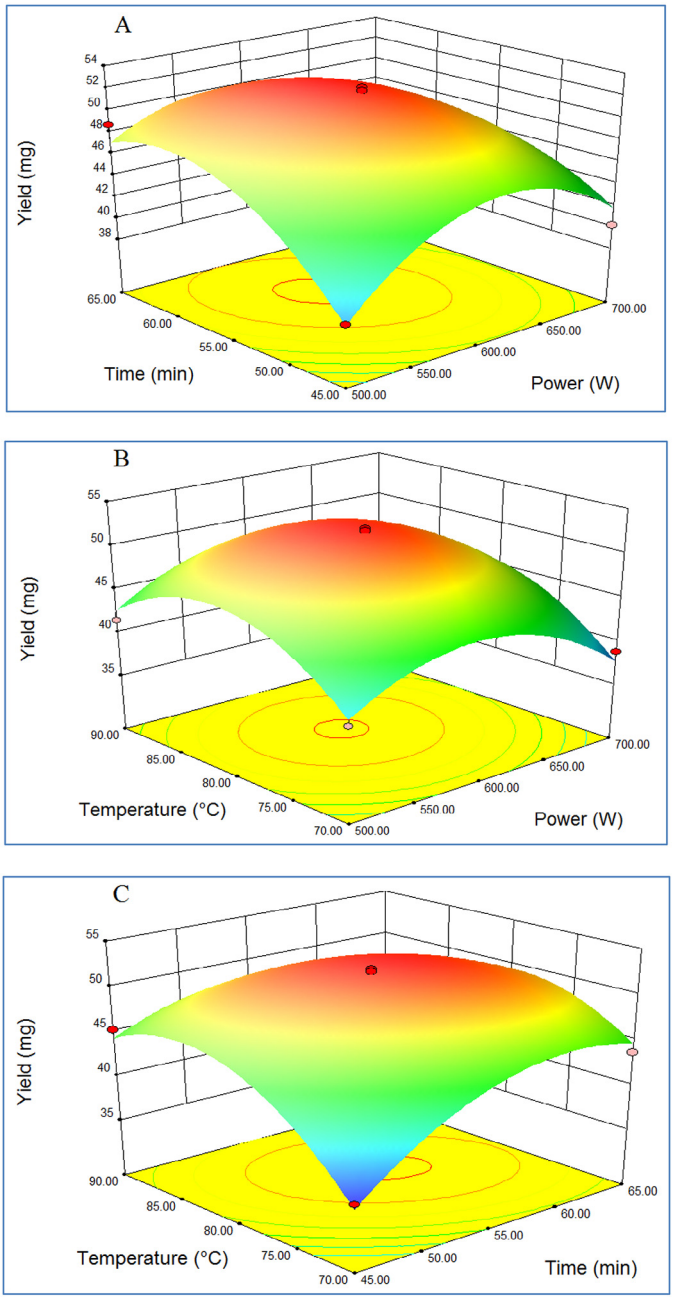

Fig. 4. RSM 3D-plots showing the effects of ultrasonic variables (A: ultrasonic power × irradiation time; B: ultrasonic power × temperature; C: irradiation time × temperature).

However, the linear coefficient of ultrasonic power X_1 and its interaction term X_1X_3 with the temperature was not significant due to the *p*-value which was more than 0.05.

3.5. The effect of ultrasonic power, irradiation time and temperature on the yield of extracted polysaccharides

The relationship between independent ultrasonic factors and the dependent variables represented by the yield of PS has been depicted in the three dimensional plots of response surfaces and the two dimensional contour plots using Design-Expert as shown in Figs. 4 and 5. These plots show the mutual interaction

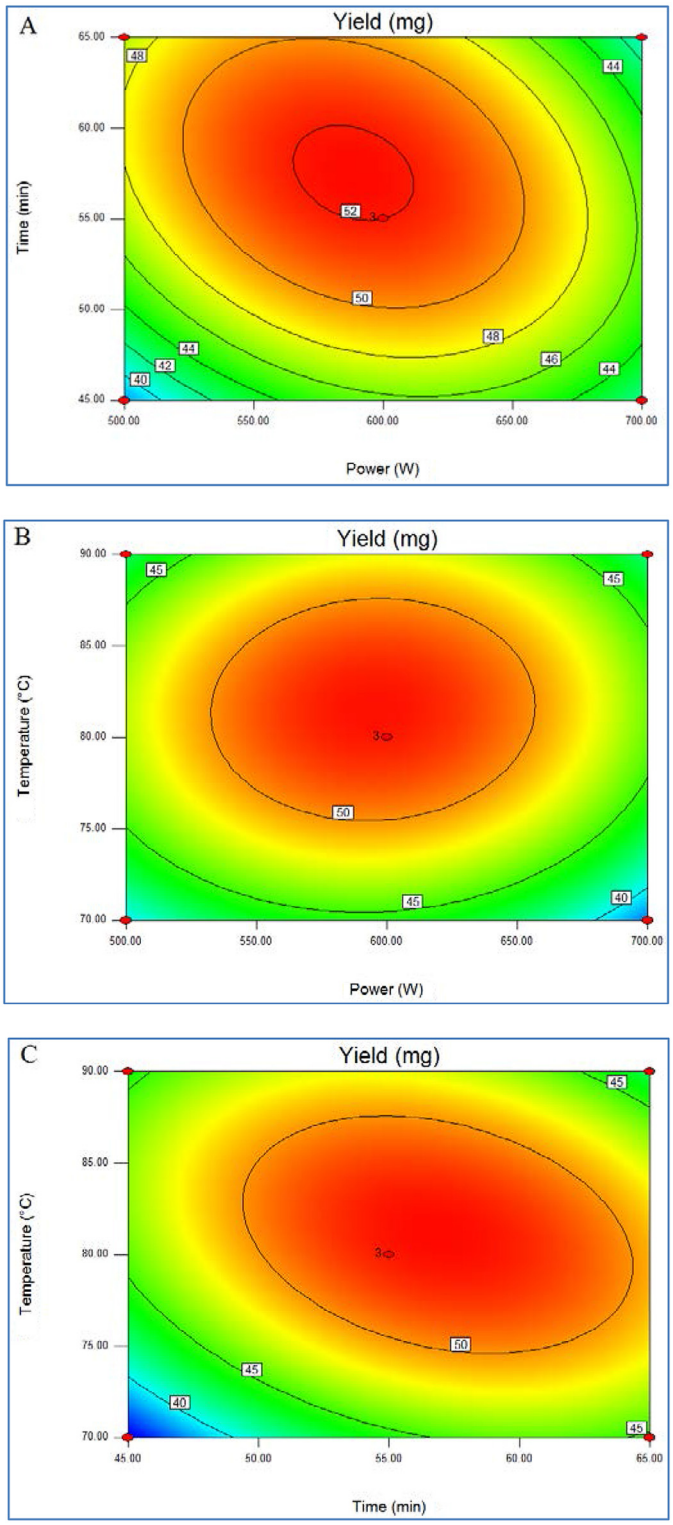

Fig. 5. The contour plots show the effects of ultrasonic variables (A: ultrasonic power × irradiation time; B: ultrasonic power × temperature; C: irradiation time × temperature) on the yield of extracted PS.

between the variables as well as their subjective effects on the yield of PS in terms of significance. In all the 3-dimensional surface plots (Fig. 4), when two investigated ultrasonic variables were within the experimental ranges, the third variable was kept constant at zero level. In the two dimensional contour plots, the elliptic plots refer to significant mutual interactions between the variables, while the circular plots mean otherwise (Fig. 5).

In Fig. 4A, when the temperature was kept at zero level, the yield increased significantly (p-value of 0.015) by increasing the irradiation time at low power levels. However, increasing the power levels beyond 600 W significantly decreased the yield of PS in interaction with the effects of irradiation time (p-value 0.023). In other words, extensive application of high levels of power ultrasound leads negatively to decomposition [47] on the extracted components. Similar response has been observed for the interaction effects of irradiation time and ultrasonic power in larger scale (2 L) of an ultrasonic extraction system [26]. The effects of three levels of ultrasonic power (400, 600 and 800 W) and extraction time (30, 40 and 50 min) have been optimized on the yield of polysaccharides from *Asparagus officinalis*. The ultrasonic power exhibited quadratic and interactive significant effects with the extraction time (p-value <0.0001) on the yield of polysaccharides for 2 L scale of extraction. Therefore, these optimized conditions could be subjected for further studies to scale-up the extraction process with the aid of ultrasound.

From Fig. 4B, it could be seen that when the irradiation time was kept constant at zero level, the temperature and power displayed quadratic effects on the yield of PS. At lower levels of temperature and power the yield increased to reach an extreme response surface at 80 °C and at 600 W. However any further increase in the power levels and temperatures resulted in decreasing the yield of PS due to the degradation of these components to their free sugars at higher temperatures and intensive power ultrasound [22,26,29,43]. According to Patist and Bates, the temperature affects the vapour pressure, surface tension, and viscosity of the liquid medium. Increasing the temperature has positive effects due to decreasing the viscosity and surface tension and hence the total cavitational bubbles. However, it negatively reduces the effects of the bubble collapse due to its cushioning and damping by higher vapour pressure [21]. Therefore, in a single factor experimental design the temperature range of 70–90 °C has been preliminary selected for further studies by multifactor experimental design of BBD-RSM, in order to find the optimal temperature that enable us to achieve the highest bubble population and least collapse cushioning/damping effects. In the single factor experimental design the effects of temperature variation on the yield was studied by fixing the other two parameters at certain levels. While in the BBD-RSM, the optimization of the effects of extraction temperature (70–90 °C) was conducted interactively with the other parameters namely ultrasonic power (500–700 W) and irradiation time (45–65 min). In this process increasing the temperature leads to an increase the concentration of polysaccharides in the extraction medium due to increasing the mass transfer of these macromolecule sugars, which then leads to the change of the rheological properties of the extraction medium represented by viscosity and surface tension. Therefore, the optimal temperature of 80 °C was high enough to decrease the viscosity of the polysaccharide solution,

form enough violent cavitation bubbles, and low enough to avoid the bubble collapse damping effects due to high vapour pressure.

From Fig. 4C, it could be noted that the ultrasonic power was kept constant at zero level. The other two parameters, temperature and ultrasonic irradiation time, showed significant quadratic effects as well as mutual interactions on the yield of PS. Remarkably, at lower extraction time, below 55 min, the yield increased by increasing the temperatures to reach the extreme point of response surface at 80 °C. Nevertheless extending the extraction time resulted in reducing the yield levels at higher temperatures due to the degradation of PS.

The elliptic plots in Fig. 5A and C refer to significant mutual interactions between the irradiation time and other variables on the yield of PS with *p*-values of 0.023 and 0.015 respectively. Remarkably the irradiation time has dominating effects on other variables as compared to the ultrasonic power and temperature of extraction (Table 5).

3.6. Experimental validation of the optimized ultrasonic conditions

In order to validate the mathematical model, the optimal theoretical conditions predicted computationally by the response surface analysis to achieve the maximum yield of extracted PS were adopted in three actual experiments with minor modifications. The optimum predicted extraction conditions (ultrasonic power: 589.62 W, temperature: 80.97 °C, irradiation time: 57.19 min) were modified to meet the operability of the ultrasonic horn system as follows: ultrasonic power: 590 W, temperature: 81 °C, irradiation time: 58 min. Under these modified conditions the actual extracted yield of PS achieved was 52.28 mg, which is consistent with the predicted value of 52.33 mg and indicated the adequacy of RSM model to reflect the optimized extraction conditions under the influence of ultrasound. According to Table 1, the optimal ultrasonic power of 590 W could be approximately equivalent to 85 W.

3.7. Comparison of ultrasonic assisted extraction with a classical method under the optimal conditions

Hot water extraction is a well-known conventional method which was conducted under the optimal conditions achieved through ultrasonic assisted extraction (81 °C and 58 min). The water bath that has been employed for this purpose has been described in our previous work [35]. The maximum yield obtained by ultrasonic assisted extraction (52.25 mg) was higher than its corresponding value (29.2 mg) obtained by hot water extraction at 81 °C and 58 min extraction time and temperature respectively. The results indicated the ability of power ultrasound to intensify the extraction rate through its thermal and mechanical effects as compared with the thermal effects of the classical method. The purpose of comparison with a conventional extraction method is to investigate the mechanical effects of ultrasound under the optimized conditions (81 °C and 58 min) by eliminating the thermal effects associated with ultrasound. The optimal temperature and time achieved were fixed to observe the quantity of polysaccharides extracted. The intensification was obviously remarkable due to an increase in the yield by 79% (from 29.2 to 52.25 mg). Not only an increase in the yield, but also a significant reduction in the energy consumption to achieve the same yield resulted by ultrasound under the optimized conditions. The energy consumed by the ultrasonic system is 0.6 kWh, while for the conventional method is 6.4 kWh which shows a 10 times reduction in the energy consumption when ultrasound was employed.

3.8. The scale-up of the optimal conditions

Upon applying the optimal conditions achieved in this study in different larger scales (1, 2, 3, 4, 6 L) [34], the efficiency of power ultrasound to intensify the extraction is reduced. The yield of extracted PS has not been enhanced only after modifying the geometry in a circulating flow reactor for scales 3 and 6 L. This could be attributed to the significant quadratic effects of power intensity (p-value <0.0001) which showed non-significant linear relationship (p-value 0.2730) with the yield of PS. In other words, extending the ultrasonic power levels has not only negative effects in 0.25 L scale but also a significant reduction at larger scales. Therefore, modifying the geometry of extraction vessel was of prime importance to eventually enhance the yield of PS at larger scales beyond 0.25 L. Another successful scale-up study employed multi-horn reactor working in a continuous flow mode at higher power density for the extraction of phenolic compound from dry clove buds. The study showed that the usage of multi-horn flow reactor has higher process intensification and better free radical scavenging effects [48].

4. Conclusions

In this study all the statistical indicators support that RSM is a successful tool to describe the ultrasonic process in extracting the water-soluble β-D-glucan PS from *G. lucidum* for the following tested ultrasonic parameters; power (500–600 W), time (45–65 min) and temperature (70–90 °C) at a frequency of 20 kHz. The response dependent variable as represented by the yield of extracted PS could be expressed by a quadratic polynomial model and according to the analysis of variance and the regression coefficients. The optimal theoretical extraction conditions were found to be: power, 590 W, irradiation time, 58 min and temperature, 81 °C. Under the optimal conditions the predicted optimal yield of extracted PS was 52.33 mg. By applying these conditions, the actual experimental value of extracted PS yield was 52.28 mg which is in agreement with the theoretically predicted one. These outcomes clearly indicate the adequacy of quadratic polynomial model to represent the ultrasonic extraction of PS for the variables within the ranges of investigation for a scale of 0.25 L. The significant non-linear effects of power ultrasound may require modification in the geometry of the extraction vessel for larger scale-up.

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