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Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology

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ABSTRACT

Ultrasonic technology was applied for polysaccharides extraction from the dried longan pulp and response surface methodology (RSM) was used to optimize the effects of processing parameters on polysaccharides yields. Three independent variables were ultrasonic power (X_1), extraction time (X_2) and ratio of water to raw material (X_3), respectively. The statistical analysis indicated that three variables and the quadratic of X_1 and X_2 had significant effects on the yields, and followed by the significant interaction effects between the variables of X_2 and X_3 ($p < .05$). A mathematical model with high determination coefficient was gained and could be employed to optimize polysaccharides extraction. The optimal extraction conditions of polysaccharides were determined as follows: Ultrasonic power 680 W, extraction time 4.5 min, ratio of water to raw material 25 mL/g. Under these conditions, the experimental yield of polysaccharides was 4.455 ± 0.093%, which was agreed closely with the predicted value (4.469%).

1. Introduction

Longan (*Dimocarpus Longan* Lour.) is an important tropical fruit in Southeast Asia, such as China, Vietnam and Thailand. It's taste, nutritional and favored by many consumers in the world. Longan has also been used as a traditional chinese medicine since ancient times, and great attentions have been paid for their great health effects (Yang, Zhao, Shi, Yang, & Jiang, 2008a), such as promoting blood metabolism, soothe nerves, relieve insomnia, etc. (Yang et al., 2008b). Polysaccharides and lignin in the Longan fruit have been considered the main functional compositions for these health effects (Yang, Jiang, Wang, Zhao, & Sun, 2009).

Polysaccharides from plant, epiphyte and animals extracts are an interesting source of additives for several industries, in particular food and drug industry (Forabosco et al., 2006). They play important roles in the growth and development of living organisms, and have been widely studied in recent years due to their unique biological, chemical and physical properties (Schepetkin & Quinn, 2006). Whereas, there have been only a few reports on longan polysaccharides and few on its functional effects. One of the reasons is the lack of high efficient extraction technology of polysaccharides from Longan pulp. Hot-water technology is the main extraction method of longan polysaccharides in recent research, which is a classical extraction of polysaccharides. It usually requires long extraction time, high temperature and extraction efficiency is low (Li, Ding, & Ding, 2007). Therefore, it's essential and desirable to find an economical and high efficient extraction method of polysaccharides from longan fruit.

Ultrasonic treatment has been employed for preparing polysaccharides from different plant materials in recent years and showed the great extraction efficiency (Hemwimon, Pavasant, & Shotipruk, 2007; Hofmann, Kappler, & Posten, 2006; Hromadkova & Ebringerova, 2003; Hromadkova, Ebringerova, & Valachovic, 1999; Wang, Cheng, Mao, Fan, & Wu, 2009). This great extraction efficiency by ultrasonic treatment is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases through a super agitation (Vinatoru et al., 1997), and the most important mechanical effects of ultrasonic treatments are microjetting and microstreaming (Tsochatzidis, Guiraud, Wilhelm, & Delmas, 2001; Velickovic, Milenovic, Ristic, & Veljkovic, 2006).

Response surface methodology (RSM) is an affective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). It is wide used in optimizing the extraction process variables, such as polysaccharides, anthocyanins, vitamin E, phenolic compounds and protein from varied materials (Cacace & Mazza, 2003; Chandrika & Fereidoon, 2005; Ge, Ni, Yan, Chen, & Cai, 2002; Lee, Kim, & Kwon, 2005; Li & Fu, 2005; Liyana-Pathirana & Shahidi, 2005a; Qiao et al., 2009). Box–Behnken design (BBD), one of RSM, only have three levels, and need fewer experiments. It's more efficient and easier to arrange

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and interpret experiments in comparison with others and widely used by many researches (Box & Behnken, 1960; Ferreira et al., 2007).

In this study, the main objective was to optimize ultrasonic technology conditions for the extraction of polysaccharides from dried Longan pulp. RSM was designed to systemic analyze the effects of extraction parameters on the yields of polysaccharides from dried Longan pulp and their interactions.

2. Materials and methods

2.1. Experimental materials and chemicals

Dried longan (*D. longan* Lour.) fruit was purchased in a local commercial market and producing area was Putian, Fujian Province, China. The dried longan fruit was peeled, seeded and air-dried at 50 °C for balancing the water. Then, the dried longan pulp was grinded by a miller (A11 basic, ZKA®-WERKE, Germany), collected and stored in desiccator at room temperature (15–20 °C) until used (less than one month). All chemicals used in this investigation were analytical grade and purchased from Beijing Chemicals Co. (Beijing, China).

2.2. Extraction of polysaccharides from dried Longan pulp with ultrasonic treatment

The process of polysaccharides extraction from dried longan pulp by ultrasonic treatment was performed in an ultrasonic cell disintegrator (JY92-II, Xinzhi Bio-technology and Science Inc., Lingbo, ZheJiang Province, China). Two grams of dried longan pulp powders were extracted with distilled water in a 100-mL beaker, then the beaker was held in the ultrasonic cell disintegrator and exposed to extract for different time at varied ultrasonic power. Ice bathing was used to ensure the temperature of solution was below 50 °C in the whole extraction processing.

2.3. Isolation and determination yield of Longan polysaccharides

After the extraction with ultrasonic treatment, the extracted slurry was centrifuged at 4200 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again for 2–3 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (Senco Technology and Science Inc., Shanghai, China) at 55 °C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4 °C. Then the solution was centrifuged at 4200 rpm/min for 20 min, washed six times with dehydrated ethanol, and the precipitate was collected as crude extract. The extract was air-dried at 50 °C until its weight was constant, and then was weighted with a balance (AY 120, SHIMADZU, Japan). The percentage polysaccharides yield (%) is calculated as follows:

$$\text{Yield }(\%) = \frac{\text{weight of dried crude extraction(g)}}{\text{weight of longan pulp powder(g)}} \times 100 \qquad (1)$$

2.4. Experimental design

A three level, three variable Box–Behnken factorial design (BBD) (Design Expert software, Trial Version 6.0.5, Stat-Ease Inc., Minneapolis, MN) was applied to determine the best combination of extraction variables for the yields of longan polysaccharides. Three extraction variables considered for this research were X_1 (ultrasonic power), X_2 (extraction time), and X_3 (ratio of water to raw material) (Li et al., 2007), and the proper range of three variables were determined on the basis of single-factor experiment for the polysaccharides production (Table 1). Table 1 listed the whole design consisted of 17 experimental points, five replicates (treatment 13–17) at the centre of the design were used to allow for estimation of a pure error sum of squares. The triplicates were performed at all design points in randomized order.

Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 0 + \sum_{i=0}^{4} \beta_i X_i 0 + \sum_{i=0}^{4} \beta_{ii} X_i^2 0 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ij} X_i X_j \qquad (2)$$

where Y is the measured response associated with each factor lever combination; β_0 is an intercept; β_i is regression coefficients computed from the observed experimental values of Y; and X_i is the coded levels of independent variables. The terms $X_i X_j$ and X_i^2 represent the interaction and quadratic terms, respectively.

2.5. Statistical analyses

Data were expressed as means standard errors (SE) of three replicated determinations. The responses obtained from each set of experimental design (Table 1) were subjected to multiple non-linear regressions using the Design Expert software (Trial Version 6.0.5, Stat-Ease Inc., Minneapolis, MN). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and the significances of the regression coefficient were checked by F-test and p-value.

3. Results and discussion

3.1. Fitting the model

A regression analysis (Table 2) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied. Predicted response Y for the yield of Longan polysaccharides could be expressed by the following second-order polynomial equation in terms of coded values:

$$\begin{aligned} Y = {} & 4.26 + 0.65X_1 + 0.096X_2 - 0.040X_3 - 0.67X_1^2 - 0.11X_2^2 \\ & + 6.250 \times 10^{-4} X_2^3 - 0.022X_1X_2 - 0.015X_1X_3 \\ & - 0.068X_2X_3 \end{aligned} \qquad (3)$$

Table 1
Box–Behnken experimental design with the independent variables.

Run	Coded variable levels			Yield of polysaccharide (%)	
	X_1	X_2	X_3	Actual values	Predicted values
1	−1	−1	0	2.750	2.720
2	1	−1	0	4.039	4.056
3	−1	1	0	2.974	2.957
4	1	1	0	4.175	4.205
5	−1	0	1	2.945	2.979
6	1	0	−1	4.315	4.301
7	−1	0	1	2.915	2.929
8	1	0	1	4.225	4.191
9	0	−1	−1	4.030	4.026
10	0	1	−1	4.372	4.356
11	0	−1	1	4.066	4.083
12	0	1	1	4.135	4.139
13	0	0	0	4.340	4.265
14	0	0	0	4.251	4.265
15	0	0	0	4.243	4.265
16	0	0	0	4.270	4.265
17	0	0	0	4.221	4.265

Table 2
Analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides.

Source	SS	DF	MS	F-value	Prob > F
Model	5.42	9	0.60	302.64	<0.0001
Residual	0.014	7	1.989×10^{-3}		
Lack of fit	5.658×10^{-3}	3	1.886×10^{-3}		
Pure error	8.266×10^{-3}	4	2.066×10^{-3}	0.91	0.5103
Cor Total	5.43	16			
	$R^2 = 0.997$	$R^2_{Adj} = 0.994$	CV = 1.14		

where Y is the yield of longan polysaccharides (g), and X_1, X_2, and X_3 are the coded variables for ultrasonic power, extraction time and the ratio of water to the raw material, respectively.

In general, exploration and optimization of a fitted response surface may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy essential (Liyana-Pathirana & Shahidi, 2005b). The F-ratio in this table is the ratio of the mean square error to the pure error obtained from the replicates at the design centre. The significance of the F-value depends on the number of degrees of freedom (DF) in the model, and is shown in the p-value column (95% confidence level). Thus, the effects lower than .05 in this column are significant (Cai, Gu, & Tang, 2008; Qiao et al., 2009).

Table 2 listed the analysis of variance (ANOVA) for the fitted quadratic polynomial model of extraction yields of longan polysaccharides. F-test suggested that model had a very high model F-value ($F = 302.64$) and a very low p-value ($p < .0001$), indicating this model was highly significant. The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As showed in Table 2, F-value and p-value of the lack of fit were .91 and .5103, respectively, which implied it was not significant relative to the pure error and indicated that the model equation was adequate for predicting the yield of longan polysaccharides under any combination of values of the variables. R^2_{adj} (adjusted determination coefficient) is the correlation measure for testing the goodness-of-fit of the regression equation. Higher it is the better degree of correlation between the observed and predicted values (Ravikumar, Ramalingam, Krishnan, & Balu, 2006). The value of R^2_{adj} for Eq. (3) was .994, which was reasonably close to 1 and implied that only less 1.0% of the total variations were not explained by model. Meanwhile, it also confirmed that the model was highly significant and indicated a high degree of correlation between the observed and predicted data. Coefficient of variation (CV) indicates the degree of precision with which the experiments are compared. A relatively low value of CV (1.14) in Table 2, which showed a better precision and reliability of the experiments carried out.

The significance of each coefficient was determined using p-value in Table 3. The p-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable (Muralidhar, Chirumamilla, Ramachandran, Marchant, & Nigam, 2001). The corresponding variables would be more significant at greater F-value and smaller p-value (Atkinson & Donev, 1992). The data in the Table 3 indicated that all the independent variables (X_1, X_2, X_3) and two quadratic terms (X_1^2 and X_2^2) significantly affected the yield of longan polysaccharides, and there was significant interaction between extraction time (X_2) and ratio of water to raw material (X_3). Meanwhile, the ultrasonic power (X_2) was the major factor affecting the yield of polysaccharides.

Table 3
Estimated regression model of relationship between response variables (yield of Longan polysaccharides) and independent variables (X_1, X_2, X_3).

Variables	DF	SS	MS	F-value	p-Value
X_1	1	3.340	3.340	1679.70	<.0001
X_2	1	0.074	0.074	37.360	.0005
X_3	1	0.013	0.013	6.480	.0384
X_1X_1	1	1.870	1.870	937.860	<.0001
X_2X_2	1	0.056	0.056	27.930	.0011
X_3X_3	1	1.645×10^{-6}	1.645×10^{-6}	8.269×10^{-4}	.9779
X_1X_2	1	1.936×10^{-3}	1.936×10^{-3}	0.970	.3567
X_1X_3	1	9.000×10^{-4}	9.000×10^{-4}	0.450	.5227
X_2X_3	1	0.019	0.019	9.370	.0183

3.2. Analysis of response surface

The 3D response surface and 2D contour plots are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant (Muralidhar et al., 2001). The relationship between independent and dependent variables was illustrated in tri-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model for yield of polysaccharides (Figs. 1–3), two variables were depicted in one tri-dimensional surface plots while the other variable kept at level zero. It is clear that the yield of polysaccharides was sensitive to minor alterations of the test variables (ultrasonic power, extraction time and ratio of water to raw material).

The interaction relationships of ultrasonic power (X_1) with the extraction time (X_2) and radio of water to material (X_3) on the yield of polysaccharides were shown in Figs. 1 and 2, respectively, and which indicated these three variables all had significant effect on the yield of longyan polysaccharides. As shown in Figs. 1 and 2, the ultrasonic power (X_1) and extraction time (X_2) had positive impact on the polysaccharides production, while the yield changed slightly when the ratio of water to material (X_3) was in the range of 20 mL/g to 50 mL/g. Yield of polysaccharides rapid enhanced with the increasing of ultrasonic power (X_1) and reached to the peak value at 668.43 W. With the farther increasing of ultrasonic power (X_1), the yield went to slight decrease. Longer extraction time (X_2) had positive effects on the yield extraction, and had a critical value at 4.6 min when at a constant ultrasonic power (668.43 W). This suggested more yield was resulted at higher ultrasonic power, longer extraction time and lower ratio of water to material.

It was considered higher extraction efficiency of polysaccharides at higher ultrasonic power due to the increase in the number of cavitation bubbles formed and enhance mass transfer rates. However, less yield was resulted at farther increasing ultrasonic power. The same result was gained by Li et al. (2007), and was considered for that a part of polysaccharides could be more depolymerize into some free sugars.

It was shown that the interactions between the ultrasonic power and other two extraction variables did not impact the yield of polysaccharides significantly (Table 3, Figs. 1 and 2), in spit of the ultrasonic power was the major factor affecting the yield of polysaccharides. This observation was in agreement with previous investigation. Li et al. (2007) researched the optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* cv. *Jinsixiaozao* by RSM, and analysed the effects of interactions of extraction variables on the yield of polysaccharides. They also indicated that the interactions between ultrasonic power and extraction time, and ultrasonic power and ratio of water to

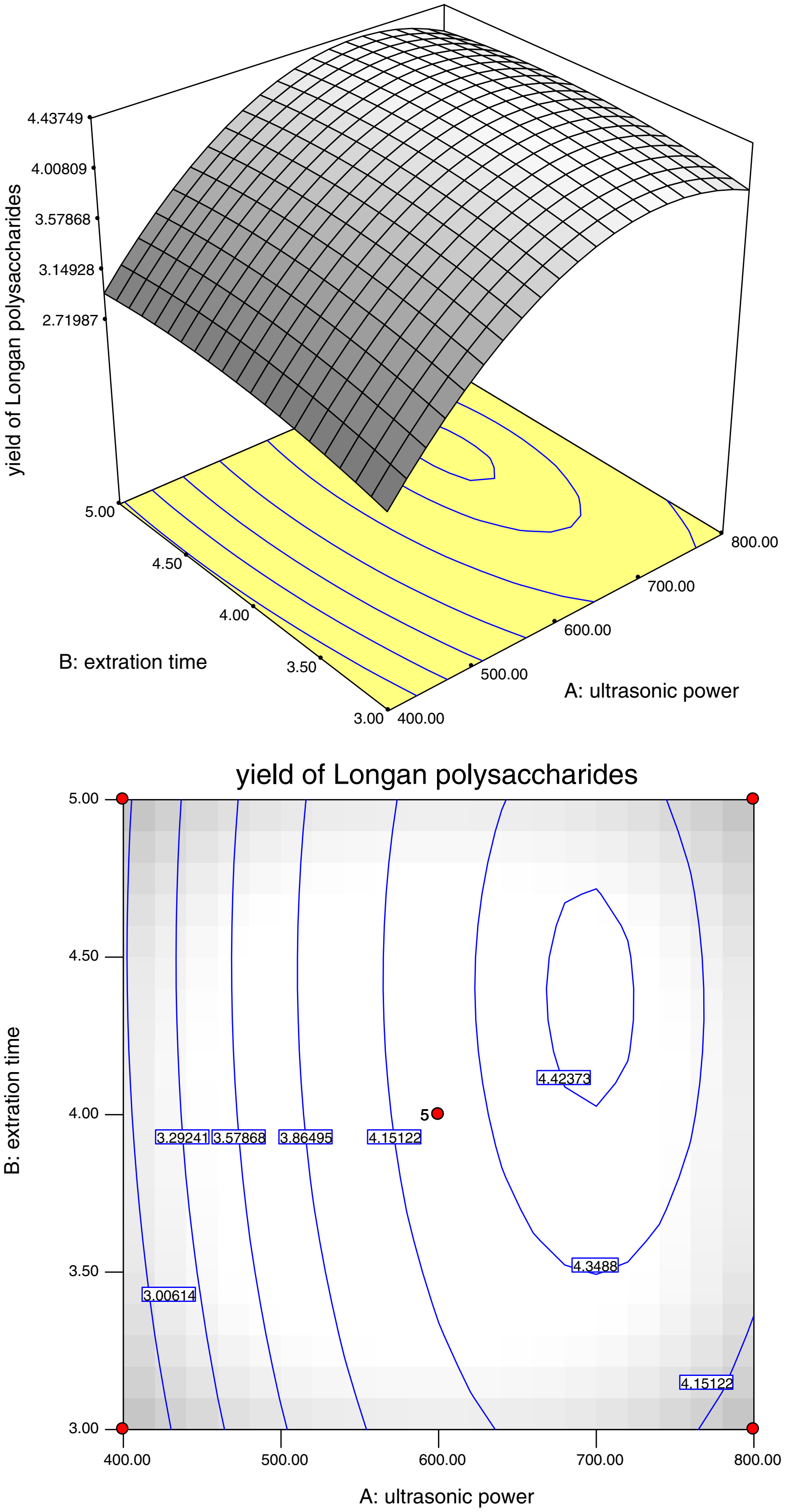

Fig. 1. Response surface plot and contour plot of ultrasonic power and extraction time and their mutual interactions on the yield of longan polysaccharides.

material caused no significant effect on the extraction yield, while ultrasonic power had significant effect.

Fig. 3 showed the response surface plot at various extraction times (X_2) and ratio of water to material (X_3). The response curves demonstrated that higher yield at longer extraction time. The response curves were comparatively smooth at lower extraction time, indicating the less effect on the increasing of the yields extraction when ratio of water to material changed in the range from 20 mL/g to 50 mL/g. However, the yield decreased with the farther enhancing of ratio of water to material at longer extraction time. This result indicated that extraction time (X_2) had a different extent of influence on extraction yield in different ratio of water to

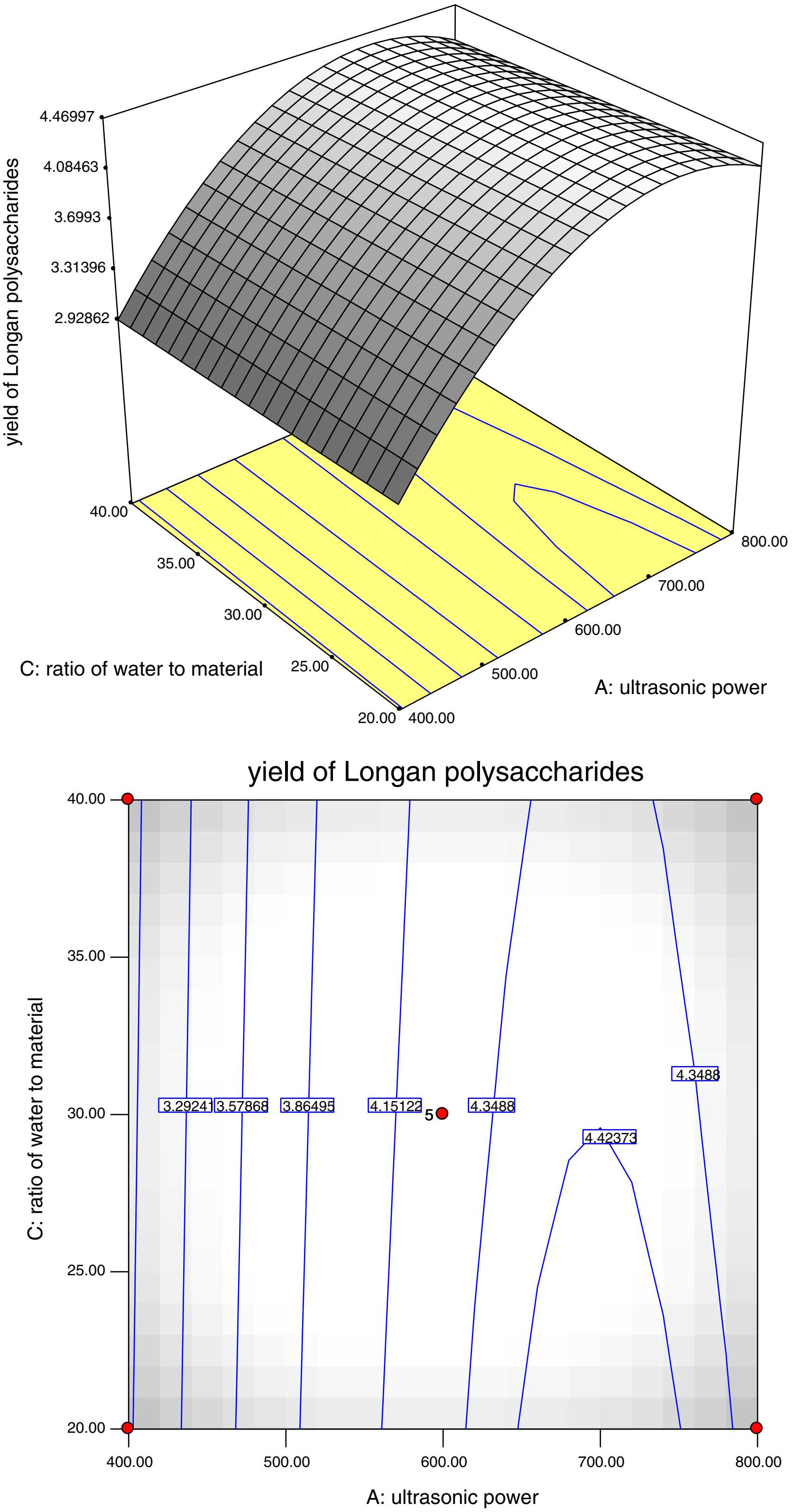

Fig. 2. Response surface plot and contour plot of ultrasonic power and the ratio of water to material and their mutual interactions on the yield of longan polysaccharides.

materials (X_3), and significant interactions were existed between extraction time (X_2) and ratio of water to raw material (X_3). Higher yields of polysaccharides were resulted at longer extraction time and lower ratio of water to material in the experimental range.

As shown in Fig. 3 and Table 3, the interactions of extraction time and ratio of water to materials had significant effect on the extraction yields, which was the same with other research results (Rodrigues, Pinto, & Fernandes, 2008; Wang et al., 2009). This con-

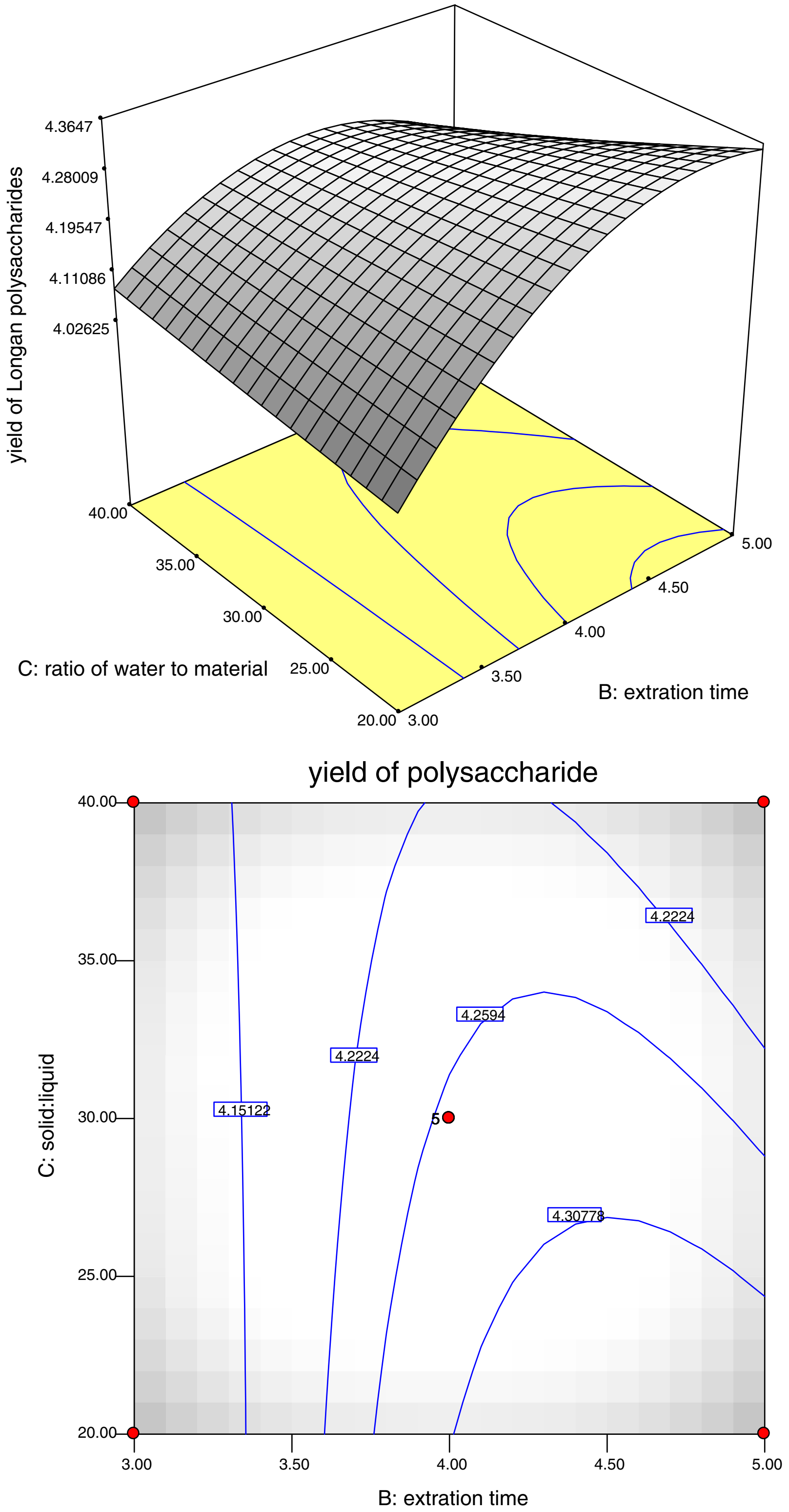

Fig. 3. Response surface plot and contour plot of extraction time and the ratio of water to material and their mutual interactions on the yield of longan polysaccharides.

clusion was inconsistent with the observation obtained by Li et al. (2007), who reported that this interaction caused no significant effect on the extraction yields at a constant ultrasonic power. This contradiction was possibly due to the large difference in parameters of sonic power. In this study, the sonic power (>400 W) were far greater than that (60 W) in Li et al. (2007).

3.3. Optimization of extracting parameters and validation of the model

The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. The maximum predicted yield and experimental yield of longan polysaccharides were given in Table 4. Additional experi-

Table 4
Optimum conditions and the predicted and experimental value of response at the optimum conditions.

	Ultrasonic power (W)	Extraction time (min)	Ratio of water to raw material (mL/g)	Yield of Longan polysaccharide (%)
Optimum conditions	668.43	4.60	24.25	4.469 (predicted)
Modified conditions	680	4.50	25	4.455 ± 0.093 (actual)

ments using the predicted optimum conditions for polysaccharides extraction were carried out: Ultrasonic power of 668.43 W, extraction time of 4.6 min, ratio of water to material 24.25 mL/g, and the model predicted a maximum response of 4.469%. To ensure the predicted result was not bias the practical value, experiment rechecking was performed using this modified optimal conditions: Ultrasonic power of 680 W, extraction time of 4.5 min, ratio of water to material 25 mL/g. A mean value of 4.456 ± 0.063% ($N = 5$) was gained, which was in agreement with the predicted value significantly ($p > .05$), obtained from real experiments, demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization (Table 4), and the model of Eq. (3) was satisfactory and accurate.

4. Conclusion

Ultrasonic technology was performed for the polysaccharides extraction from dried longan pulp in order to increase the yield extraction. Based on the single-factor experiments, Response surface methodology (RSM) was used to estimate and optimize the experimental variables-ultrasonic power (W), extraction time (min) and ratio of water to raw material (mL/g). All the independent variables, quadratic of ultrasonic power and extraction time had high significant effects on the response values, followed by the significant interaction effects between the extraction time and ratio of water to material. A high correlation of the quadratic polynomial mathematical model was gained and could be great employed to optimize polysaccharides extraction from Longan by ultrasonic technology. The optimal extraction conditions for the polysaccharides were determined as follows: Ultrasonic power 680 W, extraction time 4.5 min, ratio of water to material 25 mL/g. Under these conditions, the experimental yield of polysaccharides was 4.455 ± 0.093%, which was agreed closely with the predicted yield value.

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