E3S Web of Conferences 38, 02010 (2018) https://doi.org/10.1051/e3sconf/20183802010
ICEMEE 2018

# Optimization of extraction of chitin from procambarus clarkia shell by Box-Behnken design

*Fang* Dong, *Hailong* Qiu, *Shaoqian* Jia, *Cuiping* Dai, *Qingxin* Kong, *Changliang* Xu

Jiangsu Food & Pharmaceutical Science College, 223003, Huai'an, China

**Abstract.** This paper investigated the optimizing extraction processing of chitin from procambarus clarkia shell by Box-Behnken design. Firstly, four independent variables were explored in single factor experiments, namely, concentration of hydrochloric acid, soaking time, concentration of sodium hydroxide and reaction time. Then, based on the results of the above experiments, four factors and three levels experiments were planned by Box-Behnken design. According to the experimental results, we harvested a second-order polynomial equation using multiple regression analysis. In addition, the optimum extraction process of chitin of the model was obtained: concentration of HCl solution 1.54mol/L, soaking time 19.87h, concentration of NaOH solution 2.9mol/L and reaction time 3.54h. For proving the accuracy of the model, we finished the verification experiment under the following conditions: concentration of hydrochloric acid 1.5mol/L, soaking time 20h, concentration of sodium hydroxide 3mol/L and reaction time 3.5h. The actual yield of chitin reached 18.76%, which was very close to the predicted yield (18.66%) of the model. The result indicated that the optimum extraction processing of chitin was feasible and practical.

## 1 Introduction

Chitin owns excellent biological efficacy and diverse applications in different fields, such as textile industry, feed industry, medical materials, environmental protection, and so on [1]. For example, chitin is utilized for treating wastewater as adsorbents and flocculants [2,3], and is vital for artificial skin, artificial dialysis membrane and artificial blood vessels in medical materials field [4,5]. The shell of arthropod and fungus are rich in chitin. Such as prawns and crabs, the content of chitin is up to 58%~85%. Procambarus clarkia is one of the most popular aquatic products in China, but the shell of procambarus clarkia is usually discarded as waste of the food processing industry. Herein, the extraction of chitin from the shell of procambarus clarkia has dual significance for saving resource and protecting environment. In order to achieve the above purpose, this paper extracted chitin from the shell of procambarus clarkia by acid-alkali method, explored the vital factors influencing extraction by single factor investigation and selected the optimum processing by Box-Behnken design, one of the response surface methods.

## 2 Experimental section

### 2.1 Experimental methods

The certain weight of powder made from clean dried rocambarus clarkia shell was soaked in HCl solution with different concentrations at room temperature for certain soaking time to remove minerals. Then, HCl solution was discarded and the precipitate was washed pH to neutral by water. After that, the precipitate was reacted in NaOH solution with different concentrations in 95℃ water-bath for different reaction time to remove protein. After dumping NaOH solution and washing the precipitate to neutral by water, the crude chitin was obtained.

The crude chitin was soaked in 3g/L KMnO4 solution for 1.5h. Then, the chitin was filtered and washed to remove KMnO4. 5g/L oxalate solution mixed with chitin in 65℃ water-bath to decolor until chitin was turned white. Finally, the refined chitin dried at a lower temperature for 24h was obtained after removing oxalate solution and washing pH to neutral [6].

Yield of chitin (%) = (Weight of chitin/Weight of dried powder of rocambarus clarkia shell (30g))×100%.

### 2.2 Single factor investigation

The main factors affecting extraction of chitin were concentration of HCl solution (mol/L), soaking time (h), concentration of NaOH solution (mol/L) and reaction time (h). Therefore, the four factors were explored respectively through single factor experiment to select the optimal conditions of each influence factor.

### 2.3 Optimization of Box-Behnken design

Based on the single factor test results, the four factors (A, concentration of HCl solution; B, soaking time; C, concentration of NaOH solution; D, reaction time) at

* Corresponding author: dong1234fang@126.com

three levels was performed. According to the principle of Box-Behnken design, the low, medium and high levels of each factor as independent variable was coded -1, 0, and +1, respectively. Meanwhile, the yield of chitin was the response value (Y) as dependent variable. The factors and their levels are shown in Table 1.

**Table 1** Factors and levels of the extraction of chitin process

| Levels | Factors | | | |
|---|---|---|---|---|
| | A (mol/L) | B (h) | C (mol/L) | D (h) |
| -1 | 1 | 15 | 1.5 | 3 |
| 0 | 1.5 | 20 | 2.5 | 3.5 |
| +1 | 2 | 25 | 3.5 | 4 |

### 2.4 Statistical analysis

Design-Expert software (Version 7.0) was employed in planning Box-Behnken design and analyzing the following data. All extraction experiments were implemented in triplicate.

## 3 Result sections

### 3.1 Single factor assays

Fig.1 showed the results of single factor investigation of extraction of chitin. Fig. 1(a) indicated that the yield of chitin rapidly increased when the concentration of HCl solution reached 1.5mol/L(other factor levels: soaking time 10h, concentration of NaOH solution 0.5mol/L, reaction time 2h), but the yield of chitin did not obviously increase while concentration of HCl solution reached 2mol/L and 2.5mol/L. Similarly, Fig.1(b) showed the yield of chitin signficantly increased at soaking time 20h, while slowly increased at soaking time 25h and 30h(other factor levels: concentration of HCl solution 1.5mol/L, concentration of NaOH solution 0.5mol/L, reaction time 2h). The reason for these results was that the rocambarus clarkia shell was fully remove minerals when concentration of HCl solution reached 1.5mol/L and soaking time was 20h. The higher concentration of HCl solution and the longer soaking time could not obviously promote the yield of chitin. Fig. 1(c) illustrated that the yield of chitin reached a maximum value at concentration of NaOH solution 2.5mol/L(other factor levels: concentration of HCl solution 1.5mol/L, soaking time 20h, reaction time 2h). Fig.1(d) showed that the yield of chitin evidently increased when the reaction time was 3.5h. The reason was that the higher concentration of NaOH solution and the longer reaction time could decompose the chitin. Herein, concentration of HCl solution 1~2mol/L, soaking time 15~20h, concentration of NaOH solution 1.5~3.5mol/L and reaction time 3~4h were considered to be optimal levels in the Box-Behnken design.

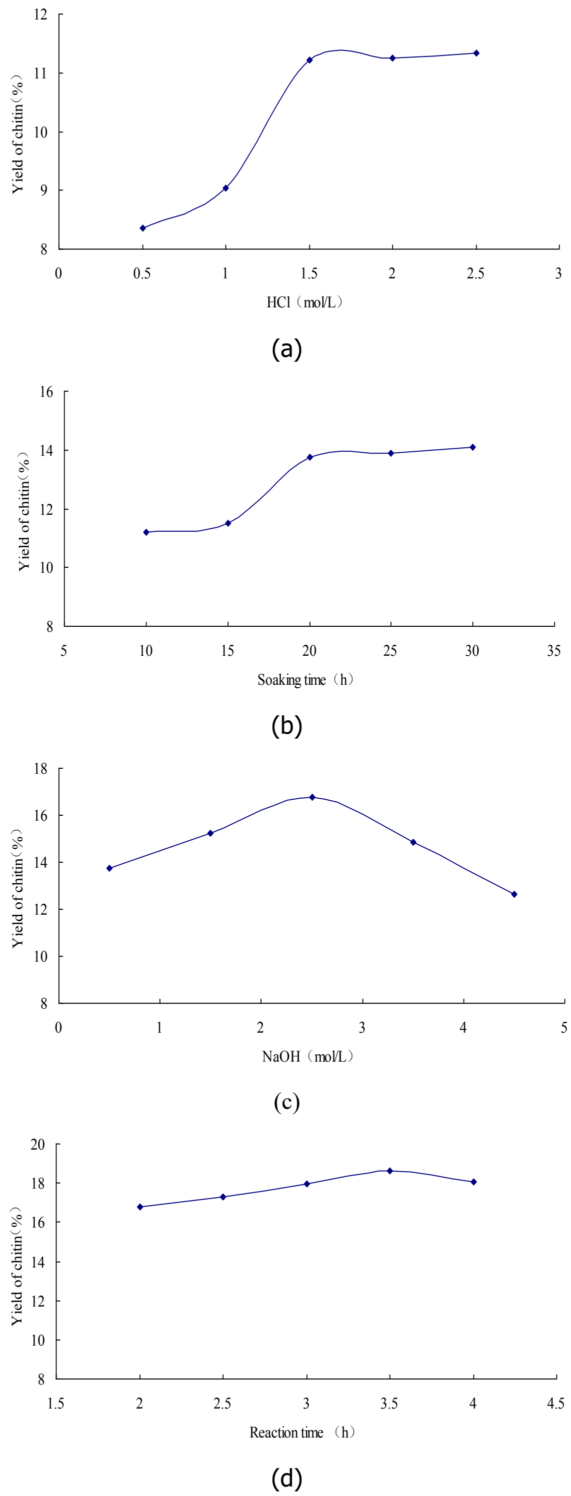



**Fig. 1.** Effect of four single factors on the extraction of chitin

### 3.2 Experimental results of the yield of chitin by Box-Behnken design

Twenty-nine experiments planned by Box-Behnken design and their results of the yield of chitin were shown in Table 2. No.1~24 was the factorial experiment, and No.25~29 was the central experiment. It could be seen from Table 2, the yield of chitin ranged from 16.18% to 18.79%.

**Table 2** Box-Behnken design and the results of yield of chitin

| No. | Factors and levels | | | | Yield of chitin (%) |
|---|---|---|---|---|---|
| | A (mol/L) | B (h) | C (mol/L) | D (h) | |
| 1 | -1 | -1 | 0 | 0 | 16.32 |
| 2 | 1 | -1 | 0 | 0 | 16.67 |
| 3 | -1 | 1 | 0 | 0 | 16.52 |
| 4 | 1 | 1 | 0 | 0 | 16.18 |
| 5 | 0 | 0 | -1 | -1 | 17.21 |
| 6 | 0 | 0 | 1 | -1 | 17.83 |
| 7 | 0 | 0 | -1 | 1 | 17.16 |
| 8 | 0 | 0 | 1 | 1 | 17.65 |
| 9 | -1 | 0 | 0 | -1 | 17.28 |
| 10 | 1 | 0 | 0 | -1 | 16.68 |
| 11 | -1 | 0 | 0 | 1 | 16.89 |
| 12 | 1 | 0 | 0 | 1 | 17.71 |
| 13 | 0 | -1 | -1 | 0 | 16.64 |
| 14 | 0 | 1 | -1 | 0 | 17.72 |
| 15 | 0 | -1 | 1 | 0 | 17.95 |
| 16 | 0 | 1 | 1 | 0 | 17.64 |
| 17 | -1 | 0 | -1 | 0 | 16.27 |
| 18 | 1 | 0 | -1 | 0 | 16.25 |
| 19 | -1 | 0 | 1 | 0 | 17.08 |
| 20 | 1 | 0 | 1 | 0 | 17.93 |
| 21 | 0 | -1 | 0 | -1 | 17.52 |
| 22 | 0 | 1 | 0 | -1 | 17.63 |
| 23 | 0 | -1 | 0 | 1 | 17.33 |
| 24 | 0 | 1 | 0 | 1 | 17.98 |
| 25 | 0 | 0 | 0 | 0 | 18.55 |
| 26 | 0 | 0 | 0 | 0 | 18.79 |
| 27 | 0 | 0 | 0 | 0 | 18.48 |
| 28 | 0 | 0 | 0 | 0 | 18.69 |
| 29 | 0 | 0 | 0 | 0 | 18.37 |

## 3.3 Model fitting and statistical significance analysis

According to the experimental results of Table 2, a second-order polynomial regression equation of extraction of chitin was obtained by Design-Expert 7 software analysis and fitting.

$$Y=18.49+0.15A+0.094B+0.53C+0.12D-0.022AB-0.038AC+0.21AD-0.048BC+0.11BD-7.500E-003CD-1.23A^2-0.80B^2-0.69C^2-0.22D^2$$

In the above formula, Y indicated the yield of chitin, A was the concentration of HCl solution, B was soaking time, C was the concentration of NaOH solution, and D was the reaction time.

Table 3 showed that yield of chitin was extremely significantly affected by linear parameters (C), quadratic parameters ($A^2$, $B^2$, $C^2$) ($P<0.01$), and significantly affected by interactive parameters (AD,BC) and quadratic parameters($D^2$) ($P<0.05$). Other parameters showed no significant effect ($P>0.05$).In addition, the R2 of chitin extraction model was 0.9157, the Adeq Precision was more than 4, and coefficient variation (C.V.) was 0.0128. These indicated that the model obtained by optimization experiment had the high degree of precision. The simulation of four factors and three levels could be used to optimize the experiment, and the accuracy of the experiment was higher.

**Table 3** Analysis of optimal process regression model for extraction of chitin

| Parameter | Sum of Squares | Df | Mean Square | Standard Deviation | F Value | P Value |
|---|---|---|---|---|---|---|
| Model | 15.24 | 14 | 1.09 | - | 10.86 | <0.0001** |
| A | 0.094 | 1 | 0.094 | 0.091 | 0.93 | 0.3502 |
| B | 0.13 | 1 | 0.13 | 0.091 | 1.28 | 0.2772 |
| C | 1.94 | 1 | 1.94 | 0.091 | 19.39 | 0.0006** |
| D | 0.027 | 1 | 0.027 | 0.091 | 0.27 | 0.6114 |
| AB | 0.12 | 1 | 0.12 | 0.16 | 1.19 | 0.2943 |
| AC | 0.19 | 1 | 0.19 | 0.16 | 1.89 | 0.1911 |
| AD | 0.50 | 1 | 0.50 | 0.16 | 5.03 | 0.0416* |
| BC | 0.48 | 1 | 0.48 | 0.16 | 4.82 | 0.0455* |
| BD | 0.073 | 1 | 0.073 | 0.16 | 0.73 | 0.4081 |
| CD | 4.225E-003 | 1 | 4.225E-003 | 0.16 | 0.042 | 0.8403 |
| $A^2$ | 9.87 | 1 | 9.87 | 0.12 | 98.51 | <0.0001** |
| $B^2$ | 3.12 | 1 | 3.12 | 0.12 | 31.15 | <0.0001** |
| $C^2$ | 1.89 | 1 | 1.89 | 0.12 | 18.87 | 0.0007** |
| $D^2$ | 0.78 | 1 | 0.78 | 0.12 | 7.82 | 0.0143* |
| Residual | 1.40 | 14 | 0.10 | - | - | - |
| Lack of Fit | 1.29 | 10 | 0.13 | - | 4.65 | 0.0759 |
| Pure Error | 0.11 | 4 | 0.028 | - | - | - |
| Cor Total | 16.65 | 28 | - | - | - | - |

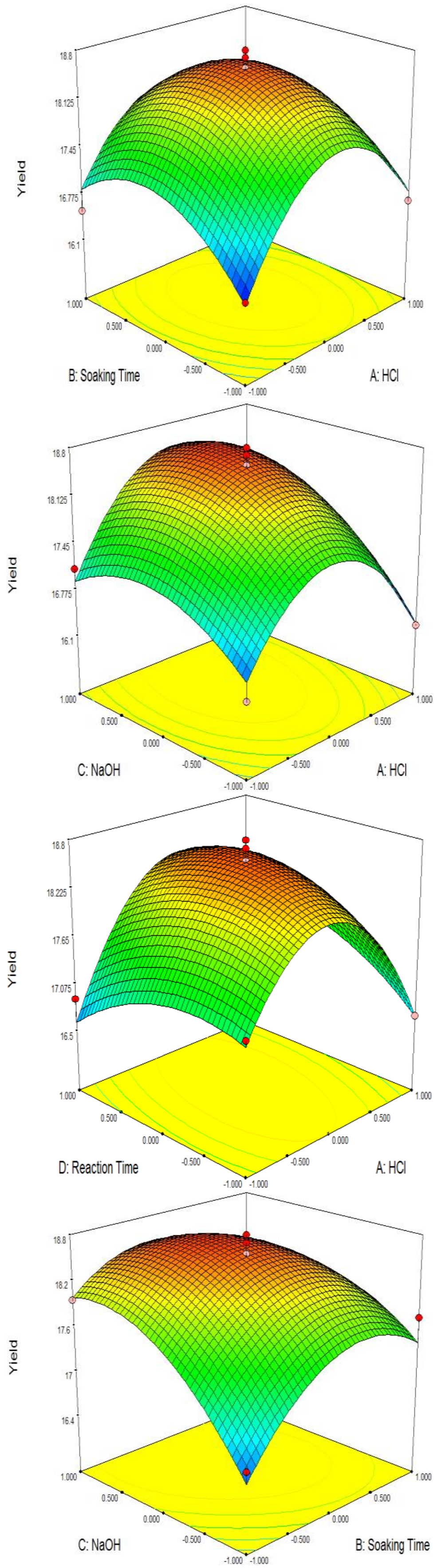
$R^2$ =0.9157, Adjusted $R^2$=0.8314, C.V.%=1.82, Adeq Precision=10.125,$^*P < 0.05$, $^{**}P < 0.01$.

## 3.4 Optimization of extraction processing of chitin

On the basis of the regression equation obtained by Design-Expert 7.0 software, the response surface plots of the yield of chitin affected by A, B, C and D were shown in Fig.2. The contour plots represented the intensity of the interaction actions. According to Table 3 and Figure 2, the interaction between concentration of HCl solution and reaction time was significant ($P<0.05$). Likewise, the interaction between soaking time and concentration of NaOH solution aslo showed the significance ($P<0.05$).

Meanwhile, the predictive optimum processing of extraction of chitin was harvested from the response surface analysis. The was shown as follows: concentration of HCl solution 1.54mol/L, soaking time 19.87h, concentration of NaOH solution 2.9mol/L and reaction time 3.54h. The maximum predicted yield of chitin was 18.66%.

 https://doi.org/10.1051/e3sconf/20183802010



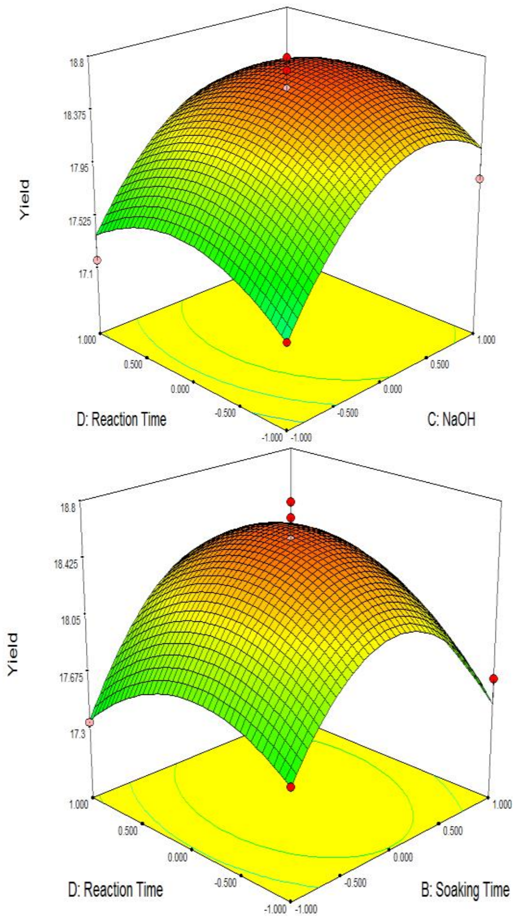




**Fig. 2.** Response surface plots (3D) of Interaction between the four factors to the yield of chitin

### 3.5 Model verification

In order to verify the accuracy and reliability of the predictive optimum processing of extraction of chitin, moreover, considering the convenience of the actual operation, the optimum processing of extraction of chitin were implemented as follows: concentration of HCl solution 1.5mol/L, soaking time 20h, concentration of NaOH solution 3mol/L, reaction time 3.5h. As Table 4 showed, the actual result was close to the predictive result.

**Table 4** Predictive and actual results of extraction of chitin

| | Concentration of HCl solution (mol/L) | Soaking time (h) | Concentration of NaOH solution (mol/L) | Reaction time (h) | Yield of chitin (%) |
|---|---|---|---|---|---|
| Predictive | 1.54 | 19.87 | 2.9 | 3.54 | 18.66 |
| Actual | 1.5 | 20 | 3 | 3.5 | 18.76 |

## 4 Conclusion

Procambarus clarkia shell is a new source of chitin. How to make full use of procambarus clarkia shell has great significance to environmental protection and new resources development. This paper investigated the optimizing extraction processing of chitin from procambarus clarkia shell by Box-Behnken design. Through single factor investigation, we found the optimal levels of four factors (concentration of HCl solution, soaking time, concentration of NaOH solution and reaction time). According to the single factor assays, Box-Behnken design planned 29 experiments to optimize of the extraction processing of chitin. The actual processing was shown as follows: concentration of HCl solution 1.5mol/L, soaking time 20h, concentration of NaOH solution 3mol/L, reaction time 3.5h. The actual yield of chitin (18.76%) was close to the predictive value (18.66%). It indicated that the model was accurate and the optimum extraction processing of chitin was feasible and practical.

## References

1. E.Tabata, A. Kashimura, S. Wakita, et al, Int. J. Mol. Sci **19**, 14(2018)
2. M Osada, C. Miura, Y.S. Nakagawa, et al, Carbohydr. Polym. **134**, 8(2015).
3. Y.Q. Li, C.Yang, X.Q. Gong, et al. Nat. Prod. Res. Dev. **28**, 4(2016)
4. Y.L. Li. Cott. Textile Technol, 3**8**, 4(2010)
5. K. Brodaczewska, K. Donskow-Lysoniewska, M. Doligalska, Acta Parasitol. **60**, 8(2015)
6. Q. L. Xu, Q. Z. Zeng. China Food Addit. 6 (2013)