



Study on utilization of lactic acid for the decalcifying of the shrimp head and shell

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ABSTRACT

In this study, lactic acid was used to extract chitin by decalcifying shrimp heads and shells of penaeus vannamei with ultrasonic -assisted. A Box–Behnken design with three variables and three levels was applied in order to approach the prediction of optimal the lactic acid concentration, the liquid-solid ratio and the reaction time on the demineralization degree with lactic acid. These optimal conditional were: with the ultrasonic assisted, the lactic acid concentration of 11.50%, the liquid-solid ratio of 26:1(mL/g),and the reaction time of 95 min allowing to obtain chitin, which ash content was predicted at 0.35 ± 0.05 (g/100g), and the chitin would be used in the food industry.

Keywords: shrimp waste; lactic acid; chitin; decalcification.

INTRODUCTION

Chitin ,the second largest renewable next to cellulose resources, it mainly exists in the arthropods ,such as shrimp, crab shell, the cell walls of fungi and algae. Chitin and its derivatives have many advantages, which are widely used in the food industry, functional materials, medicine, agriculture and textile industry[1,2], they become a research focus in the processing of aquatic products waste. Production technology of chitin mainly include acid and alkali method[3,4], enzymatic[5-7], fermentation[8,9], EDTA, etc.These methods all have their own different faults and shortcomings, such as acid-base method and EDTA can produce amounts of acid and alkali waste water, it made large pollution to environment;Enzymatic was cleaner, it made less pollution to the environment, but the cost of production was high,Fermentation disadvantage was that the production cycle was long.Ultrasonic extraction is a new extraction technology for scientific research [10], use ultrasonic cavitation effect, thermal effect and mechanical effect, which can make cell wall and the entire organism instantaneously rupture, speed up the release of substances in the cell, diffusion and dissolution to improve the efficiency of the extraction of effective components.In view of the many advantages of ultrasonic, and considering that the decalcified of microorganism fermentation was used microbes produce lactic acid to consume inorganic substances in the fermentation process.This study directly took lactic acid as decalcification reagent, sodium hydroxide as deproteinization reagent, to explore the optimal conditions of ultrasonic assisted extract chitin .

EXPERIMENTAL SECTION

Materials :Vannamei was purchased in Agricultural technology (Baoding,China) ; Shrimp heads and shells accounted for 50% of total quality shrimp, the quality ratio of shrimp head and shrimp shell was about 3:1. Lactic acid, sodium hydroxide were purchased from Tianjin Tianli Chemical Reagent Co., Ltd.(Tianjin ,China).

pre-treatment :shrimp meats were removed ,the residual wastes (hesds,shrimp-shell,tails,etc) and washed thoroughly with tap water , and finally frozen at -18□ for later use.

Deproteinization:mixed with sodium hydroxide solution at a ratio of 1:3(w/v), the concentration of sodium hydroxide was 1.5mol/L.Firstly,ultrasound for 30 min, secondly,put it in the 55 degrees of water bath oscillator ,model Jieruier THZ -82(Changzhou,China), oscillation for 90 min, filtered, rinsed solid heads and shells to neutral , dried , then crushed,set aside.

Decalcification:In order to better remove the calcium from shrimp wastes,the water-bath oscillator was chosen and the mixture was processed by an ultrasonic cleaning instrument,model Kudos SK5200H(Shanghai,China)for good contact between the suspended solids and the liquid in extraction experiment.

Use ultrasonic- assisted lactic acid soaked the processed shrimp wastes to remove calcium, filtered , dried ,then obtained coarse chitin.

Took the processed shrimp waste 1g into 100mL conical flask,added lactic acid (20-30mL) ,first mixed the liquid by ultrasound (59Hz)for 10min,then placed in a water-bath oscillator (200r/min),selected three time points (50-110min),three lactic acid concentration points(8%-12%),then filtered,last,the ash content of chitin was measured with calcinations method.

The determination of ash content : The indexes of Chitin grade were appearance, water content, nitrogen content and ash content, but the ash content was an indicator to evaluate the removed calcium degree of shrimp waste.

The determination of ash content refer to the national standard of China :The determination of ash content in foods.

Box – Behnken design: According to the results of single factor experiments, select three factors of lactic acid concentration, liquid-solid ratio and reaction time, use Box-Behnken design scheme of response surface of three factors and three levels to analysis.Factor levels and coding are shown in table 1.

Table1 Level and code of variables for box-behnken design

Variables	Symbols	Coded levels		
		-1	0	+1
Lactic acid concentration (%)	X1	8	10	12
The liquid-solid ratio (mL/g)	X2	20:1	25:1	30:1
The reaction time (min)	X3	50	80	110

Table 2:Box-Bchnken design and experimental results

Number	X1	X2	X3	Ash content
1	0	1	-1	1.08
2	0	-1	-1	1.19
3	1	0	1	0.4
4	-1	0	-1	1.22
5	-1	0	1	1.18
6	1	-1	0	0.99
7	0	0	0	0.47
8	0	0	0	0.47
9	0	1	1	0.74
10	-1	1	0	1.38
11	1	1	0	0.66
12	0	0	0	0.49
13	1	0	-1	0.68
14	0	0	0	0.45
15	0	-1	1	1.16
16	-1	-1	0	1.51
17	0	0	0	0.47

RESULTS AND DISCUSSION

Regression models of response: The Box-Behnken design was used for optimization of ratio of sauce,17 experimental runs with different combinations of three factors were carried out.The design matrix of the variables in the coded units was shown in Table2.

The regression equation of Chitin ash content (R1) on the lactic acid concentration (x_1), the liquid-solid ratio (x_2) and the reaction time (x_3) is

$$R=0.47-0.32x_1-0.12x_2-0.086x_3-0.050x_1x_2-0.0060x_1x_3-0.078x_2x_3+0.25x_1^2+0.42x_2^2+0.15x_3^2$$

It can be seen from Table 3 that The Model F-value of 691.81 implied the model was significant. Value of "Prob>F" was "<0.0500", indicating model terms were significant. The impact of the concentration, time, and solid-liquid ratio on chitin ash content was extremely significant ($P < 0.01$). In this case $x_1, x_2, x_3, x_1^2, x_2^2, x_3^2, x_1x_2, x_1x_3, x_2x_3$ were extremely significant model terms.

Lack of fit is used to assess the reliability of an equation, if equation modeling shows significant, it needs to be adjusted, if not, it can be good for data analysis in future. The "Lack of Fit F-value" of 2.87 implied the Lack of Fit was not significant relative to the pure error ($p > 0.05$). Non-significant lack of fit was good. R-Squared was 0.9989, indicating a reasonable fit of the model to the experimental data. The "Pred R-Squared" of 0.9872 was in reasonable agreement with the "Adj R-Squared" of 0.9974. Therefore, the model adequately represented the real relationship between the parameters chosen.

Table 3 Analysis of variance (ANOVA) for response surface quadratic model for the extracted chitin

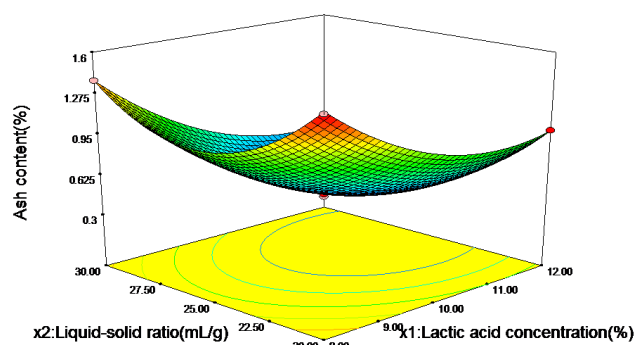
Source	S.S.	df	M.S.	F-Value	Prob >F	
Model	2.25000	9	0.25000	691.81	< 0.0001	Significant
x_1	0.82000	1	0.82000	2271.05	< 0.0001	
x_2	0.12000	1	0.12000	339.64	< 0.0001	
x_3	0.06000	1	0.06000	164.99	< 0.0001	
x_1x_2	0.01000	1	0.01000	27.72	0.0012	
x_1x_3	0.01400	1	0.01400	39.92	0.0004	
x_2x_3	0.02400	1	0.02400	66.60	< 0.0001	
x_1^2	0.26000	1	0.26000	707.82	< 0.0001	
x_2^2	0.74000	1	0.74000	2046.83	< 0.0001	
x_3^2	0.10000	1	0.10000	275.93	< 0.0001	
Residual	0.00253	7	0.00036			Not significant
Lack of Fit	0.00173	3	0.00058	2.87	0.1670	
Pure Error	0.00080	4	0.00020			
Cor Total	2.25	16				

$R^2:0.9989$; $AdjR^2:0.9974$; $Pred.R^2:0.9872$; *S.S.:Sum of square*; *M.S.:Mean square*

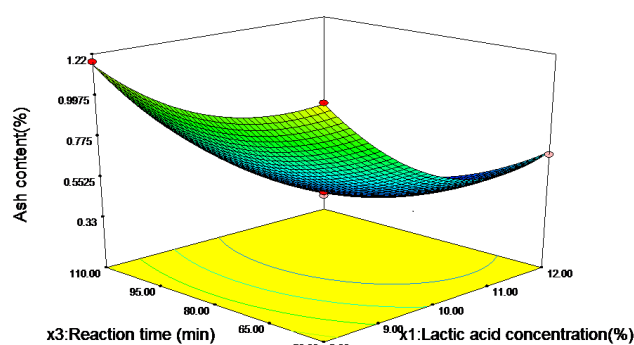
Figure 1(a) shows that in low concentration of lactic acid, chitin ash content was not reduced with the increased of the liquid-solid ratio, it may be because that lactic acid content was less, this was not good decalcified from the shrimp waste; when lactic acid concentration was higher, with the increased of liquid-solid ratio, the chitin ash content reduced and tended to a constant value. when the liquid ratio was constant, with the lactic acid concentration increased gradually, ash content of chitin reduced and tended to a constant value.

From figure 1(b), when the time was content, with the increased of lactic acid concentration, chitin ash content reduced gradually. But when lactic acid concentration was constant, with the time changed, chitin ash content had two kinds of tendency, this change depends on the lactic acid concentration. When lactic acid content was low, with time prolong, chitin gradually increased and reached a constant value. And when lactic acid content was higher than a certain value, the chitin ash content would gradually decrease with time prolong and reached a constant value. It related to the properties of calcium lactate. When it dissolved in hot water changed into a transparent or slightly turbid solution, and it can dissolve in acid solution. When lactic acid concentration was low, As reaction going, the solution of lactic acid gradually reduced, eventually led to the solution pH value increase and make the precipitation of calcium lactate adsorption on chitin, so it make the product ash content increased. When lactic acid concentration was high, the solution has been present acid, and calcium lactate no precipitation occurred, so ash content decreased gradually over time.

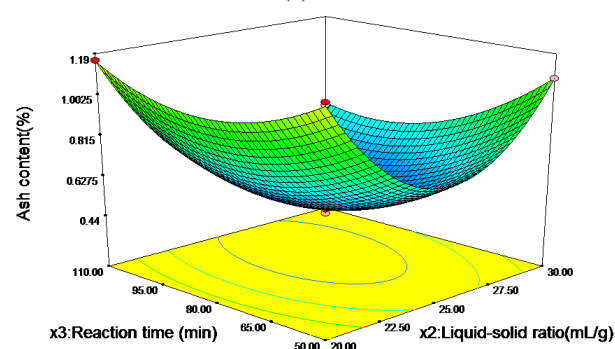
From figure 1(c), with the increased of ratio liquid, chitin ash content gradually reduced. Time The effect of time on chitin ash content under, it also affected by liquid-solid ratio. In low liquid ratio, with the increased of time, ash content increased and tended to be a constant value, when the liquid-solid ratio was higher than a certain value, with time prolonged, ash content decreased and reached a constant value. The principle was the same to the above.



(a)



(b)



(c)

Fig.1 : Surface plots for ash content of chitin.(a)figure plot to show the combination of lactic acid concentration and liquid-solid ratio;(b)figure plot to show the combination of lactic acid concentration and reaction time;(c) figure plot to show the combination of the liquid-solid ratio and reaction time

Validation of the model:The repeat experiments were conducted under optimal conditions for verification of optimization (lactic acid concentration of 11.50% and liquid –solid ratio of 26:1, the reaction time of 95 min).Combined with the actual production,there might be slight variation in experimental conditions,the minimum ash content was obtained at $0.35\pm 0.05(\text{g}/100\text{g})$.This value was found to be in agreement with the predicted value.This model can better predict the actual situation of the ash content of chitin.

CONCLUSION

In this experiment,lactic acid was used to extract chitin by decalcifying shrimp heads and shells of *Penaeus vannamei* with ultrasonic -assisted. A Box–Behnken design with three variables and three levels was applied in order to approach the prediction of optimal the lactic acid concentration, the liquid-solid ratio and the reaction time on the demineralisation degree with lactic acid.These optimal conditional were: the lactic acid concentration of 11.50%, the liquid-solid ratio of 26:1(mL/g),and the reaction time of 95 min allowing to obtain chitin,which ash content was predicted at $0.35\pm 0.05(\text{g}/100\text{g})$.

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