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Optimization of preparation of calcium acetate from eggshell by Response Surface Methodology (RSM)

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Abstract

Response surface methodology (RSM) was used to investigate the preparation-process parameters on calcium acetate from eggshell. Box-Behnken was employed for experimental design, and the optimal combination of reaction temperature, solid-liquid ratio, acetic acid concentration, primary reaction time and secondary reaction time for preparing calcium acetate was analyzed. Multiple regression analysis was used to fit the experimental data to the second-order polynomial equation, and appropriate statistical methods were used for analysis. The results showed that the optimum preparation conditions of calcium acetate were as follows: reaction temperature of 39 °C, solid-liquid ratio of 1:12, acetic acid concentration of 159%, primary reaction time 1.5 h and secondary reaction time of 80min. Under the optimum preparation conditions, the experimental yield of calcium acetate was 96.5%, which was in good agreement with the predicted value.

Keywords: calcium acetate; eggshell; response surface methodology.

Practical Application: Through the rational development and utilization of discarded eggshells, the purpose of turning waste into treasure has been realized.

1 Introduction

Calcium is one of the basic elements required by the human body, accounting for about 1.4% of the total body weight. Calcium participates in the human metabolism. Insufficient or excessive calcium in the human body will affect growth, development and health. Insufficient calcium intake could lead to calcium deficiency, which was mainly manifested as bone diseases, such as osteoporosis in adults (Mandatori et al., 2021). Excessive calcium would increase the risk of kidney stones. (Bolland et al., 2010). Some studies have pointed out that calcium could promote the accumulation of peak bone mass during childhood and early adulthood (Antoniazzi et al., 2004). In addition, it could effectively improve bone loss in the elderly (Genaro & Martini, 2010). Calcium also played the protective role in the treatment of essential hypertension (Liu et al., 2020; Nadal et al., 2018) and prevention of colon cancer (Gutierrez et al., 2019). Some scholars have conducted long-term researches on the role of calcium in the human body, and consistently pointed out that people with white, yellow or dark skin were threatened by calcium deficiency throughout their lives (Balk et al., 2018; Harinarayan et al., 2021). It was difficult for Chinese people to obtain the dietary calcium intake (DRIS) recommended by the Chinese Nutrition Society in the daily diet (Ge & Chang, 2001). Therefore, in the Chinese population, most people were generally calcium-deficient, and a few people were severely calcium-deficient (He et al., 2007).

The annual production of poultry eggs in China alone was as high as 33 million tons (Yang et al., 2018), and the by-product eggshell accounted for about 10% of the total weight of an egg (Kismiati et al., 2018). At least 4 million tons of eggshells were discarded annually, causing severe environmental pollution (Francis & Rahman, 2016; Shekhawat et al., 2019). At present, the calcium products used in the market were mainly derived from marine organisms as well as minerals. However, the content of heavy metals in eggshell raw materials and eggshell calcium products was much lower than that of marine organisms (oyster shells) and mineral sources (calcite) (Xu et al., 2020). Therefore, eggshell calcium products were safer. More than 90% of the calcium in the eggshell was $CaCO_3$, which was not easily digested and absorbed by human body (Wallot et al., 1996). However, organic calcium had good solubility in the body and was easy to be absorbed in the body, so it was widely used as a nutritional fortifier of calcium (Liu et al., 2019) and a food chelator in the international market (Fiume et al., 2014).

Therefore, many scholars have studied the transformation from inorganic calcium to organic calcium, which provides a strong theoretical basis for the eggshells as a raw material source of calcium supplements. Currently, the international calcium market is booming, and various calcium products, calcium health products and functional calcium foods emerge as the times require. Organic calcium acetate is a metabolic component of the human body and has no discomfort in the gastrointestinal tract. The US Food and Drug Administration (FDA) has determined that calcium acetate is a safe food additive. The preparation of eggshell calcium acetate could avoid environmental pollution and eggshells waste.

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Response surface methodology (RSM) is a collection of mathematical and statistical techniques widely applied in the food industry to evaluate the relationship between the predicted values of independent variables and dependent variables (Box & Wilson, 1951).The main advantage of RSM is a reduced the number of experiments to evaluate multiple variables and interactions, thus RSM was less laborious and time-compared with other approaches (Wu et al., 2007).

There are studies on the preparation of calcium acetate from egg shells by ultrasonic neutralization and suction filtration (Zhang et al., 2019), and the preparation of calcium acetate from shells by RSM (Lee et al., 2015a, b). However, the preparation of calcium acetate from eggshells through RSM has not been reported yet.

In this paper, we applied RSM to optimize the preparation conditions of calcium acetate. Moreover, the secondary reaction method was adopted in the preparation of calcium acetate, which greatly improved the reaction between eggshells and acetic acid. This research achieved the goal of turning waste into treasure, making full use of resources and promoting sustainable economic development through the rational development and utilization of eggshells. Therefore, it is necessary to study the preparation of calcium acetate from eggshells.

2 Materials and methodologies

2.1 Materials and chemical reagents

The eggshells were obtained from those commercial eggs. Distilled water came from the laboratory. Acetic acid, sodium hydroxide, ammonia water, EDTA and hydrochloric acid were purchased from Jilin Jintai Chemical Glass Co., Ltd.. Chrome black T, Naiphenol Green B, uric acid amine A and base red were purchased from Tianjin Hongyan Chemical Reagent Factory.

2.2 Preparation of calcium acetate from eggshells

At a certain temperature (20, 40, 60, 80 and 100 °C), different volumes of distilled water (solid-liquid ratio 1:6, 1:8, 1:10, 1:12, 1:14, 1:16, 1:18 and 1:20) were added to the eggshell powder separated by shell membrane, and at the same time, soybean oil was added as defoamer for neutralization reaction. In order to make the eggshell reaction complete, the secondary reaction method was selected. After different reaction times (0.5, 1, 1.5, 2, 2.5 and 3 h), acetic acid with different concentrations was added for the second reaction with different reaction times (0, 20, 40, 60, 80, 100, 120 and 140 min). Then the yield of calcium acetate was calculated as follows:

$$\text{Calcium acetate yeild } (\%) = \frac{m_1}{m} \times 100 \quad (1)$$

where m_1 is the actual yield of calcium acetate, g; m is the theoretical yield, g.

2.3 Optimization of experimental design and statistical analysis

At first, the preliminary range of the optimized variables for preparing calcium acetate was determined through single factor experiments. Then, the preparation parameters were optimized by RSM. To determine the comprehensive influence of independent variables on the reaction, a Box-Behnken design with five independent variables at three levels was designed using Design-Expert.V8.0.6 statistical analysis software.

3 Results and discussions

3.1 Single factor text

Effect of temperature on the yield of calcium acetate

The effect of temperature on the yield of calcium acetate prepared from eggshells was shown in Figure 1A. Calcium acetate was prepared at different temperatures (20, 40, 60, 80 and 100 °C) whilst other preparation parameters were solid-liquid ratio of 1:16, acetic acid concentration 160% (theoretical concentration of 100%), primary reaction time of 1h and secondary reaction time of 40 min. When the temperature reached 40 °C, the yield of calcium acetate was the highest (94.7%), and then it decreased. The increase of reaction temperature would increase the number of activated molecules and the effective collision between molecules, leading to the increase of reaction rate and the yield of calcium acetate. However, when the reaction temperature exceeded 40 °C, the yield of calcium acetate decreased, which was mainly because acetic acid was a volatile organic acid. The higher the temperature, the faster the volatilization, which affected the completion and yield of the reaction between acetic acid and eggshell. Thus, 40 °C was the best temperature for the production of calcium acetate.

Effect of solid-liquid ratio on the yield of calcium acetate

The effect of solid-liquid ratio on yield of calcium acetate prepared from eggshells was shown in Figure 1B. Calcium acetate was prepared with different solid-liquid ratios (1:6, 1:8, 1:10, 1:12, 1:14, 1:16, 1:18, 1:20). Other experimental conditions were as follows: reaction temperature of 40 °C, acetic acid concentration of 160%, primary reaction time of 1h and secondary reaction time of 40min.The yield of calcium acetate increased rapidly with the rising solid-liquid ratio, and reached the peak at 1:12 (94.5%). After that, the yields dropped. Too low solid-liquid ratio would result in incomplete reaction between eggshells and acetic acid, while too high solid-liquid ratio would cause dilution of acetic acid and affect the yield of calcium acetate.Therefore, the solid-liquid ratio of 1:12 was selected as the best condition for preparing calcium acetate from eggshells for subsequent tests.

Effect of the concentration of acetic acid on the yield of calcium acetate

To investigate the effect of the concentration of acetic acid on the yield of calcium acetate, the process was carried out with different concentrations of acetic acid: 100, 120, 140, 160, 180 and 200% (the theoretical value was 100%). Other parameters were set as follows: reaction temperature of 40 °C, solid-liquid ratio of 1:12, primary reaction time of 1h and secondary reaction time of 40 min. As can be seen from Figure 1C, the yield of calcium acetate increased with increasing acetic acid concentration, following by a decrease. When the concentration of acetic acid is 160% (the theoretical value was 100%), the yield of calcium acetate reached the maximum (94.4%). Then, with the increase of acetic acid concentration, the yield of calcium acetate decreased. Acetic acid hydrolyzed and volatilized in water. When the concentration of acetic acid was low, the reaction was incomplete, and the output of calcium acetate prepared from eggshells was reduced. Therefore, the acetic acid concentration of 160% was sufficient to obtain maximum calcium acetate.

Effect of primary reaction time on the yield of calcium acetate

The effect of primary reaction time on the yield of calcium acetate prepared from eggshells was shown in Figure 1D. The preparations were carried out at different primary reaction times (0.5, 1, 1.5, 2, 2.5 and 3 h), whilst conditions were set as follows: temperature of 40 °C, solid-liquid ratio of 1:12, acetic acid concentration of 160%, and secondary reaction time 40 min. With the increase of the primary reaction time, the yield of calcium acetate also increased, reaching the peak value (96.2%) at 1.5 h, and then, the primary reaction time and the yield of calcium acetate yield showed an opposite trend. The reaction between acetic acid and calcium carbonate is a weak acid and a weak base reaction, respectively. And the reaction speed is slow. When the primary reaction time was short, the reaction was incomplete and the yield of calcium acetate was low. However, when the reaction time was too long, the re-dissolution reaction occurred and the yield of calcium acetate decreased. Therefore, 1.5 h was selected as the primary reaction time for the preparing calcium acetate from eggshells.

Effect of secondary reaction time on the yield of calcium acetate

The effect of secondary reaction time on the yield of calcium acetate preparation was shown in Figure 1E. The secondary reaction time was set at 0, 20, 40, 60, 80,100, 120 and 140 min. Other experimental conditions were as follows: temperature of 40 °C, solid-liquid ratio of 1:12, acetic acid concentration of

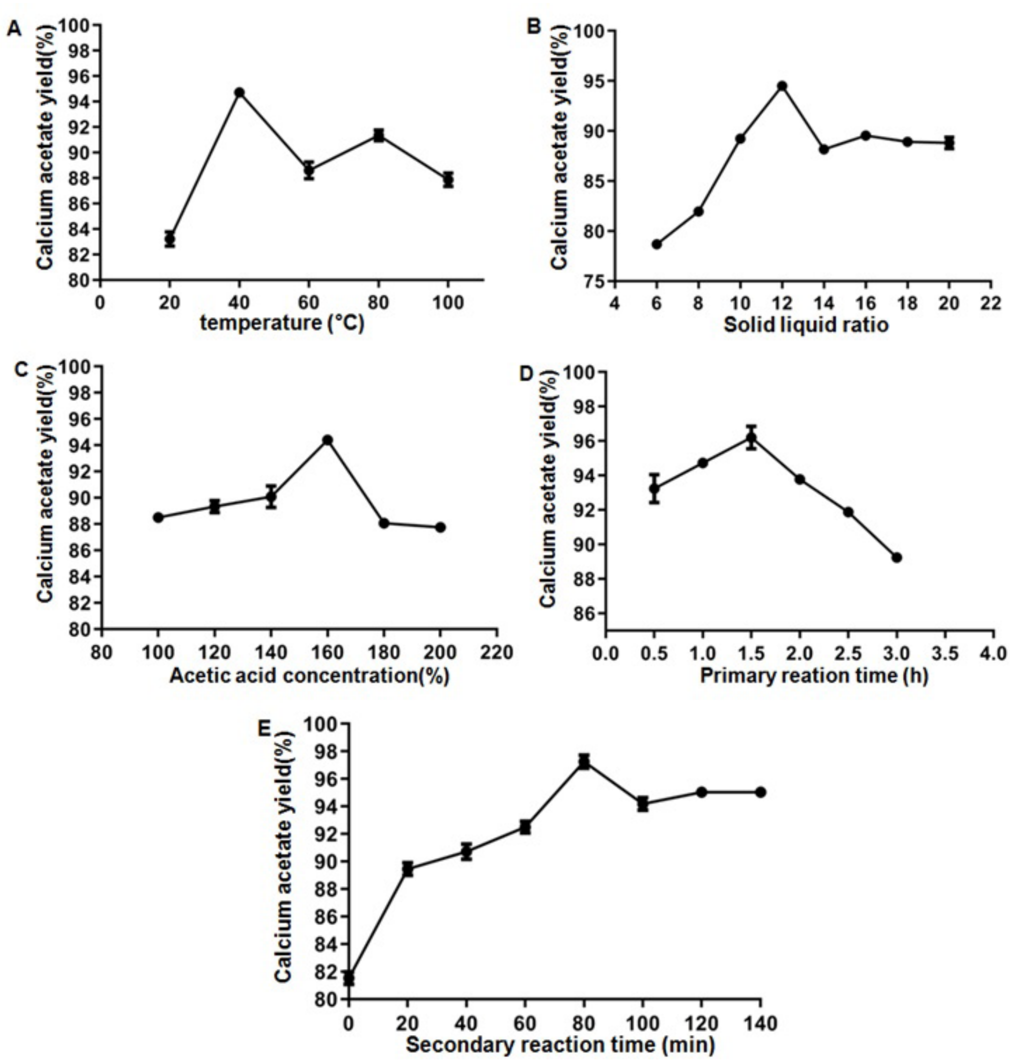

Figure 1. Effects of different (A) temperature, (B) solid-liquid ratio, (C) acetic acid concentration, (D) primary reaction time, (E) secondary reaction time on Preparation of calcium acetate from eggshell.

160%, and primary reaction time of 1.5 h.With the increase of secondary reaction time, the yield of calcium acetate increased significantly, reaching the peak value (96.4%) at 80 min, and then decreased. The low yield of the second reaction time of 0 min indicated that the eggshell and calcium acetate were not fully reacted by only one-step reaction, which proved the necessity of the second reaction. Thus, in the process of preparing calcium acetate from eggshells, the secondary reaction method was adopted to increase the yield of calcium acetate.

3.2 Optimization of calcium acetate preparation

Model fitting

The range of five independent variables was based on the results of the single-factor experiments. A total of 46 runs for five variables to optimize the preparation of calcium acetate from eggshells (A, reaction temperature, B, solid-liquid ratio, C, acetic acid concentration, D, primary reaction time, E, secondary reaction time) in the current Box-Behnken design were shown in Table 1.

The results indicated that the maximum yield of calcium acetate was 96.7% under the preparation conditions of temperature of 40 °C, solid-liquid ratio of 1:12, acetic acid concentration of 160%, primary reaction time of 1.5 h, and secondary reaction time of 81 min. The first step of optimization was to determine the appropriate model regression to match the experimental data based on the Box-Behnken design (Aslan & Cebeci, 2007; Souza et al., 2005). Based on the Box-Behnken experimental design model, through multiple regression analysis of the experimental data, the response variables and the test variables were correlated according to the following second-order polynomial Equation 1:

$$\begin{aligned} Y = &+96.63 - 0.42 \times A - 0.20 \times B - 0.48 \times C - 0.54 \times D + \\ &0.20 \times E + 0.000 \times AB - 0.93 \times AC - 0.43 \times AD + 0.13 \times AE - \\ &0.40 \times BC + 0.050 \times BD + 0.30 \times BE - 0.30 \times CD - 0.20 \times CE + \\ &0.57 \times DE - 1.13 \times A^2 - 1.94 \times B^2 - 1.40 \times C^2 - 2.45 \times D^2 - 1.55 \times E^2 \end{aligned} \quad (1)$$

where Y is the yield of calcium acetate, and A, B, C, D and E are the coding variables of reaction temperature, solid-liquid ratio, acetic acid concentration, primary reaction time and secondary reaction time, respectively.

The fitting statistics of the preparation yield (Y) of the selected secondary prediction model was shown in Table 2. The determination coefficient of quadratic regression model variance analysis (R^2 = 0.9892) showed that the model only explained 1.08% of the total variance. However, a large value of R^2 did not imply that the regression model was good. The value of the adjusted determination coefficient (Adj R^2 = 0.9806) also confirmed that the model was highly significant, which indicated the experimental value of calcium acetate yield was in good agreement with the predicted value. The results of error analysis indicated that the lack of fitting test (0.0525) was insignificant at the 95% confidence level, confirming the validity of the model. Meanwhile, a relatively low coefficient of the variation (CV = 0.21) indicated a very high degree of precision and a highly reliable experimental values (Song et al., 2011). Therefore, the model was adequate for prediction within the range of experimental variables. In addition, the model P value was extremely low (<0.00001), indicating that the model terms were significant.

3.3 Optimization of calcium acetate preparation

The effects of reaction temperature, solid-liquid ratio, acetic acid concentration, primary reaction time and secondary reaction time on the yield of calcium acetate were studied by response surface method. The regression model equation allowed the prediction of the effects of the five parameters on the yield. The interaction types among the five tested variables and the relationship between responses of each variable and the experimental level were illustrated in 3D response-surface plots and 2D contour plots of the response surfaces (Liu et al., 2014). The circular contour plot indicated that the interactions between the corresponding variables were negligible, while an elliptical contour plot was the opposite (Muralidhar et al., 2001).

Figure 2A-D showed the 3D graphic surface and contour plot of the comprehensive effects of reaction temperature and solid-liquid ratio, reaction temperature and acetic acid concentration, reaction temperature and primary reaction time, and reaction temperature and secondary reaction time on the yield of calcium acetate. It could be seen from the figure that with the increase of various factors, the yields tended to increase first and then decrease. The central points of each factor were: temperature of 35 °C - 40 °C, solid-liquid ratio of 1:11 - 1:13, acetic acid concentration of 155% - 165%, primary reaction time of 1.2 - 1.6h,and secondary reaction time of 70 - 90 min.The center of the contour line between temperature and solid-liquid ratio, temperature and primary reaction time was elliptical, relatively dense and the surface was steep, indicating that the interaction between them was obvious. The curved surface in the center of the contour plots of temperature and acetic acid, temperature and secondary reaction time was relatively flat, but it also formed an ellipse, indicated that the interaction between various factors is significant.

The effect of solid-liquid ratio and acetic acid concentration, solid-liquid ratio and primary reaction time, solid-liquid ratio and secondary reaction time on calcium acetate yield was shown in Figure 2E-G. With the increase of various factors, the yield of calcium acetate increased. The ratio of solid to liquid was 1: 11 - 1: 13, the concentration of acetic acid was 155% - 165%, the reaction time was 1.2-1.6 hours, and the second reaction time was 70-90 minutes, so that calcium acetate with higher concentration could be obtained, which decreased with the increase of various factors. The center of the contour line of acetic acid concentration, solid-liquid ratio and single reaction time, solid-liquid ratio and secondary reaction time was elliptical, relatively dense and steep in surface, indicating that the interaction between the two factors was significant.

The effect of the acetic acid concentration and primary reaction time, acetic acid concentration and secondary reaction time on the yield of calcium acetate was shown in Figure 2H-I. It could be seen from the figure that with the increase of various factors, the yield tended to increase first and then decreased. The central points

Table 1. The Box-Behnken design Design and the Response for the yield of calcium acetate.

Number	Temperature (°C)	Solid liquid ratio	Acetic acid (%)	Primary reaction time (h)	Second reaction time (min)	Yield (%)
1	30	10	160	1.5	80	94.1
2	50	10	160	1.5	80	93.4
3	30	14	160	1.5	80	93.8
4	50	14	160	1.5	80	93.1
5	40	12	150	1.0	80	93.6
6	40	12	170	1.0	80	93.1
7	40	12	150	2.0	80	93.3
8	40	12	170	2.0	80	91.6
9	40	10	160	1.5	60	93.5
10	40	14	160	1.5	60	92.4
11	40	10	160	1.5	100	93.4
12	40	14	160	1.5	100	93.5
13	30	12	150	1.5	80	94.0
14	50	12	150	1.5	80	94.9
15	30	12	170	1.5	80	94.9
16	50	12	170	1.5	80	92.1
17	40	12	160	1.0	60	93.5
18	40	12	160	2.0	60	91.2
19	40	12	160	1.0	100	93.0
20	40	12	160	2.0	100	93.0
21	40	10	150	1.5	80	93.7
22	40	14	150	1.5	80	94.1
23	40	10	170	1.5	80	93.6
24	40	14	170	1.5	80	92.4
25	30	12	160	1.0	80	93.6
26	50	12	160	1.0	80	93.6
27	30	12	160	2.0	80	93.5
28	50	12	160	2.0	80	91.8
29	40	12	150	1.5	60	93.6
30	40	12	170	1.5	60	93.1
31	40	12	150	1.5	100	94.4
32	40	12	170	1.5	100	93.1
33	30	12	160	1.5	60	94.5
34	50	12	160	1.5	60	93.4
35	30	12	160	1.5	100	94.3
36	50	12	160	1.5	100	93.7
37	40	10	160	1.0	80	92.9
38	40	14	160	1.0	80	92.4
39	40	10	160	2.0	80	91.5
40	40	14	160	2.0	80	91.2
41	40	12	160	1.5	80	96.7
42	40	12	160	1.5	80	96.7
43	40	12	160	1.5	80	96.7
44	40	12	160	1.5	80	96.5
45	40	12	160	1.5	80	96.7
46	40	12	160	1.5	80	96.5

of each factor were: acetic acid concentration of 155% - 165%, primary reaction time of 1.2-1.6 h and secondary reaction time of 70 - 90 min. Moreover, the center of the contour lines was relatively dense, and the curved surface was steep, which indicated that the corresponding effects between the two factors were obvious.

Figure 2J showed the effect of the primary reaction time and secondary reaction time on the yield of calcium acetate preparation. With the increase of the primary reaction time and the secondary reaction time, the yield of calcium acetate increased, reaching the maximum value at the primary reaction

Table 2. Regression coefficients of the predicted quadratic polynomial model.

Sources	Sum of Squares	Degrees of freedom(df)	Coefficient	Mean Square	F-value	p-value	
Model	92.15	20		4.61	114.85	<0.0001	significant
A-temperature	2.81	1		2.81	69.94	<0.0001	
B- solid-liquid ratio	0.64	1		0.64	15.95	0.0005	
C- acetic acid concentration	3.71	1		3.71	92.37	<0.0001	
D- primery reaction time	4.62	1		4.62	115.23	<0.0001	
E- secondary reaction time	0.64	1		0.64	15.95	0.0005	
AB	0.00	1		0.00	0.00	1.0000	
AC	3.42	1		3.42	85.31	<0.0001	
AD	0.72	1		0.72	18.01	0.0003	
AE	0.06	1		0.06	1.56	0.2235	
BC	0.64	1		0.64	15.95	0.0005	
BD	0.01	1		0.01	0.25	0.6220	
BE	0.36	1		0.36	8.97	0.0061	
CD	0.36	1		0.36	8.97	0.0061	
CE	0.16	1		0.16	3.99	0.0568	
DE	1.32	1		1.32	32.97	<0.0001	
A^2	11.13	1		11.13	277.38	<0.0001	
B^2	32.76	1		32.76	816.65	<0.0001	
C^2	17.00	1		17.00	423.86	<0.0001	
D^2	52.56	1		52.56	1310.27	<0.0001	
E^2	21.08	1		21.08	525.47	<0.0001	
Residual	1.00	25		0.04			
Lack of Fit	0.95	20		0.047479	4.451172	0.0525	Not significant
Pure Error	0.05	5		0.010667			
Cor Total	93.15	45					
R-Squared			0.9892				
Adj R-Squared			0.9806				
C.V. %			0.21				
PRESS			3.875				
Standard deviation			0.20				
Adeq Precision			39.318				

AB: temperature and solid-liquid ratio. AC: temperature and acetic acid concentration. AD: temperature and primary reaction time. AE: temperature and secondary reaction time. BC: solid-liquid ratio and acetic acid concentration. BD: solid-liquid ratio and primary reaction time. BE: solid-liquid ratio and secondary reaction time. CD: acetic acid concentration and primary reaction time. CE: acetic acid concentration and secondary reaction time. DE: primary reaction time and secondary reaction time. C.V.: coefficient of the variation. ^{2} means quadratic.

time of 1.2-1.6 h and the secondary reaction time of 70-90 min, and then showing the opposite trend.

In this paper, we used eggshells as raw materials to prepare calcium acetate. Through the rational development and utilization of discarded eggshells, the purpose of turning waste into treasure has been realized. In previous studies, most of the preparation of calcium acetate used shells as raw materials (Lee et al., 2015a, b). Compared with calcium sources such as shells, eggshells are less exposed to external pollution and can be regarded as a green source of calcium.

In the preparation of calcium acetate, compared with other studies (Zhang et al., 2019), we not only increased the number of preparation parameters, including temperature, solid-liquid ratio, acetic acid dosage, primary reaction time and secondary reaction time, but also used the RSM to comprehensively consider the test. The RSM fits the complex unknown function piping system in a small area with a simple one letter or quadratic polynomial model. The calculation is relatively simple, and it is an effective means to solve practical problems. In the process of optimizing the experimental conditions, the various levels of the experiment can be continuously analyzed, thereby greatly improving the yield of calcium acetate.

3.4 Verification of calcium acetate preparation model

The optimized conditions were reaction temperature of 38.69 °C, solid-liquid ratio of 1:11.92, acetic acid concentration of 159.18%, primary reaction time of 1.47 h and secondary reaction time of 80.66 min. Under the optimum conditions, the model predicted that the yield of calcium acetate was 96.71%. However, considering the feasibility of practical operation, the selected technological parameters were reaction temperature of 39.00°C, solid-liquid ratio of 1:12, acetic acid concentration of 159.00%, primary reaction time of 1.5h and secondary reaction time of 80.00 min. The results of analysis indicated that the

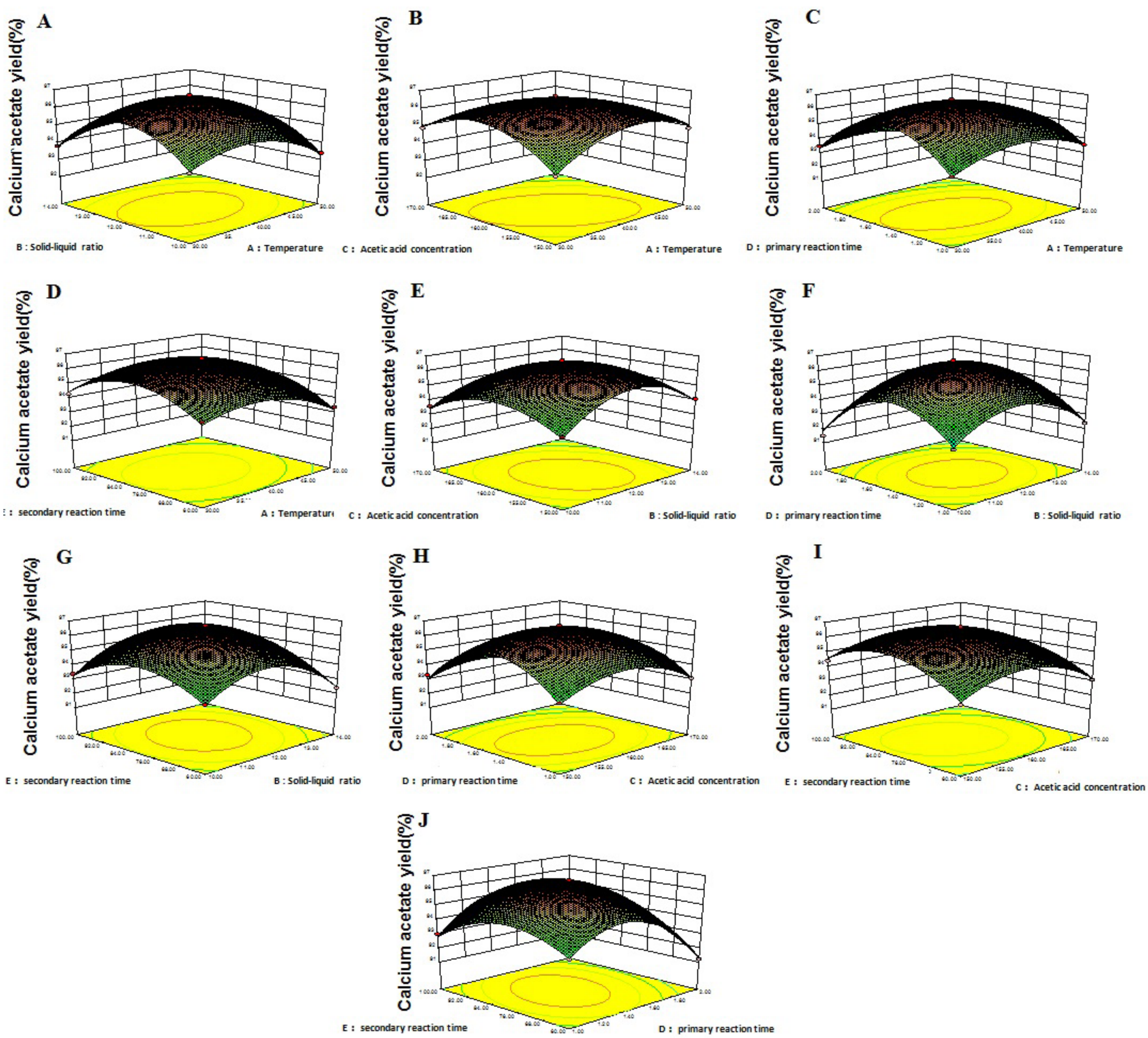

Figure 2. The effect of preparation parameters on the calcium acetate yield. (A) temperature and solid-liquid ratio, (B) temperature and acetic acid concentration, (C) temperature and primary reaction time, (D) temperature and secondary reaction time, (E) solid-liquid ratio and acetic acid concentration, (F) solid-liquid ratio and primary reaction time, (G) solid-liquid ratio and secondary reaction time, (H) acetic acid concentration and primary reaction time, (I) acetic acid concentration and secondary reaction time, (J) primary reaction time and secondary reaction time.

experimental values (96.63%,) were in good agreement with the predicted ones and consequently, which indicated that the RSM model was satisfactory and accurate.

4 Conclusions

In this paper, the preparation technology of calcium acetate from eggshells was analyzed and optimized by response surface methodology. Through the RSM, we determined the optimal process parameters for the preparation of calcium acetate: reaction temperature of 39.00 °C, solid-liquid ratio of 1:12, acetic acid concentration of 159.00%, primary reaction time of 1.5 h and secondary reaction time of 80.00 min. Under the optimum conditions, the yield of calcium acetate could reach 96.63%. Through the rational development and utilization of eggshells, this research had achieved the goal of turning waste into treasure, made full use of resources, and promoted sustainable development of economy. Eggshell, as a new resource, needs further research in development and utilization.

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