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Study on the technology of oligomeric mannose preparation

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Abstract: *Konjac is a special economic crop grown in southern China, and oligomannose is its main decomposition product. The existing clinical research results show that β-mannan hydrolase can effectively enzymatically hydrolyze konjac starch to prepare oligomannose and mannose complexes. Oligomannose has a variety of physiological functions such as promoting the specific proliferation of beneficial bacteria in the organism, reducing the production of toxic metabolites, preventing constipation, protecting the liver, fighting cancer, and enhancing the body's immunity. In addition, it also has the characteristics of not being absorbed and degraded by the human body, and does not increase blood sugar concentration. It is a new generation of functional food. β-mannanase is the most critical enzyme in the mannan degrading enzyme system. It can randomly hydrolyze β-1,4-glycosidic bonds from the inside of the main chain of mannan molecules to produce oligomannoses of different lengths. This subject experiment uses β-mannanase to hydrolyze konjac refined powder to produce oligomannose. The main detection indicators are the hydrolysis rate of konjac refined powder and the average degree of polymerization of the enzymatic hydrolyzed product. The optimal enzymolysis conditions of polymannose is under the conditions of enzyme addition 60 U/g, enzymolysis temperature 40 ℃, konjac flour concentration 30 g/L, enzymolysis time 5 h. The hydrolysis rate can reach 42%, and the average degree of polymerization of oligomannose can reach 1.71.*

Keywords: *konjac flour, oligomannose, β-mannanase, hydrolysis rate, average degree of polymerization*

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I. Introduction:

The monomer of oligomannose is D-mannose, which is connected by β-1, 4 glycosidic bonds to form the main chain, and glucose is connected to the main chain or branch to form oligomannose. The polymerization degree of this product is between 2 ~ 10, which is also known as mannose oligosaccharides.

Existing research results show that oligomannose has an excellent effect on promoting the specific proliferation of beneficial intestinal flora in the organism, and the growth-promoting effect on bifidobacteria can reach more than 50 times, and it can also inhibit the growth of pathogenic bacteria in the body. It reduces the production of toxic metabolites such as uric acid, moisturizes the intestines, relaxes bowel movements, fights cancer, improves the body's immune ability, and prolongs life and many other important physiological functions. In addition, it will not be directly absorbed and degraded by the human body, and will not increase blood sugar concentration. In October 2013, the National Health and Family Planning Commission approved it as a new food raw material, injecting new power into the research and development of health food. There is a huge market space in the market of medical health care products, functional dairy products and functional beverages.

At present, there are three main production processes of oligomannose: extraction from natural raw materials, chemical degradation and biodegradation [1]. There are very few natural raw materials containing high-concentration mannose oligosaccharides, and the mannose oligosaccharides extracted from them are usually considered to be some derivatives. The later processing is more difficult, which wastes time and greatly increases the process and operating costs. Therefore, the promotion of this method has been greatly restricted [2]. Chemical degradation is mainly based on konjac powder and other industrial and agricultural by-products containing more mannans as the main raw materials, using high temperature, acid or alkali hydrolysis to produce oligomannose. However, this production method still needs to use a large amount of acid or alkali in the later stage, and the treatment process is more complicated, the cost is relatively high, and the harmful pollution to the environment is relatively large. It happens to be contrary to the concept of the "resource-saving and environment-friendly" scientific development concept advocated by the state [2]. And biodegradation is a method of preparing oligomannose by hydrolyzing the mannan in konjac flour by using widely existing biological organisms (including organelles, cells and tissues) or biocatalysts such as enzymes. Biosynthesis catalysts are

generally derived from renewable resources, and they are also degradable, non-toxic and harmless. During use, they have a high degree of selectivity to the substrate, which improves the quality of the product. In addition, biodegradation is carried out under mild temperature, pressure and near neutral pH conditions. This condition does not require other auxiliary equipment, and at the same time reduces energy consumption; and the near-neutral pH greatly reduces the amount of acid and alkali, which not only effectively saves natural resources but also fully protects the ecological environment. In today's society, when people's awareness of environmental protection is gradually increasing, biodegradation provides a strongly attractive option [3].

The climate in southern China is mild and humid, so it is more suitable for the cultivation of citrus, lychee, longan and other economic crops. Konjac is also one of them. Its main component is konjac glucomannan. Existing studies have shown that it can be hydrolyzed into oligomannose by β-mannanase. In the research of enzymatic production of oligomannose, β-mannanase acts as a "hub". As we all know, the main chain of mannose is connected by mannose through β-1,4-glycosidic bonds. This enzyme can Act on its glycosidic bond, which is conducive to the further hydrolysis of other enzymes [4].

This subject experiment uses β-mannanase to hydrolyze konjac refined powder to produce oligomannose, continuously explore and optimize the enzymatic hydrolysis conditions, increase the enzymatic hydrolysis rate, and increase the utilization rate of konjac refined raw materials. This method is gentler than natural extraction and chemical degradation, and the post-processing is easier. This subject is of great practical significance. It can effectively promote the industrial development of konjac deep processing and functional oligosaccharides, and open up other applications of konjac in addition to being used as a food additive. The research greatly enhanced its value, and driving farmers in konjac producing areas to get rid of poverty and become rich, which has significant economic and social benefits.

II. Material and methods

2.1 Reagent: The main reagent materials used in this study and their manufacturers are as follows:

Table 2-1: Reagent name and the manufacturer

Reagent	Manufacturer
Konjac powder	Jinan Fushun Biological Technology Co., Ltd.
β-Mannanase	Jinan Fushun Biotechnology Co., Ltd.
DNS reagent	Jinan Fushun Biotechnology Co., Ltd.
D-Mannose Standard	Shanghai Macleans Biochemical Technology Co., Ltd.
Concentrated sulfuric acid (Analytical pure)	Tianjin Jindong Tianzheng Fine Chemical Reagent Factory
Sodium Hydroxide (Analytical Pure)	Jinan Xinzhenyuan Chemical Co., Ltd.

2.2. Determination of total sugar content in konjac flour

Total sugar refers to the reaction of refined konjac powder with acid, complete hydrolysis, and the sum of all reducing monosaccharides in the system (reducing sugars in this article are all calculated as mannose) [5]. The detection method is as follows: accurately weigh 1.0 g of refined konjac powder, place it in a 100 mL beaker, slowly add 50 mL of deionized water and 2.5 mL of 6 mol/L H2SO4, stir thoroughly, hydrolyze in a boiling water bath for 2 hours, and cool. Then neutralize with NaOH solution and transfer to a 100 mL volumetric flask to make a constant volume. Perform a centrifugal operation at 5000 r/min for 5 min. Take 62.5 μL of the supernatant and dilute it 16 times (the volume of the diluted solution is 1 mL). Add 1 mL DNS reagent to boiling water bath for 5 minutes to determine the sugar content, which is the total sugar content of konjac powder.

The formula for calculating the mass fraction of total sugar in refined konjac flour is [6]: Total sugar content = total reducing sugar (g) / konjac powder quality (g) × 100%.

2.3 Determination of free reducing sugar content in konjac flour

Free reducing sugar refers to the reducing sugar that the raw material only dissolves in deionized water and no other reagents are added to the system. The determination method is as follows: accurately weigh 1.0 g of konjac powder, place it in a 250 mL clean beaker, slowly add 100 mL of deionized water, stir thoroughly and centrifuge at 5000 r/min for 5 minutes, take 200 μL of the supernatant and dilute it by 5 times (The volume of the solution after dilution is 1 mL), add 1 mL DNS

reagent to boiling water bath for 5 minutes to determine the sugar content, and determine the sugar content, which is the free reducing sugar content of konjac powder.

The calculation formula is [6]: Free reducing sugar content = (total free reducing sugar (g)) / (quality of konjac flour (g)) × 100%. The calculation formula of KGM quality score is [6]: KGM=(Total amount of reducing sugar (g)-Total amount of free reducing sugar (g))/Quality of konjac flour (g) ×0.9×100%. In the formula, 0.9 is the conversion factor. The conversion factor is the ratio of the molecular weight of glucose and mannose residues in konjac glucomannan (KGM) to the molecular weight of glucose and mannose produced by the hydrolysis of KGM[6].

2.4 Determination of reducing sugar content in enzymolysis solution

Add appropriately diluted β-mannanase solution to konjac flour, carry out enzymatic hydrolysis under certain conditions, take samples, take the supernatant after centrifugation, and dilute the appropriate multiples to determine the reducing sugar content in the enzymatic hydrolysate by DNS method[5].

2.5 Determination and calculation of hydrolysis rate and average degree of polymerization

(1) Hydrolysis rate of konjac flour

The hydrolysis rate of konjac flour refers to the ratio of reducing sugar produced during enzymatic hydrolysis to the total sugar content of konjac flour [2]. The calculation formula is:

The hydrolysis rate of refined konjac flour = (reducing sugar amount of enzymatic hydrolysis solution (g)-free reducing sugar amount (g))/(KGM total sugar amount (g))×100%

The rate of hydrolysis is a common indicator that reflects the degree of polymer hydrolysis, and the rate of hydrolysis is directly proportional to the degree of hydrolysis.

(2) The average degree of polymerization of the product

The average degree of polymerization (DP) is also used to judge the degree of hydrolysis of konjac endosperm powder. It refers to the ratio of the total sugar content in the konjac refined powder to the reducing sugar content in the enzymatic hydrolysate. Its value (DP>1). The smaller the hydrolysis of the refined konjac flour, the more thorough the degree of polymerization of the industrialized production of oligomannose should generally be controlled between 1.8 and 1.9 [6].

2.6 Study on the conditions of making oligomannose by enzymatic method

The main factors affecting the enzymatic hydrolysis of konjac flour include the temperature in the enzymatic hydrolysis process, the enzymatic hydrolysis time period, the concentration of the substrate, and the amount of enzyme additives used. In this study, based on the conclusions drawn by Zhao Mei and Wang Chunjuan's experiments [5], the four-factor five-level enzymatic factor level table is shown in Table 2-2:

Table 2-2 Enzymatic hydrolysis factor level table

Level	Factors			
	Enzyme addition amount (U/g)	Enzymatic hydrolysis temperature (℃)	Substrate concentration (g/L)	Enzymatic hydrolysis time/h
-2	40	30	10	1
-1	50	35	20	3
0	60	40	30	5
1	70	45	40	7
2	80	50	50	9

Take enzyme addition (A), enzymolysis temperature (B), substrate concentration (C) and enzymolysis time (D) as independent variables, and respond to the hydrolysis rate of konjac flour (Y1) and average degree of polymerization (Y2) Value, use Design-Expert 11 design software for response surface experimental design. The design table is as follows:

Table 2-3 Response surface experimental design table

Group number	A: Enzyme addition amount (U/g)	B: Enzymatic hydrolysis temperature/℃	C: Substrate concentration (g/L)	D: Enzymatic hydrolysis time/h
1	60	30	30	5
2	50	35	20	3
3	70	35	20	3
4	50	35	40	3
5	70	35	40	3
6	50	35	20	7
7	70	35	20	7
8	50	35	40	7
9	70	35	40	7
10	60	40	30	1
11	60	40	30	9
12	40	40	30	5
13	80	40	30	5
14	60	40	10	5
15	60	40	50	5
16	60	40	30	5
17	60	40	30	5
18	60	40	30	5
19	60	40	30	5
20	60	40	30	5
21	60	40	30	5
22	50	45	20	3
23	70	45	20	3
24	50	45	40	3
25	70	45	40	3
26	50	45	20	7
27	70	45	20	7
28	50	45	40	7
29	70	45	40	7
30	60	50	30	5

III. Results and discussion:

3.1 Determination of sugar content in konjac powder

Detect and calculate the mass fractions of total sugar, free sugar and konjac glucomannan (KGM) in konjac powder according to the methods and formulas described in 2.2 and 2.3. The results of 5 parallel determinations are shown in Table 3- 1.

Table 3-1 Mass fraction of total sugar, free sugar and KGM in refined konjac flour

Measurement times	Total sugar mass fraction (%)	Free reducing sugar mass fraction (%)	KGM mass fraction (%)
1	56.57	18.65	34.16
2	56.71	18.67	34.25
3	56.52	18.77	34.15
4	56.58	18.70	34.21
5	56.62	18.71	34.23
Average	56.6	18.7	34.2

3.2 Determination of reducing sugar content, hydrolysis rate and average degree of polymerization of the product in the enzymatic hydrolysis solution

According to the method described in 2.4 above and the formula described in 2.5, the reducing sugar content, the hydrolysis rate of konjac flour and the average degree of polymerization of the product in each experimental group in the response surface experimental design table in Table 2-4 are obtained as shown in the following Table 3-2:

Table 3-2 Reducing sugar content, hydrolysis rate and average degree of polymerization of the product in the enzymatic hydrolysis solution

Group number	Reducing sugar content (g/L)	Hydrolysis rate/%	Average degree of polymerization
1	7.38	17.25	2.3
2	5.19	21.20	2.29
3	5.72	28.95	2.08

4	10.28	15.74	2.21
5	10.30	20.61	2.20
6	6.37	38.45	1.78
7	6.40	38.89	1.77
8	11.66	30.56	1.95
9	11.54	29.68	1.97
10	8.57	28.85	2.08
11	7.79	38.25	1.78
12	7.11	14.62	2.31
13	7.46	18.03	2.28
14	3.18	38.30	1.78
15	14.53	30.29	1.95
16	9.64	39.78	1.76
17	9.82	41.01	1.75
18	9.78	40.64	1.74
19	9.93	42.10	1.71
20	9.92	41.01	1.73
21	9.72	40.06	1.71
22	5.29	22.66	2.14
23	5.65	27.92	2.08
24	10.59	22.73	2.14
25	10.73	23.76	2.11
26	5.49	25.58	2.06
27	5.12	20.18	2.17
28	10.96	25.44	2.11
29	10.11	19.22	2.24
30	7.04	13.94	2.42

3.3 Response surface analysis of hydrolysis rate

Through the measurement results in Table 3-2, the regression equation is obtained as follows: Y_1=40.77+0.5696A-1.80B-2.17C+2.63D-1.09AB-0.5781AC-1.94AD+1.61BC-3.61BD-0.0194CD-5. 98A ^2-6.16B^2-1.49C^2-1.67D^2. Perform variance analysis on the model. From Table 3-3, we can see that the model P<0.01, the lack of fit term P=0.0980>0.05, which is not significant, indicates that the established model is successful, and the model regression coefficient R2=0.9896, the adjustment coefficient of determination R2 adj= 0.9798. In addition, the decomposition and conversion temperature of enzymes, the concentration of substrate addition, the primary and secondary terms of the enzymatic conversion time, the secondary terms of the amount of enzyme additives, the interaction terms between the amount of enzyme additives and the temperature of enzymatic hydrolysis, and the use of enzyme additives The interaction terms between the amount and the enzymolysis conversion time, the enzyme decomposition conversion temperature and the solid substrate concentration, the interaction terms between the enzymolysis temperature and the substrate enzymolysis conversion time all reached extremely significant levels (P<0.01). However, the interaction terms between the amount of enzyme additives used, the amount of enzyme additives used and the addition concentration of a given substrate, and the interaction terms between a given substrate addition concentration and the enzymolysis conversion time of the substrate are not significant. A comparison of the absolute value F of the coefficient of the primary term shows that the order of the influence of the four factors on the hydrolysis rate is: enzymatic hydrolysis time>substrate concentration>enzymatic hydrolysis temperature>enzyme addition amount.

Table 3-3 Analysis of variance table of the regression equation of hydrolysis rate

Variance source	Sum of squares	Degree of freedom	Sum of mean square	F value	P value	Significance
Model	2470.51	14	176.46	101.67	＜0.0001	**
A- Enzyme addition amount	7.79	1	7.79	4.49	0.0513	
B- enzymolysis temperature	77.80	1	77.80	44.82	＜0.0001	**
C-substrate concentration	113.14	1	113.14	65.19	＜0.0001	**
D- enzymolysis time	166.58	1	166.58	95.98	＜0.0001	**
AB	19.16	1	19.16	11.04	0.0046	**
AC	5.35	1	5.35	3.08	0.0996	
AD	59.95	1	59.95	34.54	＜0.0001	**

BC	41.31	1	41.31	23.80	0.0002	**
BD	208.30	1	208.30	120.01	＜0.0001	**
CD	0.0060	1	0.0060	0.0035	0.9539	
A^2	981.16	1	981.16	565.331	＜0.0001	**
B^2	1041.96	1	1041.96	600.34	＜0.0001	**
C^2	60.77	1	60.77	35.01	＜0.0001	**
D^2	76.93	1	76.93	44.32	＜0.0001	**
Residual	26.03	15	1.74			
Lack of Fit	22.64	10	2.26	3.33	0.0980	Not obvious
Pure error	3.40	5	0.6790			
Sum	2496.54	29				

Note: *. The impact is significant (P<0.05); **. The impact is extremely significant (P<0.01).

3.4 Analysis of factor interaction

Use Design-Expert 11 to create a response surface diagram composed of A, B, C, and D to the response value Y1 (hydrolysis rate) and the contour diagram formed by its projection, as shown in the following diagrams. The contour line can more directly reflect the influence of the interaction terms of the two factors on the response value. The significance of the interaction between the two main factors can be accurately judged by the contour line shape. If a circular contour line appears on the graph, it shows that the interaction between the two factors is not significant. If the contour is elliptical or horseshoe-shaped, the interaction is significant[7]. Within a certain range of response values, the distance between contour lines is also related to the significance of the interaction term. The larger the contour line distance is, the more significant the interaction is. Conversely, the smaller the interaction is, the less significant the interaction is [8,9]. The steeper the surface graph, the more significant the effect of influencing factors [10].

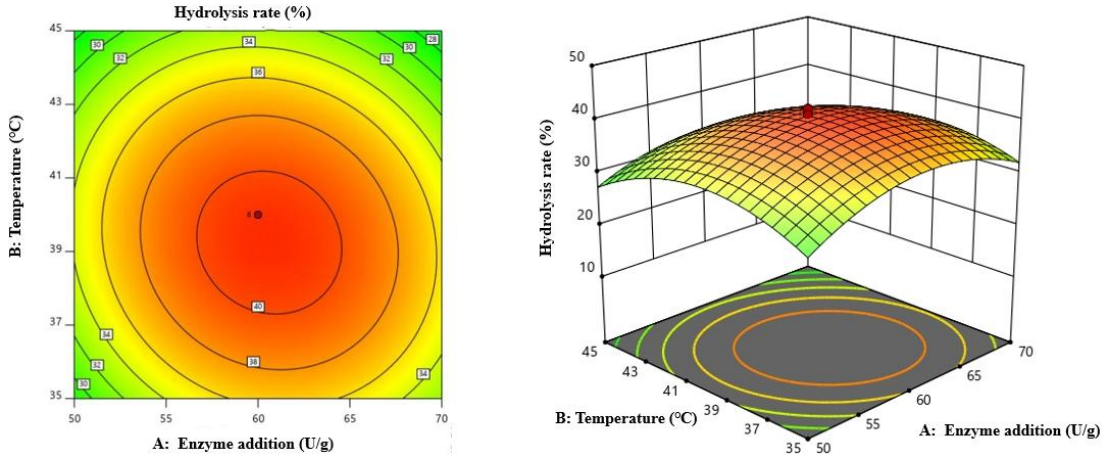

Figure 3-1 Curved surface and contour analysis diagram of enzyme addition amount and enzymolysis temperature

Figure 3-1 is the surface and contour analysis graph of the hydrolysis rate of konjac flour when the substrate concentration is 30 g/L, the enzymatic hydrolysis conversion time is 5 h, the amount of enzyme additives and enzymatic hydrolysis conversion temperature are used to analyze the hydrolysis rate of konjac flour. When the amount of enzyme added does not change, the hydrolysis rate first increases and then decreases with the increase of enzymatic hydrolysis temperature, and reaches the maximum value at 0 level. This is because during the heating process, the activity of β-mannanase first increases and then decreases, and the degree of hydrolysis of konjac flour first increases and then decreases. When the conversion temperature of

enzymatic hydrolysis is constant, the hydrolysis rate first increases and then decreases with the increase of enzyme addition, and reaches the maximum value at 0 level. This is because if the amount of enzyme added is insufficient, the substrate hydrolysis is insufficient, and when the amount is too large, the promotion effect on the enzymatic hydrolysis process is not obvious.

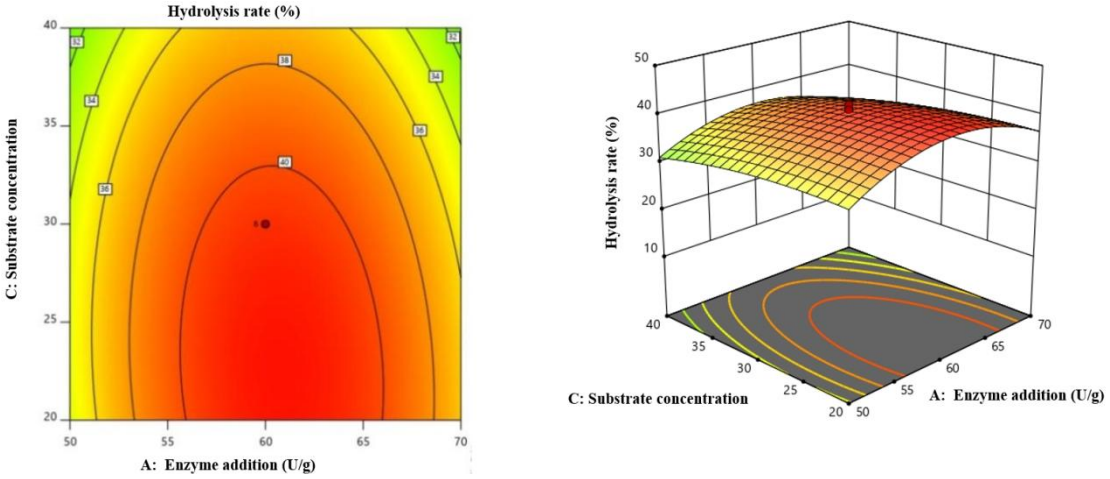

Figure 3-2 Curved surface and contour analysis diagram of enzyme addition amount and substrate concentration

Figure 3-2 shows the surface and contour analysis diagram of the hydrolysis rate of konjac flour by the amount of enzyme additives and the concentration of substrate added when the enzymatic conversion temperature is 40 ℃ and the enzymatic hydrolysis time is 5 h. When the amount of enzyme added does not change, the hydrolysis rate first increases and then decreases with the increase of the substrate concentration. This is because when the required amount of the system substrate is not saturated, the hydrolysis rate will increase with the increase of the reaction substrate, and the increasing concentration of the substrate will increase the viscosity of the reaction system and hinder the enzyme and konjac powder. Contact, thereby reducing the enzymatic hydrolysis [5]. As a result, the rate of hydrolysis is reduced. When the substrate concentration is constant, the hydrolysis rate first increases and then decreases with the increase of the amount of enzyme added.

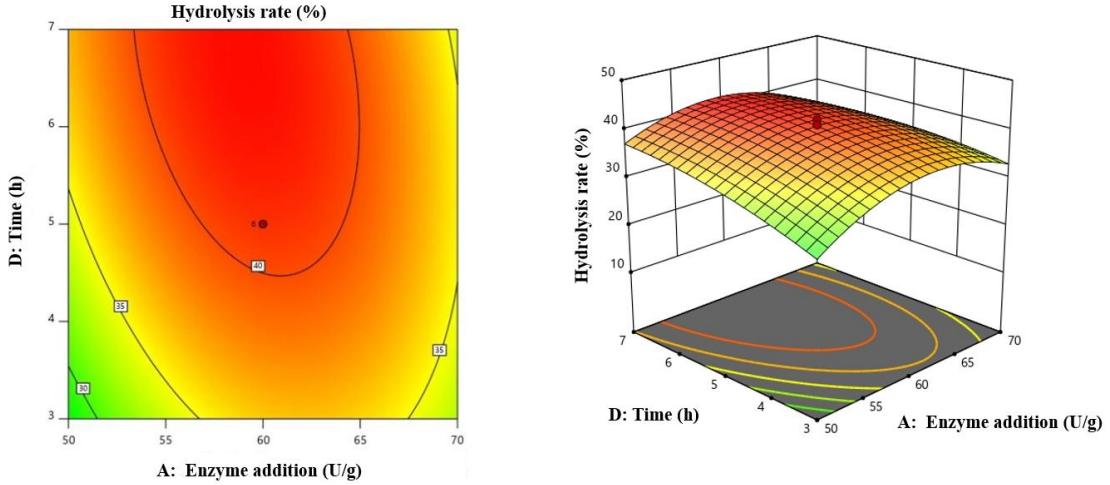

Figure3-3 Curve and contour analysis of enzyme addition amount and enzymatic hydrolysis time

Figure 3-3 shows the surface and contour analysis graphs of the hydrolysis rate of konjac flour by enzymatic conversion temperature of 40 ℃ and solid substrate concentration of 30 g/L, the amount of enzyme additives and the time of enzymatic hydrolysis. When the amount of enzyme added does not change, the hydrolysis rate first increases and then slightly decreases with the increase of enzymatic hydrolysis time. This is mainly because β-mannanase always has a preferential selectivity to long-chain mannans, and its activity is

proportional to the chain length[41]. When the enzymatic hydrolysis time remains unchanged, the hydrolysis rate first increases and then decreases with the increase in the amount of enzyme additives used.

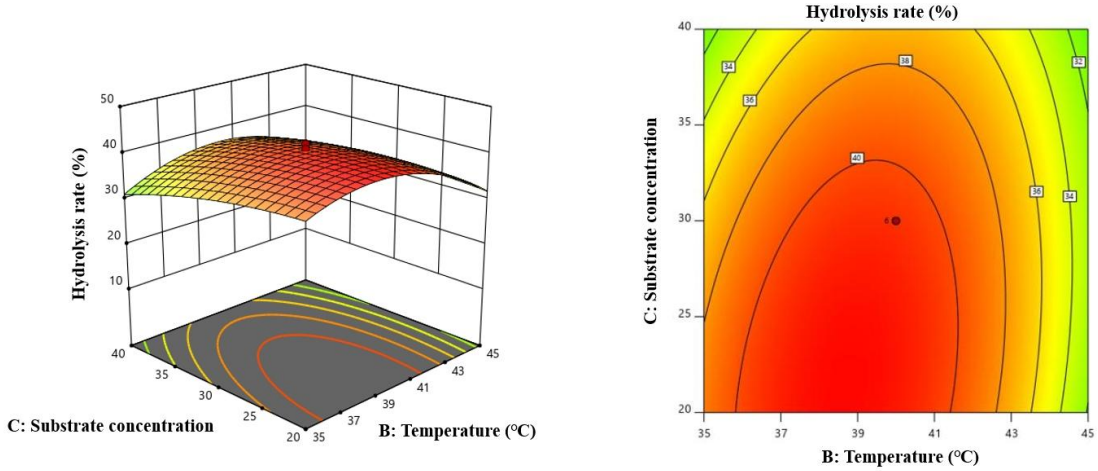

Figure 3-4 Surface and contour analysis of enzymolysis temperature and substrate concentration

Figure 3-4 shows the surface and contour analysis diagrams of the hydrolysis rate of konjac flour by the conversion temperature of enzyme hydrolysis and the concentration of substrate addition when the dosage of enzyme additives is 60 U/g and the enzymatic hydrolysis time is 5 hours. When the enzymatic hydrolysis conversion temperature is constant, the hydrolysis rate first increases and then decreases with the increase of the substrate concentration, and reaches the maximum when the substrate concentration is 30 g/L. When the substrate concentration is constant, the hydrolysis rate increases in the range of 30-40 ℃, and decreases in the range of 40-50 ℃.

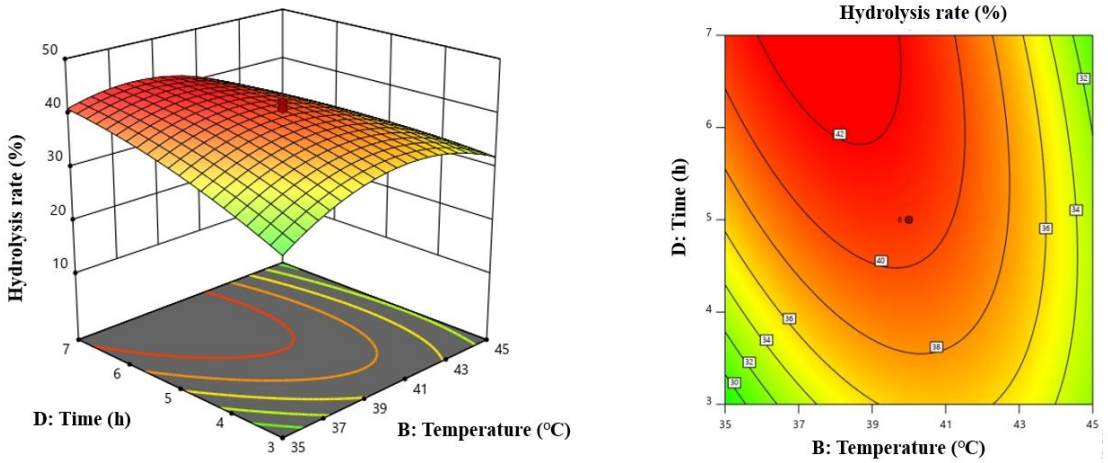

Figure 3-5 Curved surface and contour analysis diagram of enzymolysis temperature and enzymolysis time

Figure 3-5 shows the analysis of the surface and contour curves of the hydrolysis rate of konjac flour by enzymolysis temperature and enzymolysis time when the enzyme dosage is 60 U/g and the substrate concentration is 30 g/L. When the conversion temperature of enzymatic hydrolysis does not change, the hydrolysis rate first increases and then tends to level off with the increase of enzymatic hydrolysis time. When the enzymatic hydrolysis time is constant, the hydrolysis rate first increases and then decreases with the increase of the enzymatic hydrolysis system temperature, and reaches the maximum at 40 ℃.

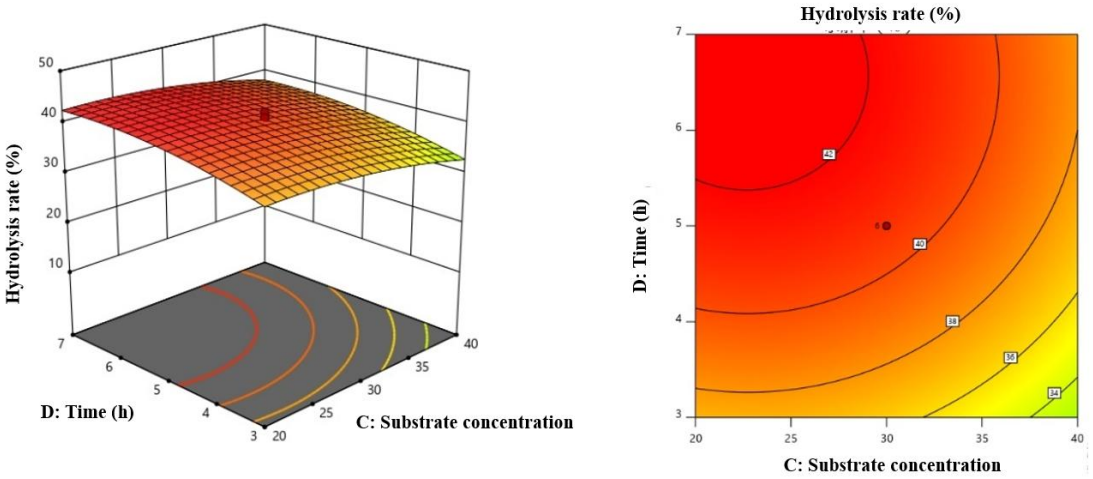

Figure 3-6 Curved surface and contour analysis diagram of substrate concentration and enzymolysis time

Figure 3-6 shows the surface and contour analysis diagram of the response value Y1 of the substrate concentration and enzymolysis time when the enzyme addition amount is 60 U/g and the enzymolysis temperature is 40 ℃. When the substrate concentration is constant, the hydrolysis rate first increases and then tends to level off with the increase of enzymatic hydrolysis time. When the enzymatic hydrolysis time is unchanged, the hydrolysis rate increases in the range of 10-30 g/L of substrate concentration, and decreases in the range of 30-50 g/L.

3.5 Response surface analysis of average polymerization degree

Through the measured data in Table 3-2, the regression equation is as follows:

$$Y_2 = 1.73 - 0.0050\ A + 0.0433\ B + 0.0375\ C - 0.0750\ D + 0.0225\ AB + 0.0175\ AC + 0.0350\ AD - 0.0162\ BC + 0.0888\ BD + 0.0263\ CD + 0.1352\ A^2 + 0.1515\ B^2 + 0.0277\ C^2 + 0.0440\ D^2$$

. Perform variance analysis on the model. Table 3-4 shows that the model P<0.01, the lack-of-fit term P=0.0657>0.05, which is not significant, indicating that the established model is successful, and the model regression coefficient R2=0.9859, the adjustment coefficient of determination R2adj= 0.9728. In addition, the temperature of the enzymatic hydrolysis system, the concentration of substrate addition, the primary and secondary terms of the enzymatic hydrolysis reaction time, and the secondary term of the amount of enzyme additives used, the interaction term of the amount of enzyme additives used and the time of enzymatic hydrolysis, and the temperature of the enzymatic hydrolysis system The interaction terms of enzymolysis reaction time reached a significant level (P<0.01); the interaction terms of enzyme additive usage and enzymolysis system temperature, and the interaction term of substrate addition concentration and enzymolysis reaction time reached a significant level (P<0.05). The interaction term between the amount of enzyme additives, the amount of enzyme additives used and the concentration of substrate addition, and the interaction terms of enzymatic hydrolysis reaction temperature and the concentration of substrate addition are not significant. By comparing the absolute value of the regression coefficient F, it can be seen that the order of the influence of the four factors on the average degree of polymerization is: enzymatic hydrolysis time> enzymatic hydrolysis temperature> substrate concentration> enzyme addition amount.

Table 3-4 The analysis of variance of the average degree of polymerization regression equation

Variance source	Sum of squares	Degree of freedom	Sum of mean square	F value	P value	Significance
Model	1.38	14	0.0988	75.12	＜0.0001	**
A- Enzyme addition amount	0.0006	1	0.0006	0.4563	0.5097	
B- enzymolysis temperature	0.0451	1	0.0451	34.27	＜0.0001	**
C-substrate concentration	0.0337	1	0.0337	25.67	0.0001	**
D- enzymolysis time	0.1350	1	0.1350	102.66	＜0.0001	**
AB	0.0081	1	0.0081	6.16	0.0254	*
AC	0.0049	1	0.0049	3.73	0.0727	
AD	0.0196	1	0.0196	14.90	0.0015	**

BC	0.0042	1	0.0042	3.21	0.0932	
BD	0.1260	1	0.1260	95.84	＜0.0001	**
CD	0.0110	1	0.0110	8.38	0.0111	*
A^2	0.5014	1	0.5014	381.32	＜0.0001	**
B^2	0.6292	1	0.6292	478.48	＜0.0001	**
C^2	0.0211	1	0.0211	16.01	0.0012	**
D^2	0.0530	1	0.0530	40.31	＜0.0001	**
Residual	0.0197	15	0.0013			
Lack of Fit	0.0176	10	0.0018	4.12	0.0657	
Pure error	0.0021	5	0.0004			
Sum	1.40	29				

Note: *. The impact is significant (P<0.05); **. The impact is extremely significant (P<0.01).

3.6 Analysis of factor interaction

Use Design-Expert 11 to create a response surface diagram of A, B, C, and D to the response value Y2 and form a contour diagram from its projection, as shown in the following diagrams.

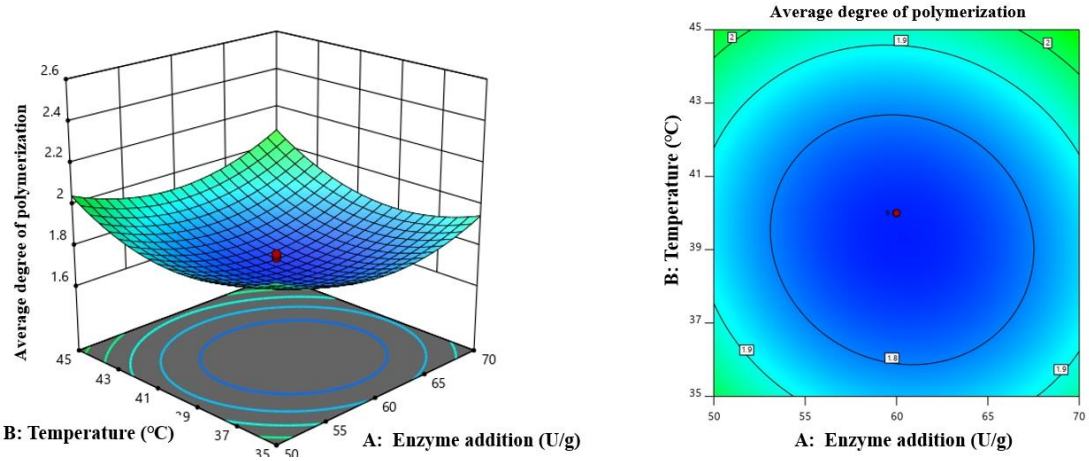

Figure 3-7 Curved surface and contour analysis diagram of enzyme addition amount and enzymolysis temperature

Figure 3-7 is the surface and contour analysis diagram of the average degree of polymerization of the enzymatic hydrolysate product by the amount of enzyme added and the enzymatic hydrolysis temperature when the substrate concentration is 30 g/L and the enzymatic hydrolysis reaction time is 5 h. When the amount of enzyme added does not change, the average degree of polymerization gradually decreases during the enzymatic hydrolysis temperature from 30 ℃ to 40 ℃, and reaches the minimum at 40 ℃. At this time, the degree of hydrolysis is the largest. Within the range of 40-50 ℃, the average degree of polymerization gradually increased again, which may be due to the fact that as the temperature increased, the optimum reaction temperature of β-mannanase was exceeded, and the enzymatic hydrolysis gradually weakened, with the maximum degree of enzymatic hydrolysis at 40 ℃. When the enzymatic hydrolysis reaction temperature is constant, the average degree of polymerization decreases first and then increases with the increase of enzyme addition. The lowest point in the middle is the minimum average degree of polymerization under this condition.

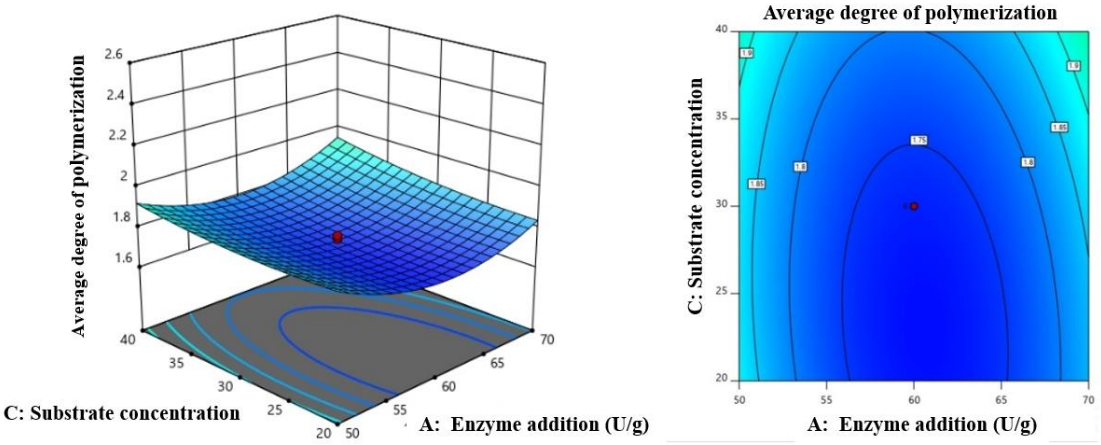

Figure 3-8 Curved surface and contour analysis diagram of enzyme addition amount and substrate concentration

Figure 3-8 is the surface and contour analysis diagram of the average degree of polymerization of the enzymatic hydrolysate product by the amount of enzyme additives and the concentration of substrate added when the enzymatic hydrolysis temperature is 40 ℃and the enzymatic hydrolysis reaction time is 5 h. When the amount of enzyme added does not change, the average degree of polymerization decreases when the substrate concentration is within the range of 10-30 g/L, and increases within the range of 30-50 g/L. This is because before reaching the optimal reaction concentration, the hydrolysis of konjac flour will increase with the increase of the concentration, but when the optimal enzymatic hydrolysis concentration is reached, increasing the substrate will oversaturate the reaction system and increase the viscosity of the system. In a sense, it will seriously hinder the contact between the enzyme and other substrates, thereby hindering the enzymatic hydrolysis reaction. When the substrate concentration does not change, the average degree of polymerization will decrease when the amount of enzyme is added from 40 U/g to 60 U/g; when it is added from 60 U/g to 80 U/g, the average degree of polymerization Increase. When the amount of enzyme added is about 60 U/g, the degree of enzymatic hydrolysis is the largest.

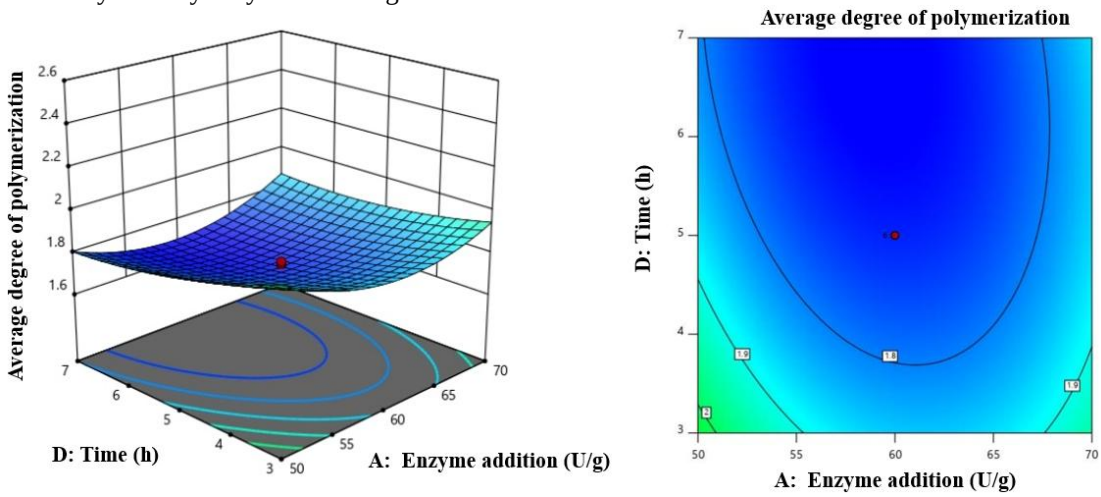

Figure 3-9 Curved surface and contour analysis diagram of enzyme addition amount and enzymolysis time

Figure 3-9 shows the surface and contour analysis diagrams of the average degree of polymerization of the enzymatic hydrolysate products when the enzymatic hydrolysis temperature is 40 ℃ and the substrate concentration is 30 g/L. When the amount of enzyme added does not change, with the increase of enzymolysis time, the average degree of polymerization first decreases and then increases. According to related information, when β-mannanase reacts with long-chain mannans, its activity is far greater than that of short-chain sugars. Within a certain range, its activity is proportional to the chain length. With the passage of reaction time, after the long-chain mannan in the system is hydrolyzed, the enzyme gradually reacts with the short-chain oligosaccharides. This process greatly increases the production of reducing monosaccharides. Therefore, in

order to fully improve the utilization rate of fine konjac powder substrate [5], the enzymolysis time should not be too long. When the enzymatic hydrolysis time is constant, the average degree of polymerization first decreases and then increases with the increase of enzyme addition, and reaches the minimum value at 0 level. Under this condition, the degree of hydrolysis is the largest.

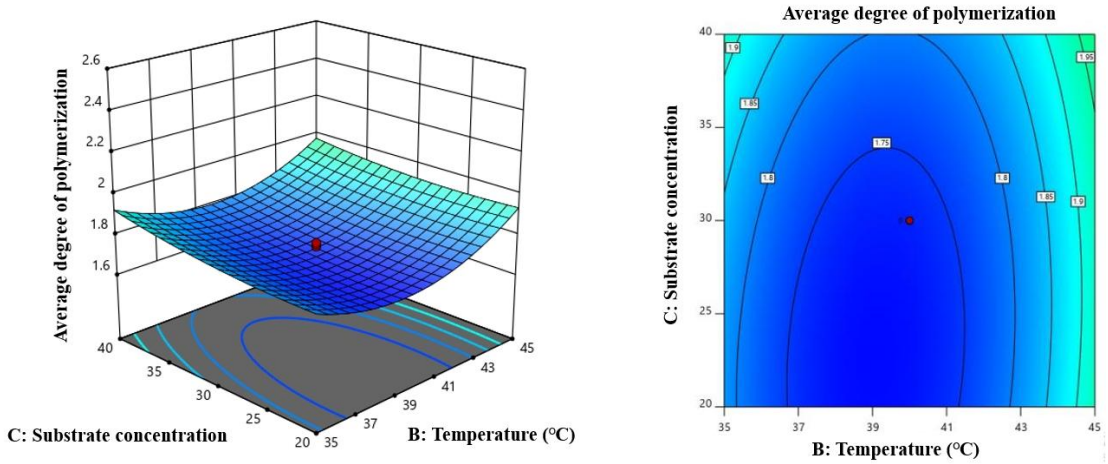

Figure 3-10 Curved surface and contour analysis diagram of enzymatic hydrolysis temperature and substrate concentration

Figure 3-10 is the analysis graph of the surface and contour lines of the enzyme hydrolysis temperature and substrate concentration versus the average degree of polymerization of the enzymatic hydrolysate product when the enzyme addition amount is 60 U/g and the enzymatic hydrolysis reaction time is 5 h. When the enzymatic hydrolysis reaction temperature is constant, the substrate concentration increases from 10 g/L to 30 g/L, the average degree of polymerization decreases, and the degree of enzymatic hydrolysis gradually increases; and then increases from 30 g/L to 50 At g/L, the average degree of polymerization gradually increases. When the concentration of the substrate is unchanged, the average degree of polymerization decreases when the enzymatic hydrolysis time increases from 1 h to 5 h, and the degree of enzymatic hydrolysis gradually increases; when it increases from 5 h to 9 h, the average degree of polymerization decreases again.

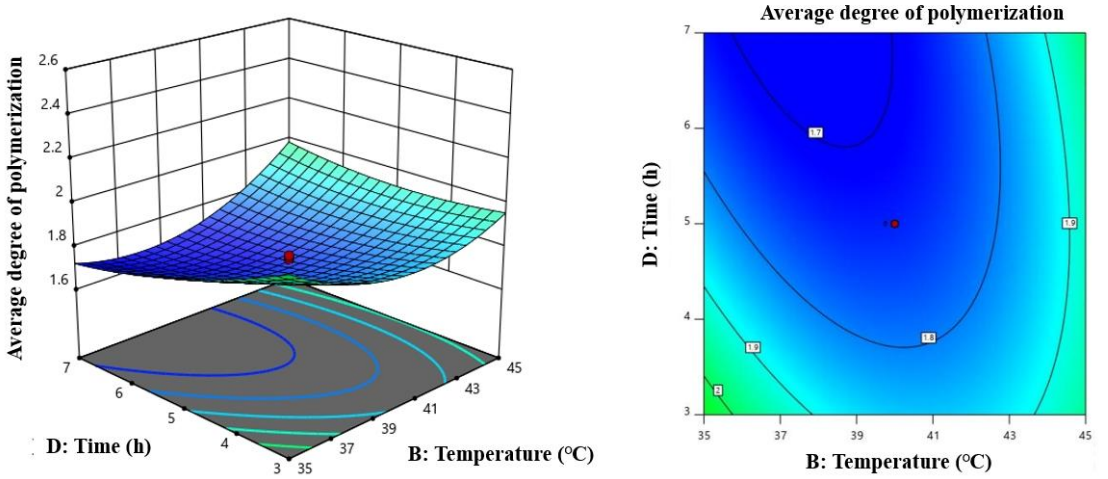

Figure 3-11 Surface and contour analysis diagram of enzymolysis temperature and enzymolysis time

Figure 3-11 shows the curves and contour curves of enzyme hydrolysis temperature and enzymolysis time versus the average degree of polymerization of the enzymolysis products when the enzyme addition is 60 U/g and the substrate concentration is 30 g/L. When the enzymolysis temperature is unchanged, the average degree of polymerization decreases first and then increases when the enzymolysis time increases from 1 h to 9 h. The degree of hydrolysis is the best when the reaction time is 5 h. When the enzymolysis time is constant, the average degree of polymerization decreases first and then increases when the enzymolysis temperature rises

from 30 ℃to 50 ℃, reaching the minimum value at the 0 level (40 ℃), and the degree of hydrolysis is the largest at this time.

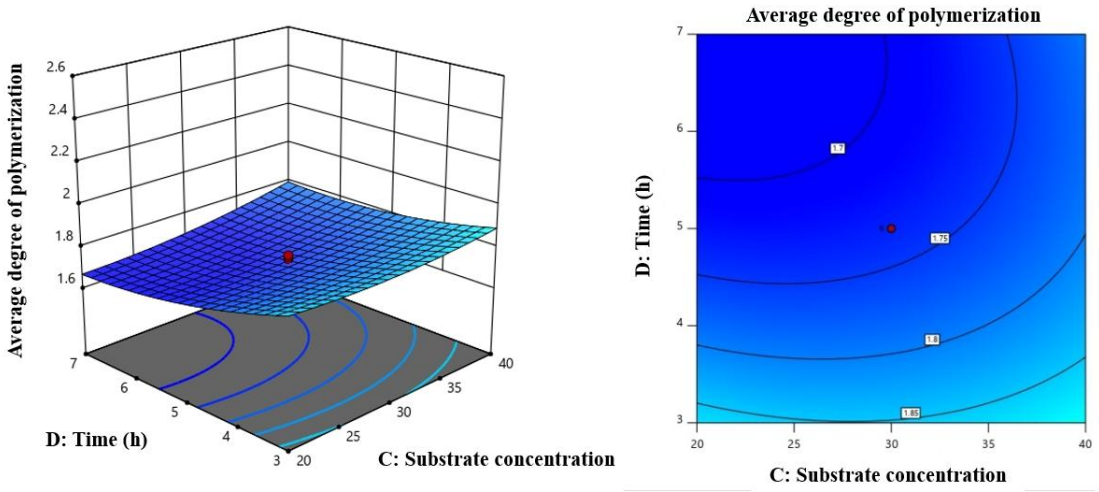

Figure 3-12 Curved surface and contour analysis diagram of substrate concentration and enzymolysis time

Figure 3-12 shows the analysis of the surface and contour curves of the average degree of polymerization of the enzymatic hydrolysate product by the substrate concentration and enzymatic hydrolysis time when the enzyme dosage is 60 U/g and the enzymatic hydrolysis temperature is 40 ℃. When the substrate concentration is constant, the average degree of polymerization increases first and then tends to be flat with the increase of enzymolysis time. When the enzymolysis time is constant, the average degree of polymerization decreases when the substrate concentration is in the range of 10-30 g/L, and increases in the range of 30-50 g/L.

IV. Conclusion:

In this experiment, the hydrolysis rate of refined konjac flour and the average degree of polymerization of the enzymatic hydrolysis products were used as detection indicators, and the influence of four factors on the enzymatic hydrolysis process was explored. The optimal process conditions for producing oligomannose by hydrolyzing konjac powder by β-mannanase were obtained. The main research results are as follows:

(1) Based on relevant literature, combined with the current research situation, and designed response surface experiments, it is concluded that the optimal process conditions for the production of oligomannose by β-mannanase enzymatic hydrolysis of konjac flour are: enzyme addition amount 60 U/g, The enzymolysis temperature is 40 ℃, the concentration of konjac powder is 30 g/L, and the enzymolysis time is 5 h. Under these conditions, the hydrolysis rate of refined konjac flour can reach about 42%, and the average degree of polymerization of oligomannose can reach 1.71.

(2) Combining the analysis of variance in the regression equation of the hydrolysis rate of konjac refined flour and the average degree of polymerization of the product, we can see that:

①The order of the effect of the four factors on the hydrolysis rate is: enzymatic hydrolysis time>substrate concentration>enzymatic hydrolysis temperature>enzyme addition amount.

②The order of the influence of the four factors on the average degree of polymerization is: enzymatic hydrolysis time> enzymatic hydrolysis temperature> substrate concentration> enzyme addition amount.

Acknowledgement

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