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Research Article

Plackett–Burman Combined with Box–Behnken to Optimize the Medium of Fermented Tremella Polysaccharide and Compare the Characteristics before and after Optimization

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The purpose of this study was to improve the yield of fermented Tremella polysaccharide (FTP) by optimizing the medium and to explore the effect of optimization on the structure. In this paper, the Plackett–Burman design and response surface method were used to optimize the fermentation medium of Tremella spore GT2# for FTP production. The results of the Plackett–Burman design showed that potato extract, glucose, and peptone were the main factors. The Box–Behnken experiment and regression analysis determined that the optimal concentrations of potato extract, glucose, and peptone were 22.01%, 29.38 g/L, and 2.61 g/L, respectively. After optimization, the fermentation period shortened from 5 days to 3 days, and the yield of FTP increased from 10.75 g/L to 12.50 g/L, which was in good agreement with the predicted value (12.36 g/L). Characterization of FTP obtained before and after optimization was carried out, and the results showed that medium optimization had significant effects on the primary structure of FTP, including molecular weight, monosaccharide composition, and proportion. After optimization, the content of acid polysaccharide increased, and the glycoside bonds changed from a-configuration to ß-configuration.

1. Introduction

Tremella polysaccharide (TP) is a kind of macromolecular substance with multiple biological activities. It is obtained from the fruit body, mycelia, or fermentation broth of *Tremella fuciformis*. TP has many physiological activities such as anti-inflammatory [1], immune protection [2–4], antioxidation [5, 6], adjuvant treatment of diabetes [7], moisturizing [8], and gelation [9]. TP of high purity sells for up to 200000 RMB/g. Commercially available TP is mainly obtained from the mature fruit body, but the quality and yield are easily affected by the environment [10]. High-density fermentation of *Tremella fuciformis* has many advantages such as easy control of the fermentation conditions, greatly increased yield, reduced production cycle [11], and meeting up with the market demand of fermented Tremella polysaccharides (FTPs).

At present, the research on Tremella polysaccharides by deep fermentation cannot be promoted on a large scale. The main reasons include large investment and low polysaccharide yield and purity. Therefore, related research has focused on the breeding of high-yielding Tremella spore polysaccharide strains and the optimization of fermentation conditions to increase the spore biomass and the production of exosporous polysaccharides. The yield of fermented Tremella polysaccharides has stabilized at about 4 g/L in the past 10 years. In Dong's research [12], after 6 days of fermentation of Tremella spores, intracellular polysaccharides and extracellular polysaccharides were extracted at the same time, and the total yield reached 3.42 g/L. Zhu [13] controlled the pH of the fermentation environment and added different carbon sources to make the bacteria propagate rapidly, and the yield of Tremella polysaccharide reached

4.4 g/L after 120 h fermentation, while fermentation of *Tremella fuciformis* spores by the method of controlling pH in three stages provided a polysaccharide yield of 3.8 g/L [14]. Fermentation of Tremella mycelium has not yet been industrialized on a large scale because of the low yield and darker color of Tremella mycelium polysaccharides. Wang fermented the mycelium of *Tremella fuciformis*, and the concentration of acid *Tremella fuciformis* polysaccharide in the fermentation broth reached 1.9 g/L [15].

According to previous studies, Tremella spore is more suitable for the deep fermentation to produce FTP than Tremella mycelium. Also, the integrated potato medium is a low-nitrogen [16], high-sugar [17], and highly inorganic salt [18] medium which is suitable for *Tremella fuciformis* fermentation [19]. Medium optimization will have a great impact on FTP output and product quality [16, 20, 21]. The optimization of the fermentation medium plays an important role in the industrial production of microorganisms, which is a necessary link from laboratory to industrial production. Whether a good fermentation medium can be designed is a very important step in the successful industrialization of fermentation products. In this study, Plackett–Burman and Box–Behnken response surface methods were used to optimize the fermentation medium. Factors affecting FTP yield were analyzed by glucose metabolism before and after optimization. The characterization differences of FTP before and after optimization were compared, and the effect of the optimized medium on the quality of polysaccharides from Tremella spores was evaluated. This paper will conduct an exploratory study on the influence of medium formulation on the structure of FTP. This research is expected to promote the industrialization of FTP.

2. Materials and Methods

2.1. Strain Culture. The *Tremella fuciformis* strain GT2# used in this experiment was isolated from the laboratory of Shanghai Institute of Technology. The strain was inoculated at 28°C for 24 h in an integrated potato medium slant (glucose 20 g/L; potato extract 20%, peptone 2 g/L, KH$_2$PO$_4$ 3 g/L, MgSO$_4$·7H$_2$O 1.5 g/L, Vb$_1$ 8 mg/L, and agar 20 g/L) until the spores on the surface turned into a smooth round colony, and the slants were stored at 4°C. All media were sterilized at 121°C for 20 min.

2.2. Seed Preparation. The liquid seed medium formula is glucose 20 g/L, potato extract 20%, peptone 2 g/L, KH$_2$PO$_4$ 3 g/L, MgSO$_4$·7H$_2$O 1.5 g/L, and Vb$_1$ 8 mg/L. The volume of seed liquid was 20% of the conical flask volume, and the culture conditions were as follows: 25 °C, 150 r/min, and 24 h. The optical density of spores was measured at 560 nm with 50 times diluted fermentation broth, and the seed preparation was completed when the absorbance reached 0.2.

2.3. Fermentation Culture. The fermentation medium is the same as the liquid seed medium, which is the integrated the potato medium. The inoculation dose was 2%. The culture condition was 25°C, 250 r/min, and the culture was carried

out on a shaking table for 7 days. The fermentation broth was centrifuged at $10000 \times g$ for 15 min. The supernatant was incubated to 100 mL with distilled water, and the total sugar content was measured and recorded as T_1 (g/L) and the reducing sugar content as R_1 (g/L). FTP yield records to Y (g/L) and calculated by equation (1). The utilization rate of reducing sugar is recorded as U(%), which represents the substrate consumption, calculated by equation (2).

$$Y \text{ (g/L)} = T1 - R_1, \tag{1}$$

$$U \text{ (\%)} = \frac{(R0 - R1)}{R0} \times 100\%, \tag{2}$$

where T_1 (g/L): total sugar content in the fermentation broth. R_1 (g/L): residual reducing sugar in the fermentation broth. R_0 (g/L): initial reducing sugar content.

2.4. Single-Factor Experiments. Various factors (glucose, potato extract, peptone, KH$_2$PO$_4$, MgSO$_4$·7H$_2$O, and V_{b1}) in the integrated potato medium were experimented at different concentrations. The optimal concentration range of each factor was preliminarily determined by the single-factor experiment. The experimental design is shown in Table 1.

2.5. Plackett–Burman Test. In order to rank the influence of various factors in the integrated potato medium on FTP yield, six independent variables at two levels with 15 runs were organized in accordance with the Plackett–Burman design. The low level (−1) of the Plackett–Burman test was set as the optimal value of the single-factor experiment, and the high level (+1) was about 1.25 times of the low level. The experimental level of each factor is shown in Table 2.

2.6. Steepest Ascent Experiment. According to the Plackett–Burman experiment, potato extract, glucose, and peptone were selected for the climbing experiment. The actual step size of potato extract was set to 1%, and the actual step size of glucose and peptone is −0.84 g/L and 0.06 g/L, respectively.

2.7. Response Surface Methodology (RSM). The response surface methodology was used to optimize potato extract, glucose, and peptone further. As presented in Table 3, variables were designed as three-level (low, middle, and high) experiments and coded as −1, 0, and 1. According to the coding design, Design-Expert software generated a three-factor, three-level, 17-run experimental design scheme, using the yield of FTP (Y) and the reducing sugar utilization (U) rate as the response value at the end of fermentation. The interaction effect of variables on the bioresponse surface isogram was used to illustrate the interaction relationship between factors [19].

2.8. Regression Model Fitting and Variance Analysis. Design-Expert 7.0.1 software was used to output the results of variance analysis of the Plackett–Burman experiment and

TABLE 1: Design of the single-factor experiment for a basic culture medium.

Trial run	1	2	3	4	5
Potato extract (%)	10.0	15.0	20.0	25.0	30.0
Glucose (g/L)	15.0	20.0	25.0	30.0	35.0
Peptone (g/L)	1.0	1.5	2.0	2.5	3.0
KH_2PO_4 (g/L)	2.0	2.5	3.0	3.5	4.0
$MgSO_4 \cdot 7H_2O$ (g/L)	0.5	1.0	1.5	2.0	2.5
V_{b1} (mg/L)	6.0	7.0	8.0	9.0	10.0

TABLE 2: Experimental level of the Plackett–Burman design.

Code	Factor	Units	Low level (−1)	High level (1)
A	Potato extract	%	20.00	25.00
B	Glucose	g/L	25.00	31.00
C	KH_2PO_4	g/L	3.00	3.75
D	$MgSO_4 \cdot 7H_2O$	g/L	1.50	1.90
E	V_{b1}	mg/L	7.00	9.00
F	Peptone	g/L	2.50	3.10

TABLE 3: Factors and levels for the Box–Behnken design.

Factor	Name	Units	Low level (−1)	Middle level (0)	High level (1)
A	Potato extract	%	21.00	22.00	23.00
B	Glucose	g/L	28.48	29.32	30.16
C	Peptone	g/L	2.56	2.62	2.68

Box–Behnken experiment and carry out regression analysis of the results of the Box–Behnken experiment to simulate the prediction equation.

2.9. Fermentation in a 5 L Fermentor.

The results of medium optimization were verified in a 5 L stirred-tank reactor (siso-bio-5l, Shanghai, China). Fermentation was carried out under the following conditions: temperature 25°C, inflation rate 2 vvm, stirring speed 200 rpm, initial pH 7, working capacity 3 litres, and fermentation time 7 days.

In a fermentation cycle, 10 mL fermentation broth was taken every 24 h and centrifuged at $10000 \times g$ for 15 min. The contents of reducing sugar and total sugar were determined, respectively, after the supernatant constant volumed to 10 mL. The precipitate was washed 3 times, then freeze-dried, and recorded as the dry weight. The fermentation time was taken as the abscissa, and the reducing sugar content, total sugar content, and dry weight of the bacteria were used as the ordinates to draw the curves. The results were compared with the control group (before optimization).

2.10. Preparation and Extraction of FTP.

Based on previous studies [22], freeze-thaw combined with the dual aqueous phase method was used to extract crude FTP from fermentation broth before and after optimization. The 10 mg/mL crude FTP was eluted in a gradient on the DEAE-Sepharose fast flow column. The eluent was NaCl solution with a concentration gradient of 0, 0.2, 0.4, 0.6, and 0.8 mol/L. The eluent flow rate was 1.5 mL/min. A total of 100 tubes were collected, each for 5 minutes. According to the total sugar content in the separated polysaccharide peaks, the recovery rate (equation (3)) of the purified fraction in the crude FTP and the proportion of FTP-1, FTP-2, and FTP-3 (equation (4)) in the purified fraction were calculated.

$$\text{The recovery rate (\%)} = \frac{W1}{W0} \times 100\%, \tag{3}$$

$$\text{Proportion (\%)} = \frac{Tn}{W1} \times 100\%, \tag{4}$$

where W_0 (mg): weight of the crude FTP. W_1 (mg): the sum of the weights of FTP-1, FTP-2, and FTP-3. T_n (mg): total sugar content in FTP-n ($n = 1, 2, 3$).

2.11. Determination of Polysaccharides, Reducing Sugar, and Protein Content.

The total sugar in fermentation broth was determined by the phenol-sulfuric acid method [23]. The reducing sugar in fermentation broth was determined by the DNS method [24]. The protein content was measured using the Bradford method with bovine serum albumin as a standard [25].

2.12. Infrared Analysis.

The FTP was determined by infrared spectroscopy with the KBr tablet pressing method. The blank KBr tablets were used as the control group and scanned in the range of 4000-400 cm^{-1} [26]. The spectral resolution is 4 cm^{-1}.

2.13. Molecular Weight Determination. The molecular weight of FTP (0.2 mg/mL) was determined by high-performance size exclusion chromatography (HPSEC) [27]. The chromatographic column was a Waters Ultrahydrogel ™ linear (7.8 × 300 mm, 10 μm) gel column, the mobile phase was 0.1 mol/L NaNO$_3$, the flow rate was 0.9 mL/min, the column temperature was 45°C, and the loading volume was 20 μL.

2.14. Monosaccharide Composition Analysis. HPAEC-PAD (high-performance anion-exchange pulsed amperometric detection chromatography) [28] was performed to determine the monosaccharide composition and the molar ratio of FTP before and after optimization.

Sample hydrolysis: 5 mg/mL FTP (100 μL) was hydrolyzed with 4 mol/L trifluoroacetic acid (100 μL) at 110°C for 2 h. Trifluoroacetic acid was rinsed with methanol (200 μL), and residual methanol was rinsed with nitrogen. The residue was dissolved to 5 mL for sample analysis by ion chromatography (Waters600 liquid chromatograph, Waters, United States).

Chromatographic conditions: Dionex ICS5000 system; column: CarboPac PA20 (ID 3 × 150 mm); mobile phase: A, H$_2$O; B, 250 mmol/L NaOH; and C, 1 mol/L NaAc, ternary gradient elution; flow rate: 0.5 mL/min; pulsed amperometric detector (PAD); Au working electrode; Ag/AgCl reference electrode; injection volume: 20 μL; and column temperature: 30°C.

2.15. Data Analysis. All experiments in this study were conducted in triplicates, and the data were presented as mean. Differences between groups were assessed using a *t*-test. A *P* value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Single-Factor Experiments. The results (Figure 1) of the single-factor experiment have shown that the optimum range of components of the FTP medium should be as follows: potato extract 20%, glucose 25.0 g/L, peptone 2.5 g/L, KH$_2$PO$_4$ 3.0 g/L, MgSO$_4$·7H$_2$O 1.5 g/L, peptone 2.5 g/L, and V_{b1} 7.0 mg/L.

3.2. Plackett–Burman Test. The design and results of the Plackett–Burman experiment are shown in Table 4. The variance analysis of Plackett–Burman experimental results in Table 5 proves the reliability of the experimental results. The Plackett–Burman experimental model is significant ($p < 0.0001$), and the missing item is not significant ($p = 0.3437$), indicating that the measured and predicted values of FTP have a good fitting degree and can truly reflect the experimental results. Potato extract ($p < 0.0001$), glucose ($p < 0.0001$), and peptone ($p = 0.0005$) have significant effects on the yield of FTP. Figure 2 also implies that potato extract, glucose, and peptone have significant effects on FTP yield, and the influence degree of each factor

is ranked. The order of effects from high to low was as follows: potato extract, glucose, peptone, KH$_2$PO$_4$, V_{b1}, and MgSO$_4$·7H$_2$O. Therefore, potato extract (A), glucose (B), and peptone (F) should be selected as the main factors for the steepest ascent experiment. Among them, potato extract (A) and peptone (F) have negative effects on FTP yield, which means that the following optimization should start from the low level of the Plackett–Burman experiment. Glucose (B) has a positive effect on the output of FTP, which means that the higher the glucose content is, the more conducive it is to the production of FTP, and the follow-up optimization should start from the high level of the Plackett–Burman experimental design.

According to the results of the Plackett–Burman experiment, Design-Expert 7.0.1 also outputs the prediction equation of FTP yield.

$$Y \text{ (yield of FTP, g/L)} = 5.11 - 2.7A + 1.89B - 1.32F, \quad (5)$$

where A: potato extract, %; B: glucose, g/L; and F: peptone, g/L.

In equation (5), corresponding to Figure 2, the coefficient of the independent variable represents its effect on the output of FTP. The positive coefficient of the independent variable indicated that the factor is not a risk factor, and the higher factor value would more easily promote the production of FTP. The coefficients of the factors determine the direction of the optimal pathways. According to the abovementioned results, the initial value of the climbing experiment should be set as potato extract (20%), glucose (31 g/L), and peptone (2.5 g/L).

3.3. Steepest Ascent Experiment. The experimental design and results are shown in Table 6. It is apparent that the yield of FTP showed a peak value when the concentrations of potato extract, glucose, and peptone were 22%, 29.32 g/L, and 2.68 g/L, respectively. At this point, the yield of FTP was close to the maximum reaction zone, so the data set was selected as the central value of the response surface optimization design.

3.4. Box–Behnken Design. The RSM design and results are recorded in Table 7. Figure 3 shows the interaction between the various factors in the medium when the yield of FTP and glucose utilization are, respectively, used as responses through contour plots.

The effects on FTP production of changes in the concentration of potato extract and glucose are shown in Figure 3(a). High FTP production was recorded when the concentration of potato extract and glucose was from 21.5% to 22.5% and 28.4 g/L to 29.74 g/L, respectively. Similarly, from the contour plot (Figure 3(b)), it can be seen that potato extract and peptone approximately yield FTP between 22% and 23% and 2.56 g/L and 2.65 g/L, respectively. In the contour plot (Figure 3(c)), FTP production has a simultaneous optimal region in the concentrations of glucose and peptone from 28.48 g/L to 29.32 g/L and 2.56 g/L to 2.65 g/L, respectively.
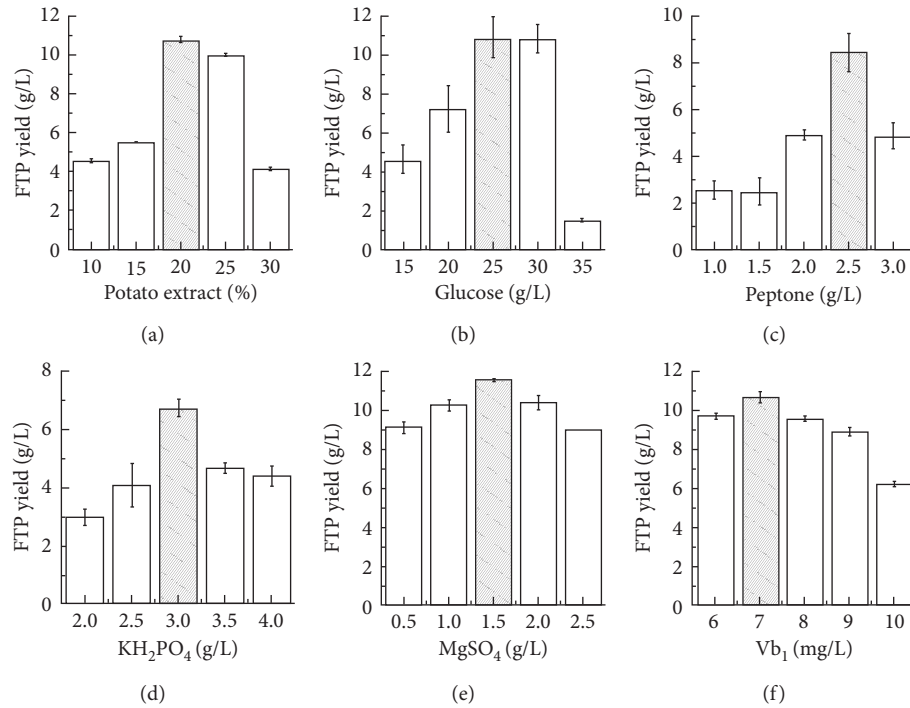
Figure 1: The bar graph of each single factor in the basic fermentation medium.

Table 4: Experimental design and results of the Plackett–Burman experiment.

Trial run	Potato extract (%)	Glucose (g/L)	KH_2PO_4 (g/L)	$MgSO_4 \cdot 7H_2O$ (g/L)	V_{b1} (mg/L)	Peptone (g/L)	Y (g/L)
1	−1	1	1	−1	1	1	8.28
2	1	1	−1	1	1	1	3.03
3	0	0	0	0	0	0	4.71
4	1	1	1	−1	−1	−1	4.51
5	1	−1	1	1	1	−1	1.87
6	−1	−1	1	−1	1	1	3.35
7	0	0	0	0	0	0	5.91
8	−1	1	−1	1	1	−1	11.42
9	0	0	0	0	0	0	4.91
10	1	−1	1	1	−1	1	0.05
11	−1	1	1	1	−1	−1	10.94
12	−1	−1	−1	−1	−1	−1	8.75
13	1	−1	−1	−1	1	−1	1.15
14	1	1	−1	−1	−1	1	3.87
15	−1	−1	−1	1	−1	1	4.17

Table 5: Variance analysis results of the Plackett–Burman experiment.

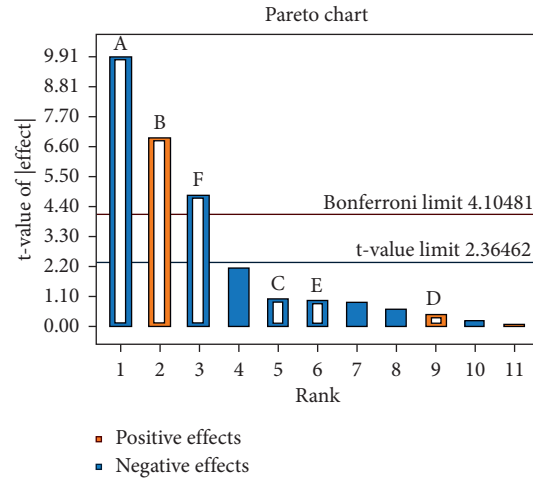| Source	Sum of squares	df	Mean square	F value	p value prob > F	
Model	151.6	3	50.53	61.19	<0.0001	Significant
A- potato extract	87.62	1	87.62	106.1	<0.0001	
B- glucose	42.94	1	42.94	52	<0.0001	
F- peptone	21.04	1	21.04	25.47	0.0005	
Curvature	9.68×10^{-3}	1	9.68×10^{-3}	0.012	0.9159	Not significant
Residual	8.26	10	0.83			
Lack of fit	7.43	8	0.93	2.25	0.3437	Not significant
Pure error	0.83	2	0.41			
Cor total	159.86	14				

FIGURE 2: Pareto chart of the Plackett–Burman design for the FTP yield. (A) Potato extract, (B) glucose, (C) KH_2PO_4, (D) $MgSO_4 \cdot 7H_2O$, (E) V_{b1}, and (F) peptone.

TABLE 6: Design and results of the steepest ascent experiment.

Test no.	1	2	3	4	5
Potato extract(%)	20.00	21.00	22.00	23.00	24.00
Glucose(g/L)	31.00	30.16	29.32	28.48	27.64
Peptone(g/L)	2.50	2.56	2.62	2.68	2.74
Yield(g/L)	12.05	12.60	13.92	11.54	11.48

TABLE 7: Experimental design and results of Box–Behnken.

| Trial run	Potato extract	Glucose	Peptone	Y (g/L)	U (g/L)
1	0	1	−1	11.83	86.88
2	1	−1	0	12.23	81.53
3	1	0	1	11.59	88.63
4	1	0	−1	12.14	82.75
5	0	0	0	12.20	79.28
6	−1	−1	0	11.86	83.88
7	0	0	0	12.44	77.30
8	0	0	0	12.36	76.90
9	−1	0	−1	11.57	89.29
10	−1	0	1	11.40	85.72
11	0	−1	−1	12.41	79.37
12	−1	1	0	10.68	87.38
13	1	1	0	12.00	84.20
14	0	1	1	11.40	82.77
15	0	0	0	12.42	76.74
16	0	0	0	12.35	77.98
17	0	−1	1	12.04	80.04

As shown in Figure 3, the effect of factors on glucose utilization was complex. When the concentration of potato extract was in the range of 21% ∼ 21.75% and glucose was in the range of 29 g/L∼30 g/L, an increase in the glucose utilization ratio was observed in Figure 3(d). Also, potato extract and peptone approach the maximum reaction center of glucose utilization between 21%∼22% and 2.56 g/L to 2.65 g/L, respectively (Figure 3(e)). In the contour plot (Figure 3(f)), the glucose utilization ratio has an optimal region in the concentrations of glucose and peptone from 29 g/L to 30 g/L and 2.56 g/L to 2.65 g/L, respectively.
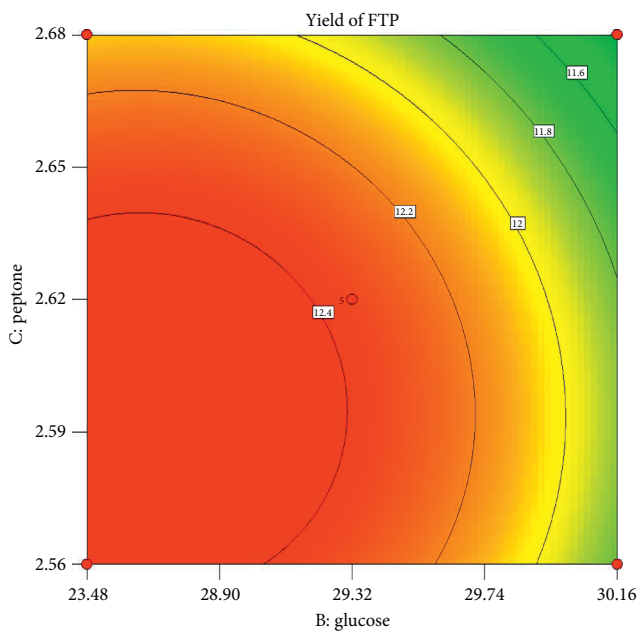
3.5. Regression Model Fitting and Variance Analysis. As shown in Table 8, when the yield of FTP and glucose utilization were used as the response values, the probability of the regression model > F value was 0.0004 and 0.0027, respectively. The results indicated that this model could
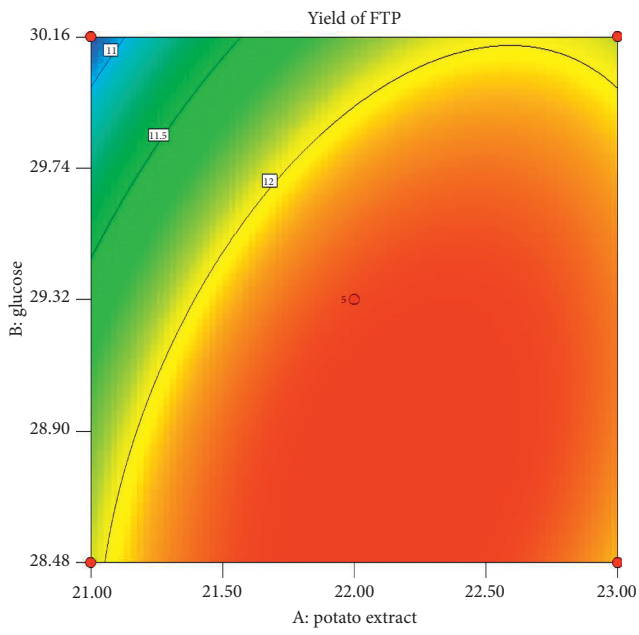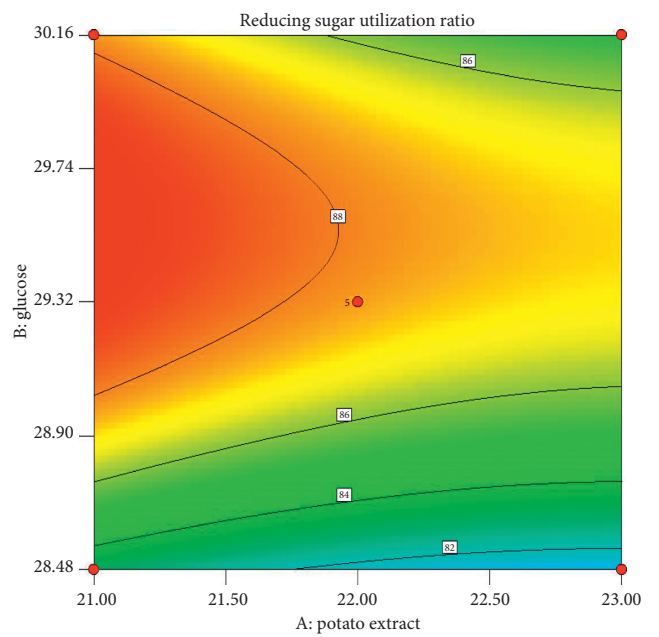
(a)
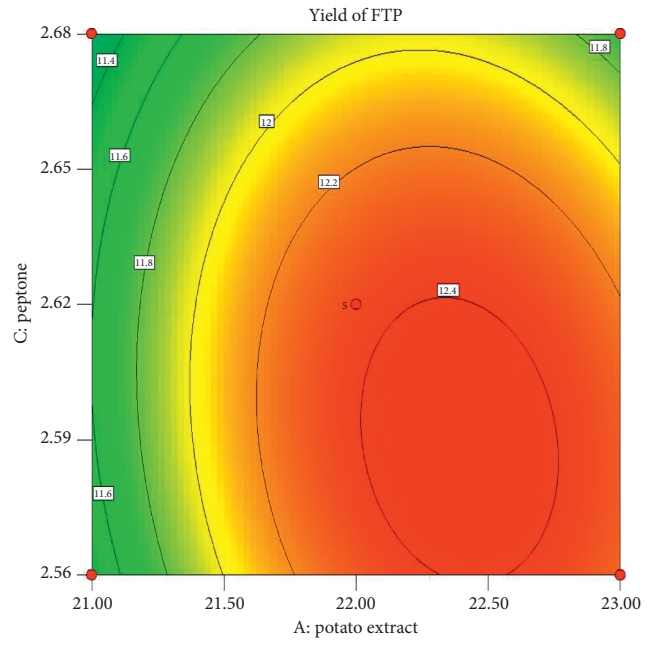
(b)
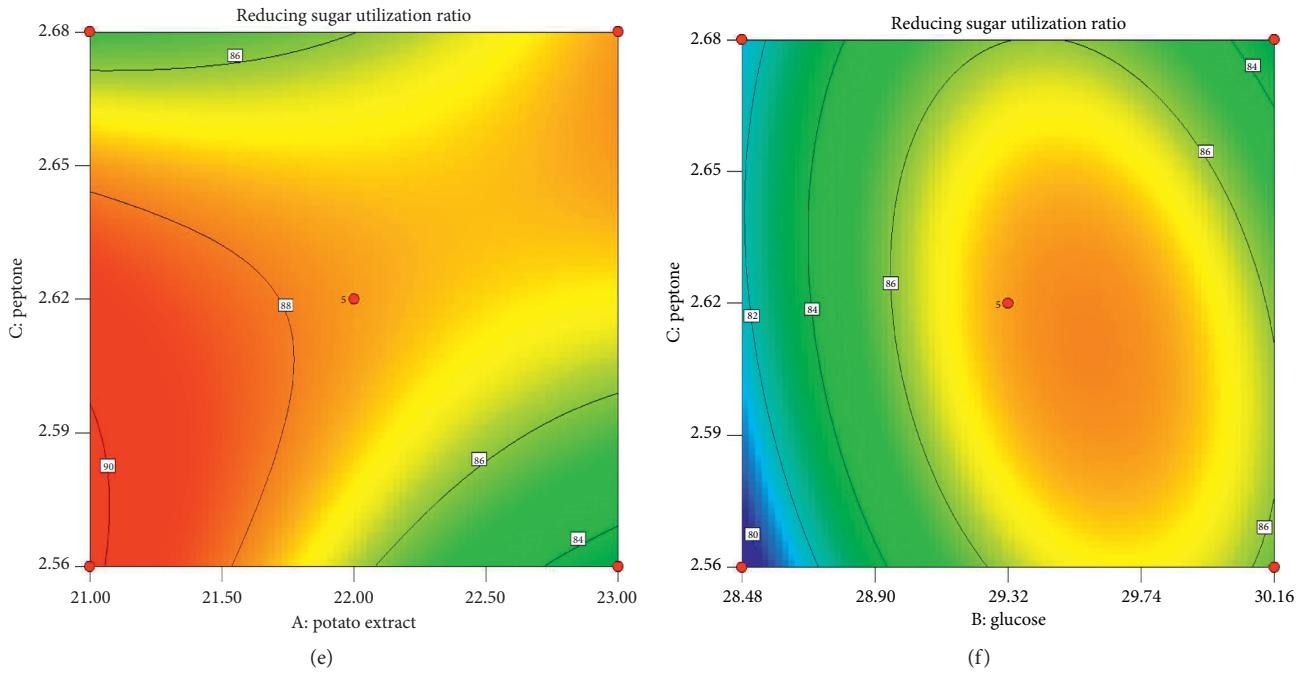
(c)

(d)

Figure 3: Continued.

(e)

(f)

Figure 3: (a~c) Contour plots of yield of FTP showing interactions between potato extract and glucose (a), potato extract and peptone (b), and glucose and peptone (c). (d~f) The interaction effect of variables on reducing the glucose utilization ratio.

Table 8: ANOVA for the response surface polynomial model of all independent variables.

Factor	FTP yield (Y)			Glucose utilization ratio (U)		
	SS	F value	Prob > F	SS	F value	Prob > F
Model	3.55	18.42	0.0004	147.8	10.49	0.0027
A- potato ex.	0.75	35.22	0.0006	10.51	6.72	0.0359
B- glucose	0.86	40.21	0.0004	33.59	21.47	0.0024
C- peptone	0.29	13.57	0.0078	0.17	0.11	0.7537
AB	0.22	10.43	0.0145	0.17	0.11	0.7492
AC	0.036	1.67	0.2377	22.3	14.25	0.0069
BC	8.04×10^{-4}	0.038	0.8519	5.72	3.65	0.0976
A^2	0.86	40.33	0.0004	0.93	0.59	0.4666
B^2	0.18	8.52	0.0224	62.83	40.15	0.0004
C^2	0.22	10.12	0.0155	9.6	6.13	0.0424
Residual	0.15			10.95		
Lack of fit	0.11	4.11	0.1027	6.68	2.08	0.2452
Pure error	3.70×10^{-2}			4.28		

predict the FTP yield and the utilization rate of reducing sugar reliably. The model prediction equations are given as follows:

$$Y = 12.35 + 0.31 \times A - 0.33 \times B - 0.19 \times C + 0.24 \times A \times B - 0.094 \times A \times C - 0.014 \times B \times C - 0.45 \times A2 - 0.21 \times B2 - 0.23 \times C2, \tag{6}$$

$$U = 87.64 - 1.15 \times A + 2.05 \times B - 0.14 \times C - 0.21 \times A \times B + 2.36 \times A \times C - 1.20 \times B \times C + 0.47 \times A2 - 3.86 \times B2 - 1.51 \times C2, \tag{7}$$

where Y is the FTP yield and U is the glucose utilization ratio and *A*, *B*, and *C* are the coded values of potato extract, glucose, and peptone, respectively.

When *Y* and *U* are at their maximum, the concentrations of *A*, *B*, and *C* are the optimal concentrations of the medium.

When the *p* value is greater than 0.1, it is an insignificant factor (Table 8). After removing the insignificant factors, the following equations are obtained:

$$Y = 12.35 + 0.31 \times A - 0.33 \times B - 0.19 \times C + 0.24 \times A \times B - 0.45 \times A2 - 0.21 \times B2 - 0.23 \times C2, \qquad (8)$$

$$U = 87.64 - 1.15 \times A + 2.05 \times B + 2.36 \times A \times C - 1.20 \times B \times C - 3.86 \times B2 - 1.51 \times C2. \qquad (9)$$

The optimal predicted value can be obtained from equations (8) and (9). When the reducing sugar utilization rate (87.73%) and the yield of FTP (12.36 g/L) reached the maximum at the same time, the concentration of potato extract (A/22.01%), glucose (B/29.38 g/L), and peptone (C/2.61 g/L) was the optimal combination.

3.6. Production of Crude FTP in a 5L Fermentor.

The results of the scale-up fermentation experiments are presented in Figure 4. The maximum biomass of the optimized medium reached 1.52 g/L, which was 0.61% lower than that of the control group. However, the FTP production increased from 10.75 g/L to 12.50 g/L after 72 h of fermentation, which was 116.27% higher than the control. Moreover, the yield of FTP (12.50 g/L) was consistent with the predicted value of 12.36 g/L. Furthermore, the fermentation cycle was significantly shorter than before optimization. The optimized group ushered in an important turning point after fermented for three days. At this point, the consumption rate of reducing sugar began to decline (Figure 4(a)), FTP accumulated rapidly (Figure 4(b)), and GT2# multiplied rapidly (Figure 4(c)); all of these phenomena indicated that Tremella spores grow and metabolize very vigorously on the third day of fermentation. After the medium was optimized, the fermentation was finished on the third day, two days earlier than the control, which shortened the fermentation cycle greatly.

3.7. Purification of FTP.

The results of homogeneous polysaccharide components obtained from the purification of crude FTP are shown in Figure 5. The crude FTP contains three components, among which the neutral polysaccharide components eluted by distilled water were in tubes 15~22, the weakly acidic polysaccharide components were in tubes 37~50, and the acidic polysaccharide components were collected in tubes 58~75, which are recorded as FTP-1, FTP-2, and FTP-3 in turn (Figure 5(a)). The optimized FTP peak position shifted slightly to the left, indicating that the proportion of neutral polysaccharide increased. In addition, the three peaks of the optimization group were all symmetrical single peaks with obvious boundaries, indicating that the separation effect was significantly higher than that of the control group.

As shown in Figure 5(b), the total sugar content of FTP-1, FTP-2, and FTP-3 only accounts for 84.69% of the total mass of the purified FTP components, indicating that the control group has many impurities and contains components that are difficult to be separated. After optimization, the FTP recovery rate was 30.07%, which is 21% higher than that of the control group (24.78%). Among them, the proportion of acid polysaccharide components was 66.18%, which was 1.17 times higher than that before optimization (56.46%). Current studies have shown that acidic polysaccharides are the main active components of FTP. Even in the optimized medium, the content of acid polysaccharide produced by Tremella spores was still lower than that of mycelia (75.32%) and frustums (94.66%) [15]. However, the total output (8.19 g/L) can make up for its deficiency in content, so it is feasible to produce highly active FTP using the optimized medium.

3.8. Total Sugar and Protein Content Change.

The total sugar and protein contents of FTP-3 before and after optimization are summarized in Figure 6. After optimization, the protein decreased and the total sugar increased. Therefore, the optimization had a high production of total sugar, and the purification efficiency can be improved by reducing protein production.

3.9. Effect of Medium Optimization on M_w.

The M_W of the FTP before and after optimization was measured (Table 9). The size and distribution of the molecular weight of FTP in the optimized group changed. M_w and M_n increased to some extent. The parameter M_w/M_n indicates the distribution range of the molecular weight of the sample, while M_w/M_n of the optimized group becomes smaller, indicating that the molecular weight range of FTP in the optimized group is narrower, that is, it has better uniformity. The parameter (%/area) indicates that the FTP in the control group contains impurities of different molecular weights, which may be caused by the protein (7.20 ± 0.27%) it contains (Figure 6). However, the protein content in the optimized group was very small (1.17 ± 0.15%), so the molecular weight distribution was not affected obviously (Figure 6). The molecular weight of FTP obtained by submerged fermentation is much smaller than that of the polysaccharides of Tremella fruit bodies reported in the literature (7.8×105) [29]. Small molecules of Tremella polysaccharides can play a more important role in drug delivery systems.
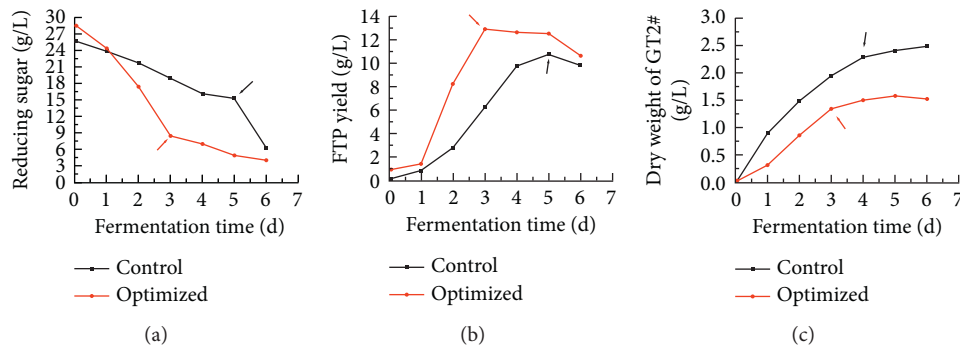
FIGURE 4: Comparison of fermentation in an optimized and preoptimized medium in a 5 L fermentor. (a) RG (reducing sugar), (b) FTP yield, and (c) dry weight of GT2#.
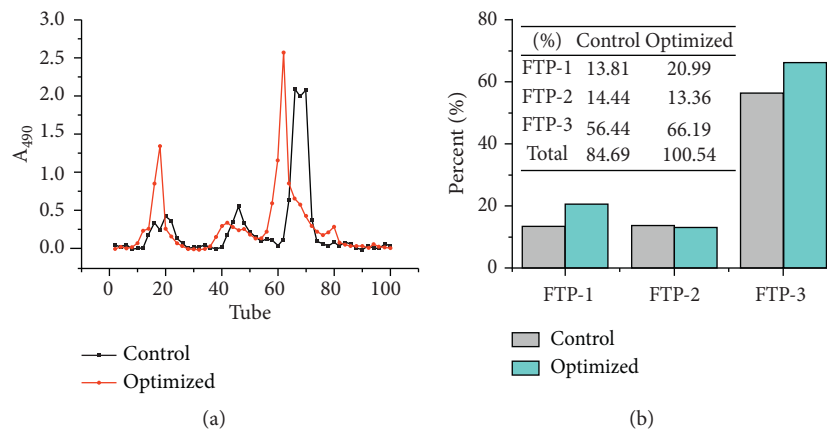

FIGURE 5: Isolation and purification of crude FTP. (a) The DEAE-Sepharose fast flow ion exchange chromatography elution curve for FTP in the control and optimized group. (b) The proportion of FTP-1, FTP-2, and FTP-3 in all purified components.
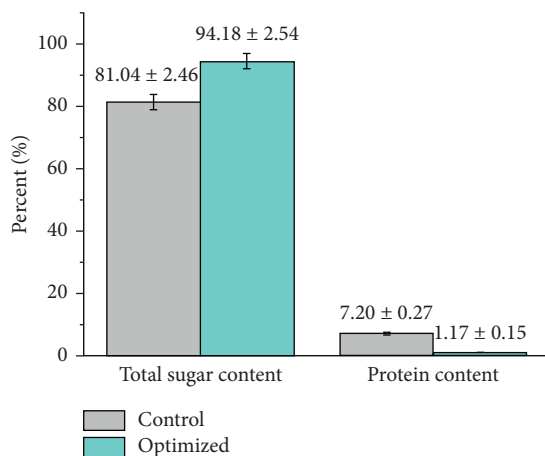

FIGURE 6: The change of total sugar content and protein content.

3.10. Infrared Spectrum Analysis.

Infrared spectra showed that FTPs in the control and optimized group had a similar structure except for the peak at 850 cm^{-1} (Figure 7). The optimization group is taken as an example to illustrate the analytical infrared spectrum. A strong and wide absorption peak at 3700–3000 cm^{-1} indicates the

TABLE 9: Molecular weight.

Sample	M_w	M_n	M_w/M_n	%/area
Control	16236	9760	1.66	94.99
Optimized	17994	11866	1.52	100.00

existence of the O-H stretching bond. The weak C-H stretching vibration peak at 2932 cm^{-1} is the characteristic absorption peak of carbohydrates, indicating that methylene is contained, and the asymmetric C=O stretching vibration peak at 1648 cm^{-1} indicates that the sample has a pyran ring structure. The band at 1730.0 cm^{-1} indicates the presence of methyl ester groups, while the band at 1650 cm^{-1} indicates the presence of aminoacyl groups [30]. The strong absorption peak between 1500 cm^{-1} and 1700cm-1 is the characteristic absorption peak of polygalactose [31]. The peak at 1100 cm^{-1} was superposed by angular vibration absorption peaks of alcohol hydroxyl-OH and C-O stretching vibration. The absorption peak of the optimized group at 902 cm^{-1} means that it has a ß-glycosidic bond structure. Also, in the control group, the characteristic absorption peak of the a-glycosidic bond is at 850 cm^{-1}.

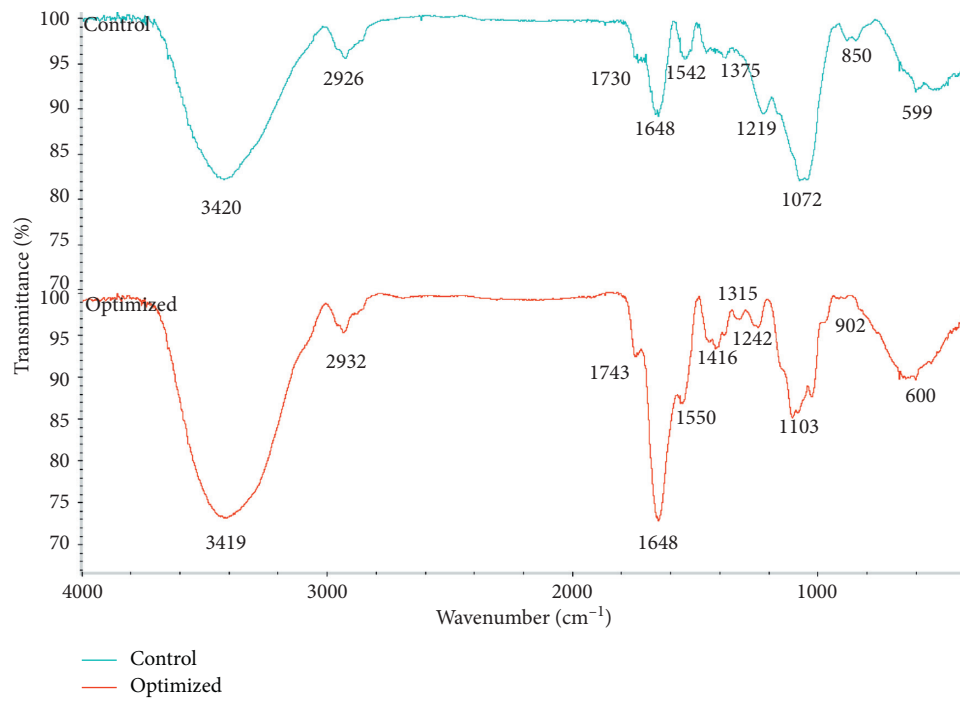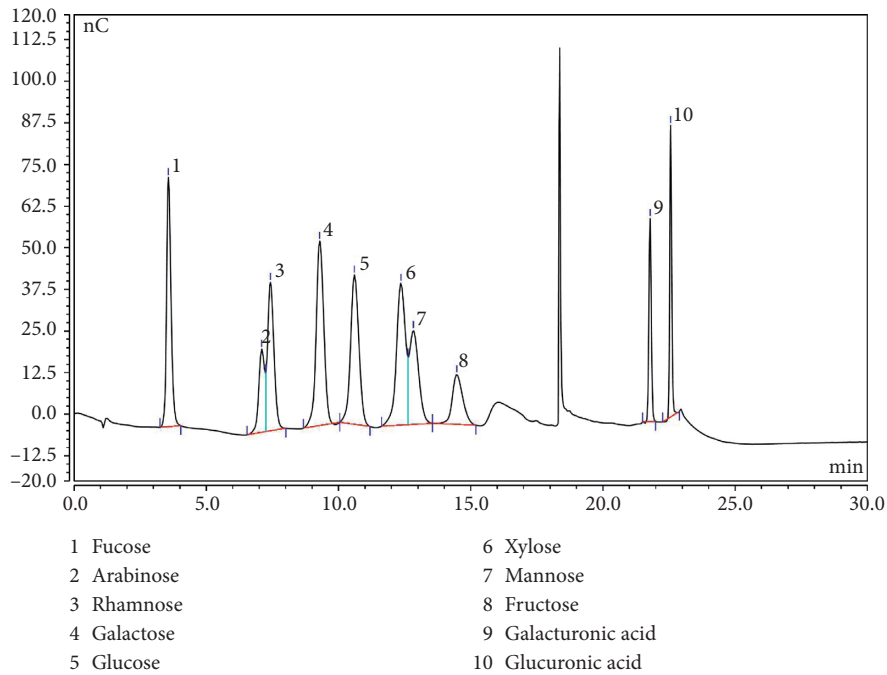Figure 7: FT-IR spectra of FTP in the control and optimized group.

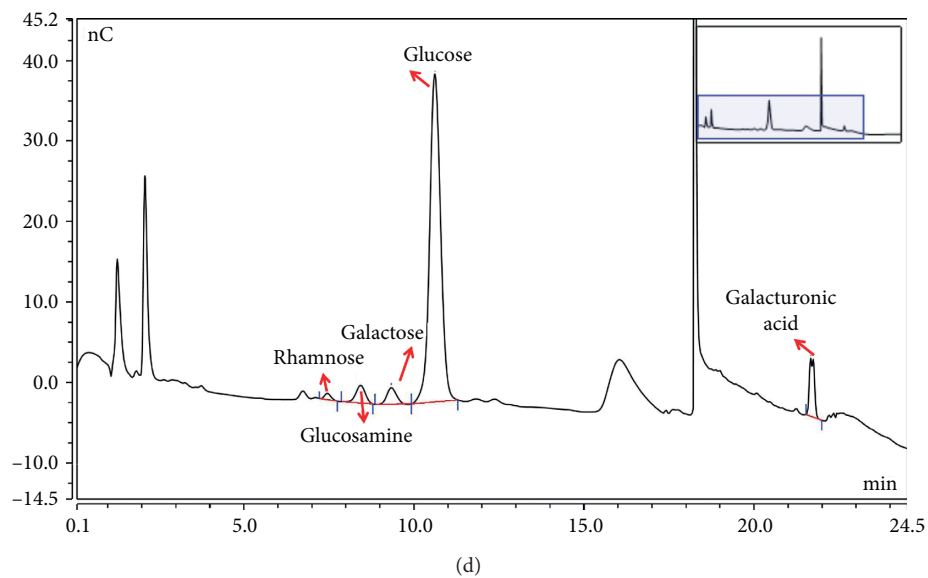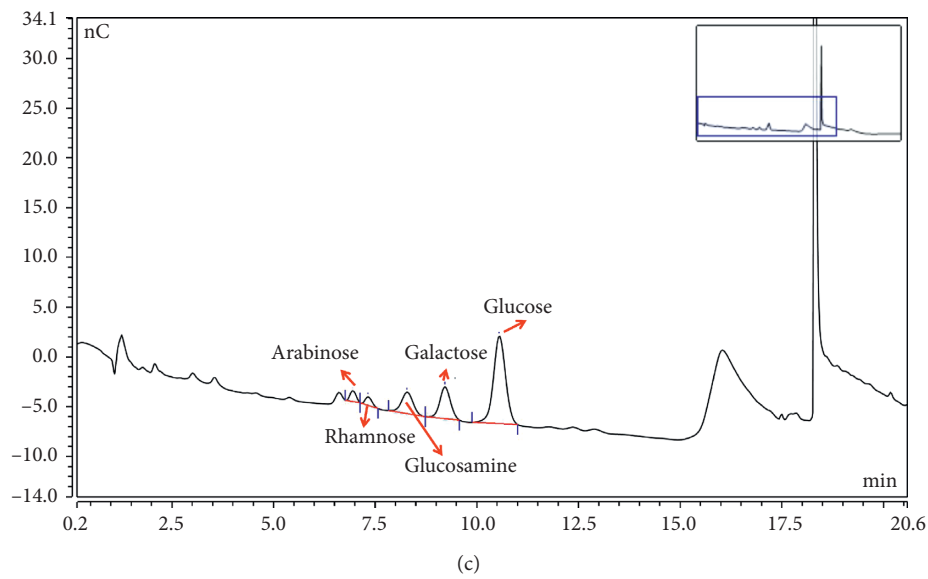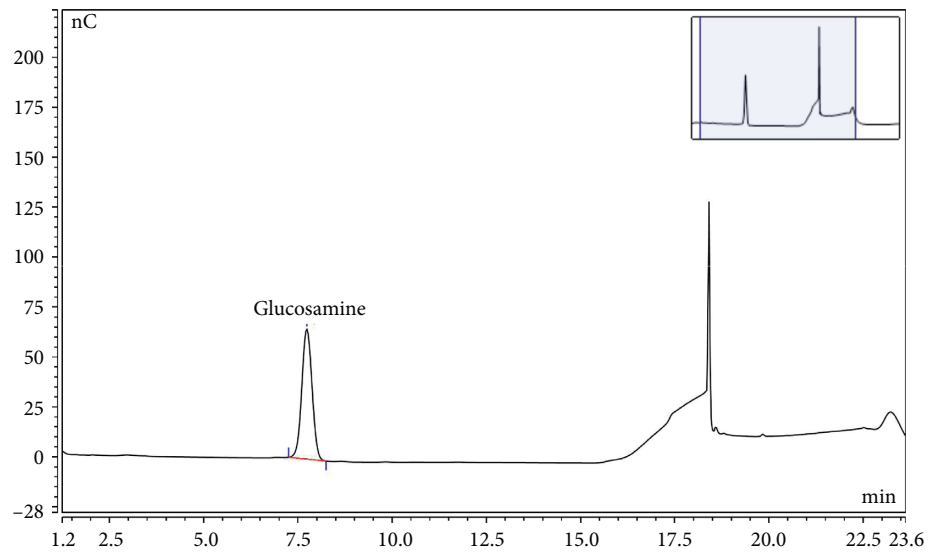| 1 Fucose | 6 Xylose |
2 Arabinose	7 Mannose
3 Rhamnose	8 Fructose
4 Galactose	9 Galacturonic acid
5 Glucose	10 Glucuronic acid

(a)

Figure 8: Continued.

Figure 8: Monosaccharide composition of FTP. (a) 10 standard monosaccharide profiles. (b) Chromatogram of glucosamine standard. (c) The monosaccharide composition of the control group. (d) The monosaccharide composition of the optimized group.

3.11. Monosaccharide Composition Change. The monosaccharide composition of FTP in the control and optimized group is shown in Figure 8. The monosaccharides in the control group were arabinose, rhamnose, glucosamine, galactose, and glucose. The monosaccharide composition of the optimization group was glucosamine, rhamnose, galactose, glucose, and galacturonic acid. Compared with the control group, the specificity of the optimized group was manifested in the presence of a galacturonic acid structure and the absence of arabinose, which was correlated with the increase of acid polysaccharide content in the optimized group (Figure 5). The monosaccharide types in the control group and the optimized group were similar, but the proportion was different. The content of glucosamine and galactose is decreased, and that of glucosamine and glucuronic acid is increased. This result implies that the change of the culture medium formula can affect the primary structure of FTP.

4. Conclusions and Prospect

The optimized medium combination of Tremella GT2# was determined to be potato extract 22.01%, glucose 29.38 g/L, peptone 2.61 g/L, KH_2PO_4 3 g/L, $MgSO_4 \cdot 7H_2O$ 1.5 g/L, and Vb_1 7 mg/L. The optimized comprehensive potato medium has high yield of FTP and a high utilization rate of reducing sugar to reduce resource waste. Compared with the existing level of FTP (4 g/L), the production was improved to 12.36 g/L [12–14]. Also, the fermentation period was reduced from 120 h to 72 h. The increase in production and the shortening of the fermentation cycle were an important breakthrough for the industrialization of FTP. Potato extract, glucose, and peptone were the factors that have the greatest impact on the productivity of FTPs. In addition, the composition and structure of FTPs have also been significantly affected. The FTPs obtained after optimization have a higher acidic polysaccharide content (66.19%) and a lower protein content (1.17%). The molecular weight and functional groups of FTP did not change significantly before and after optimization, but the composition of monosaccharides changed, and the configuration of polysaccharides changed from *a*-configuration to *ß*-configuration.

Previous studies have proved that acidic Tremella polysaccharide has a variety of biological activities, including immune activity [32], moisture retention [8], and anti-obesity activity [33]. Therefore, the optimized medium is of great significance in the production of active Tremella polysaccharide and fermentation-enabled wellness foods and functional fermented foods [34]. Regarding the reasons for the changes in the FTP structure, a detailed research plan will be proposed in the future work. In addition, the relationship between the biological activity of FTP and its structure should also receive further attention.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

Xia Ma, Meng Yang, and Yan He are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

All the authors have made substantive intellectual contributions to the study and given approval to the final version of the manuscript. Xia Ma and Meng Yang mainly contributed to the study design, manuscript revision, experimental studies, and statistical analysis; Yan He contributed to the supervision and project administration; Chengliang Li contributed to the funding acquisition; and Chuntao Zhai polished and revised the first draft.

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