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Effects of Main Nutrient Sources on Improving *Monascus* Pigments and Saccharifying Power of *Monascus purpureus* in Submerged Fermentation

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Abstract: *Hong Qu* (HQ), obtained through fermentation of various grains using *Monascus* spp., has been widely utilized as the main and characteristic initial saccharification and traditional fermentation starter in the food brewing industry. The quality, color, and flavor of HQ and HQ wine are closely related to the saccharifying power (SP) and *Monascus* pigments (MPs) of *Monascus* spp. In this study, to optimize the culture medium in submerged fermentation by *M. purpureus* G11 for improving SP and MPs, the effects of carbon source, nitrogen source, inorganic salts, and vitamins on SP activity and biosynthesis of MPs were explored through single-factor analysis and response surface Box–Behnken experiments. The results showed that the optimal medium composition was 6.008% rice powder, 1.021% peptone, 0.0049% $CuSO_4$, and 0.052% vitamin B1. Validation experiments performed under the optimized fermentation conditions showed a significant increase in MPs and SP by 14.91% and 36.24%, with maximum MPs and SP reaching 112.61 and 365.12 u/mL, respectively. This study provides a theoretical basis for enhancing MPs and SP in *M. purpureus* for HQ production, to improve the production efficiency and shorten the production cycle of HQ-related fermentation products.

Keywords: *Hong Qu*; saccharification starter; *Monascus* pigments; response surface methodology; culture medium optimization

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1. Introduction

Hong Qu (HQ) is one of the four traditional fermentation starters used in China, and is the main and characteristic initial saccharification and fermentation agent for fermented foods, such as *Hong Qu* glutinous rice wine (HQ wine), vinegar, and soy sauce [1–3]. HQ wine is the second most distinctive yellow rice wine in China. Unlike other types of yellow rice wine, HQ wine does not require the addition of colorants because the *Monascus* strains incorporated into the HQ wine during the brewing process can produce *Monascus* pigments (MPs), which give the wine a unique natural red–orange color. *Monascus* is widely used in the field of food fermentation, which can produce various important metabolites, including MPs, monacolin-like substances, and γ-aminobutyric acid, as well as various enzymes, such as amylase, glucoamylase, protease, pectinase, and esterification enzymes [4,5]. HQ used in the brewing industry must possess high saccharifying power (SP) and esterifying power [6,7]. The quality and safety of HQ play crucial roles in determining the flavor, color, functional activity, and overall quality of HQ wine [8]. Hence, enhancement of HQ quality is an important strategy for the development of the HQ wine industry. Although HQ has been widely used in the wine-making industry, the availability of only a few varieties of HQ starters and low fermentability significantly limit its applications [9].

Wine fermentation starters are the primary driving force for brewing yellow rice wine. Studies on HQ wine brewing have mainly focused on the microbial communities of the

starters, fermentation processes, active substances, and volatile flavor components [10–12]. In recent years, there has been a gradual increase in research on esterification enzymes and saccharifying enzymes of *Monascus* spp., especially on the isolation and identification of α-amylase (liquefying power) and its fermentation optimization, analysis of genes related to esterification enzymes and their biosynthetic pathways, enhancement of production efficiency, and shortening of the production cycle of HQ-related fermentation foods [13–17]. Although most of the studies on saccharifying enzymes of *Monascus* spp. have mainly focused on its applications, only a few studies have investigated their fundamental characteristics [18]. Saccharifying enzymes can fully convert starch into fermentable sugar and provide necessary substrates for the fermentation process in the food brewing industry [19]. Although many researchers have attempted to use saccharifying fermentation agents directly in the saccharification stage of the wine-making process and obtained good outputs, the agent can cause rapid hydrolysis of starch, resulting in microecological imbalance during the fermentation process, which can decrease the wine quality and create a bitter taste [20]. Thus, improving the SP of HQ has gained increasing attention in recent years [21]. Because *Monascus* plays an important role in the HQ brewing industry, it is particularly critical to obtain *Monascus* strains that not only have high SP but also adequate quantities of MPs. The use of such efficient *Monascus* strains in brewing HQ can improve the quality of HQ, enhance the color and flavor of HQ wine, and reduce the amount of red HQ used in brewing. Although a representative strain, *Monascus purpureus* G11, which can produce high yield of MPs and strong SP, was isolated in our previous study, the fermentation process was optimized.

It is well known that optimization of medium composition can increase the production of beneficial metabolites, such as MPs and lovastatin, in *Monascus* spp., and reduce the generation of harmful products, such as citrinin [22,23]. Many researchers have studied the effects of changes in carbon and nitrogen sources as well as the addition of vitamins and inorganic salts on the metabolism of *Monascus* spp. For instance, Yang Dongcheng et al. found that Fe^{2+} ions can increase the production of MPs in *Monascus* spp. [24]. However, the effects of medium optimization on SP and MPs in *M. purpureus* have not been extensively investigated. Response surface methodology (RSM) has been used for optimization of fermentation conditions based on the sophisticated interactions among multiple process variables [25,26]. However, the use of RSM for the optimization of fermentation conditions for improving SP is limited.

In the present study, RSM was employed for the optimization of fermentation conditions for both SP activity and the production of MPs in *M. purpureus*. Submerged liquid fermentation (SmF), with the advantages of short cultivation period, easy control of conditions, and good reproducibility, was employed to investigate systematically the effects of several categories of main nutrient sources on the brewing strain, *M. purpureus* G11. The medium composition was optimized for improving SP and MPs of liquid seed production and solid-state fermentation by identifying the preferred nutrients, including carbon sources, nitrogen sources, inorganic salts, and vitamins (and precursor substances). A mathematical model was established using the response surface Box–Behnken experimental design to optimize the culture medium and further improve SP and MPs. The main indicators of HQ quality, namely, SP and color value, were used as the evaluation standard. The results of this study provide systematic technical support for research on liquid seed production and solid-state fermentation processes for brewing HQ.

2. Materials and Methods

2.1. Strain and Culture Conditions

M. purpureus G11, which can produce both a high yield of MPs and SP activity, was isolated from HQ collected from HQ wine manufacturers and preserved in our laboratory and at the China Center for Type Culture Collection (CCTCC, Wuhan, China; CCTCC No. M 2023550). For the initial growth, *M. purpureus* G11 was inoculated onto MEA slant (6% malt extract and 2% agar; pH 6–7; sterilized at 121 °C for 30 min) and incubated at 35 °C for

10 d. The seed and liquid media consisted of 6% glucose, 3% soluble starch, 1% peptone, 0.15% KH_2PO_4, 0.2% K_2HPO_4, 0.2% $MgSO_4$, and 0.1% $NaNO_3$ without pH adjustment, and were sterilized at 121 °C for 30 min. The seed culture was prepared as described previously, and 6% of the seed was inoculated onto 50 mL of aseptic liquid fermentation medium and incubated at 35 °C and 180 rpm for 96 h [27].

2.2. Metabolite Detection

Color value (yield of MPs, u/mL) was determined according to the Chinese National Standard (GB1886.19-2015), with three biological replicates. The fermentation broth in each flask (0.05 mL) was mixed well and then extracted with 25 mL of 70% ethanol at 60 °C for 1 h. After filtration and cooling to room temperature, the extracted liquid or filtrate was serially diluted (A times), and the optical density (OD) was measured using an ultraviolet–visible spectrophotometer (UV-240, Shimadzu, Japan) against a 70% ethanol blank at 505 nm. The yield of MPs was calculated as follows: MPs = A × OD × V × 25, where A is the number of dilutions, OD is the optical density, and V is the total volume of the fermentation broth after fermentation (mL).

SP (u/mL) was determined according to the Chinese National Standard (QB/T 5188-2017), with three biological replicates. One unit (U) of enzyme activity was defined as the ability of 1.0 g of HQ to convert soluble starch into 1 mg of glucose in 1 h at 40 °C and pH 4.6. First, the fermentation broth in each flask (3 mL) was mixed well and then extracted with 22 mL of acetic acid–sodium acetate buffer solution at 35 °C for 2 h. After filtration and cooling to room temperature, the extracted liquid was used as the sample. Second, both 2% soluble starch solution (25 mL) and acetic acid–sodium acetate solution (5 mL) were added to two 50 mL colorimetric tubes in bottles A and B at 40 °C for 10 min. The extracted liquid sample (2 mL) was added to bottle A at 40 °C for 30 min, then 20% sodium hydroxide (0.2 mL) was added and mixed well, and cooled at 1–3 °C for 2 min. In bottle B (blank bottle), 20% sodium hydroxide (0.2 mL) was added and mixed well, cooled to room temperature, then 2 mL of the extracted liquid sample was added. Third, the solutions in bottles A and B (5 mL) were mixed well with iodine solution (10 mL) and 0.1 mol/L of sodium hydroxide (15 mL) for dark reaction (15 min), then 2 mol/L of sulfuric acid (2 mL) was added and the solution titrated with sodium thiosulfate solution until the blue color disappeared.

SP was calculated as follows: $SP = (V - V_1) \times C_1 \times 90.05 \times \frac{32.2}{5} \times \frac{1}{2} \times N \times 2$, where V (mL) is the volume of thiosulfate sodium solution in the blank in bottle B, V_1 (mL) is the volume of thiosulfate sodium solution in the sample in bottle A, C_1 (mol/L) is the concentration of thiosulfate sodium standard solution, 90.05 (mg) is the mass of glucose equivalent to 1.00 mL of thiosulfate sodium, 32.2 (mL) is the total volume of the reaction solution, 5 (mL) is the volume of the reaction solution used, 1/2 is the sample volume conversion factor from 2 mL to 1 mL, N is the dilution factor (25 mL/3 mL), and 2 is the reaction time conversion factor from 30 min to 1 h.

2.3. Effects of Carbon and Nitrogen Sources on MPs and SP by M. purpureus G11 in SmF

The medium composition was optimized for improving high MPs and SP in *M. purpureus* G11 by identifying the preferred nutrients. Accordingly, the effects of different carbon and nitrogen sources on *M. purpureus* G11 were determined using single-factor analysis by incubating *M. purpureus* G11 in basal medium containing different carbon sources (6%) (glucose, sucrose, corn starch, lactose, fructose, glycerin, maltose, rice powder, and galactose) and nitrogen sources (1%) (soybean powder, sodium glutamate, beef extract, fish meal, KNO_3, peptone, yeast extract, soy powder, NH_4NO_3, $(NH_4)_2SO_4$, and corn steep liquor), respectively, with the other factors remaining constant.

2.4. Effect of Inorganic Salt Sources on MPs and SP by M. purpureus G11 in SmF

Inorganic salts, including $CuSO_4$ (0.001% and 0.005%), $FeSO_4$ (0.005% and 0.01%), $LaCl_3$ (0.04% and 0.08%), $MnCl_2$ (0.005% and 0.01%), $MgSO_4$ (0.1%), KH_2PO_4 (0.2%), $CoCl_2$

(0.005%), $ZnSO_4$ (0.05% and 0.1%), and $CaCl_2$ (1% and 1.5%), were added to the basal medium to ascertain their effects on MPs and SP in *M. purpureus* G11 using single-factor analysis. After determining the optimal inorganic salt source, the effect of inorganic salt concentration (0.001%, 0.0025%, 0.005%, 0.0075%, and 0.01%) on MPs and SP in *M. purpureus* G11 was investigated.

2.5. Effect of Vitamins on MPs and SP by M. purpureus G11 in SmF

Vitamins, including vitamin B1 (VB1; 0.03% and 0.08%), vitamin B3 (VB3; 0.03% and 0.08%), vitamin B5 (VB5; 0.03% and 0.08%), vitamin B6 (VB6; 0.03% and 0.08%), vitamin B9 (VB9; 0.07% and 0.1%), erythorbic acid (EVC; 0.03% and 0.05%), and vitamin H (H; 0.03% and 0.08%), were individually added to the basal medium to investigate their effects on MPs and SP in *M. purpureus* G11 using single-factor analysis. After determining the optimal vitamin, the effect of vitamin concentration (0.01%, 0.03%, 0.05%, 0.07%, and 0.09%) on MPs and SP in *M. purpureus* G11 was analyzed.

2.6. Optimization of Medium Composition Using RSM

Based on the abovementioned results, the four selected medium components (rice powder, peptone, Cu^{2+}, and VB1) were further optimized through RSM to achieve high MPs and SP in *M. purpureus* G11 (Table 1).

Table 1. Factors and their code levels optimized using RSM experimental design.

Independent Factors	Symbol	Range and Code Levels		
		−1	**0**	**1**
Rice powder (%)	A	5	6	7
Peptone (%)	B	0.5	1	1.5
Cu^{2+} (%)	C	0.003	0.005	0.007
VB1 (%)	D	0.03	0.05	0.07

2.7. Statistical Analysis

All data were analyzed using SPSS and ANOVA to determine significant differences. All the experiments were performed in triplicate, and the average value was employed. The data were plotted using Origin Pro 8.6.

3. Results and Discussion

3.1. Effects of Carbon Source on MPs and SP by M. purpureus G11

The carbon source is one of the most important components in the fermentation medium, which mainly provides the carbon skeleton for the microbial cells and synthesized products and supplies energy for metabolic activities [28,29]. The properties and utilization limitations of carbon sources can directly affect *M. purpureus* metabolism, which in turn can influence material synthesis and degradation, having a certain impact on the production of MPs [30,31]. The results of the present study indicated that the type of carbon source had a significant impact on MPs and SP by *M. purpureus* G11 (Figure 1). The presence of fructose, maltose, and rice powder as carbon sources in *M. purpureus* G11 culture caused higher production of MPs and faster mycelial growth, with the yield of MPs reaching 95.81, 96.2, and 104.15 u/mL, respectively. Furthermore, cultivation of *M. purpureus* G11 in the presence of rice powder as carbon source caused higher SP (267.8 u/mL), which was significantly higher than that noted in the presence of other carbon sources ($p < 0.05$).

Glycosylation capacity is one of the important indicators reflecting the enzymatic ability of *M. purpureus*. The level of glycosylation capacity directly reflects the ability of *M. purpureus* to produce fermentable sugars, which ultimately affects wine production. *M. purpureus* exhibited the best saccharifying ability as well as higher production of MPs (104.15 u/mL) when cultivated in the presence of rice powder as carbon source. This obvious increase in MPs and SP in the presence of rice powder could possibly be owing to

the ability of *M. purpureus* G11 to completely utilize the various nutrients in rice powder during cultivation. Therefore, rice powder was selected as the suitable carbon source for achieving high MPs and SP in *M. purpureus* G11.

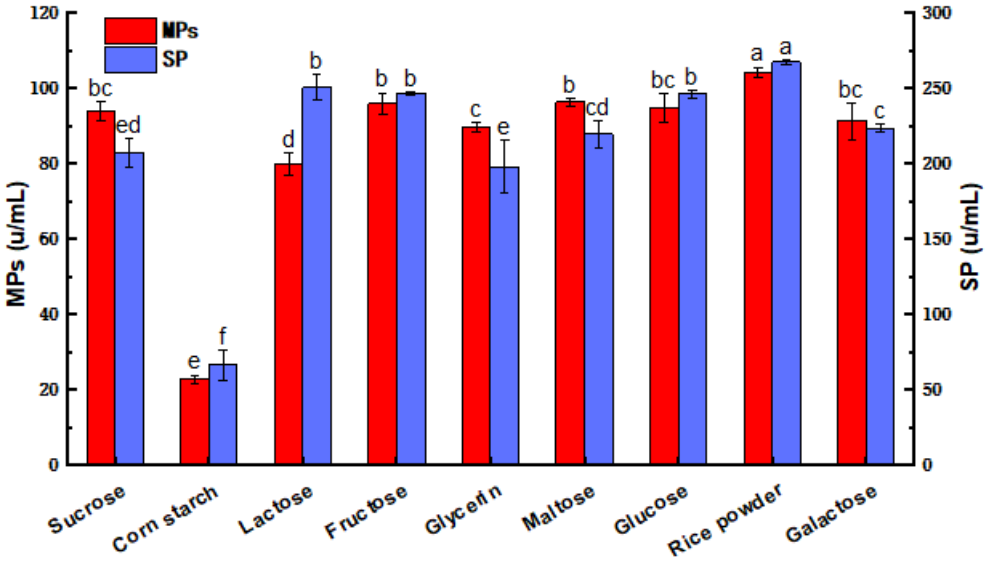

Figure 1. Effects of different carbon sources on MPs and SP by *M. purpureus* G11. Values are expressed as means ± SD (n = 3). Different lowercase letters (a, b, c, d, e and f) in the figure indicate a statistically significant difference ($p < 0.05$).

It is worth noting that when corn starch was used as the carbon source, both MPs and SP were significantly low. We speculated that except for corn starch and rice flour, all the other carbon sources are small molecules that can be quickly dissolved and absorbed as nutrients. Only corn starch and rice powder are mixed nutrients, and rice powder contains abundant fast nutrients, such as monosaccharides and disaccharides, which can also provide the necessary nutrients for the growth and metabolism of *M. purpureus* G11. However, corn starch is the only pure long-chain starch among the carbon sources without fast nutrient components. The strain cannot grow rapidly, thus affecting the production of MPs and SP activity. In addition, the addition of corn starch results in a semisolid and viscous state of the culture medium, leading to low dissolved oxygen levels, which also restricts the initial growth of the strain. Therefore, neither the composition of corn starch nor the state of the culture medium can improve the growth of the strain and promote production of MPs and SP activity.

3.2. Effects of Nitrogen Source on MPs and SP by M. purpureus G11

A nitrogen source is essential for microbial growth and synthesis of metabolites, and plays a crucial role in the process of MPs and SP by *M. purpureus* during fermentation [32]. The optimal nitrogen source for *M. purpureus* can vary depending on the strain [33–35]. As shown in Figure 2, the type of nitrogen source had a significant impact on the production of MPs and the saccharifying ability of *M. purpureus* G11. Among them, peptone and corn steep liquor had higher saccharification ability than other nitrogen sources, with SP of 263.55 and 258.55 u/mL, respectively, with no significant difference ($p > 0.05$). By contrast, production of MPs by *M. purpureus* G11 in the presence of peptone and corn steep liquor was 103.04 and 15.48 u/mL, respectively, with peptone causing significantly higher yield of MPs than corn steep liquor ($p < 0.05$). Hence, peptone was selected as the suitable nitrogen source for obtaining high MPs and SP in *M. purpureus* G11.

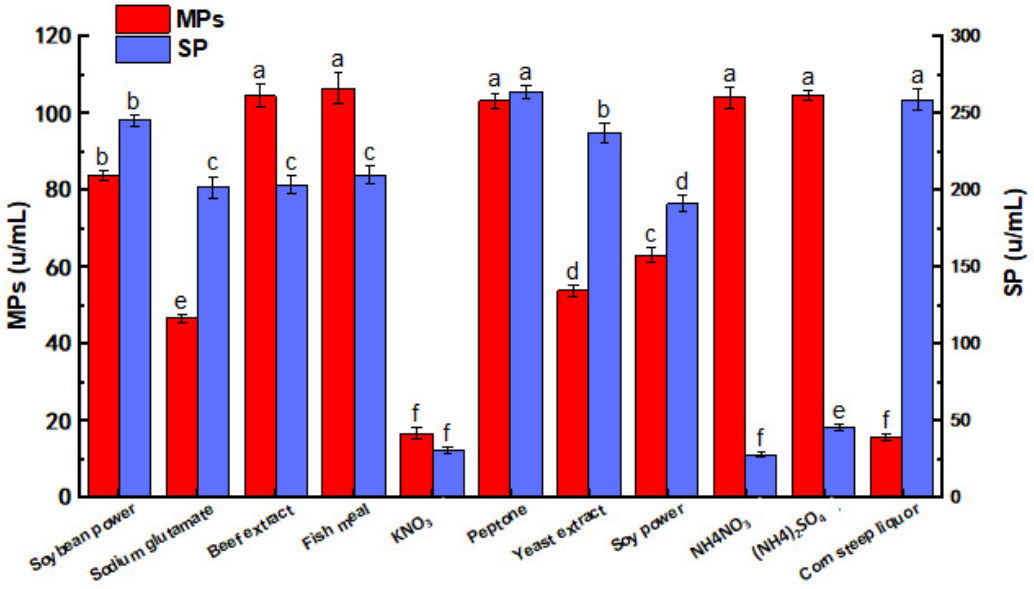

Figure 2. Effects of different nitrogen sources on MPs and SP by *M. purpureus* G11. Values are expressed as means ± SD (n = 3). Different lowercase letters (a, b, c, d, e and f) in the figure indicate a statistically significant difference ($p < 0.05$).

3.3. Effects of Inorganic Salts on MPs and SP by M. purpureus G11

Inorganic salts are essential for microbial growth and reproduction, and metal ions also play an important regulatory role in the secondary metabolism of microorganisms [36]. Different metal ions have different effects on the production of MPs and the saccharifying activity in *M. purpureus*. Accordingly, in the present study, the optimal inorganic salt and its appropriate concentration for achieving high MPs and SP in *M. purpureus* G11 was screened among Cu^{2+}, Fe^{2+}, La^{3+}, Mn^{2+}, Mg^{2+}, K^{+}, Co^{2+}, Zn^{2+}, and Ca^{2+} as exogenous metal ion additives [37]. Based on the results of preliminary experiments, $CuSO_4$ (0.001% and 0.005%), $FeSO_4$ (0.005% and 0.01%), $LaCl_3$ (0.04% and 0.08%), $MnCl_2$ (0.005% and 0.01%), $MgSO_4$ (0.1%), KH_2PO_4 (0.2%), $CoCl_2$ (0.005%), $ZnSO_4$ (0.05% and 0.1%), and $CaCl_2$ (1% and 1.5%) were added to the culture medium of *M. purpureus* G11 to explore their effects on MPs and the saccharifying capacity of the strain.

As shown in Figure 3a, the type of inorganic salt had a significant effect on MPs and the saccharifying ability of *M. purpureus* G11. Addition of 0.001% Cu^{2+} significantly promoted the SP activity (309.62 u/mL) of *M. purpureus* G11 in comparison with other inorganic salts ($p < 0.05$). Moreover, 0.001% Cu^{2+} addition achieved higher yield of MPs (104.2 u/mL) in *M. purpureus* G11, which was only slightly lower than those noted with the addition of 0.1% Mg^{2+} and 0.2% K^{+}, but did not show any significant difference ($p > 0.05$). Based on these results, Cu^{2+} was selected as the optimal exogenous metal ion additive for achieving high MPs and SP in *M. purpureus* G11.

Subsequently, the optimal Cu^{2+} concentration for achieving high MPs and SP in *M. purpureus* G11 was determined by adding different concentrations of Cu^{2+} (0.001%, 0.003%, 0.005%, 0.007%, and 0.009%) to the culture medium. The blank control comprised *M. purpureus* G11 culture medium without Cu^{2+} addition. As shown in Figure 3b, the yield of MPs decreased with the increase in Cu^{2+} concentration, and the highest yield of MPs (105.86 u/mL) was achieved with the addition of 0.003% Cu^{2+}. Similarly, the SP activity decreased with the increase in Cu^{2+} concentration, and the highest SP activity (335.2 u/mL) was observed in the presence of 0.005% Cu^{2+}. Based on these results, 0.005% Cu^{2+} was selected as the optimal Cu^{2+} concentration for subsequent experiments.

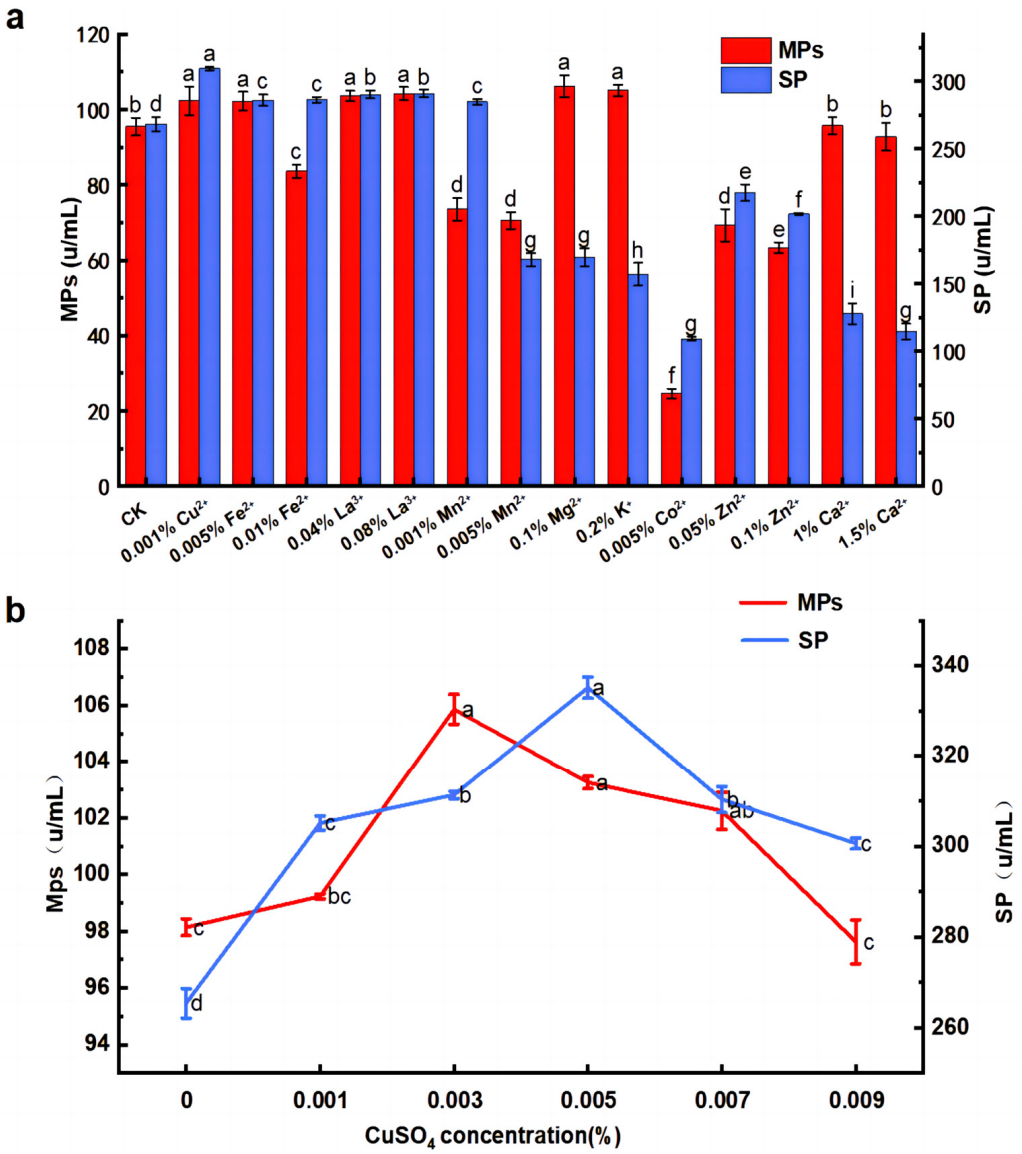

Figure 3. Effects of (**a**) different inorganic salts and (**b**) Cu^{2+} concentrations on MPs and SP in *M. purpureus* G11. Values are expressed as means ± SD (n = 3). Different lowercase letters (a, b, c, d, e, f, g, and h) in the same figure indicate a statistically significant difference ($p < 0.05$).

3.4. Effects of Vitamins on MPs and SP by M. purpureus G11

Based on the results of preliminary experiments, VB1 (0.03% and 0.08%), VB3 (0.03% and 0.08%), VB5 (0.03% and 0.08%), VB6 (0.03% and 0.08%), VB9 (0.07% and 0.1%), EVC (0.03% and 0.05%), and H (0.03% and 0.08%) were selected as exogenous vitamin additives and added to the culture medium to determine their effects on MPs and SP in *M. purpureus* G11. As shown in Figure 4a, vitamins had a significant effect on the production of MPs and SP activity in *M. purpureus* G11. In particular, 0.03% VB1 and 0.08% VB1 significantly increased SP, reaching 316.5 and 320.21 u/mL, respectively, in comparison with other vitamins ($p < 0.05$). Similarly, addition of 0.03% VB1 and 0.08% VB1 increased the yield of MPs to 99.16 and 100.09 u/mL, respectively. Therefore, VB1 was selected as the optimal vitamin for achieving high MPs and SP in *M. purpureus* G11.

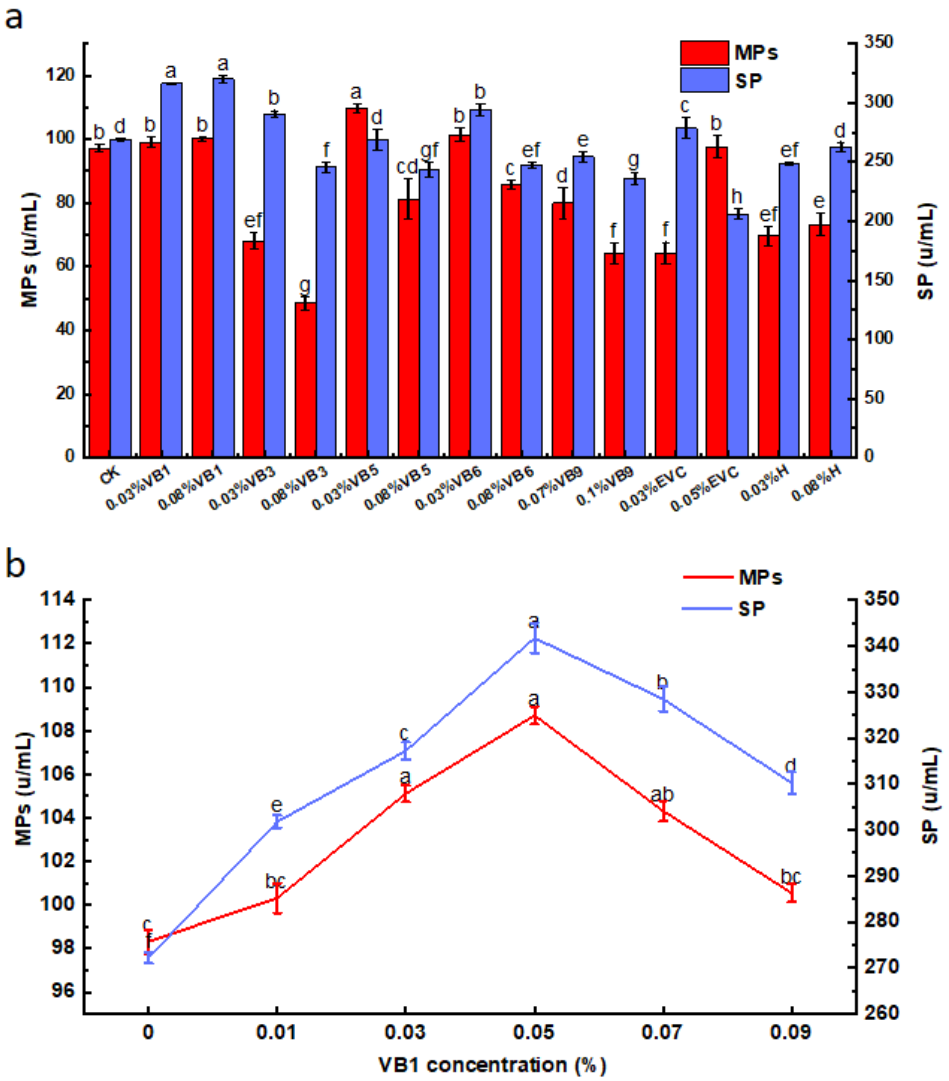

Figure 4. Effect of (**a**) vitamins and (**b**) different concentrations of VB1 on MPs and SP by *M. purpureus* G11. Values are expressed as means ± SD (n = 3). Different lowercase letters (a, b, c, d, e, f, g, and h) in the same figure indicate a statistically significant difference ($p < 0.05$).

Subsequently, the optimal concentration of VB1 for obtaining high MPs and SP in *M. purpureus* G11 was ascertained by adding different concentrations of VB1 (0.01%, 0.03%, 0.05%, 0.07%, and 0.09%) to the culture medium. The blank control comprised *M. purpureus* G11 culture medium without VB1 addition. As shown in Figure 4b, the yield of MPs first increased and then decreased with the increasing VB1 concentration. The yield of MPs was the highest (108.7 u/mL) in the presence of 0.05% VB1. Similarly, the SP activity first increased and then decreased with increasing VB1 concentration. The highest SP activity of 341.75 u/mL was achieved with the addition of 0.05% VB1. Therefore, 0.05% VB1 was chosen as the optimal concentration for subsequent experiments.

3.5. Analysis of RSM Results for MPs and SP by M. purpureus G11

Based on the abovementioned results, the four selected medium components (rice powder, peptone, Cu^{2+}, and VB1) were further optimized using response surface Box–Behnken experiments by establishing regression equations between nutrient sources and MPs and SP, with yield of MPs and SP activity employed as response variables. The design and results of the response surface Box–Behnken experiments are presented in Table 2. The quadratic polynomial regression equations for MPs (Y1) and SP (Y2) were fitted using Design-Expert 13 software as follows:

Table 2. Design and results of response surface Box–Behnken experiments.

No.	Rice Powder/%	Peptone/%	Cu^{2+}/%	VB1/%	Observed MP Yield/(u/mL)	Observed SP Activity/(u/mL)
1	5	0.5	0.005	0.05	106.4	339.56
2	7	0.5	0.005	0.05	105.6	344.63
3	5	1.5	0.005	0.05	106.2	347.17
4	7	1.5	0.005	0.05	104.4	349.78
5	6	1	0.003	0.03	106.5	323.66
6	6	1	0.007	0.03	102.5	327.63
7	6	1	0.003	0.07	106.2	334.25
8	6	1	0.007	0.07	103.2	340.14
9	5	1	0.005	0.03	103.1	329.45
10	7	1	0.005	0.03	103.2	332.64
11	5	1	0.005	0.07	105.7	338.16
12	7	1	0.005	0.07	105.2	341.57
13	6	0.5	0.003	0.05	107.5	332.72
14	6	1.5	0.003	0.05	106.2	337.80
15	6	0.5	0.007	0.05	103.4	337.84
16	6	1.5	0.007	0.05	104.4	342.25
17	5	1	0.003	0.05	107.5	334.42
18	7	1	0.003	0.05	106.5	337.80
19	5	1	0.007	0.05	103.8	339.63
20	7	1	0.007	0.05	102.9	342.76
21	6	0.5	0.005	0.03	102.1	327.04
22	6	1.5	0.005	0.03	101.5	332.16
23	6	0.5	0.005	0.07	103.1	336.59
24	6	1.5	0.005	0.07	103.2	341.77
25	6	1	0.005	0.05	113.42	364.15
26	6	1	0.005	0.05	114.2	365.86
27	6	1	0.005	0.05	113.5	360.85
28	6	1	0.005	0.05	113.2	364.26
29	6	1	0.005	0.05	114.2	358.13

$$Y1 = -0.4083A - 0.1833B - 1.68C + 0.6417D - 0.25AB + 0.025AC - 0.15AD + 0.575BC + 0.175BD + 0.25CD - 3.89A^2 - 4.7B^2 - 3.87C^2 - 5.76D^2,$$

$$Y2 = 1.73A + 2.71B + 2.47C + 4.99D - 0.6140AB - 0.0633AC + 0.056AD - 0.1668BC + 0.0167BD + 0.481CD - 8.77A^2 - 9.8B^2 - 14.6C^2 - 17.83D^2.$$

Table 3 shows the variance analysis of the MPs' regression model using Design-Expert 13 software. Factors C (Cu^{2+}), A^2, B^2, C^2, and D^2 had highly significant effects on the yield of MPs ($p < 0.01$), whereas factor D (VB1) had a significant effect on MPs ($p < 0.05$). However, factors A (rice powder), B (peptone), AB, AC, AD, BC, BD, and CD had no significant effects on the yield of MPs ($p > 0.05$). Based on the magnitude of the first-order coefficients in the quadratic regression equation, the order of the factors in terms of their effects on the yield of MPs was Cu^{2+} > VB1 > rice powder > peptone, with Cu^{2+} having the most significant effect on the yield of MPs. With the yield of MPs as the response variable, the model presented $p < 0.01$, indicating that the quadratic equation model was highly significant. Meanwhile, the lack of fit of the model was not significant ($p = 0.07 > 0.05$), suggesting that the experimental results and mathematical model fitted well, and that the model selection was appropriate. Therefore, this model can be used to predict the test results. The coefficient of determination, R^2, of the regression equation was 0.9722, implying that 97.22% of the variation in color value could be attributed to the selected variables.

Table 3. ANOVA results of the MPs' regression model.

Source	Sum Sq	Df	Mean Sq	*F* Value	*p* Value	Significant
Model	405.76	14	28.98	34.93	<0.0001	**
A	2	1	2	2.41	0.1428	
B	0.4033	1	0.4033	0.4861	0.4971	
C	34	1	34	40.98	<0.0001	**
D	4.94	1	4.94	5.95	0.0286	*
AB	0.25	1	0.25	0.3013	0.5917	
AC	0.0025	1	0.0025	0.003	0.957	
AD	0.09	1	0.09	0.1085	0.7468	
BC	1.32	1	1.32	1.59	0.2274	
BD	0.1225	1	0.1225	0.1476	0.7066	
CD	0.25	1	0.25	0.3013	0.5917	
A^2	97.92	1	97.92	118.02	<0.0001	**
B^2	143.15	1	143.15	172.54	<0.0001	**
C^2	97.29	1	97.29	117.26	<0.0001	**
D^2	215.23	1	215.23	259.41	<0.0001	**
Residual	11.62	14	0.8297			
Lack-of-fit	10.75	10	1.07	4.95	0.0685	
Pure error	0.8683	4	0.2171			
Cor. total	417.38	28				

Note: **, highly significantly different ($p < 0.01$), * significantly different ($p < 0.05$). A: Rice powder; B: Peptone; C: Cu^{2+}; D: VB1.

The ANOVA results of the SP regression model obtained using Design-Expert 13 software are shown in Table 4. Factors B (peptone), C (Cu^{2+}), D (VB1), A^2, B^2, C^2, and D^2 had a highly significant effect on SP activity ($p < 0.01$), whereas factor A (rice powder) had a significant effect on SP activity ($p < 0.05$). By contrast, factors AB, AC, AD, BC, BD, and CD had no significant effect on SP activity ($p > 0.05$). Based on the magnitude of the first-order coefficients of the quadratic regression equation, the order of the factors in terms of their effects on SP activity was VB1 > peptone > Cu^{2+} > rice powder, with VB1 having the greatest effect on SP activity. When the SP activity was used as the response variable, the model presented $p < 0.01$, indicating that the quadratic equation model was highly significant. At the same time, the lack of fit of the model was not significant ($p = 0.97 > 0.05$), suggesting that the experimental results and mathematical model fitted well, and that the model was suitable for predicting the test results. The coefficient of determination, R^2, of this regression equation was 0.9832, implying that 98.32% of the variation in enzyme activity could be attributed to the selected variables.

3.6. Model Validation and Confirmation

The response surface graph clearly showed the interaction between the different factors, with steeper slope indicating higher impact of the factors on the response value, and shallower slope denoting lower impact of the factors on the response value [38]. Figure 5 illustrates the interactive effects of various factors on the yield of MPs, with steep response surfaces indicating a significant influence of the interaction between factors on the yield of MPs. Figure 6 reveals the interactive effects of various factors on SP activity. The steep response surfaces (Figure 6b–e) implied that the SP activity increased and then decreased with the increasing levels of rice powder and Cu^{2+}, rice powder and VB1, peptone and Cu^{2+}, peptone and VB1, and VB1 and Cu^{2+}, denoting a significant influence of the interaction between factors on SP activity. By contrast, the relatively flat response surface (Figure 6a) suggested that the interaction between peptone and rice powder had little effect on SP activity.

Table 4. ANOVA results of the SP regression model.

Source	Sum Sq	DF	Mean Sq	*F* Value	*p* Value	Significant
Model	3621.32	14	258.67	58.41	<0.0001	**
A	36.01	1	36.01	8.13	0.0128	*
B	88.23	1	88.23	19.92	0.0005	**
C	73.08	1	73.08	16.5	0.0012	**
D	298.98	1	298.98	67.51	<0.0001	**
AB	1.51	1	1.51	0.3405	0.5688	
AC	0.016	1	0.016	0.0036	0.9529	
AD	0.0125	1	0.0125	0.0028	0.9583	
BC	0.1112	1	0.1112	0.0251	0.8763	
BD	0.0011	1	0.0011	0.0002	0.9877	
CD	0.9254	1	0.9254	0.209	0.6546	
A^2	498.87	1	498.87	112.65	<0.0001	**
B^2	623.57	1	623.57	140.81	<0.0001	**
C^2	1383.4	1	1383.4	312.39	<0.0001	**
D^2	2063.08	1	2063.08	465.88	<0.0001	**
Residual	62	14	4.43			
Lack-of-fit	23.23	10	2.32	0.2396	0.9696	
Pure error	38.77	4	9.69			
Cor. total	3683.32	28				

Note: **, highly significantly different ($p < 0.01$), * significantly different ($p < 0.05$). A: Rice powder; B: Peptone; C: Cu^{2+}; D: VB1.

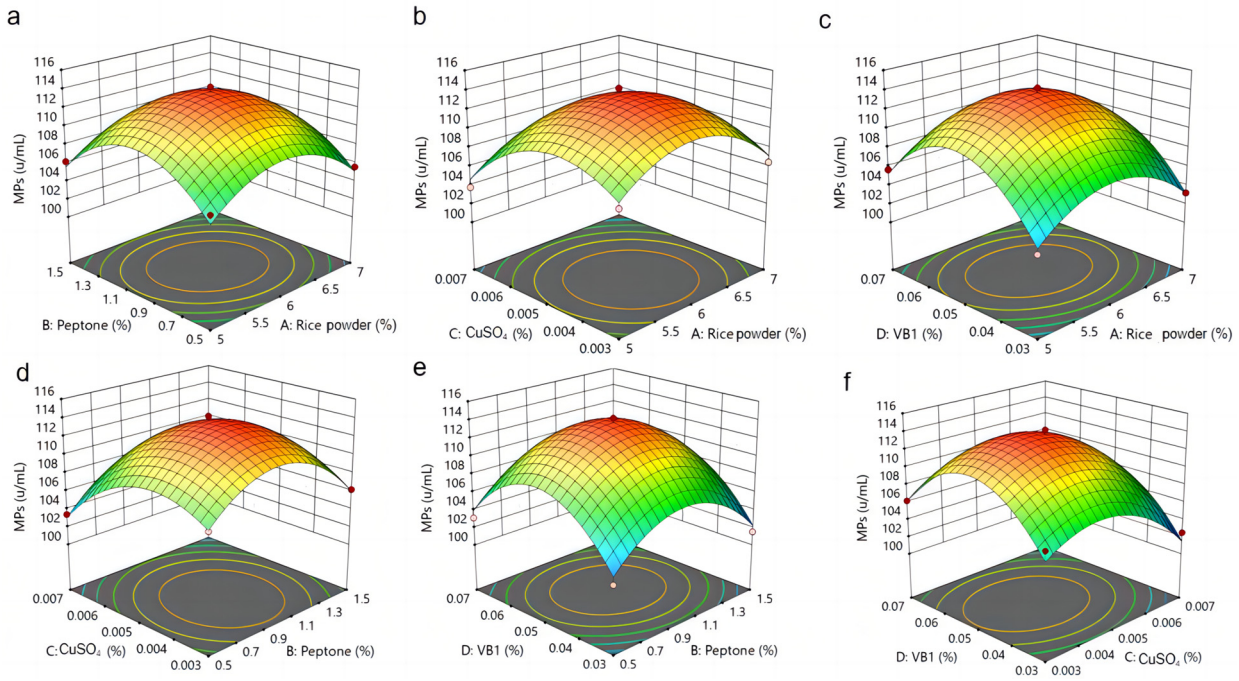

Figure 5. Response surface diagram of the influence of factors interaction (A: Rice powder; B: Peptone; C: $CuSO_4$; D: VB1) on the response of the yield of MPs.

Through optimization using Design-Expert 13 data analysis software, the optimal process parameters for obtaining a high yield of MPs and SP activity in *M. purpureus* G11 were determined to be 6.008% rice powder, 1.021% peptone, 0.0049% Cu^{2+}, and 0.052% VB1. Under these optimal conditions, the theoretical yields of MPs and SP were 113.77 and 362.91 u/mL, respectively. However, considering the operability, the optimal conditions were adjusted to 6% rice powder, 1% peptone, 0.005% Cu^{2+}, and 0.05% VB1, and the adjusted parameters were validated through experiments. The MPs and SP under these adjusted conditions were 112.61 and 365.12 u/mL, respectively, which were close to the

theoretical predicted values, indicating that the equation obtained through RSM optimization had practical significance and that the model can correctly predict the production of MPs and SP activity in *M. purpureus* G11.

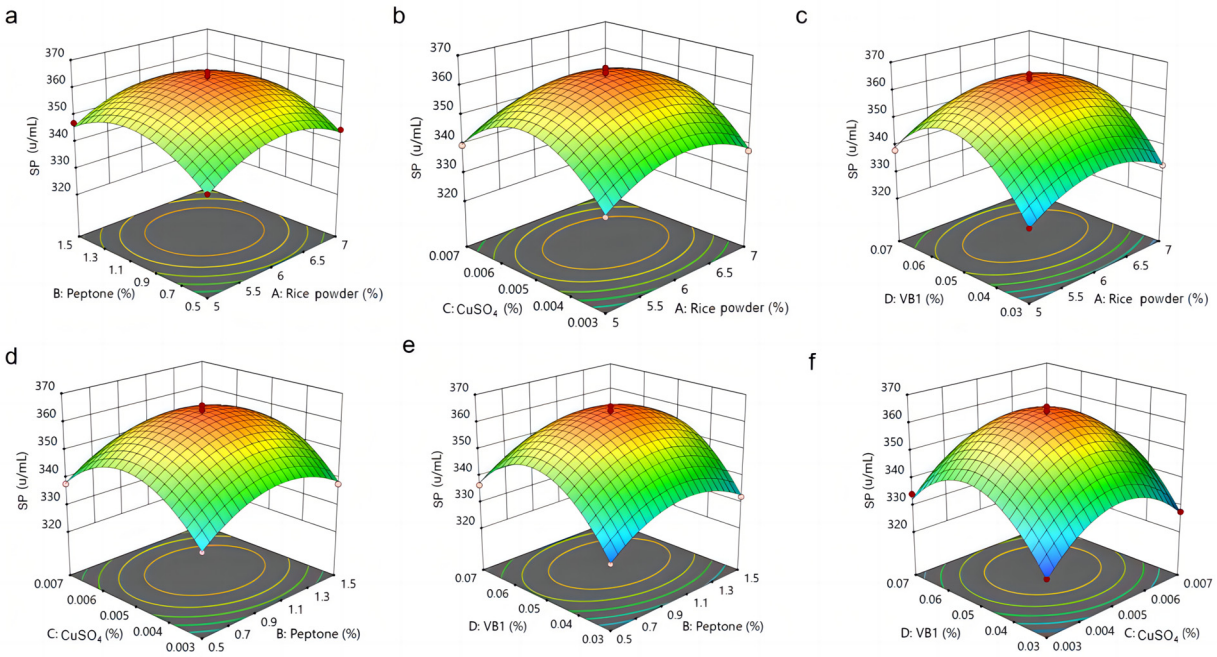

Figure 6. Response surface diagram of the influence of factors interaction (A: Rice powder; B: Peptone; C: $CuSO_4$; D: VB1) on the response of on SP activity.

4. Conclusions

SP activity is one of the most important indicators for assessing the quality and brewing process of HQ. The SP directly determines the utilization rate of raw materials and yield of HQ wine. *Monascus* is one of the key fungal genera that affects the saccharification ability and color of HQ. The difference in the SP activity and production of MPs can lead to variations in the characteristics of HQ, which in turn can affect its fermentation quality. In this study, different medium compositions were examined using single-factor experiments to select the optimal concentrations of medium components for liquid-state fermentation. RSM revealed that the optimum medium components for achieving a high yield of MPs and SP activity were 6.008% rice powder, 1.021% peptone, 0.0049% Cu^{2+}, and 0.052% VB1, which were verified by a liquid-state fermentation experiment. Under these optimal conditions, the yield of MPs and SP activity of 112.61 and 365.12 u/mL, respectively, were achieved. The results of this study provide systematic technical support for HQ production and further research on solid-state fermentation technology. Moreover, exploration and development of production technology for achieving high-quality standardized HQ can offer a theoretical basis and technical guarantee for upgrading the products in HQ-related industries.

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