RESEARCH ARTICLE

Optimization of preparation technology and study of properties of *Monascus* polysaccharides

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***Monascus* polysaccharides (MPS), a key metabolite of *Monascus*, possess multiple health-promoting activities such as antioxidant and immunomodulatory effects. However, their limited production yield restricts industrial applications. This study aimed to optimize the submerged fermentation process for MPS production and characterize their physicochemical properties and antioxidant activities. Single factor experiments and response surface methodology (RSM) were used to evaluate the effects of carbon sources, nitrogen sources, inorganic salts, initial pH, culture time, inoculation amount, and inoculum age on MPS yield. The structure of MPS was analyzed *via* monosaccharide composition and molecular weight determination, and antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. The optimized conditions were determined as 41.15 g/L sucrose, 12.81 g/L yeast extract powder, 0.8 g/L $MgSO_4 \cdot 7H_2O$, 0.8 g/L KH_2PO_4, 1.6 g/L $K_2HPO_4 \cdot 3H_2O$, pH 5.34, inoculation amount of 5%, and inoculum age of 36 h. Under these conditions, the MPS yield significantly increased to 6.15 g/L. The polysaccharides exhibited DPPH and ABTS radical scavenging rates of 79.47% and 71.46%, respectively. This study provided a theoretical basis for enhancing MPS production and promoted their potential applications in functional food and pharmaceutical industries.**

Keywords: *Monascus* polysaccharides; optimization; physical properties; antioxidant activity.

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Introduction

Monascus is a filamentous saprophytic fungus belonging to the phylum *Eumycophyta*, subphylum *Ascomycotina*, class *Plectomycetes*, order *Eurotiaes*, and family *Monascaceae* [1]. The metabolites of *Monascus* are highly concerned including primary metabolites such as ethanol, acids, esters, enzymes, and other aroma components, as well as secondary metabolites such as *Monascus* polysaccharides (MPS), *Monascus* pigments, γ-aminobutyric acid, monacolin K, ergosterol, citrinin, *etc*. [2]. Polysaccharides are considered as an effective component in the traditional Chinese medicine Hongqu, which is widely used in the field of traditional Chinese medicine and has various effects such as anti-inflammation and promoting

digestion [3]. As a complex polysaccharide structure, MPS has various linkage patterns among monosaccharides, forming straight chains and branches, and there are α and β isomers as well as pyran ring and furan ring isomers [4].

The MPS from Hong Qu mycelium has been successfully separated into four sub-fractions including MPS-1 (18.0%), MPS-2 (27.1%), MPS-3 (12.6%), and MPS-4 (14.7%), and the structure is relatively complex [5]. Due to the complexity of the study, it had not received sufficient attention in the past. However, MPS exhibits bioactivities such as anti-cancer [6], antioxidant [7], antibacterial [8], and immunomodulatory activities [9]. Numerous factors influence its bioactivities including its structure, molecular weight, functional groups, and monosaccharide composition. Therefore, MPS has become one of the current research hotspots. Although the research on MPS has initially shown signs of systematization, there is still a lack of studies to increase MPS yield, which restricts the development of MPS. To overcome these limitations and promote the development of MPS, further research and exploration are of particular importance. It has been found that the ways to improve polysaccharide production include optimized media composition, strain screening and mutagenesis, exogenous additives, gene knockout and overexpression techniques. The production of MPS by liquid submerged fermentation technology greatly shortens the production cycle and reduces the production costs [10]. Polysaccharides exhibit an intricate relationship with both the production process and the composition of the culture medium, which is not only fundamental to the biosynthesis of polysaccharides but also exerts a profound influence on their yield [11]. Feng *et al*. optimized the fermentation medium to enhance the polysaccharide yield of *Ganoderma lucidum*, and the yield of the *Ganoderma lucidum* polysaccharide reached 2.03 g/L [12]. Chen *et al*. optimized the concentrations of the carbon and nitrogen sources to obtain the maximum yield of exopolysaccharides (EPS) and intracellular polysaccharides (IPS) production of truffles *Tuber borchii* with 80 g/L sucrose and 20 g/L yeast extract for the maximum yields of 0.70 g/L EPS and 1.76 g/L IPS, respectively [11]. Yang *et al*. obtained the maximum production of antifungal metabolites of *Streptomyces* sp. KN3 by optimizing the addition amount of carbon and nitrogen sources. The antifungal effect of the optimized strain fermentation dilution was significantly enhanced [13]. Therefore, optimizing the composition of culture medium is an important way to improve the production of fermentation products. In addition, adding exogenous substances is also a way to promote the production of fermentation products. Xie *et al*. found that genistein could promote the production of MPS and enhance the immunomodulatory activity of MPS [14, 15]. Yang *et al*. enhanced the yield of MPS by optimizing culturing conditions and adding flavonoids [16]. In addition, using genetic engineering techniques to modify *Monascus* to improve its polysaccharide synthesis ability is also a potential method [17].

According to previous investigations, key factors such as carbon source, nitrogen source, inorganic salts, initial pH value, and culture time have an important influence on the yield of polysaccharide. In this study, the single factor experiment was used to obtain the parameters affecting the influencing factors of the MPS production of submerged fermentation of *Monascus*, and the response surface analysis were used to study the influencing factors and their interactions. The monosaccharide composition and molecular weight of MPS were studied, and its antioxidant bioactivity was evaluated. The results of this study provided a novel approach to enhance MPS production through process optimization, which might facilitate large-scale industrial production and expand the application of MPS in functional foods, nutraceuticals, and pharmaceuticals.

Materials and methods

Fermentation process

The seed medium contained 30 g/L glucose, 2 g/L KH_2PO_4, 3 g/L $NaNO_3$, 0.5 g/L KCl, 0.5 g/L $MgSO_4·7H_2O$ was inoculated into the fermentation medium at a ratio of 1:20 (v/v), while the fermentation medium contained 20 g/L sucrose, 2 g/L yeast extract powder, 0.5 g/L KH_2PO_4, 1 g/L $K_2HPO_4·3H_2O$, 1 g/L $MgSO_4·7H_2O$. Fermentation was performed using *Monascus purpureus* YY-1, *Monascus purpureus* M2, *Monascus purpureus* ZS, *Monascus pilosus* FJ-1, *Monascus albidus* FM-23 preserved in our laboratory in THZ-200C shaking incubator (Shanghai Boxun Industrial Co., Ltd., Shanghai, China) at 30°C and 180 rpm for 48 h. Each experiment was repeated three times.

Determination of MPS Yield

Monascus fermentation broth was filtered, separated, and centrifuged at 10,000 g for 15 min at 4°C. The supernatant was concentrated to volume of 1/4 by vacuum centrifugal concentrator (Beijing Jiaimu Technology Co., Ltd., Beijing, China). After adding 4 times volume of anhydrous ethanol, the precipitation was centrifuged after 24 h at 4℃ and dried to constant weight. Through accurate weighing and conversion, the yield of MPS (g/L) was calculated [18].

Extraction process and single factor experiments

The effects of single factor on the production were investigated with carbon sources as water-soluble starch, dextrin, glucose, fructose, sucrose, lactose, maltose (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), nitrogen sources as yeast extract, ammonium sulfate, sodium nitrate, urea, protein peptone (Aladdin Reagent Co., Ltd, Shanghai, China), $MgSO_4·7H_2O$, KH_2PO_4, $K_2HPO_4·3H_2O$, absolute ethyl alcohol, and pH of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0. In the process of optimizing factors, it was guaranteed that only one factor was changed, while the other factors remained unchanged.

Determination of *Monascus* Pigments

Monascus pigments were detected using a method based on Chinese standard GB 1816.15-2015 with minor modifications. Briefly, 1 mL of centrifuged supernatant was taken and appropriately diluted with 70% (v/v) ethanol aqueous solution. Absorbance values for yellow, orange, and red pigments in the respective ethanol solutions were measured at 410 nm, 470 nm, and 510 nm using a UV-2600 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with 70% ethanol as the negative control. Pigment concentrations were expressed as corresponding OD units (absorbance × sample dilution factor).

Response surface experiment

Box-Behnken design was used to optimize selected factors [19]. The experimental schemes of 3-level-3-factor were designed for analysis of variance, 3D response surface, and contour map using Design Expert 13 software (https://www.statease.com/). The three factors involved were sucrose addition amount (A), yeast extract powder addition amount (B), and the initial pH (C), each of which had three different levels as the three levels of sucrose addition amount being 30, 40, 50 g/L, the yeast extract powder addition amount being 10, 12, 14 g/L, and the initial pH being 5.0, 5.5, 6.0, respectively. The MPS production was taken as the dependent variable.

Molecular weight (Mw) determination

MPS samples were dissolved in ultrapure water at a concentration of 1 mg/mL, filtered through a 0.22 μm membrane, and then sent to Beijing Zhongke Baice Company (Beijing, China) for high-performance gel permeation chromatography (HPGPC) analysis.

Monosaccharide composition determination

Ion chromatography (IC) was performed using a Dionex ICS-5000+ system (ThermoFisher Scientific, Waltham, MA, USA) with a CarboPac PA10 column. In the determination of monosaccharide composition, it was prerequisite that the sample was degraded into monosaccharides initially [20]. Under an ice-bath condition, 5 mg of the MPS sample was accurately weighed and placed in a reaction

vessel. Subsequently, 0.5 mL of 12 M sulfuric acid was carefully added. Magnetic stirring was then initiated at an appropriate rate and maintained for 30 minutes before 2.5 mL of ultrapure water being added to the reaction mixture. The entire mixture was then transferred to a 110°C oil-bath and allowed to react for 3 hours. After the reaction, the solution was diluted to 50 mL with ultrapure water. After mixing 1 mL of the solution with 4 mL of deionized water, the sample was passed through a 0.22 μm water-based filter membrane before being injected for detection. The standard curve was drawn with 8 monosaccharide standards including glucose (Glc), rhamnose (Rha), xylose (Xyl), galactose (Gal), arabinose (Ara), fructose (Fru), fucose (Fuc), and mannose (Man) (Sigma-Aldrich, St. Louis, MO, USA).

Determination of antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was employed following the method of Merve *et al*. with slight modifications [21]. MPS samples were diluted into an equal concentration gradient solution in the experiment. The reaction mixtures included 2 mL DPPH solution, 1.5 mL deionized water, and 0.5 mL MPS solution at the specified concentration. After dark incubation for 0.5 h, OD_{517} was measured using a UV-2600 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Vitamin C (Vc) acted as the positive control with 95% ethanol as the negative control. The DPPH scavenging effect was calculated as below.

$$\text{DPPH·scavenging rate (\%)} = [A_0-(A_2-A_1)]/A_0\times100\%$$

where A_0 was 2 mL DPPH + 2 mL water. A_1 was 2 mL ethanol + 0.5 mL MPS solution + 1.5 mL water. A_2 was 2 mL DPPH + 0.5 mL MPS + 1.5 mL water. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) scavenging assay was performed following the method of Fan *et al*. [22]. The $ABTS^+$ radical reaction solution was prepared by mixing 5 mL of 7 mmol/L ABTS and 5 mL of 2.45 mmol/L potassium persulfate followed by dark storage for 12 h. Prior to use, the solution was diluted with 0.1 mol/L PBS (pH 7.4) to adjust the OD_{734} to 0.70 ± 0.02. The sample solution was identical to that used for the DPPH scavenging ability assay. 40 μL of sample solution was mixed with 970 μL of the $ABTS^+$ working solution, incubated in the dark for 6 min, and the OD_{734} was measured using UV-2600 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The calculation formula for $ABTS^+$ scavenging activity was as follows.

$$ABTS^+\text{·scavenging rate (\%)} = (1 - A_1/A_0) \times 100\%$$

where A_0 was the $ABTS^+$ free radical reaction solution. A_1 was the sample group.

Data analysis

All the experiments were conducted in triplicates. The statistical significance was assessed by the multiple comparisons Tukey post-hoc analysis of variance (ANOVA) using Origin 21.0 software (Origin Lab Corporation, Northampton, MA, USA). The differences of the results were considered statistically significant at *P* values less than 0.05.

Results and discussion

The effect of strains on the yield of MPS and *Monascus* pigment

The impact of strains on MPS yield demonstrated that, as fermentation time elapsed, the MPS yields of different strains initially exhibited an upward trend followed by a decline (Figure 1A). Notably, significant variations in MPS yields were observed among different strains, and the fermentation durations required to attain maximum yields also diverged. It was speculated that, in the initial stage, the consumption of nutrients in the medium enabled the strains to synthesize secondary metabolites. Subsequently, the depletion of nutrients in the medium and cellular apoptosis were likely to be the contributing factors leading to the reduction in MPS yield during the later fermentation period. *Monascus purpureus* YY-1 showed the highest MPS yield of 2.78 g/L among the five strains

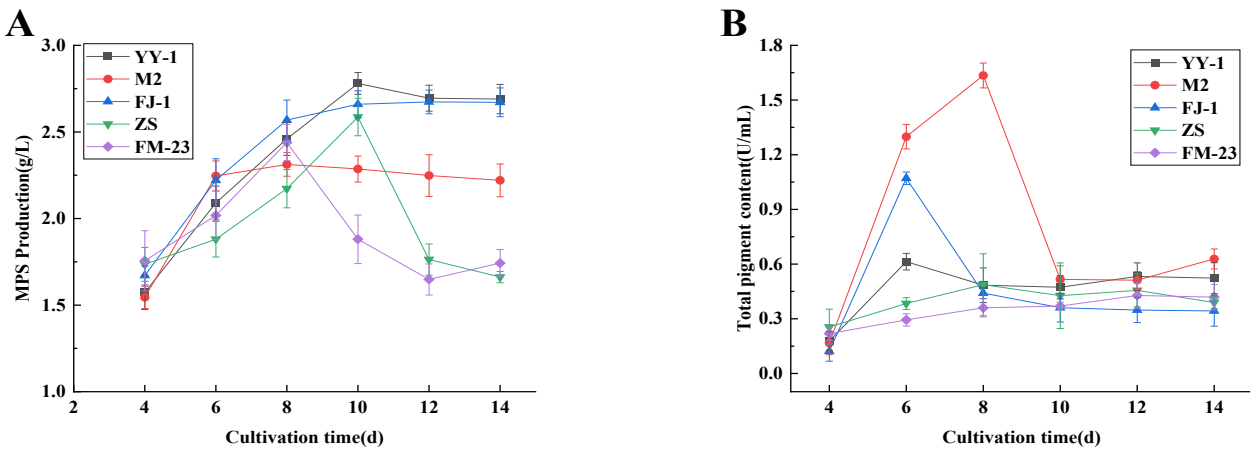

Figure 1. Effects of different strains on MPS production and pigment production. **A.** Effect of different strains on MPS production. **B.** Effect of different strains on the production of total pigment production.

investigated. To subsequent separate and purify MPS, it was essential to employ strains exhibiting relatively lower pigment yields. Among the five strains, the total pigment content produced by *Monascus purpureus* YY-1 was 0.43 U/mL, which remained at a relatively low level in the experimental group (Figure 1B). Therefore, *Monascus purpureus* YY-1 had good development and application potential and was the most suitable strain for subsequent research and optimization.

Optimization of carbon source types

The growth of microorganisms and the secretion of secondary metabolites are affected by carbon sources [23]. When *Monascus purpureus* YY-1 utilized sucrose as the carbon source, MPS production was optimal and reached 2.80 g/L (Figure 2A). The large molecular weight and branched structure of starch led to limited mass transfer and poor suspension in the fermentation system. In addition, the slow decomposition rate of starch led to prolonged reaction time, which affected the efficiency of polysaccharide production and caused a decrease in MPS production. Compared with the MPS production using sucrose as the carbon source, when different culture media were used, the MPS production decreased by 6.32%, 10.28%, and 45.46% when dextrin, lactose, and starch were added, respectively. Therefore, sucrose was selected as the carbon source for subsequent experiments.

Optimization of nitrogen source types

Nitrogen source is an essential nutrient for the growth and metabolism of *Monascus*. Substantial *differences were observed in the ability of Monascus* to produce secondary metabolism when employing inorganic and organic nitrogen sources [24]. When yeast extract powder was employed as the nitrogen source and 20 g/L sucrose as the carbon source, yeast extract powder exhibited the most robust ability to produce MPS, reaching 2.84 g/L (Figure 2B). Compared with yeast extract powder, the productions of MPS with ammonium sulfate, protein peptone, sodium nitrate, and urea as nitrogen sources were all reduced, which indicated that yeast extract powder was the most appropriate nitrogen source and was selected for subsequent experiments.

Optimization of sucrose addition amount

Sucrose stood as an incontrovertible and preeminent carbon source in the production of MPS and showed the best sugar production effect when used as the carbon source. As the adding amount of sucrose increased from 10 g/L to 50 g/L, the yield of MPS initially increased and then decreased, and reached the maximum value of 4.20 g/L at 40 g/L (Figure 2C). Compared with

2.84 g/L at 20 g/L, the output value of MPS increased by 40.89%. As a key nutrient for microbial growth and metabolism, carbon source was not only a component of mycelium and its metabolites, but also an important source of energy. The concentration of the carbon source had a significant impact on the growth rate of microorganisms, metabolic processes, product synthesis efficiency, and oxygen transfer. When the carbon source concentration was too high, it might trigger a metabolic inhibition effect, resulting in obvious limitations on polysaccharide synthesis. Conversely, insufficient carbon source would reduce the growth rate of the product synthesis. Meanwhile, the excessive consumption of the carbon source by the mycelium during growth would restrict the biosynthesis of polysaccharides [25]. Consequently, sucrose with a concentration of 40.0 g/L was selected as the carbon source for subsequent experiments.

Optimization of yeast extract powder addition amount

Yeast extract powder is an organic nitrogen source, which is hydrolyzed from components such as proteins and nucleic acids in the yeast cell wall. It is rich in proteins, amino acids, polypeptides, nucleotides, B vitamins, growth factors, and trace elements, *etc*. with a balanced nutrition, making it suitable for microbial fermentation culture. Its components had a significant impact on the production of MPS and were a key nutritional factor in the fermentation process. The adding amount of yeast extract powder directly affected the yield of polysaccharides [26]. When the sucrose was set at the optimized concentration of 40 g/L, during the adding of yeast extract powder from 2 g/L to 14 g/L, the yield of MPS of the strain first increased and then decreased (Figure 2D). The results showed that, when the concentration was 12 g/L, *Monascus purpureus* YY-1 had the best ability to produce MPS, reaching 5.21 g/L, which was an increase of 24.05% compared with 4.20 g/L at the initial addition amount of 2 g/L. Consequently, yeast extract powder with a concentration of 12 g/L was selected as the nitrogen source.

Optimization of the addition amount of $MgSO_4 \cdot 7H_2O$

Carbon source, nitrogen source, and inorganic salt are the three most important components in the fermentation medium [27]. As one of the six major nutritional elements required for the growth of microorganisms, the concentration of inorganic salt had been proven to significantly affect the growth of mycelial cells and the formation of *Monascus* pigments, which provided the required elements or precursors for the formation of fermentation metabolites [28]. As the adding amount of $MgSO_4 \cdot 7H_2O$ increased from 0.6 g/L to 1.4 g/L, the yield of MPS of the strain first increased and then decreased. When the concentration was 0.8 g/L, *Monascus purpureus* YY-1 had the best ability to produce MPS, reaching 5.54 g/L, which was an increase of 6.3% compared with 5.21 g/L at the initial adding amount of 1 g/L (Figure 2E). Consequently, 0.8 g/L $MgSO_4 \cdot 7H_2O$ had been determined as the optimal amount.

Optimization of KH_2PO_4/K_2HPO_4 addition amount

KH_2PO_4 and $K_2HPO_4 \cdot 3H_2O$ were added at ratios of 0.4/0.8, 0.5/1.0, 0.6/1.2, 0.7/1.4, 0.8/1.6, 0.9/1.8 g/L, while the MPS yields were measured. As the adding amount of KH_2PO_4 increased from 0.4 to 0.9 g/L and the adding amount of K_2HPO_4 increased from 0.8 to 1.8 g/L, the yield of MPS of the strain first increased and then decreased with the yield of MPS reaching the maximum value of 5.8 g/L when the ratio of KH_2PO_4/K_2HPO_4 was 0.8/1.6 g/L (Figure 2F). The results showed an increase of 4.7% compared with the yield of MPS at the initial adding amount. Overall, the ratio of KH_2PO_4/ K_2HPO_4 was 0.8/1.6 g/L, which had been determined as the optimal addition amount.

Optimization of the initial pH Value, inoculation amount and inoculum age

The pH value is one of the important factors determining the growth and metabolic activity of microorganisms. Studies have shown that, when

Table 1. Analysis of variance of *Monascus purpureus* YY-1 fermented MPS model.

Source	Sum of squares	Freedom	Mean square	*F* value	*P* value
Model	11.63	9	1.29	50.77	< 0.0001
Sucrose (A)	0.6441	1	0.6441	25.31	0.0015
Yeast extract powder (B)	2.51	1	2.51	98.57	< 0.0001
Initial pH (C)	0.3081	1	0.3081	12.11	0.0103
AB	0.0930	1	0.0930	3.66	0.0975
AC	0.0016	1	0.0016	0.0629	0.8092
BC	0.1260	1	0.1260	4.95	0.0614
A^2	4.20	1	4.20	165.11	< 0.0001
B^2	2.32	1	2.32	90.96	< 0.0001
C^2	0.7043	1	0.7043	27.67	0.0012
Residual	0.1782	7	0.0255		
Omission item	0.0931	3	0.0310	1.46	0.3519
Not significant	0.0851	4	0.0213		
Net error	11.81	16			

the environmental pH value is lower than 2.0 or higher than 8.5, the growth of microorganisms will be inhibited. During the fermentation process, the production of metabolites depends on the synergistic action of various enzymes. The fluctuation of the pH value can affect the dissolution and transport of nutrients in the medium, the activity of enzymes, the production of by-products, and the progress of redox reactions, thus having a significant impact on MPS synthesis. *Monascus* prefers a slightly acidic growth environment and has a particular preference for lactic acid [29]. Lactic acid was added to the medium to adjust the pH to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, respectively, to measure the yield of MPS. The results showed that, as the pH value increased from 3.0 to 5.5, the yield of MPS increased (Figure 2G). When the pH value is 5.5, the yield of MPS of *Monascus purpureus* YY-1 reached the optimal value of 5.89 g/L. An acidic environment inhibited the accumulation of polysaccharides, which was consistent with the conclusion that *Monascus* preferred a weakly acidic environment [30]. The seed liquid was inoculated into the fermentation medium at the concentrations of 2%, 5%, 8%, 11%, 14% (v/v) with the cultural times (seed age) of 24 h, 36 h, 48 h, 60 h, 72 h, respectively. The results demonstrated that, as the inoculation quantity was incrementally elevated from 2% to 14%, the production of MPS exhibited a trend of initially rising and subsequently declining. Specifically, the peak production yield of 5.95 g/L for MPS was attained when the inoculation quantity was set at 5% (Figure 2H). As the seed age gradually increased from 24 h to 72 h, the yield of MPS first increased and then decreased (Figure 2I), which indicated that the longer incubation time of seed solution might not result in the higher yield of MPS. The ability to produce polysaccharides increased from 24 h to 36 h, and significantly weakened at 48 h. The polysaccharide production at an inoculum time of 36 h was 6.10 g/L, therefore, choosing a seed age of 36 h had advantages both in terms of MPS production and time saving.

Response surface test results and analysis

According to the results of the single-factor experiment, the amounts of sucrose, yeast extract powder, and initial pH were determined. A response surface experiment with three factors and three levels was performed. The Box-Behnken design for the response surface was used to determine the factor levels for the fermentation production of MPS by *Monascus purpureus* YY-1 and the influence of the interactions among them on the MPS yield. The relationship between the yield of MPS and the amounts of sucrose, yeast extract powder, and the initial pH was shown in Table 1. The simulated correlation coefficient R^2 of the MPS yield was

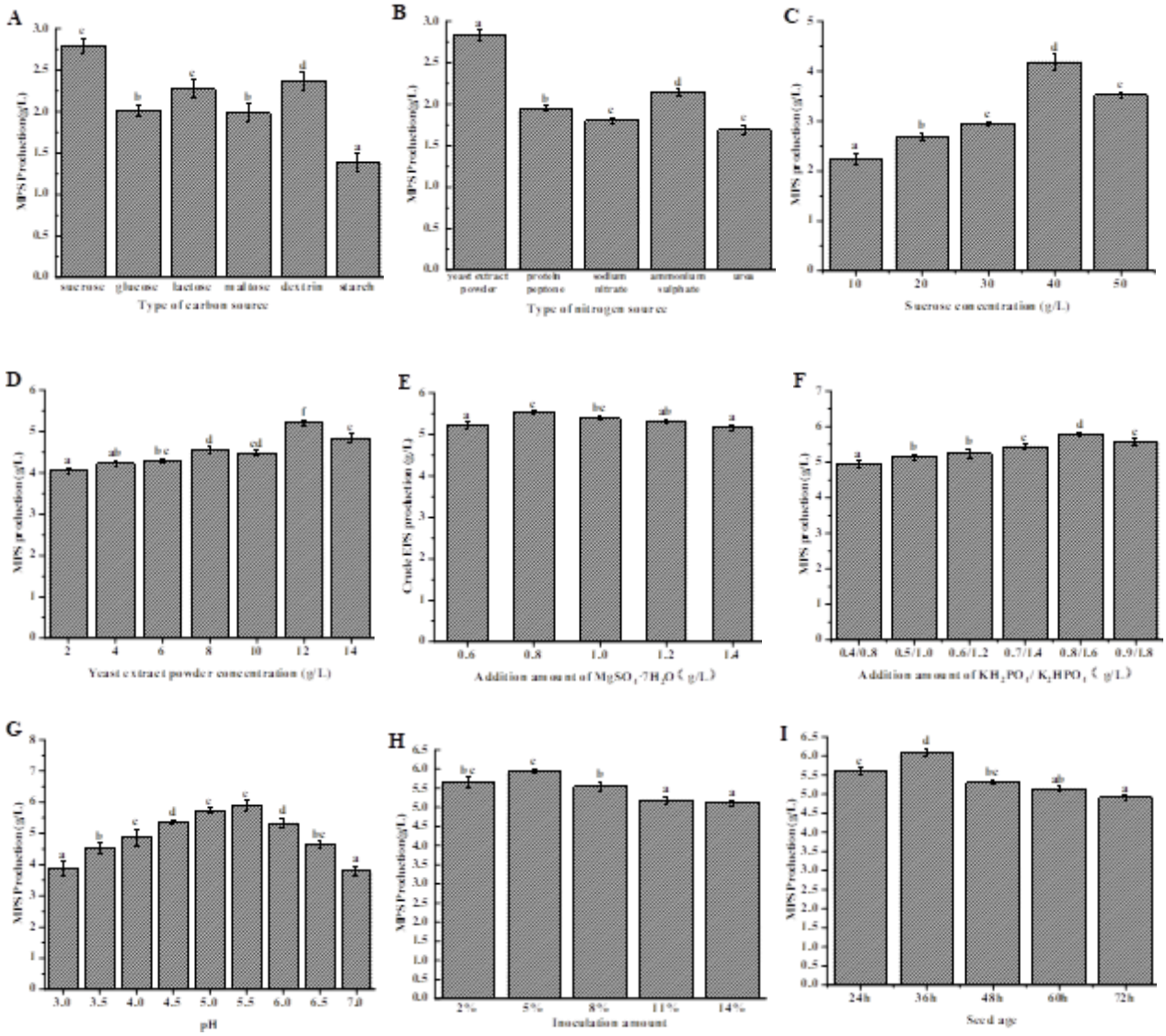

Figure 2. Optimization of MPS production preparation conditions. **A.** Effect of different types of carbon source on MPS production. **B.** Effect of different types of nitrogen source on MPS production. **C.** Effect of sucrose on MPS production. **D.** Effect of yeast powder on MPS production. **E.** Effect of $MgSO_4 \cdot 7H_2O$ on MPS production. **F.** Effect of KH_2PO_4/ K_2HPO_4 on MPS production. **G.** Effect of initial pH on MPS production. **H.** Effect of inoculation amount on MPS production. **I.** Effect of seed age on MPS production. The different letters represented the significant differences ($P < 0.05$).

0.9849, indicating a good response relationship between each factor and the value of MPS. The *P* values of three factors sucrose (A), yeast extract powder (B), and initial pH (C) were all less than 0.05, indicating that all of them had significant effects on the MPS. The results showed that the influence of single factors on the yield of MPS was yeast extract powder > sucrose > initial pH. The quadratic polynomial equation was then obtained through regression fitting as follows.

$$Y = 5.85 + 0.2838A + 0.56B - 0.1962C - 0.1525AB - 0.02AC - 0.1775BC - 0.999A^2 - 0.7415B^2 - 0.409C^2$$

Interaction analysis of different factors

3D response surface and contour map could effectively demonstrate the pairwise interactive effects of the three factors A, B, C on the yield of MPS (Figures 3). In a three-dimensional graph, the closer the contour lines were to an ellipse, the steeper the response surface was, and the

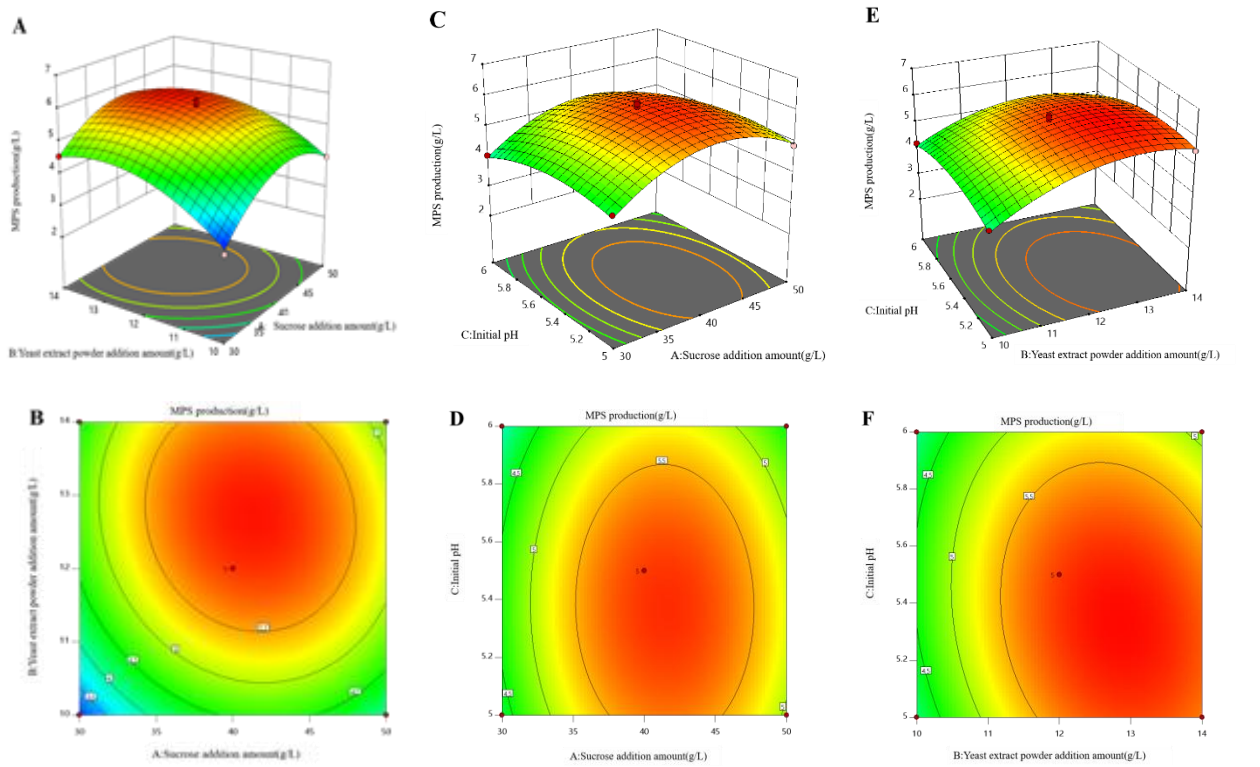

Figure 3. 3D response surface and contour maps of interaction between two factors. **A and B**: Effect of the interaction between sucrose and yeast extract powder. **C and D**: Effect of the interaction between sucrose and initial pH. **E and F**: Effect of the interaction between yeast extract powder and initial pH.

more obvious the interaction was, and vice versa [31]. The order of the influence of each factor on the yield of MPS was consistent with the results of the analysis of variance. The predicted optimal process conditions were 41.15 g/L sucrose, 12.81 g/L yeast extract powder, and 5.34 of initial pH. Under these conditions, the predicted yield of MPS reached 6.06 g/L. A fermentation verification experiment was carried out according to the optimal process, and the yield of polysaccharides produced by the liquid-state fermentation of *Monascus purpureus* YY-1 was 6.15 g/L, which had a small difference from the predicted value. Therefore, this model could accurately predict the yield of MPS.

Molecular weight of MPS

The molecular weight is an important parameter of polysaccharides [32], which not only reflects the length of the molecular chain but is also closely related to biological activity. Changes in the molecular weight may enhance or weaken the biological activity by influencing the water solubility and viscosity [33]. Therefore, the biological activity of polysaccharides will vary with the change of the molecular weight. The results of gel permeation chromatography (GPC) showed that MPS was a heteropolysaccharide (HePS) containing two components as MPS-1 and MPS-2 with their molecular weights of 45.45 kDa and 6.48 kDa, respectively. Polysaccharides with low molecular weights usually have stronger antioxidant activities and often enhance the immune function [34], while polysaccharides with high molecular weights may inhibit the inflammatory response [35]. Moreover, polysaccharides with high molecular weights may limit their *in vivo* absorption due to poor water solubility and high viscosity [36].

Monosaccharide composition of MPS

Polysaccharides are composed of different monosaccharides, from which their unique structures and functions are formed [37]. The

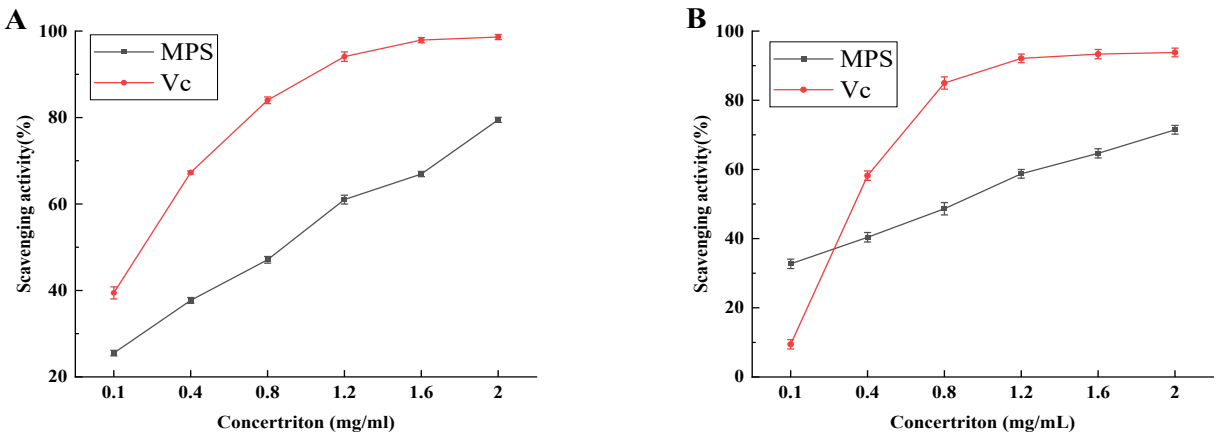

Figure 4. Effect of MPS on DPPH and ABTS radical scavenging rates in each group. **A.** The effect of various MPS on DPPH radical scavenging rate. **B.** The effect of MPS from different groups on ABTS free radical scavenging rate.

monosaccharide composition of MPS was determined by ion chromatography (IC) and showed that it was composed as follows.

Fucose : Arabinose : Glucose : Xylose : Mannose = 13.3 : 2.93 : 10.78 : 7.95 : 44.2

where fucose was endowed with anti-inflammatory attribute, while arabinose was potentially involved in the modulation of the immune system [7]. The differences in monosaccharides among various groups might determine their biological activities [38], and a high content of Man might account for the relatively high DPPH radical scavenging rate and ABTS radical scavenging rate.

Antioxidant activity

Due to the complexity of the chemical components in MPS, it was reasonable to choose at least two methods for evaluating antioxidant activity to ensure authenticity of results. Both DPPH radical and $ABTS^+$ radical scavenging assays were employed in this study. Upon the acceptance of an electron or a hydrogen radical, DPPH undergoes transformation into a stable diamagnetic species. In its free-radical form, DPPH manifests a broad absorption band with the maximal absorption occurring at 517 nm. When protonated by an anti-free radical agent, DPPH forfeits this distinct absorption characteristic [39]. The results indicated that, within the polysaccharide concentration range of 0.1 to 2 mg/mL, the scavenging rate of DPPH radical scavenging by MPS increased with the increase of the polysaccharide concentration (Figure 4A). The scavenging rate of DPPH free radicals increased from 25.52% to 79.47%, and the IC_{50} value was less than 1.2 mg/mL. MPS demonstrated a strong ability to scavenge DPPH radical scavenging. The scavenging rate of $ABTS^+$ free radicals by MPS was shown in Figure 4B. The results showed that, when the polysaccharide concentration increased from 0.1 mg/mL to 2.0 mg/mL, the scavenging rate of $ABTS^+$ free radicals by MPS increased with the increase of the polysaccharide concentration, rising from 34.56% to 71.46%, and the IC_{50} value was greater than 0.8 mg/mL. The scavenging rate of $ABTS^+$ free radicals by MPS was lower than that of DPPH free radicals, but it still remained at a relatively high level (71.46%).

Conclusion

This study primarily delved into the impact of diverse factors within the MPS production process on the MPS yield. By employing a three-factor and three-level response surface experimental design, the variables of sucrose addition quantity, yeast extract powder addition

quantity, and initial pH were selected. Through in-depth analysis, the optimal extraction conditions were 41.15 g/L sucrose, 12.81 g/L yeast extract powder, and the initial pH of 5.34. Under the optimized conditions, the MPS yield reached as high as 6.15 g/L. Subsequently, an analysis was conducted on the interrelationships among molecular weight, monosaccharide composition, and antioxidant activity. The results showed that a high content of Man might be responsible for the relatively elevated DPPH radical scavenging rate (79.47%) and ABTS radical scavenging rate (71.46%). The proposed optimization method in this study provided a robust theoretical basis, offering an important perspective for the development and comprehensive utilization of MPS in industrial production.

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