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OPTIMISATION OF FERMENTATION CONDITION FOR PRODUCTION OF HIGH MONACOLIN K (MK) YIELD BY *MONASCUS*-STRAINS

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Abstract

Monacolin K (MK),produced by *Monascus* fermentation, has significant hypolipidemic effects.In this study, under solid-state fermentation (SSF) quinoa was used as substrate to investigate the effect of the MK production by*Monascus*species quinoa. The results of the single factor experimentshowed that the amount of the substrate loaded, addition ofwater to the substrate, and fermentation temperature change time have a significant effect on the MK production of *Monascus*-fermented quinoa. It was found that neither *M. pilosus*-fermented quinoa nor*M. ruber*-fermented quinoa produced citrinin. Consequently, these three factors were selected for the response surface optimization to determine the optimal fermentation process. The results showed that the optimal fermentation process of *M. pilosus*-fermented quinoa wasas follows: quinoa loading amount of 30 g/300 ml, addition of 20 ml water to substrate, seed liquid inoculation volume of 10%, cultured at 30°C for 2 days, followed by a temperature change to 25°C and continued cultivation until 12 days. Under this fermentation process, the yield of MK reached 2.51 mg/g and without production of any citrinin. The optimal fermentation process of *M. ruber*-fermented quinoa was as follows: quinoa loading amount of 31 g/300 ml, water addition of substrate of 20 ml, seed liquid inoculation volume of 20%, cultured at 30°C for 2 days, followed by a temperature change to 25°C and continued cultivation until 12 days. Under this fermentation process, the yield of MK reached 3.22 mg/g and without any citrinin production.

Introduction

Monascus is a traditional edible fungus in East Asia, known for its production of valuable secondary metabolites, including monacolin K (MK), *Monascus* pigments, and γ-aminobutyric acid (GABA) (Chen *et al.* 2015, Wang *et al*. 2016). MK, a physiologically active polyketone, was first isolated from a *Monascus* culture (Endo 1979). Currently, MK is regarded as one of the most effective substances for lipid regulation. In addition, MK also has the functions such as anti-cancer, neuroprotective, anti-inflammatory, and antibacteria, and is wildly used in industries such as health food and medicine(Xiong *et al*. 2019).

Rice is a commonly utilized substrate for the solid-state fermentation (SSF) of *Monascus*. For instance (Zhang *et al*. 2017), conducted a fermentation process with *M. purpureus* on rice, achieving an optimized MK yield of 2.58 mg/g after refining the fermentation conditions.

In recent years, there has been a growing interest in the fermentation of *Monascus* using a variety of grain substrates, including purple rice(Prodpran *et al*. 2017), buckwheat(Ou 2012), highland barley(Hao *et al*. 2021), and beans(Chen and Chen 2016). Zhang *et al*. carried out solid fermentation of *M. ruber* using barley as the substrate, and the MK content of the fermentation product was 2.69 mg/g (Zhang *et al.* 2018).

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Quinoa is a coarse grain crop originating in the Andes of South America, which is distributed in Xizang, Qinghai, Gansu, Yunnan, Jiangxi, Zhejiang, Jilin,etc. in China (Ren *et al*. 2019 and 2023). Quinoa is rich in macronutrients such as carbohydrates, proteins and lipids, as well as physiological active substances such as phenols and sterols, and is called a "super grain" by nutritionists(Dakhili *et al*. 2019, Jiang *et al*. 2021).

In this study, *M. ruber* CICC 5018 and *M. pilous* CICC 5045 were used as test strains to investigate the effects of solid-state fermentation (SSF) conditions (including substrate loading amount, addition of water to substrate, substrate soaking time, seed liquid inoculation volume, fermentation temperature change time and fermentation time) on the production of MK and citrinin in *Monascus*-fermented quinoa (MFQ). On the basis of single factor optimization, the response surface design method was used to optimize the optimal fermentation process for MFQ with high-yield MK.

Materials and Methods

The *M. pilous* strain CICC 5045, *M. purpureus* strain CICC 5046 and *M. ruber* strain CICC 5018 were both purchased from China Center of Industrial Culture Collection (CICC).

Monascus was transferred to wort medium plate (Qingdao Haibo Biotechnology Co., LTD.) and cultured at 28°C for 7 days. After that, *Monascus* blocks were taken by a hole puncher and inserted into seed liquid medium. at 28°C, 180 r/min, and incubated by oscillating for 48 h (Zhang *et al*. 2019).

10, 20, 30, 40, 50 and 60g of quinoa were added to a 300ml fermentation flask, and then was added 20ml of sterile water. After autoclave sterilization, the seed solution was diluted to 1×10^7 /ml of spores ,which was added into the medium, and then 10% the seed solution inoculated. Four replicates were set for each group. After incubation at 28°C for 15 days, the fermentation products were dried at 55°C to constant weight, crushed and screened with 100 mesh to detect the contents of MK and citrinin.

The loading amount of quinoa was 30 g/300 ml, and addition of water to substrate was set at 8 levels (10, 15, 20, 25, 30, 35, 40 and 45 ml). The remaining fermentation conditions and sample processing methods are the same as above

The loading amount of quinoa was 30 g/300 ml, and the soaking time of substratewas set at 7 levels (0, 2, 4, 6, 8, 10 and 12 hrs). The remaining fermentation conditions and sample treatment methods were the same as above.

The loading amount of quinoa was 30 g/300 ml, and the inoculation volume of seed liquid was set at 7 levels (5, 10, 15, 20, 25, 30 and 35%). The remaining fermentation conditions and sample treatment methods were the same as above.

The loading amount of quinoa was 30 g/300 ml, and the fermentation temperature change time was set at 7 levels, which were cultured at 30°C for 1, 2, 3, 4, 5 and 6 days, respectively, and then transferred to 25°C for further culture until 15 days. The remaining fermentation conditions and sample treatment methods are the same as above.

The loading amount of quinoa was 30 g/300 ml, and the fermentation time was set at 7 levels (6, 9, 12, 15, 18 and 21 days). The remaining fermentation conditions and sample treatment methods were the same as above.

According to the above single-factor experimental results, factors that have a greater impact on MK yield (substrate loading amount, addition of water to substrate, and fermentation temperature change time) are selected as independent variables. Response surface optimization software Design Expert 12 is adopted, and according to the Box-Behnken principle, three factors

and three levels of response surface experiments were designed for the solid-state fermentation conditions of *Monascus pilosus*-fermented quinoa (MPFQ) and *Monascus ruber*-fermented quinoa (MRFQ), respectively. The levels of factors were showed in Tables 1 and 2.

Table 1. Factors and levels of response surface experiment of MPFQ.

Levels	Factors		
	A:Water added to substrate (ml)	B: Fermentation temperature change time (days)	C: Substrate loaded (g/300 ml)
-1	10	1	30
0	15	2	40
1	20	3	50

Table 2. Factors and levels of response surface experiment of MRFQ.

Levels	Factors		
	A:Water added to substrate (ml)	B: Fermentation temperature change time (days)	C: Substrate loaded (g/300 ml)
-1	15	1	20
0	20	2	30
1	25	3	40

Following the method of Huang *et al.*(Huang *et al.* 2024), the MPFQ and MRFQ samples were processed and the contents of MK and citrinin were detected, respectively.

Detection was performed using HPLC-quadrupole time-of-flight tandem mass spectrometry (Q-TOF). The mobile phase consisted of 0.1% formic acid in water - acetonitrile (45:55, v/v). The flow rate was 1.0 ml/min, the chromatographic column was Eclipse Plus C18(100mm × 4.6mm, 3.5 μm), the detection wavelength was 228 nm, and the column temperature was 30°C. The sample volume was 10 μl. The nebulizing gas pressure was set at 40 psi, the drying gas flow rate was 10 L/min, and the drying gas temperature was maintained at 350°C. ESI(+) mode was adopted, and the scanning range was 200-800 m/z.

The Statistical Product and Service Solutions (SPSS) data processing system (IBM SPSS Statistics 26) was used for Variance analysis and significance testing of the experimental data. The Origin 2018 was used for mapping.

Results and Discussion

The HPLC chromatogram of MFQ sample to be tested is shown in Fig. 1. The *M. ruber*, *M. purpureus and M. pilosus* are three kinds of *Monascus* commonly used in *Monascus* fermentation(Dai *et al.* 2021). In the preliminary experiment of this study, the *M. ruber* CICC 5018, *M. purpureus* CICC 5046 and *M. pilosus* CICC 5045 were used for SSF experiment. The results showed that the MK yield of CICC 5018 and CICC 5045 was significantly higher than that of CICC 5046, which was only about 10% of the other two strains. Therefore, CICC 5018 and CICC 5045 were selected as the test strains in this study.

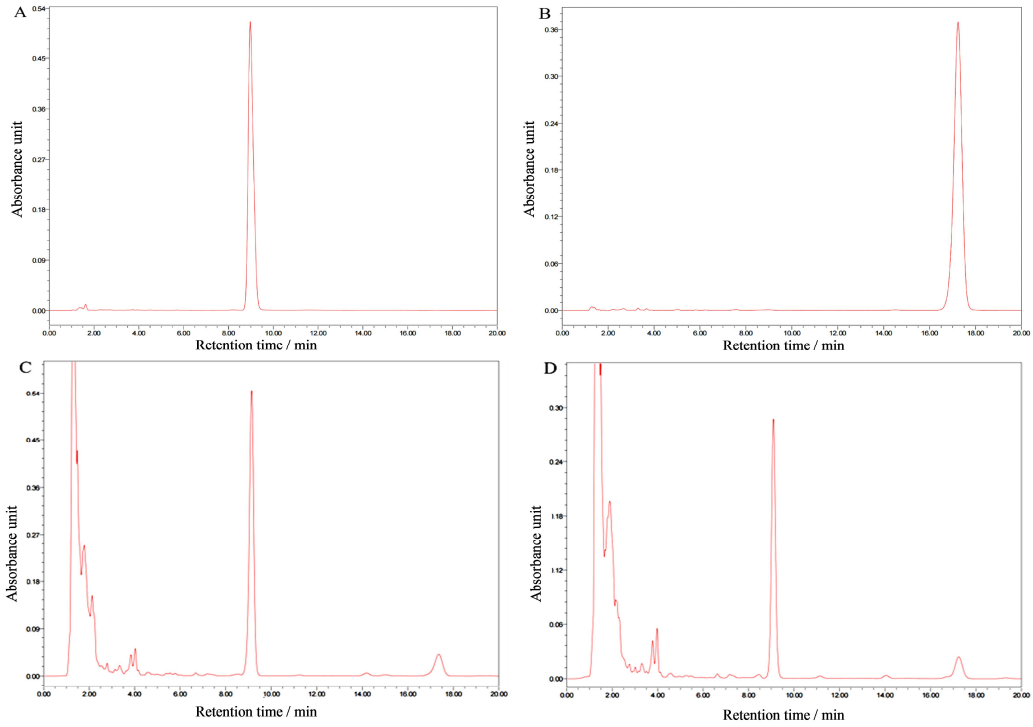

Fig. 1. The HPLC chromatogram of MK standard and MFQ samples. A. Acid MK standard, B. Lactone MK standard, C. Red yeast quinoa test sample, D. Red yeast quinoa test sample.

As illustrated in Fig. 2, the production of MK in MFQ exhibited a trend of initial increase followed by a decline with the augmentation of substrate loading. Specifically, the MK yield for MPFQ peaked when the substrate loading amount was at 40 g/300 ml or 50 g/300 ml.When the substrate loading was 60 g/300 ml, the MK production decreased significantly. When the substrate loading amount was 30 g/300 ml or 40 g/300 ml, the MK yield of MRFQ reached the highest. These findings suggest an optimal range for substrate loading amount to maximize MK yield in the fermentation process.

As shown in Fig. 2, the MK production of MPFQ and MRFQ both showed a trend of first increasing and then decreasing with the increase of the quinoa loading amount. This may be due to the limited nutrients provided by quinoa substrate when the amount of quinoa is too low, which is not conducive to the growth of *Monascus* mycelium. Excessive loading of quinoa can affect the oxygen circulation and heat dissipation of the substrate (He *et al*. 2020, Hao *et al*. 2021).

Referring to Fig. 3, it is observed that the MK yield for both MPFQ and MRFQ initially rose and then fell as the water content in the substrate increased. For MPFQ, the peak MK yield was recorded at 1.43 mg/g with the addition of 15 ml of water to the substrate. Beyond this volume, a continued increase in water led to a decline in MK yield. In the case of MRFQ, the MK yield remained relatively stable and high within the range of 10-25 ml of water added to the substrate, with no significant variation observed between 20 ml and 25 ml (yielding 1.92 to 2.10 mg/g of MK). However, a further increase in water addition beyond this point resulted in a marked decrease in MK production. These results indicate a critical threshold for water content in the substrate that is essential for optimizing MK yield during the fermentation process. As shown in

Fig. 3, the MK production of MPFQ and MRFQ both first increased and then decreased with the increase of substrate water addition. It is speculated that when the moisture content of the solid substrate is too low, the substrate is too dry, which is not conducive to the growth of *Monascus*mycelium. However, when the moisture content is too high, it is easy to lead to the substrate agglomeration, porosity reduction, affecting the mass transfer process such as gas exchange, thereby affecting the MK synthesis of *Monascus* (Lu *et al.* 2013, He *et al.* 2020).

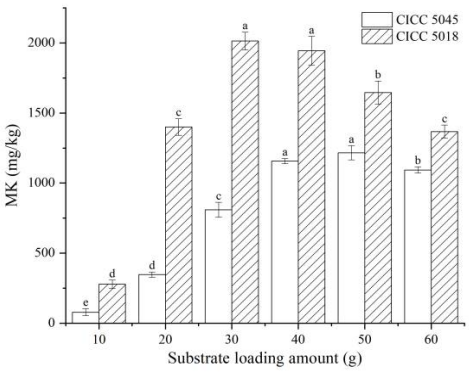

Fig. 2 Effect of substrate loading amount on MK production of MFQ. Different letters in different strains indicate significant differences ($p < 0.05$).

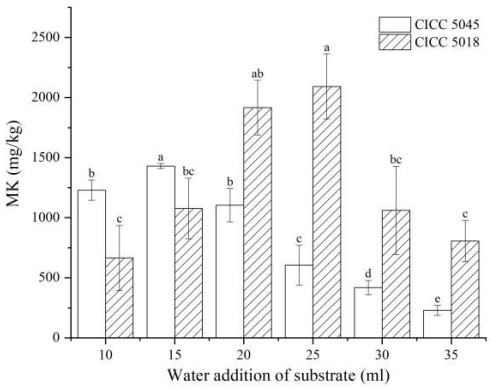

Fig. 3. Effect of water addition to the substrate on MK production of MFQ. Different letters in different strains indicate significant differences ($p < 0.05$).

During the fermentation process of *Monascus*, the substrate is usually soakedbefore fermentation, but there are few studies on effects of substrate soaking onMK production. Therefore, this study explored the influence of substrate soaking time on the yield of MK by selecting different soaking times (0 , 2 , 4 , 6 , 8 , 10 and 12 hrs). The results shown that whether the substrate is soaked and different soaking times have no significant effect on the MK content of MFQ (Fig. 4).

In this study, the *Monascus* seed liquid was inoculated into a sterilized solid culture medium across a range of inoculum volumes, specifically at 5, 10, 15, 20, 25, 30 and 35%. The results are described in Fig. 5. For MPFQ, the MK yield was found to be higher when the seed liquid inoculation was at 10, 15, and 20%, with no significant variation observed between these levels (ranging from 1.36 to 1.37 mg/g). However, a marked decline in MK yield was noted when the inoculation volume reached 25%.

The MK yield increased progressively with the seed liquid inoculation rate from 5% to 25% for MRFQ. Notably, there was no significant divergence in MK yield between inoculation rates of 20% and 25% (with yields between 1.48 to 1.60 mg/g). Nonetheless, further increments in the seed liquid inoculation rate resulted in a subsequent decrease in MK production. These findings provide critical insights for determining the optimal inoculum volume to maximize MK yield during the fermentation process, highlighting the importance of precision in inoculation rates for the efficiency of *Monascus* fermentation.

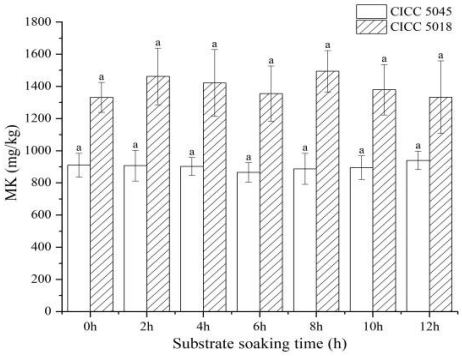

Fig. 4. Effect of substrate soaking time onMK production of MFQ. Different letters in different strains indicate significant differences ($p < 0.05$).

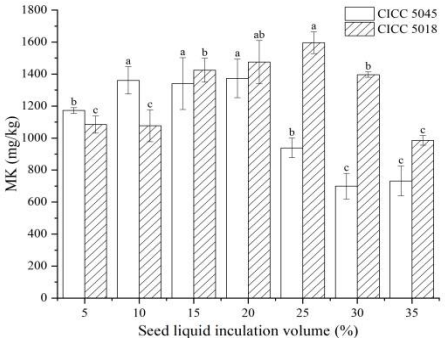

Fig. 5. Effect of seed liquid inoculation volume on MK production of MFQ. Different letters in different strains indicate significant differences ($p < 0.05$).

In this experiment, different temperature change times were set to explore the effects of MPFQ and MRFQ on MK yield of MFQ under the temperature changing conditions of 30 to 25°C. From Fig. 6, it can be seen that the MK yield of MPFQ and MRFQ was lower when cultured at constant temperature (25 or 30°C), while the MK yield was significantly increased when cultured at variable temperature. *Monascus* was first cultured at 30°C for 2 days, and then switched to 25°C for further cultivation until 15 days. When the MK production reached its maximum, the MK production of MPFQ and MRFQ cultured under these conditions reached 2.43 and 2.83 mg/g, respectively. However, as the time of temperature change passed backward, the MK production continued to decline.These results underscore the importance of temperature modulation as a critical factor in enhancing the production of MK during the fermentation process.

From Fig. 6, it can be seen that variable temperature culture (30°C for 2 days, then 25°C) can significantly increase the MK yield of MFQ, which is consistent with the research results of Lin *et al.*(Lin *et al.* 2017). The reason may be that higher temperature (30°C) in the early stage of fermentation can promote the growth of mycelia and shorten the growth cycle. When the mycelium transition into the secondary metabolic stage, changing the temperature to a lower temperature (25°C) is conducive to the production of MK(Su *et al.* 2019). If the temperature change time is too early, the mycelia have not grown in large quantities, resulting in lower MK yield. If the temperature change time is too late, the mycelium growth will be excessive, and the nutrients will be consumed in advance, which is not conducive to the later synthesis of MK.

As described in Fig. 7, the yield of MK in the fermentation products of MPFQ and MRFQ exhibited an initial increase followed by a stabilization trend with the extension of fermentation time. During the initial phase of fermentation (6-9 days), both MPFQ and MRFQ yielded relatively lower amounts of MK. The MK yield for both strains notably increased when the fermentation duration was extended to 12 days, achieving peak yields of 1.50 mg/g for MPFQ and 1.41 mg/g for MRFQ, respectively. Beyond this point, there was no significant further increase in MK production, indicating that the yield had reached a plateau. These findings suggest that an optimized fermentation period is crucial for achieving maximum MK yields in the fermentation process involving these two strains of *Monascus*.

The regression equations for water addition of substrate (A), fermentation temperature change time (B), and substrate loading amount (C) were obtained through multiple regression fitting of the corresponding levels of factors in Table 1 and the experimental results in Table 3: $Y(MK) = 1.85+0.44A-0.055B-0.35C-0.0225AB-0.2525AC+0.0225BC-0.2503A^2-0.4953B^2-0.0653C^2$.

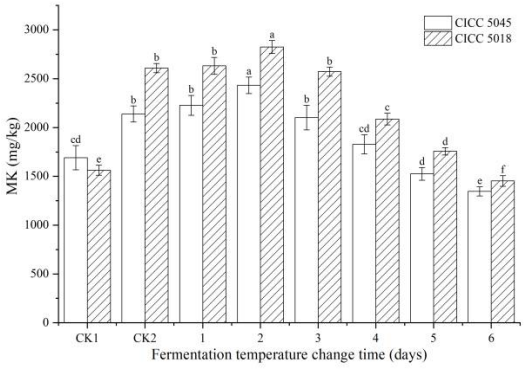

Fig. 6. Effect of fermentation temperature change time on MK production of MFQ. CK1. 30 °C for 15 days, CK2. 25 °C for 15 days. Different letters in different strains indicate significant differences ($p < 0.05$).

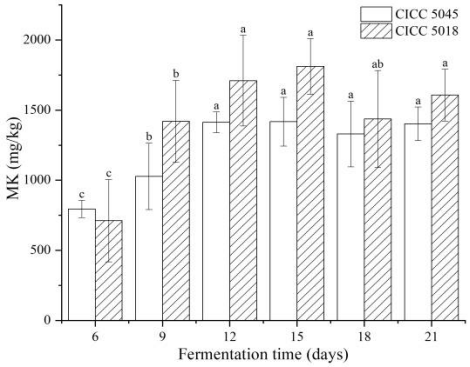

Fig. 7. Effect of fermentation time on MK production of MFQ. Different letters in different strains indicate significant differences ($p < 0.05$).

Regression model variance analysis was performed on the experimental results in Table 3, and the results were shown in Table 4. The $p < 0.001$ of this model is very significant. The missing item was 0.2104, which was not significant. The coefficient of determination R^2=0.9781, indicating that the model can explain 97.81% of the variation of the response value. The composite correlation coefficient R^2_{adj} = 0.9499 indicates that 94.99% of the experimental results are affected by experimental factors. In summary, the model fits well with the actual situation, and can reflect the relationship between the water addition of substrate, the fermentation temperature change time, the substrate loading amount and the MK yield of MPFQ. The interaction term AC and the secondary term A^2 had significant effects on the MK yield of MPFQ ($p < 0.01$). The effects of primary terms A, C and secondary terms B^2 on MK yield of quinoa were significant ($p < 0.001$). By comparing the F value, it can be inferred that the influence degree of each factor on the MK yield of Quinoa was in the order of A > C > B, that is, water addition of substrate > substrate loading amount > fermentation temperature change time.

The response surfaces and contours of the interaction between the additionwater to substrate, the fermentation temperature change time, and the substrate loading amount according to the multiple regression equation are shown in Fig. 8. The surface inclination of the water added to the substrate and the content of the substrate is high, and the contours are sparse, which indicates that the interaction between the addition of water to substrate and the content of the substrate has a significant effect on the MK yield of MPFQ, and the influence of the water addition of substrate is more significant than the other two factors. By observing the interaction between the water addition of substrate and the temperature change time, as well as the surface slope and contour density of the interaction between the temperature change time and the substrate loading amount, it was found that the interaction between the water addition of substrate and the temperature change time and the substrate loading amount had no significant effect on the MK yield of MPFQ. The result is consistent with the analysis of variance.

Using MK yield as evaluation index, the optimal fermentation conditions for MPFQ were predicted by Design Expert software as follows: water addition of substrate 20 ml, fermentation temperature change time 1.899 d, substrate loading amount 30 g/300 ml, and MK yield predicted value 2.58 mg/g. The theoretical predicted value was combined with the actual operation, and the

final optimization results were as follows: quinoa loading amount of 30 g/300 ml, addition of water to substrate of 20 ml, seed liquid inoculation volume of 10%, and the fermentation temperature changed to 25°C until 12 days total after fermentation at 30°C for 2 days. Under these conditions, the MK yield of MPFQ was 2.51 mg/g, which was close to the predicted value, indicating that the optimal fermentation conditions obtained by response surface optimization were stable and reliable.

Table 3. Response surface test design and results of MPFQ.

Group	A: Water added to substrate (ml)	B: Fermentation temperature change time (days)	C: Substrate loaded (g/300 ml)	MK content (mg/g)
1	10	1	40	0.79
2	20	1	40	1.57
3	10	3	40	0.68
4	20	3	40	1.37
5	10	2	30	1.16
6	20	2	30	2.69
7	10	2	50	0.88
8	20	2	50	1.40
9	15	1	30	1.65
10	15	3	30	1.54
11	15	1	50	0.99
12	15	3	50	0.97
13	15	2	40	1.88
14	15	2	40	1.93
15	15	2	40	1.88
16	15	2	40	1.86
17	15	2	40	1.69

Table 4. Variance analysis of regression model of MPFQ.

Source of variance	Sum of squares	Degrees of freedom	Mean square	F-value	P-value	Significance
Model	4.22	9	0.4684	34.72	<0.0001	significant
A	1.55	1	1.55	114.81	<0.0001	
B	0.0242	1	0.0242	1.79	0.2223	
C	0.9800	1	0.9800	72.65	<0.0001	
AB	0.0020	1	0.0020	0.1501	0.7099	
AC	0.2550	1	0.2550	18.90	0.0034	
BC	0.0020	1	0.0020	0.1501	0.7099	
A^2	0.2637	1	0.2637	19.55	0.0031	
B^2	1.03	1	1.03	76.55	<0.0001	
C^2	0.0179	1	0.0179	1.33	0.2868	
Residual	0.0944	7	0.0135			
Misfit term	0.0606	3	0.0202	2.38	0.2102	not significant
Pure error	0.0339	4	0.0085			
Total	4.31	16	R^2=0.9781		R^2_{adj}=0.9499	

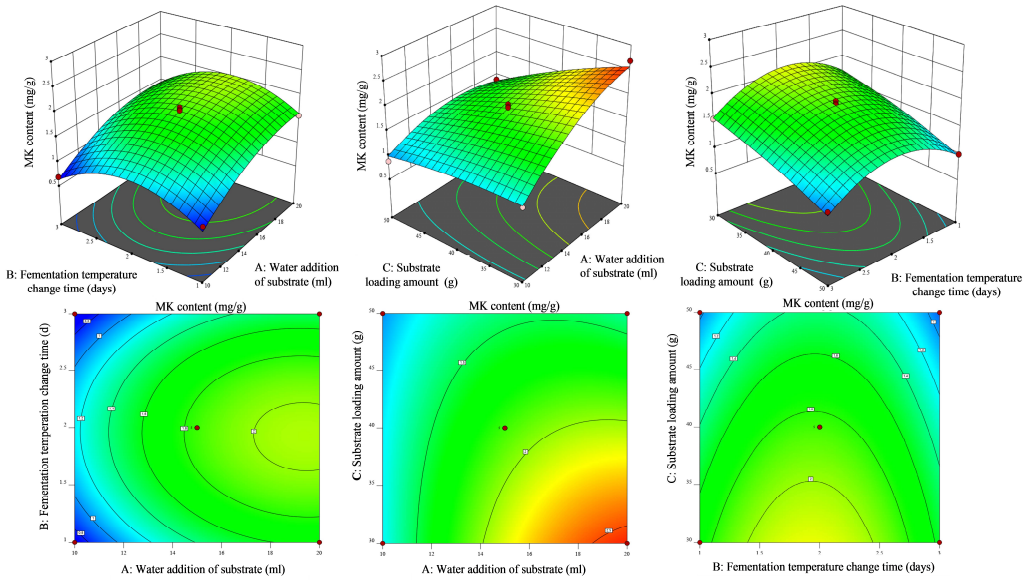

Fig. 8. Response surface plots and contour plots for effects of interaction between various factors on MK yield of MPFQ.

Table 5. Response surface test design and results of MRFQ.

Group	A: Water added to substrate (ml)	B: Fermentation temperature change time (days)	C: Substrate loaded (g/300 ml)	MK content (mg/g)
1	15	1	30	2.62
2	25	1	30	2.79
3	15	3	30	2.67
4	25	3	30	2.51
5	15	2	20	3.10
6	25	2	20	1.49
7	15	2	40	2.30
8	25	2	40	2.95
9	20	1	20	2.48
10	20	3	20	2.15
11	20	1	40	2.64
12	20	3	40	2.62
13	20	2	30	3.18
14	20	2	30	3.33
15	20	2	30	3.35
16	20	2	30	3.20
17	20	2	30	3.46

Table 6. Variance analysis of regression model of MRFQ.

Source of variance	Sum of squares	Degrees of freedom	Mean square	F-value	P-value	Significance
Model	3.91	9	0.4346	17.60	0.0005	significant
A	0.1128	1	0.1128	4.57	0.0699	
B	0.0420	1	0.0420	1.70	0.2332	
C	0.2080	1	0.2080	8.42	0.0229	
AB	0.0272	1	0.0272	1.10	0.3286	
AC	1.28	1	1.28	51.71	0.0002	
BC	0.0240	1	0.0240	0.9730	0.3568	
A^2	0.4711	1	0.4711	19.08	0.0033	
B^2	0.4366	1	0.4366	17.68	0.0040	
C^2	1.09	1	1.09	44.27	0.0003	
Residual	0.1728	7	0.0247			
Misfit term	0.1195	3	0.0398	2.99	0.1589	not significant
Pure error	0.0533	4	0.0133			
Total	4.08	16	R^2=0.9577		R^2_{adj}=0.9033	

The regression equations for water addition of substrate (A), fermentation temperature change time (B), and substrate loading amount (C) were obtained through multiple regression fitting of the corresponding levels of factors in Table 2 and the experimental results in Table 5: $Y(MK) = 3.30-0.1188A-0.0725B+0.1613C-0.0825AB+0.5650AC+0.0775BC-0.3345A^2-0.3220B^2-0.5095C^2$

According to the regression model method analysis in Table 6, $R^2 = 0.9577$, $R^2_{adj} = 0.9033$, and $p < 0.001$ of the model, which is extremely significant, and the missing item >0.05, which is not significant, indicate that the regression equation is well fitted, and the model can reflect the relationship between experimental factors and the response value MK yield. The primary terms C had significant effects on the MK yield of MRFQ ($p < 0.05$), the secondary terms A^2 and B2 had significant effects on the MK yield of MRFQ ($p < 0.01$), and the interaction terms AC and C^2 had extremely significant effects on the MK yield of MRFQ ($p < 0.001$). By comparing the F value, it can be seen that the influence degree of each factor on the MK yield of MRFQ is as follows: C > A > B, that is, substrate loading amount > addition of water to substrate > fermentation temperature change time.

The response surfaces and contours of the interaction between the addition of water to substrate, the fermentation temperature change time, and the substrate loading amount according to the multiple regression equation are shown in Fig. 9. The surface incline of the water content and the water content of the substrate is high, and the contour lines are elliptical, which indicates that the interaction between the water content and the water content of the substrate has a significant influence on the MK yield of MRFQ, and the influence of the substrate loading amount is more significant than the other two factors. The surface slope and contours of the interaction between the water addition of substrate and the fermentation temperature change time, as well as the interaction between the fermentation temperature change time and the substrate loading amount, found that the slope of the surface was low, and the contour shape was more circular, indicating that the interaction between the addition of water to substrate and the fermentation temperature change time, and the interaction between the fermentation temperature change time and the substrate loading amount had no significant effect on the MK yield of MRFQ. The result is consistent with the analysis of variance.

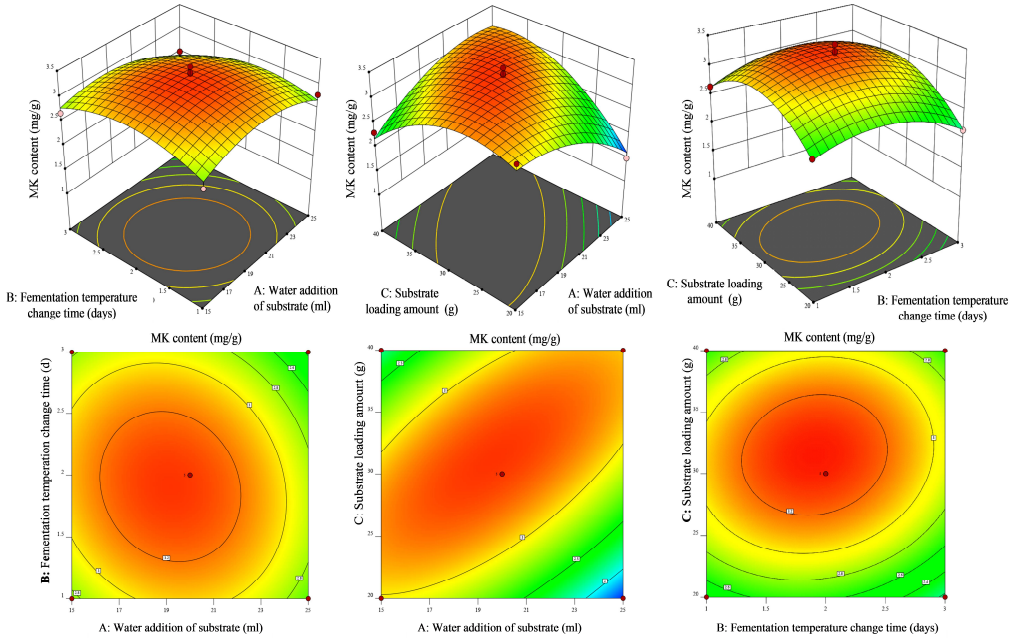

Fig. 9. Response surface plots and contour plots for effects of interaction between various factors on MK yield of MRFQ.

Taking the MK yield as the response value, the optimal fermentation conditions for MRFQ were predicted by the response surface software Design Expert analysis: 19.633 ml of water addition of substrate, 1.911 d of fermentation temperature change time, 31.112 g/300 ml of substrate loading amount, and 3.32 mg/g of MK yield predicted. Combined with the theoretical predicted value and the actual operation, the final optimization results were as follows: quinoa loading amount of 31 g/300 ml, water addition of substrate of 20 ml, seed liquid inoculation volume of 20%, fermentation at 30°C for 2 days, then the temperature changed to 25°C and continued fermentation until 12 days total. Under this fermentation condition, the MK yield of MRFQ was 3.22 mg/g, and the actual value was close to the predicted value, indicating that the fit was good and the model was stable and reliable.

The mass spectrometry identification results of MK in MFQ are shown in Fig.10. In the positive ion mode (Fig. 10 A-B), the mass-charge ratio after acid MK+ sodium ion of quinoa is 445. The mass charge ratio of lactone MK+ hydrogen ion and sodium ion is 405 and 427, respectively. In the negative ion mode (Fig. 10 C-D), the mass-charge ratio of MFQ after acid Mk-hydrogen ion is 421. The mass charge ratio of lactone MK + carboxyl group is 449. The results of mass spectrometry showed that MFQ contained acid MK and lactone MK.

In this study, the fermentation process of two *Monascus* strains CICC 5045 and CICC 5018 were optimized by single factor experiment and Box-Behnken response surface optimization, with the yield of MK as the index. The results showed that the optimal fermentation process of MPFQ was as follows: 30 g/300 ml of quinoa loading amount, 20 ml of water addition of substrate, 10% of seed liquid inoculation volume, cultured at 30°C for 2 days and then at 25°C until 12 days, the yield of MK could reach 2.51 mg/g under this fermentation process, and no citrinin was detected.

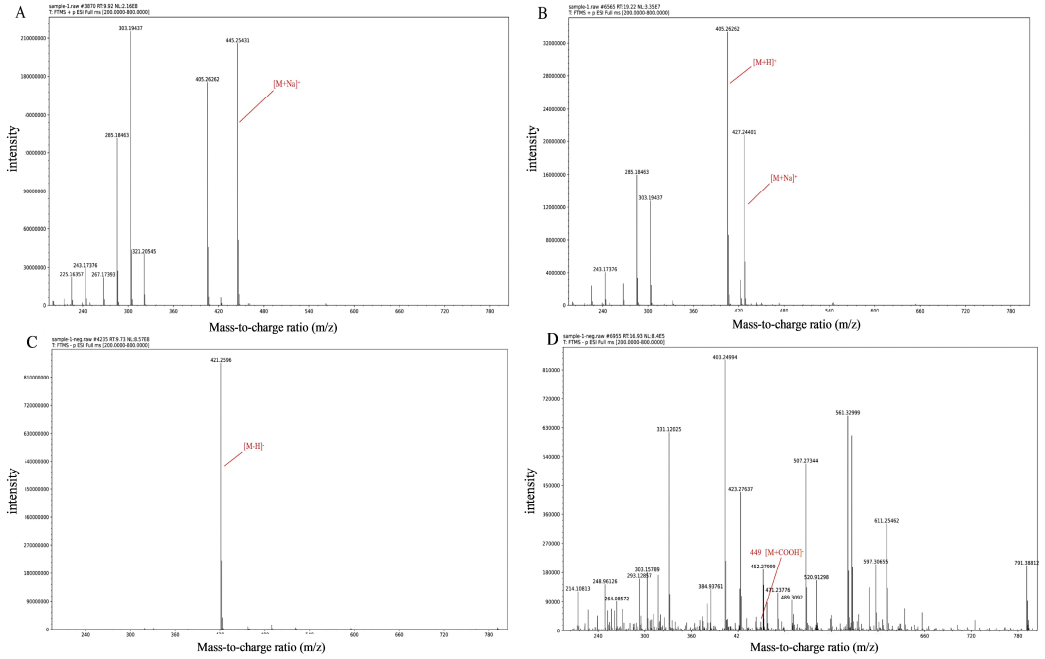

Fig. 10 LC-MS detection profile of MK in MFQ. (A) Acid MK $(M+Na)^+$; (B) Lactone MK $(M+Na)^+$ and $(M+H)^+$; (C) Acid MK $(M-H)^-$; (D) Lactone MK $(M+COOH)^-$.

The optimal fermentation process for MRFQ was as follows: 31 g/300 ml of quinoa loading amount, 20 ml addition of water to substrate, 20% of seed liquid inoculation volume, and cultured at 30°C for 2 days and then at 25°C until 12 days. Under this fermentation process, MK yield could reach 3.22 mg/g, and no citrinin was detected.

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