

Optimisation of konjac glucomannan extraction conditions using response surface methodology

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Abstract: Konjac glucomannan (KGM), derived from konjac tubers, is a novel functional plant polysaccharide that plays a significant role in antioxidant activity, anti-tumour effects and blood glucose regulation. This study aimed to develop an optimal extraction method for KGM. The extraction yield of KGM was surveyed quantitatively analysed using a response surface central composite design (CCD) to maximise the yield. A quadratic regression model was created to illustrate the interactions between the factors influencing KGM extraction yield. The findings indicated that the effects of extraction temperature and the liquid-to-solid ratio had a greater impact on the extraction yield of KGM than the extraction time and extraction frequency. The optimal extraction conditions for KGM were determined to be an extraction temperature of 100 °C, an extraction time of 2 h, an extraction frequency of 2, and a liquid-to-solid ratio of 113 : 1. Under these optimal conditions, the extraction yield of KGM reached 15.31%.

Keywords: extraction rate; extraction frequency; liquid-solid ratio; extraction temperature; extraction yield

Konjac is a perennial herb belonging to the *Araceae* family, primarily distributed in China, Japan, Myanmar, and Indonesia. To date, 115 species of konjac have been identified worldwide, with 19 species recorded in China (Hu et al. 2019). White and flower konjac production is highest in the southern and southwestern provinces of China, accounting for one-third

of global production (Kapoor et al. 2024). The edible part of konjac is the tuber, which grows mainly underground. The tuber contains many active ingredients, such as polysaccharides, amino acids, vitamins, proteins and others (Danalakoti et al. 2023). Therefore, konjac is processed into a variety of foods for human consumption.

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Konjac glucomannans (KGM), also known as konjac polysaccharides, are natural macromolecular polysaccharides with strong water solubility. They are extracted from the tubers of *Amorphophallus konjac*, and can be classified into two types: homogeneous mannan and isomannan (Guerreiro et al. 2023). Homogeneous mannan is a branched polysaccharide composed of D-mannose residues linked by β -1,4 mannoside bonds, while isomannan is a branched heteropolysaccharide composed of various monosaccharide residues linked by glycosidic bonds, primarily consisting of D-monosaccharides. Research has shown that KGM is mainly composed of β -1,4 linked D-mannose and D-glucose units (Guo et al. 2022).

As early as 1994, KGM was recognised by the U.S. Food and Drug Administration as a food additive. In food processing, KGM is widely used as a gelling agent and stabiliser, to improve food quality, enhance stability, and increase production efficiency. In the food industry, KGM is primarily utilised in beauty foods, bionic foods, gel foods, food additives, and preservatives (Hou et al. 2022). Research has shown that KGM possesses numerous beneficial properties that help prevent metabolic dysfunction, such as reducing cholesterol levels and regulating hyperlipidaemia and hyperglycaemia (Hu et al. 2025). Recently, KGM has received considerable attention due to its high nutritional value, including its role in boosting immunity and preventing tumours. Furthermore, it has been demonstrated that KGM can assist obese individuals, both children and adults, in managing their weight.

KGM is primarily exported from China to Europe and the United States, because of the high production of konjac in China. In-depth research into the edible, healthcare, medicinal, and other functions of KGM suggests that demand for it will continue to increase both domestically and internationally (Li et al. 2022). However, due to the lack of optimal KGM extraction technology, there are few high-value-added konjac products in China. Previous research have identified several KGM extraction methods, including water extraction, enzymatic extraction, acid extraction, and ethanol extraction (Liang et al. 2024). However, acid extraction introduces heavy metal ions that contaminate the KGM, rendering it inedible. Enzymatic extraction of KGM may result in hydrolysis by β -mannanase present in starch hydrolase and in the konjac tuber itself, leading to low KGM yield. Ethanol extraction causes the KGM molecular

chains to undergo stretching, crimping, rearrangement, winding, and interweaving. Consequently, the surface state and aggregation structure change, resulting in decreased solubility (Liu et al. 2023). In summary, in terms of food safety and application, water extraction of KGM is safer, while other methods are more suitable for industrial use of KGM (Ma et al. 2020). The KGM content is influenced by factors such as the solid-to-liquid ratio, extraction time, frequency of extraction, and number of extraction steps during the KGM extraction process (Ouyang et al. 2022). The KGM content is an important index for measuring the purity of konjac powder (Pan and Tang 2023). Higher purity KGM is required for molecular studies to determine molecular characteristics, conformation, and related pharmacological activity mechanisms (Ran and Yang 2022). Recently, the pharmacological activity of KGM has become a hot topic, including its roles in obesity prevention, anti-aging effects, resistance to oxidation, and regulation of intestinal microorganisms.

Response surface methodology (RSM) is a widely used and effective statistical technique for exploring the relationships between multiple explanatory variables and one or more response factors (Tong et al. 2024). RSM is commonly applied to optimise processing conditions, such as the extraction parameters of various polysaccharides, bovine plasma protein, and in food and drug processing technologies.

The objective of this study is to investigate the influence of extraction temperature, extraction time, extraction frequency, and solid-to-liquid ratio on the extraction efficiency of KGM and to determine the optimal extraction conditions using response surface methodology. The interactions between the factors affecting extraction efficiency were also examined. We believe that the information obtained from this study will be highly valuable in developing a fast, cost-effective KGM extraction process with high yield and purity, improving the utilisation rate of KGM, and minimising its loss during industrial production. Furthermore, this study provides a theoretical foundation for the further extraction and separation of KGM and other polysaccharide components.

MATERIAL AND METHODS

Material. Konjac was sourced from the experimental base of Hubei University for Nationalities in Enshi, China. Ethanol, concentrated sulfuric acid, and phenol were all bought from Sigma-Aldrich (USA).

Preparation of konjac powders. Konjac powders were prepared following a previously described method with some modifications (Wu et al. 2024). Konjac tubers were washed, peeled, and sliced into pieces 3–5 mm thick. These were then soaked in a 0.1% citric acid solution for 30 min, dried at 70 °C for 4 h, and then frozen at –20 °C for 24 h. After freezing, the material was ground for 30 s and finally sieved through a 120-mesh sieve.

The konjac tubers were cleaned, cut into slices, crushed and dried using a 50-mesh sieve, and then stored in a dryer at room temperature.

Extraction of KGM. The effects of extraction temperature, extraction time, extraction frequency, and solid-to-liquid ratio were examined to optimise the conditions for KGM extraction (Yin et al. 2020). In summary, 2 g of KGM powder was mixed with distilled water and extracted in a hot water bath as detailed in Table 1. The mixture was then centrifuged at 4 000 rpm for 15 min to separate the liquid supernatant. The remaining residue was extracted three additional times under the same conditions. The combined supernatants were concentrated to 200 mL using rotary evaporation. This concentrated solution was then precipitated with 95 % ethanol. The resulting sediment was centrifuged at 4 000 rpm for 15 min and subsequently freeze-dried in a freezer to obtain pure KGM. The extraction yield of KGM was calculated using Equation 1:

$$W = \frac{C \times V \times P}{M} \times 100\% \quad (1)$$

where: W – KGM extraction yield; C – KGM concentration; V – dilution ratio; P – KGM solution volume; M – KGM powder weight.

Measurement of polysaccharide content. Standard glucose curve. A glucose standard curve was created using the phenol-sulfuric acid method (Yin et al. 2020). First, 100 mg of anhydrous glucose was precisely weighed and dissolved in distilled water to prepare a glucose standard solution with a concentration of 1 g·L^{–1}. Then, 10 mL of the standard glucose solution of glucose of concentration 1 g·L^{–1} was dissolved in distilled water to obtain a standard glucose solution of concentration 0.1 mg·mL^{–1}. Next, volumes of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the standard glucose solution were placed into six separate test tubes, respectively and each tube of which was then filled with distilled water up

to a total volume of 1.0 mL. To each tube, 6% phenol and 5 mL of concentrated sulfuric acid were added sequentially, mixed thoroughly, and allowed to cool at room temperature for 15 min. The tube containing 0 mL of glucose solution was used as the blank, and the absorbance of the other tubes was measured at 490 nm using a UV spectrophotometer. These measurements were used to plot the glucose standard curve and develop a linear regression model with one variable (Equation 2).

$$A = C_1 X + C_2 \quad (2)$$

where: A – the value of the absorbance; X – the mass concentration of glucose; C_1 and C_2 – constant regression coefficients of the model.

Measurement of KMG content. KGM, prepared as described in Table 2, was diluted to a final volume of 10 mL with distilled water. Then, 2 µL of this solution was mixed with 6% phenol and 5 mL of concentrated sulfuric acid, shaken thoroughly, and allowed to cool to room temperature. The sample was subsequently analysed using an ultraviolet spectrophotometer at 490 nm.

Experimental design and statistical analysis. Four factors including extraction temperature, extraction time extraction frequency, and solid-to-liquid ratio were identified as key variables. The range of levels for these factors was determined based on preliminary single-factor experiments and relevant literature (Zhang et al. 2023). The independent variables to be optimised were extraction temperature (80, 90, and 100 °C), extraction time (1.5, 2, and 2.5 h) extraction frequency (1, 2, and 3), and solid-to-liquid ratio (1:80, 1:100, and 1:120). The response variable measured was the extraction yield of KGM (Zhang et al. 2021). Experiments were conducted using a centre composite design (CCD) of RSM, with Design Expert Software (version 8.0.6.1, Stat-Ease, Inc., USA) establishing the RSM-CCD model. This model included centre points, 16 factorial points, and 8 axial points to explore the interactions between factors and optimal KGM extraction conditions, as shown in Tables 1 and 2. Each factor was coded with values of –2, –1, 1, 0, 1, and 2 in Table 1, where ± 2 represented the axial points. To reduce errors, a total of 30 experimental runs were carried out, including six replicates at the centre point (0) as shown in Table 2. The extraction yield

Table 1. Process parameters including coded and levels in factorial design

Variables	Levels				
	–2	–1	0	1	2
Extraction temperature (°C)	70	80	90	100	110
Extraction time (h)	1	1.5	2	2.5	3
Extraction frequency	0	1	2	3	4
Liquid-to-solid ratio	60 : 1	80 : 1	100 : 1	120 : 1	140 : 1

of KGM (W) served as the response variable. A second-order polynomial equation for the response value was formulated to analyse the relationship between the probable optimum conditions and the response, as presented in Equation 3.

$$\begin{aligned}
 Y = & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 \\
 & + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 \\
 & + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 \\
 & + b_{34}X_3X_4
 \end{aligned} \quad (3)$$

where: Y – the predicted response represents the extraction yield of KGM; b_0 – b_{34} – the regression coefficients, representing the intercepts, linear, quadratic, and interaction terms of the model, respectively; X_1 , X_2 , X_3 , and X_4 – the independent variables.

The effects and regression coefficients of the individual linear, quadratic and interaction terms were assessed through analysis of variance (ANOVA). The statistical significance of all the terms in the mathematical model was confirmed statistically by computing the P -value differences with $P < 0.05$ were considered statistically significant. The model's goodness of fit was evaluated using the coefficient of determination R^2 .

Optimisation conditions of KGM. To maximise the extraction yield of KGM, numerical and graphical optimisation methods were performed using Design Expert Software (version 8.0.6.1) on the independent variables and response. The maximum extraction level and the relationship between the actual and predicted values were evaluated using the conventional graphical method (Chang et al. 2024). The optimal conditions for KGM extraction-including temperature, time frequency, and solid-to-liquid ratio, were determined using response surface plots and the results of numerical optimisation.

Table 2. Experimental matrix with factorial design, experimental response values, and predicted response values

Run number	Levels of factors				Experimental W	Predicted W	Student residual
	X_1	X_2	X_3	X_4			
1	–1	–1	–1	–1	7.67	7.58	0.090 8
2	1	–1	–1	–1	8.54	7.94	0.600 8
3	–1	1	–1	–1	8.35	8.09	0.257 5
4	1	1	–1	–1	9.16	9.40	–0.237 5
5	–1	–1	1	–1	8.02	8.28	–0.257 5
6	1	–1	1	–1	8.87	9.29	–0.422 5
7	–1	1	1	–1	7.11	6.78	0.329 2
8	1	1	1	–1	8.86	8.74	0.119 2
9	–1	–1	–1	1	9.93	8.72	1.21
10	1	–1	–1	1	10.12	10.62	–0.504 2
11	–1	1	–1	1	9.77	9.52	0.247 5
12	1	1	–1	1	13.95	12.37	1.58
13	–1	–1	1	1	10.89	10.83	0.062 5
14	1	–1	1	1	14.45	13.38	1.07
15	–1	1	1	1	10.34	9.62	0.724 2
16	1	1	1	1	12.85	13.12	–0.265 8
17	–2	0	0	0	8.45	9.21	–0.755 0
18	2	0	0	0	12.67	13.06	–0.395 0
19	0	–2	0	0	9.89	10.24	–0.346 7
20	0	2	0	0	9.68	10.48	–0.803 3
21	0	0	–2	0	6.45	7.50	–1.05
22	0	0	2	0	8.84	8.94	–0.103 3
23	0	0	0	–2	6.43	6.10	0.335 0
24	0	0	0	2	10.13	11.62	–1.49
25	0	0	0	0	14.54	14.56	–0.016 7
26	0	0	0	0	13.98	14.56	–0.576 7
27	0	0	0	0	15.67	14.56	1.11
28	0	0	0	0	14.78	14.56	0.223 3
29	0	0	0	0	13.55	14.56	–1.01
30	0	0	0	0	14.82	14.56	0.263 3

W – extraction yield of konjac glucomannan; X_1 – extraction temperature; X_2 – extraction time; X_3 – extraction frequency; X_4 – liquid-to-solid ratio

RESULTS AND DISCUSSION

Changes in the extraction factors (extraction temperature, extraction time extraction frequency, and solid-to-liquid ratio) affected the variation in KGM extraction yield. RSM was used to explore the levels of these rela-

tive factors and to establish a second-order polynomial equation to determine the optimal extraction conditions for KGM. The degree of fit of the second-order polynomial equation represented the relationships between the factors and the response values.

Glucose standard curve plots. The standard curve was plotted with the glucose mass concentration (X) on the x-axis and the absorbance (A) on the y-axis (Chen et al. 2022). A was measured at 490 nm. The linear regression equation for one variable was then derived to describe the relationship between glucose mass concentration and absorbance (Equation 2). The coefficient of determination R^2 was 0.989 8, indicating a strong linear correlation between X and A within the linear range of 1.13–20.11 $\mu\text{g}\cdot\text{mL}^{-1}$ (Figure 1).

$$A = 8.9017X + 0.0169 \quad (4)$$

where: A and X – absorbance and mass concentration of glucose.

Optimisation of results for the KGM extraction. Compared with conventional one-factor optimisation experiments, RSM can collect a large amount of experimental data in a short time (Chen et al. 2024). Additionally, this method optimises the number of experiments to minimise the use of consumables. In the present study, RSM was successfully used to optimise the KGM extraction conditions, including temperature, time, frequency, and liquid-to-solid ratio, to maximise KGM extraction yield. These parameters were selected based on previous single-factor experiments. The design matrix, along with the corresponding responses in terms of KGM extraction yield for 30 runs, was established using CCD and is presented in Table 2.

The maximum W value of 15.67% for the KMG extraction yield of KMG was observed in experimental run 27 (Table 2), which was significantly higher than the minimum W value of 6.43% recorded in experimental run 23 ($P < 0.05$). Interestingly, it was found that there were three identical parameter values (extraction temperature, extraction time, and extraction frequency) were identical in both runs 23 and 27, with the only difference being the liquid-to-solid ratio. A higher liquid-to-solid ratio resulted in more complete dissolution of KGM. The extraction yield of KGM depended on the liquid-solid ratio in these two runs, indicating that the liquid-to-solid ratio was a major factor in the KGM extraction process. This finding is therefore valuable for the industrial extraction of KGM.

The data collected from the experimental runs were analysed using RSM Design-Expert software to fit nonlinear quadratic models for the extraction yield of KGM (Table 3, Equation 3). The results of the analysis of variance (ANOVA) are presented in Table 3 and validate the model. This model accurately reflects the KGM extraction conditions, as the variation in the response value closely corresponds to the variation in the factors. The F -value of the regression model in Table 3 is 15.36, with a very low probability value ($P < 0.0001$), indicating that the model is highly significant. There is only a 0.01% chance that an F -value this large could occur due to noise. P -values less than 0.05 indicate that the model terms are significant. As shown in Table 3, X_1 , X_4 , X_1^2 , X_2^2 , X_3^2 , and X_4^2 are significant model terms ($P < 0.05$), however, values greater than 0.1000 indicate that the model terms are not significant. Therefore, X_2 , X_3 , X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_2X_4 , and X_3X_4 were not significant within the test-

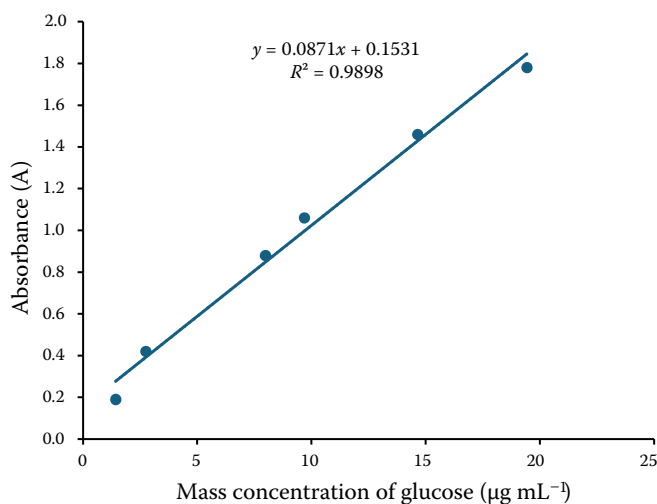


Figure 1. Standard glucose curve plot calculating konjac glucomannan extraction amount

Table 3. The results and reduced quadratic model of the extraction yield of konjac glucomannan response surface through analysis of variance (ANOVA)

Source	df	Sum of squares	Mean square	F-value	Prob > F
Model	14	207.76	14.84	15.36	< 0.000 1
X_1	1	22.35	22.35	23.13	0.000 2
X_2	1	0.091 3	0.091 3	0.094 5	0.762 8
X_3	1	3.14	3.14	3.25	0.091 6
X_4	1	45.71	45.71	47.31	< 0.000 1
X_1X_2	1	0.893 0	0.893 0	0.924 3	0.351 6
X_1X_3	1	0.429 0	0.429 0	0.444 1	0.515 3
X_1X_4	1	2.37	2.37	2.45	0.138 0
X_2X_3	1	4.04	4.04	4.18	0.058 8
X_2X_4	1	0.081 2	0.081 2	0.084 1	0.775 8
X_3X_4	1	1.97	1.97	2.04	0.173 4
X_1^2	1	20.07	20.07	20.77	0.000 4
X_2^2	1	30.19	30.19	31.25	< 0.000 1
X_3^2	1	68.83	68.83	71.25	< 0.000 1
X_4^2	1	55.73	55.73	57.68	< 0.000 1
Residual	15	14.49	0.966 1		
Lack of fit	10	11.79	1.18	2.18	0.201 8
Pure error	5	2.70	0.541 0		
Corrected total	29	222.25			
SD	0.982 9	R^2	0.934 8		
Mean	10.63	Adjusted R^2	0.873 9		
CV		9.25	Pred R^2	0.677 0	
PRESS		71.79	Adequate precision	12.174 6	

Values of 'Prob > F' less than 0.050 0 indicate model terms are significant; X_1 – extraction temperature; X_2 – extraction time; X_3 – extraction frequency; X_4 – liquid-to-solid ratio; PRESS – predicted sum of square

ed range ($P > 0.05$). This indicates that the equation for coded factors in Equation 3 accurately reflects changes in response values. Based on the magnitude the F -values in the regression model, the order of influence of all factors is liquid-to-solid ratio > extraction temperature > extraction frequency > extraction time. The model was proven to fit well when the lack-of-fit value was greater than 0.05. In the present study, the lack-of-fit F -value was 2.18, which was not significant relative to pure error, with a P -value of 0.201 8. This implies that there is a 20.18% chance that a lack of fit F -value this large could occur due to noise. A non-significant lack-of-fit indicates that the regression model fits the data well and accurately represents the actual situation. Equation 5 presents the regression equation showing the effect of the extraction conditions on the extraction yield of KGM in terms of the actual values of the variables.

$$\begin{aligned}
 Y = & 14.56 + 0.965X_1 + 0.0617X_2 + 0.3617X_3 \\
 & + 1.38X_4 - 0.8554X_1^2 - 1.05X_2^2 - 1.58X_3^2 \\
 & - 1.43X_4^2 + 0.2363X_1X_2 + 0.1638X_1X_3 \\
 & + 0.385X_1X_4 - 0.5025X_2X_3 + 0.0713X_2X_4 \\
 & + 0.3513X_3X_4
 \end{aligned} \quad (5)$$

where: X_1 , X_2 , X_3 , and X_4 – extraction temperature, extraction time, extraction frequency, and liquid-solid ratio respectively, in the coded factors of the multivariate nonlinear equation.

A determination coefficient R^2 of 0.998 3 indicates that the regression model is highly significant. The adjusted R^2 of 0.873 9 shows that more than 87% of the variation in the response values is explained by changes in the experimental factors included in the model. Furthermore, the predicted R^2 of 0.677 0 is reasonably close to the adjusted R^2 of 0.873 9, with a difference of less than 0.2. The adequate precision value meas-

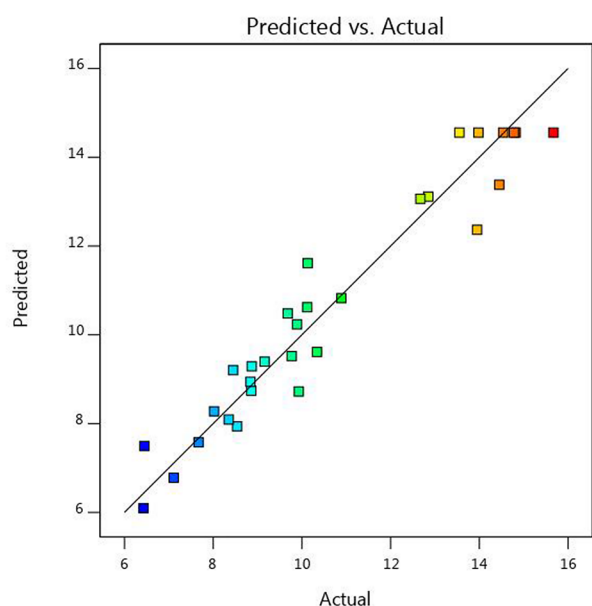


Figure 2. Scattergram of the predicted response versus experimental weighted values of the extraction yield of konjac glucomannan, where the extraction conditions include extraction temperature, extraction time, extraction frequency, and liquid-to-solid ratio

Colours indicate clustered; W – value ranges

ures the signal-to-noise ratio, where a desirable signal-to-noise ratio is greater than 4, in the present model,

the adequate precision value of 12.175 indicates an adequate signal, confirming that this model can be used to navigate the design space. The reliability and precision of the experiments are further supported by a low coefficient of variation (CV) of 9.25. The ANOVA analysis therefore assumes that the relationship between the KGM extraction yield and the four extraction factors is well fitted by the regression model within the limits of experimental factors. Figure 2 shows the similarities between the predicted and actual values of the experiment. As can be seen in Figure 2, the values are very close to each other. The extraction parameters (extraction temperature, extraction time, extraction frequency, and liquid-to-solid ratio) were optimised numerically to find the point that maximises extraction yield of KGM. The optimal conditions for the extraction yield of KGM are an extraction temperature of 100 °C, an extraction time of 2 h, an extraction frequency of 2, and a liquid-to-solid ratio of 113 : 1. Under these conditions, Figure 3 shows that the maximum extraction yield of KGM is 15.31%.

Effects of extraction process parameters on extraction yield of KGM. The maximum KGM extraction yield was achieved through the interaction of all extraction factors. As shown in Figure 4, it was hypothesised that there was a degree of interaction between the two factors, as indicated by the

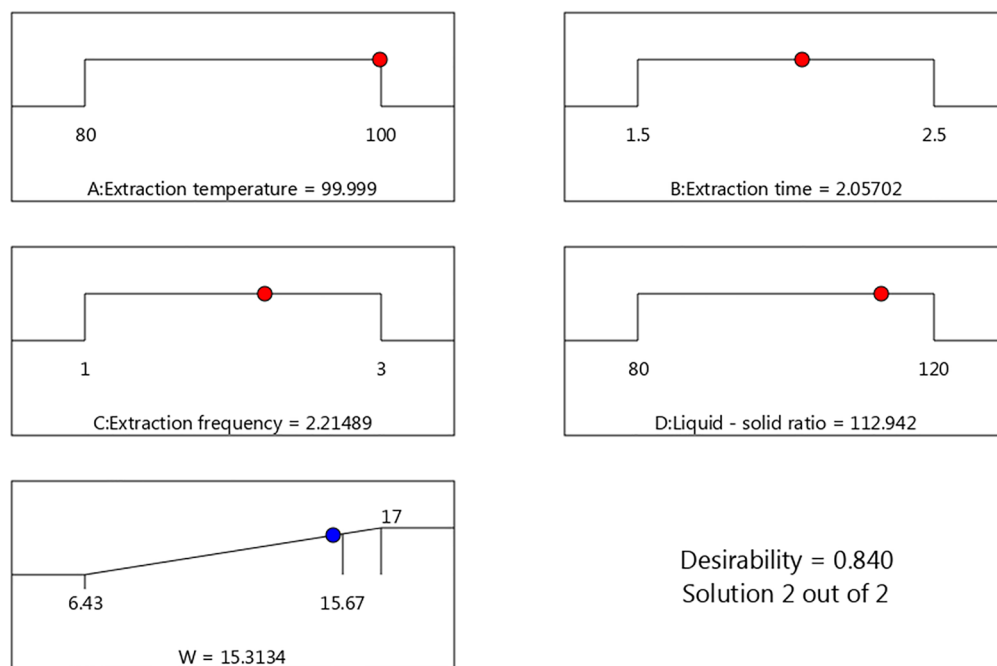


Figure 3. The theoretical optimisation results of the extraction yield of konjac glucomannan (KGM) under the above extraction conditions obtained by response surface methodology

W – extraction yield of KGM

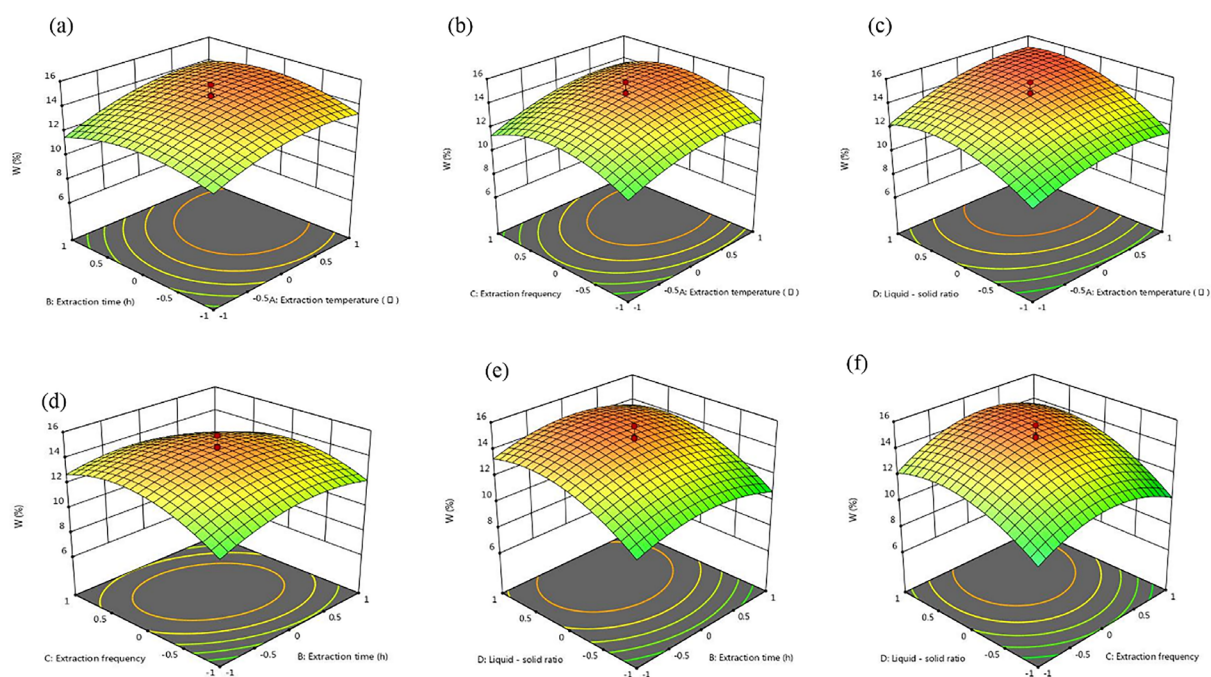


Figure 4. Three-dimensional contour response surface plots displaying interactions between factors

(A) extraction temperature and extraction time; (B) extraction temperature and extraction frequency; (C) extraction temperature and liquid-to-solid ratio; (D) extraction time and extraction frequency; (E) extraction time and liquid-to-solid ratio, and (F) extraction frequency and liquid-to-solid ratio; W – extraction yield of konjac glucomannan

steepness of the response surface and the shape of the three-dimensional contour. The coefficients in Equation 5 also intuitively reflected the interaction between the two factors. A positive coefficient implied that the extraction yield of KGM increased with an increase in extraction temperature, extraction time, extraction frequency, and liquid-to-solid ratio. However, the presence of positive interaction terms between extraction temperature and extraction time, extraction temperature and extraction frequency, extraction temperature and liquid-to-solid ratio, extraction time and liquid-to-solid ratio, and extraction frequency and liquid-to-solid ratio predicted that an increase in these levels would decrease the extraction yield of KGM. The negative coefficient between extraction time and extraction frequency indicated that there was no interaction, and increase in their levels decreased the extraction yield of KGM. The liquid-to-solid ratio is an important parameter in the KGM extraction process. The greater the amount of solvent, the higher the extraction yield of KGM. When the amount of solvent reaches a certain value, the extraction yield of KGM is essentially complete (Ding et al. 2023).

Response surface plots (Figures 4A–F) were generated to fit the model effectively as a function of two

variables while keeping the third variable at its central point. These plots clearly illustrate the combined influence of the four factors on the extraction yield of KGM. The extraction yield of KGM initially increased gradually and then decreased during the extraction process with respect to temperature, extraction time, and extraction frequency (Figures 4A–C).

Meanwhile, the extraction yield of KGM increased rapidly in the early stages of the extraction process. After this, the extraction yield of KGM slowed down gradually as the saturation degree of the konjac powder solution approached saturation with increasing liquid-to-solid ratio (Figures 4D–F). It has been demonstrated that the liquid-to-solid ratio is a critical factor affecting the extraction yield of polysaccharides (Du et al. 2021). As the liquid gradually increased, the extraction rate of polysaccharides increased in line with the increase in water-soluble polysaccharides. However, once the water-soluble polysaccharides reached a saturated state, the extraction rate declined in the later stages (Fang 2021).

Within a certain range, the extraction yield of KGM increased with an increase in the extraction times (Figures 4A and 4D) and also with a corresponding increase in the extraction frequency (Figures 4B and 4D). However, beyond this range, the extraction rate decreased,

possibly due to structural and property changes in the structures and properties of KGM with increased extraction time. Increasing the extraction frequency would not enhance the extraction yield of KGM if its structure were damaged. As can be seen in Figures 3A–C, the extraction yield of KGM increased significantly with rising extraction temperature throughout the entire extraction process. This may be because the high-viscosity KGM could not fully dissolve at lower temperature; a higher temperatures could facilitate complete dissolution. These findings are consistent with those reported by (Yin et al. 2020).

Model validation experiments. As shown in Figure 3, the theoretical optimal conditions for KGM extraction were an extraction temperature of 100 °C, an extraction time of 2 h, an extraction frequency of 2 and a liquid-to-solid ratio of 113:1. Under these conditions, the maximum KGM extraction yield was 15.31%. To verify the mathematical model's predictive accuracy, verification experiments were conducted under the aforementioned optimised conditions in quintuplicate to further validate the model. The KGM extraction yield was 14.97%, which is comparable to the predicted value of 15.31%. The extraction temperature, time, frequency, and liquid-to-solid ratio were all reasonable. This indicates that the measured extraction yield of KGM is in good agreement with the predicted value. Therefore, the values obtained for extraction temperature, extraction time, extraction frequency, and liquid-to-solid ratio by RSM were real and valid. From the above discussion, it can be concluded that the model can be used to assess the extraction yield of KGM and that RSM is valuable for optimising extraction conditions to maximise KGM yield.

CONCLUSION

In this study, we investigated the effects of extraction temperature, extraction time, extraction frequency, and liquid-to-solid ratio on the yield of KGM extraction, using RSM to determine the optimal conditions. The study concluded that the liquid-to-solid ratio and extraction temperature were the most significant factors influencing the extraction yield of KGM during the extraction process followed closely by extraction frequency and time. The steepness of the response surface and the shape of the three-dimensional contours plots suggested interactions among the factors in the response surface plots. RSM was found to enhance the extraction yield of KGM by incorporating the in-

teractive effects of the examined factors. These findings indicate that RSM is effective in optimising the parameters of the KGM extraction process.

ANOVA showed that the factors involved in the extraction process were statistically significant. A second-order polynomial model was developed to predict the extraction yield of KGM. Optimising the following parameters extraction temperature at 100 °C, extraction time of 2 h, extraction frequency of 2, and a liquid-to-solid ratio of 113:1 provided optimal conditions that resulted in a significantly higher extraction yield of 15.31% compared to other experimental groups. Under these conditions, a high-yield KGM extract can be obtained. Future studies will investigate the protective effects of KGM on cells and organs through proteomic analyses of its immune regulation mechanisms.

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