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Optimisation of polyphenol extraction from Chinese Baijiu distillers' spent grains: Stability and antioxidant capacity

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Abstract: Chinese Baijiu distillers' spent grains (DSGs), a major byproduct of liquor production containing valuable polyphenols, face disposal challenges because of their high moisture content and rapid spoilage. In this study, an optimised cellulase-assisted extraction process was developed for DSG polyphenols (DGPs), and their stability and antioxidant capacity were comprehensively characterised. The extraction yield of DGP was determined as the primary response variable to evaluate the effectiveness of the process. A central composite design (CCD) was employed to optimise key operational parameters: enzyme concentration, enzyme temperature and liquid–solid ratio. Results demonstrated that the optimal process conditions were a cellulase dosage of 4.0%, an enzyme temperature of 50 °C and a liquid–solid ratio of 40 mL·g^{-1}, obtaining a polyphenol yield of 4.20 ± 0.10 mg·g^{-1}. Stability assessment indicated that DGP retained 68.9 ± 1.8% of the phenolic content after 7 days of frozen storage at −18 °C, exhibiting better preservation than storage under refrigeration (47.9 ± 2.1%) and room temperature (45.5 ± 3.2%) conditions. Antioxidant assays showed concentration-dependent (0.50–8.0 μg·mL^{-1}) scavenging capacities for ABTS (IC_{50} = 6.0 μg·mL^{-1}) and DPPH (IC_{50} = 2.8 μg·mL^{-1}). These findings offer valuable insights for the transformation of distillery byproducts into functional food ingredients while simultaneously addressing the challenges of solid waste management in alcoholic beverage production.

Keywords: Chinese Baijiu byproduct; phenolic compounds; response surface methodology; storability; radical scavenging activity

As the world's most consumed distilled spirit, the annual output of Chinese Baijiu exceeds 236.7 million hectolitres (National Bureau of Statistics of China 2023; Lee et al. 2025). Sorghum (Sorghum bicolor L. Moench) serves as the primary raw material for its production. As the fifth most important cereal crop globally, sorghum holds a prominent position in Chinese grain production. Red sorghum varieties from Northeast China, characterised by a high starch content and moderate tannin levels (1.0–2.0%), have become a key raw material for leading distilleries such as Maotai and Wuliangye (Kassara et al. 2022; Shi et al. 2024). Sorghum grains are rich in diverse polyphenolic compounds, mainly phenolic acids (e.g. ferulic acid, p-coumaric acid, and syringic acid), flavonoids, and alkylresorcinols (Meenaa et al. 2022). These compounds are predominantly located in the bran layer and exhibit remarkable antioxidant, anti-inflammatory, and anticancer properties, making them important targets in nutritional and pharmacological research.

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The production of Chinese Baijiu typically involves solid-state fermentation using a starter culture called Daqu, followed by distillation. During this process, sorghum and other grains undergo simultaneous saccharification and fermentation in pits or tanks, where microorganisms convert starch into ethanol and various flavour compounds. After spirit is obtained through distillation, the residual solid material, known as distiller's spent grains (DSGs) or Jiuzao, constitutes a major byproduct. The brewing process generates substantial quantities of DSG, a nutrient-rich byproduct of fermentation. As of June 2023, China's annual DSG output exceeded 25 million tonnes. DSG contains valuable bioactive compounds, including proteins, dietary fibre, phenolic compounds and polysaccharides (Sha et al. 2017; Hu et al. 2020; Yang et al. 2021; Zeng et al. 2021), making it a promising resource for the food industry. However, its high moisture content (60–70%), combined with its acidic nature and susceptibility to rapid spoilage, poses significant storage challenges and environmental risks (Espana-Gamboa et al. 2011). Currently, only a small portion of DSG is valorised, primarily through the extraction of functional ingredients for applications in food, biodegradable films, packaging materials and cosmetics (Prabhakumari et al. 2018; DeRose et al. 2019). Meanwhile, the majority of DSG is utilised as low-value animal feed or discarded as waste (Jara-Palacios et al. 2019; Sancho-Galán et al. 2020; Liu et al. 2023). This situation underscores the urgent need to develop high-value utilisation pathways for DSG to align with the circular economy principles.

Polyphenols, which are widely present in cereals, fruits, vegetables and tea, exhibit considerable structural diversity and bioactivity. Cereal-derived polyphenols, predominantly comprising phenolic acids, flavonoids, and alkylresorcinols (Shanmugam 2024), have attracted increasing interest because of their notable antioxidant, anti-inflammatory, and anticancer properties (Tian et al. 2019). Sorghum, in particular, contains substantial amounts of flavonoids and phenolic acids, contributing to its high antioxidant capacity. Given that DSG is rich in bioactive polyphenols (DGPs) derived from raw grains, it represents a promising source of natural antioxidants.

Conventional methods for extracting DGP, such as solvent extraction (Wang et al. 2019) and ultrasound-assisted extraction (Alonso-Riaño et al. 2020), are often hampered by drawbacks such as presence of solvent residues, complex procedures, and relatively low extraction efficiency. To overcome these drawbacks, this study utilised an enzymatic hydrolysis approach to improve the recovery of DGP. The extraction conditions were systematically optimised using a combination of single-factor experiments and response surface methodology. Additionally, the stability and antioxidant activity of DGP were investigated. This work aims to establish an efficient and environmentally friendly DGP extraction protocol, thereby promoting the high-value utilisation of brewing byproducts, creating new conversion pathways for functional food ingredients, reducing the environmental impact of Baijiu production waste, and ultimately contributing to a more sustainable beverage industry.

MATERIAL AND METHODS

Material and chemicals

DSG was procured from Shenyang Tianjiang Old Longkou Brewing Co., Ltd. (China). The samples were pre-dried and derived from a locally specific Northeast Chinese red sorghum, with the following typical composition: > 68.0% starch, 9.0–11.0% crude protein, 3.0–4.0% fat and 6.0–9.0% total dietary fibre. This composition supports solid-state fermentation and ensures sample consistency. Gallic acid standard (HPLC purity > 98%), cellulase (CAS: 9012-54-8, derived from Trichoderma viride; enzyme activity ≥ 600 $U \cdot mg^{-1}$; pH 4.0–5.5; and optimal temperature 40–60 °C), and Folin–Ciocalteu reagent were sourced from Shanghai Jinshui Biotechnology Co., Ltd. (China). 1,1-Diphenyl-2-trinitrophenylhydrazine (DPPH) was purchased from Shanghai Huhui Biotechnology Co., Ltd. (China); 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS) was obtained from Hefei Bomei Biotechnology Co., Ltd. (China); and Trolox was sourced from Anhui Coolsaint Biological Technology Co., Ltd. (China). Distilled water was used for all the experiments.

Pretreatment of DSG

Dry DSGs were crushed and then sieved through a 60 mesh sieve (Theertha and Suresh 2025). A specific amount of the resulting powder was accurately weighed and mixed with petroleum ether (boiling point at 60–90 °C) at a liquid–solid ratio of 40 $mL \cdot g^{-1}$. The mixture was placed in an oscillator at room temperature and defatted for 4 h at a rotation speed of 150 rpm. After defatting, the mixture was filtered, and the filtrate was discarded. The remaining residue was dried at a low temperature to obtain defatted DSG. Finally, the defatted DSG was stored at 4 °C for preservation.

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Extraction process for DGP

A buffer solution with a pH of 4.8 was preheated to 50 °C and added to the defatted DSG, followed by thorough stirring. Afterwards, an appropriate amount of enzyme was added and mixed again. The mixture underwent enzymatic digestion at the preset temperature. Subsequently, the enzyme was inactivated in a water bath at 90 °C for 3 min. The mixture was centrifuged at 4 000 rpm for 15 min, and the supernatant was diluted to an appropriate concentration for determining the total phenolic content.

Determination of total phenolic content

The total phenolic compound concentration was determined via the Folin–Ciocalteu reagent method (Kovacev et al. 2020; Tariq et al. 2023) with minor modifications. Briefly, 0.5 mL gallic acid standard solution or diluted sample extract was combined with 1 mL Folin–Ciocalteu reagent and 3 mL 8.0% Na_2CO_3 solution in a 10 mL volumetric flask. Following dilution to the mark, the mixture was incubated in dark for 1 h prior to measuring its absorbance at 760 nm. A standard curve was generated using gallic acid standards, whose concentration ranged from 1.05 to 6.65 mg·L^{-1}, fitting the linear equation $y = 0.117\,6x + 0.026\,2$ ($R^2 = 0.999\,2$). The total phenolic content was calculated as gallic acid equivalents (GAE) using Equation 1:

$$Y = \frac{C \times V \times N}{m} \quad (1)$$

where: Y – the total phenol extraction (mg·g^{-1}); C – the DGP concentration (mg·L^{-1}); V – the volume of extract (mL), N – the dilution factor; m – the mass of the defatted DSG (g).

Single-factor experiment design

Based on the preliminary experimental results, the influence of four factors – the liquid–solid ratio (10.0–70.0 mL·g^{-1}), enzyme concentration (1.0–6.0%), enzyme temperature (30–80 °C), and enzyme time (30–120 min) – on the DGP extraction yield was individually investigated using the DGP extraction yield as the index. During each single-factor test, the other conditions were fixed at 50 °C, 3.0% enzyme addition, a 40 mL·g^{-1} liquid–solid ratio, and a 40 min enzyme time. Moreover, the parameter ranges for each factor in the subsequent optimisation experiments were determined.

Response surface experiment design. The single-factor experiment results showed that the enzyme concentration (X_1), enzyme temperature (X_2), and liquid–solid ratio (X_3) significantly affected DGP extraction. To optimise the extraction process, a three-factor and three-level central composite design (CCD) was used, with the DGP extraction yield utilised as the response variable (Y) (Sun et al. 2023). The factor-level coding is shown in Table 1.

Table 1. Factor level coding table by central composite design (CCD) experimental design

Level	Factors		
	X_1 (enzyme concentration, %)	X_2 (enzyme temperature, °C)	X_3 (liquid–solid ratio, mL·g^{-1})
–1	1.0	30	20
0	3.0	50	40
1	5.0	70	60

Stability study of DGP. Following the methods reported by Al et al. (2023) and Salazar-Orbea et al. (2023) with slight modifications, the DGP test solution was prepared via the optimal extraction process and diluted to polyphenol extracts at 1.0, 2.0, and 5.0 μg·mL^{-1}. After storing under different conditions [room temperature (20.0 °C), room temperature without light (20 °C), refrigeration (4 °C), and freezing (–18 °C)], samples were taken at time points ranging from 1 to 168 h to monitor changes in the polyphenol content. The rate of change was calculated using Equation 2:

$$Change\ of\ DGP\ (\%) = \frac{C_0 - C_t}{C_0} \times 100\% \quad (2)$$

where: C_t – the post-treatment DGP content (μg·mL^{-1}); C_0 – the pre-treatment DGP content (μg·mL^{-1}).

Antioxidant capacity

ABTS radical scavenging assay. A method reported by Wang et al. (2017) was adopted with modifications. Before use, the ABTS solution was prepared, and its concentration was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm (A_0). Afterwards, 4.0 mL of this solution was mixed with 40 μL diluted sample solution and reacted in dark for 6 min. Subsequently, the absorbance (A_s) of the resulting solution was measured. The free-radical scavenging ability of the sample was calculated using Equation 3. A standard curve was constructed by plotting the ABTS scavenging rate against different concentrations of Trolox, ranging from 10 to 500 μg·mL^{-1}. The resulting linear equation was $y = 0.148\,1x + 5.940\,6$ ($R^2 = 0.999\,4$). The results of the ABTS assay were expressed as Trolox equivalents.

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$$ABTS\ radical\ scavenging\ rate\ (\%) = (1 - \frac{A_s}{A_0}) \times 100\% \quad (3)$$

DPPH radical scavenging assay. In accordance with Lu et al. (2016) and with slight modifications, the assay was performed as follows. A 2.0 mL aliquot of the diluted sample solution was mixed with 2.0 mL 0.2 mmol·L^{-1} DPPH ethanol solution and incubated in the dark at room temperature for 1 h. The absorbance of the resulting solution was then measured at 517 nm (A_s). Control measurements were performed by replacing the sample with anhydrous ethanol (A_b) and replacing the DPPH solution with anhydrous ethanol (A_c). The radical scavenging rate was calculated using Equation 4. A standard curve was established using Trolox solutions, whose concentration ranged from 2.0 to 25.0 μg·mL^{-1}. The fit of the linear equation was $y = 3.505\ 3x + 5.746\ 7$ ($R^2 = 0.999\ 8$), and the results were expressed as Trolox equivalents.

$$DPPH\ radical\ scavenging\ rate\ (\%) = (1 - \frac{A_s - A_c}{A_b}) \times 100\% \quad (4)$$

Statistical analysis. The results were analysed using one-way ANOVA with SPSS 26.0 (IBM, USA). The experimental design was evaluated by Design Expert 8.0.6 (StatEase, USA), and Prism 9.0 (GraphPad, USA) was employed for data visualisation. All experiments were conducted thrice, and all measured values are presented with one decimal place to maintain consistency, unless otherwise specified for particular statistical analyses requiring higher precision.

RESULTS AND DISCUSSION

Single-factor experiment. As shown in Figure 1, single-factor experiments demonstrated the influence of key factors on polyphenol extraction.

Enzyme concentration (X_1). Cellulase addition significantly influenced polyphenol release through cell wall hydrolysis. An enzyme concentration of 3.0–5.0% maximised the extraction efficiency; meanwhile, excessive amounts (> 5.0%) led to substrate saturation without further yield improvement.

Enzyme temperature (X_2). The temperature had a bi-directional effect on DGP extraction. Moderate temperatures (30–70 °C) accelerated enzymatic reactions and increased the extraction amount, whereas temperatures exceeding 70 °C caused thermal degradation of bioactive compounds.

Liquid–solid ratio (X_3). An initial increase in the liquid–solid ratio accelerated molecular diffusion and increased the polyphenol extraction rate. Meanwhile, when the substrate was limited, the enzyme concentration was decreased and polyphenols were fully released; additionally, a further increase in the ratio had a minimal effect on extraction. A ratio in the range of 40–60 mL·g^{-1} struck a balance between the efficiency and cost.

Enzyme time. A prolonged enzyme activity time caused polyphenol oxidation. After 40 min, the enzymes fully extracted polyphenols while avoiding structural damage. Consequently, this parameter was excluded from the primary factors in the subsequent optimisation.

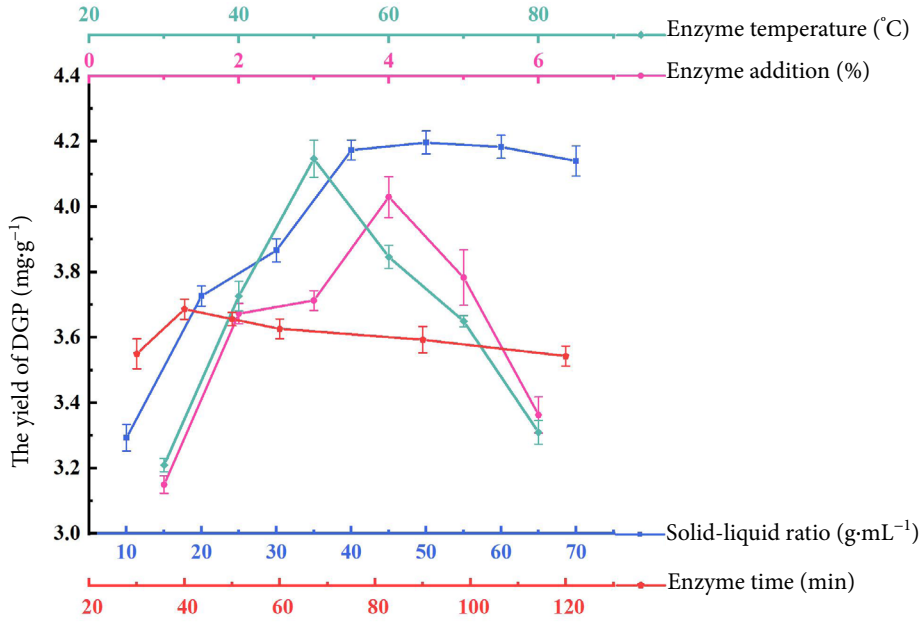

Figure 1. Effect of single factor on polyphenol extraction of DSG: enzyme temperature (30–80 °C); enzyme addition (1.0–6.0%); liquid–solid ratio (10–70 mL·g^{-1}); enzyme time (30–120 min)

DSG – distillers' spent grains; DGP – DSG polyphenols

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Table 2. Central composite design (CCD) experimental design and response values

Test number	Factors			DGP extraction yield ($mg \cdot g^{-1}$)
	X_1 (enzyme concentration, %)	X_2 (enzyme temperature, °C)	X_3 (liquid–solid ratio, $mL \cdot g^{-1}$)	
1	0	0	0	4.40
2	0	0	0	3.90
3	1	1	0	3.50
4	0	–1	1	3.70
5	–1	0	–1	3.10
6	0	1	–1	3.50
7	0	0	0	4.10
8	0	0	0	4.20
9	–1	0	1	2.70
10	–1	1	0	2.20
11	–1	–1	0	2.80
12	0	–1	–1	3.10
13	0	1	1	2.60
14	1	0	–1	3.10
15	1	–1	0	3.20
16	0	0	0	4.10
17	1	0	1	3.90

Optimisation of extraction process. The results of the CCD design are shown in Table 2. Response surface regression analysis was performed on the experimental data using Design Expert 8.0 to establish a multiple quadratic regression model (Table 3). The derived equation is as follows:

$$Y = 4.14 + 0.42X_1 - 0.14X_2 - 0.07X_3 + 0.22\,X_1X_2 + 0.43\,X_1X_3 - 0.39\,X_2X_3 - 0.69\,X_1^2 - 0.52\,X_2^2 - 0.38\,X_3^2 \quad (5)$$

where: Y – the extraction yield; X_1 – enzyme concentration (%); X_2 – temperature (°C); X_3 – liquid–solid ratio ($mL \cdot g^{-1}$).

This quadratic equation describes the individual effects of each factor, their quadratic curvilinear relationships, and the interaction effects. Y exhibited a positive linear relationship with X_1 (coefficient +0.42), whereas it demonstrated negative linear relationships with X_2 (–0.14) and X_3 (–0.07). Owing to the presence of negative quadratic terms ($-0.69X_1^2$, $-0.52X_2^2$, and $-0.38X_3^2$), all factors displayed a quadratic relationship (inverted U shape), indicating that their effects first peak before declining. Interaction effects were also observed: X_1X_2 (+0.22) and X_1X_3 (+0.43) represent positive interactions, signifying that simultaneous increases in both factors enhance the yield. Meanwhile, X_2X_3 (–0.39) denotes a negative interaction, indicating that a concurrent increase in these two factors reduces the yield.

As presented in Table 3, model's P value was 0.000 1, indicating statistical significance. The lack-of-fit term's P value was 0.846 9, which exceeded 0.05, suggesting no significant lack of fit between the regression equa-

Table 3. Regression model ANOVA table

Project	Sum	*df*	Mean^2	*F*	*P*	Significance
Model	7.330	9	0.810	50.27	< 0.000 1	**
X_1	1.390	1	1.390	86.09	< 0.000 1	**
X_2	0.150	1	0.150	9.34	0.018 4	*
X_3	0.039	1	0.039	2.42	0.163 7	
X_1X_2	0.200	1	0.200	12.50	0.009 5	**
X_1X_3	0.740	1	0.740	45.66	0.000 3	**
X_2X_3	0.610	1	0.610	37.56	0.000 5	**
X_1^2	2.030	1	2.030	125.02	< 0.000 1	**
X_2^2	1.150	1	1.150	71.24	< 0.000 1	**
X_3^2	0.600	1	0.600	37.24	0.000 5	**
Residual	0.110	7	0.016	–	–	–
Lack of fit	0.019	3	0.006 3	0.27	0.846 9	not significant
Pure error	0.150	4	0.024	–	–	–
Total error	5.320	16	–	–	–	–

* and **significance levels at 0.05 and 0.01, respectively

X_1 – enzyme concentration (%); X_2 – enzyme temperature (°C); X_3 – liquid–solid ratio ($mL \cdot g^{-1}$)

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tion and actual results. These results show that the model can be employed to analyse the DGP yield. The coefficient of determination R^2 = 0.984 8 and adjusted coefficient of determination R_{adj}^2 = 0.965 2 indicate a strong correlation of the model. The coefficient of variation CV% was 3.75, suggesting high accuracy and reliability of the experimental data.

X_2 had a significant effect on the DGP yield (P < 0.05). Moreover, the main effects of X_1 and the interaction terms X_1X_2, X_1X_3, and X_2X_3 as well as quadratic terms X_1^2, X_2^2, and X_3^2 were extremely significant ($P < 0.01$). The F value of the regression model indicates that the factors affect the extraction of DGP in the following order: enzyme concentration (X_1) > enzyme temperature (X_2) > liquid–solid ratio (X_3).

The optimal extraction parameters were as follows: an enzyme concentration of 4.0%, an enzyme temperature of 47.69 °C, and a liquid–solid ratio of 40.72 mL·g^{-1}, potentially resulting in a DGP yield of 3.96 mg·g^{-1}. To facilitate experimental operations, the process parameters were adjusted to an enzyme concentration of 4.0%, an enzyme temperature of 50 °C, and a liquid–solid ratio of 40 mL·g^{-1}. Multiple parallel experiments were carried out under these optimised conditions, obtaining an actual DGP yield of 4.20 ± 0.10 mg·g^{-1}. The prediction error was 1.0%, indicating that the parameters obtained from the regression equation are accurate and reliable and the model has good predictive performance.

Response surface analysis. Figure 2 illustrates the interactive effects of the three factors on the DGP yield. The slope of the response surface serves as an indicator of the sensitivity of the response value to changes in the factors. A steeper slope implies that the response value is highly sensitive to variations in the factors. In contrast, a gentler slope suggests relatively low sensitivity of the response value to such changes. The contour plot, which represents the projection of the response surface, reflects the magnitude of the interaction between factors. An elliptical contour shape indicates a significant interaction between two factors.

As shown in Figure 2A, the extraction yield of DGP initially increased and then decreased as the enzyme concentration and temperature increased. The steep response surface and elliptical contour lines indicate a significant interaction between X_1 (enzyme concentration) and X_2 (enzyme temperature) ($P < 0.05$). Figure 2B reveals that the extraction yield of DGP initially increased and then decreased with increasing enzyme concentration and liquid–solid ratio. The steep response surface and elliptical contour lines indicate a significant interaction between X_1 and X_3 (liquid–solid ratio) ($P < 0.05$). Figure 2C shows that the extraction yield of DGP first increased and then plateaued as the enzyme temperature and liquid–solid ratio increased. These results suggest a significant interaction between X_2 and X_3 ($P < 0.05$).

Data diagnostics for analogue. As depicted in Figures 3A and 3B, the residuals follow a normal distribution, with the data points closely aligning along a straight line. This implies a strong agreement between the predicted and actual values of the experiment. Figures 3C and 3D illustrate relationships among the predicted values, residuals, and experimental runs.

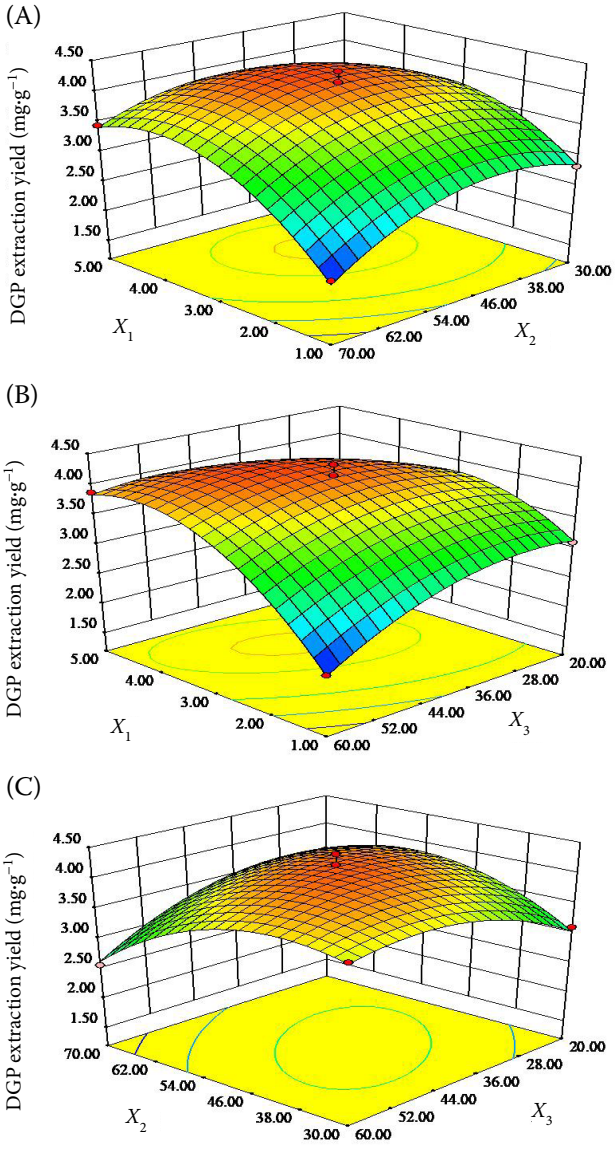

Figure 2. Response surface analysis of the effects of key parameters on DGP extraction yield

X_1, X_2, X_3 – enzyme concentration (1.0–5.0%), enzyme temperature (30–70 °C), liquid–solid ratio (20–60 mL·g^{-1}), respectively; DGP – distillers' spent grain polyphenols

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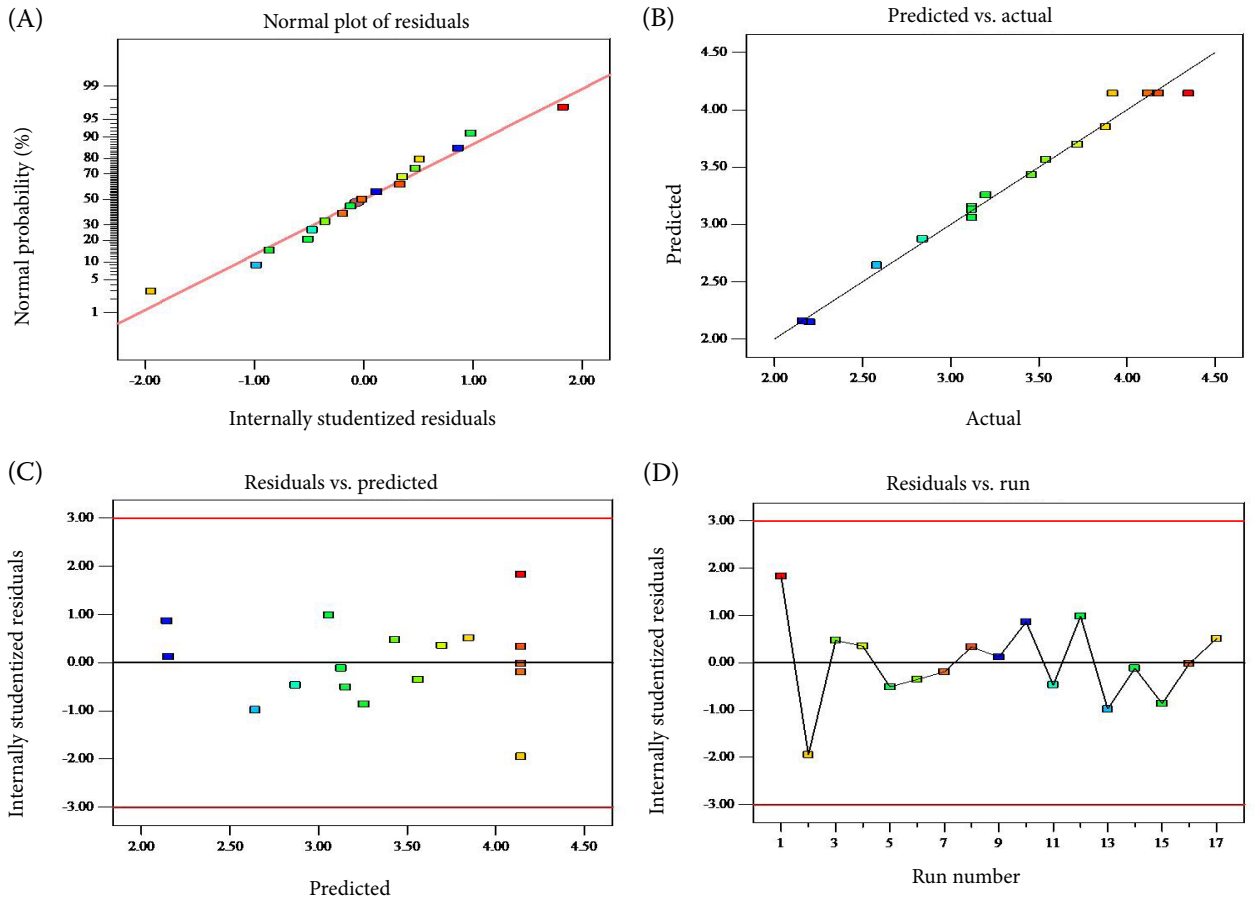

Figure 3. (A) Normal probability plot of residuals; (B) predicted vs. actual values; (C) residuals vs. predicted values; (D) residuals vs. run order

The scattered and irregular distribution of points indicates a good fit between the predicted and measured values, accompanied by minimal prediction errors. These findings demonstrate that the model accurately and reliably predicts and analyses the experimental outcomes.

Results of DGP stability measurements. As shown in Figure 4, the stability of DGP was affected by the temperature and storage time. The DGP amount in samples at different concentrations decreased over time under the four storage conditions. After 7 days of storage at room temperature (both under light and dark conditions) and under refrigeration (4 °C), a significant degradation (rate > 50%) of DGP was observed, with the highest degradation rate (~ 80%) occurring at room temperature. In contrast, under freezing conditions, DGP degradation was the slowest, with the polyphenol content remaining at approximately 70% after 7 days. The samples with higher DGP concentrations degraded more slowly and consequently were more stable. Overall, freezing is the best storage condition for polyphenols, and higher DGP concentrations enhance the storage stability. Thus, the storage temperature and polyphenol concentration must be carefully considered for optimal storage (Benhur et al. 2025).

Determination of antioxidant capacity. As shown in Figure 5, the scavenging rates of DGP against DPPH and ABTS radicals linearly increased with increasing concentration. For a DGP concentration of 8.0 μg·mL^{-1}, the sample exhibited a maximum scavenging rate of 93.0% for both radicals, demonstrating a substantial increase in the rate with the increasing addition of DGP. The results of antioxidant activity evaluation demonstrated that the radical scavenging abilities of DGP for both ABTS and DPPH increased in a concentration-dependent manner within the range of 0.5–8.0 μg·mL^{-1}. Specifically, for the ABTS radical, DGP exhibited an IC_{50} value of 6.0 μg·mL^{-1}, equivalent to 50.0 mg Trolox·g^{-1} DW, while for the DPPH radical, it demonstrated an IC_{50} value of 2.8 μg·mL^{-1}, corresponding to 4.5 mg Trolox·g^{-1} DW. This phenomenon can be attributed to abundant hydroxyl groups in the phenolic struc-

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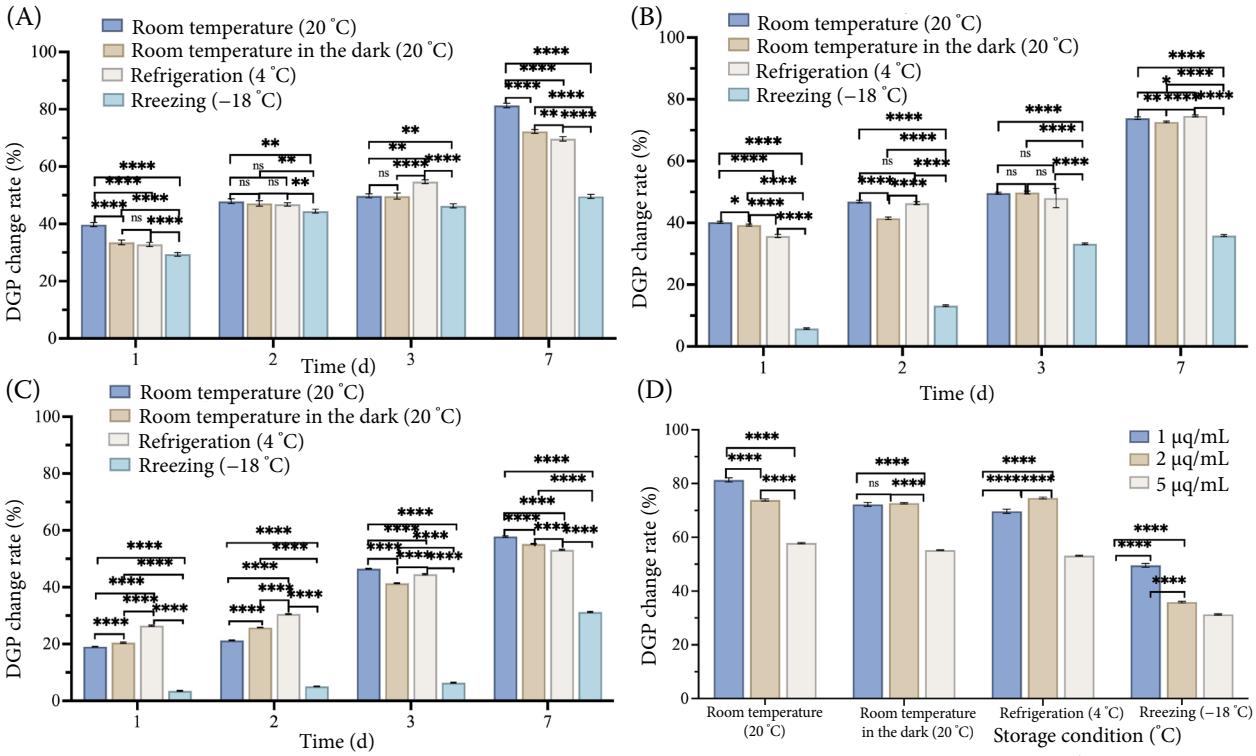

Figure 4. Changes in DGP concentration versus (A, B, C) time for initial loads of 1.0, 2.0, and 5.0 μg·mL^{-1} and (D) the corresponding 7-day change rate

* and ****significance levels at 0.05 and 0.01, respectively; DGP – distillers' spent grain polyphenols

ture in samples with high DGP concentrations, which significantly increase the antioxidant activity.

CONCLUSION

Chinese baijiu DSGs, organic by-products generated during the production of alcoholic beverages, are primarily used as animal feed despite their untapped potential. In this study, an efficient cellulase-mediated approach was employed to extract polyphenols from defatted DSG derived from a specific Northeast Chinese red sorghum variety, whose distinct compositional traits are shaped by local agroclimatic conditions. The optimised extraction parameters resulted in high

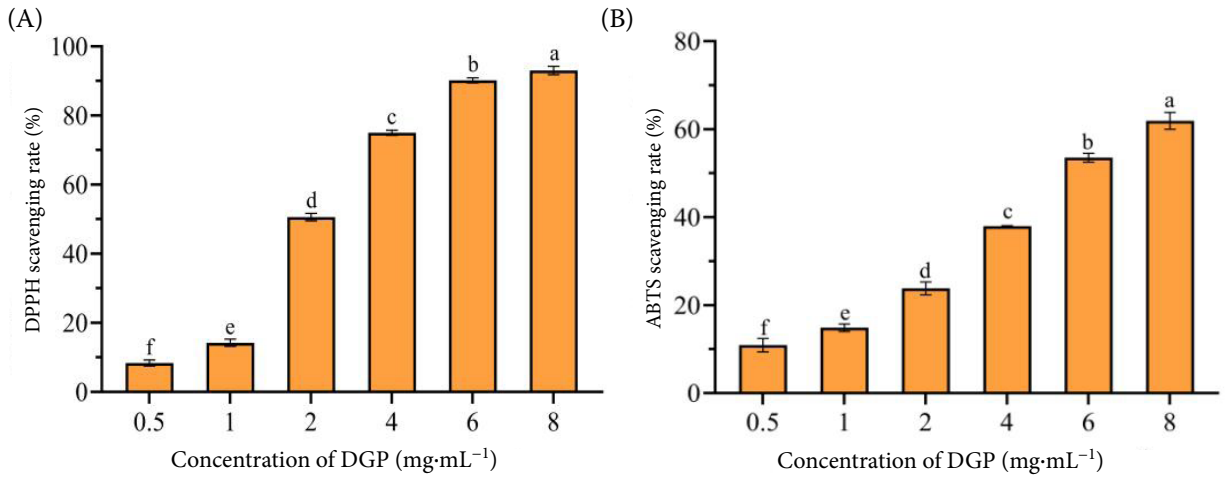

Figure 5. Scavenging abilities of polyphenols from distillers' spent grain (0.5–8.0 μg·mL^{-1}) against (A) DPPH and (B) ABTS radicals

DGP – distillers' spent grain polyphenols

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yields with minimal variability. Stability tests revealed that higher concentrations of polyphenols and storage under freezing conditions were the most favourable for their retention. Moreover, polyphenols demonstrated strong antioxidant activity, which was positively correlated with their concentration.

In conclusion, this research presents an efficient method for extracting polyphenols from a well-characterised DSG source, ensuring internal validity and providing a reference baseline for future comparative studies. It also underscores DSG's potential as a valuable resource for secondary utilisation, enhancing economic value and promoting sustainability within the Baijiu industry. We acknowledge that the polyphenol profile and extraction efficiency may vary across sorghum varieties or sources because of genetic and environmental factors. Future work should focus on refining the extraction process, expanding its applications across the food, pharmaceutical, and cosmetic sectors, and investigating the effects of extraction conditions on specific polyphenol subclasses to fully elucidate the compositional and functional properties of DSG.

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