EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title: EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya
OPEN ACCESS

Volume-8 | Issue-2 | May-Jun; 2026 | DOI: https://doi.org/10.36349/easjnfs.2025.v08i02.006

Original Research Article

Optimization of Blackening Process for *Lycium barbarum* L. Using Response Surface Methodology and Its Impact on Bioactive Components and Antioxidant Activity

Yu Zhang[1], Muhammad Aamer Mehmood[1], Hui Zhu[1]*, Ning Wang[1,3], Hang Zhong[1], Heyue Li[1], Tao Xiong[2], Leilei Guo[1], Lingyu Zhao[1]

[1]Sichuan Province Engineering Technology Research Center of Liquor-Making Grains, School of Food and Liquor Engineering, Sichuan University of Science and Engineering, Yibin 644000, China
[2]Cuiping District Agricultural and Rural Bureau, Yibin 644000, China
[3]Yibin Sichuan University of Science and Engineering Industrial Technology Research Institute, Yibin 644000, China

Article History
Received: 15.04.2026
Accepted: 08.06.2026
Published: 09.06.2026

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: In this study, high-temperature blackening (pile fermentation), a technique originally used for dark tea processing, was applied for the first time to the processing of goji berries (*Lycium barbarum* L.) to enhance their functional components and antioxidant activity. Single-factor experiments were conducted to examine the effects of blackening temperature (60–80 °C), time (40–60 h) and water replenishment (25–45 %) on total phenolic content, flavonoid content, reducing sugar content, DPPH radical scavenging capacity and sensory quality. On this basis, a three-factor, three-level Box-Behnken response surface design was employed to optimize the process, using DPPH radical scavenging capacity as the response value. The results showed that temperature was the dominant factor affecting the quality of blackened goji berries, with the order of influence strength being temperature > water replenishment > time. The established quadratic regression model was significant ($P = 0.0011$) with a coefficient of determination $R^2 = 0.9473$. The optimal process parameters were determined as follows: dried goji berries with a moisture content of 10–13 % were selected, cleaned, supplemented with water to 35 %, and then blackened at 72 °C for 50 h. Under these optimized conditions, the DPPH radical scavenging capacity reached 21.7 μmol/mL, while total phenolic and flavonoid contents remained high, and the sensory evaluation was excellent. This study provides a scientific basis and an industrially applicable technical solution for the high-value processing of goji berries.

Keyword: Goji Berry (*Lycium Barbarum* L.), High-Temperature Blackening (Pile Fermentation), Response Surface Methodology, Antioxidant Activity, DPPH Radical Scavenging Capacity, Flavonoids.

1. INTRODUCTION

Goji berry (*Lycium barbarum* L.) is a traditional Chinese medicinal and edible plant, first recorded in *Shennong's Classic of Materia Medica*, and is known for nourishing the liver and kidneys and improving eyesight. Modern research has shown that goji berries are rich in various bioactive components, including goji polysaccharides, flavonoids, carotenoids and betaine, and exhibit multiple physiological functions such as antioxidant, immunomodulatory, neuroprotective and microcirculation-improving effects [1–4]. However, current goji berry processing is still dominated by primary drying, which results in low product conversion rates and low added value, restricting the sustainable development of the goji berry industry [5].

Blackening (pile fermentation) is a traditional solid-state fermentation technique that originated from dark tea processing. Through the synergistic action of microbial community metabolism and hydrothermal effects, it achieves deep biotransformation of raw material components, creating unique colour, aroma and taste characteristics [6, 7]. In dark tea production, blackening is a key step for quality formation.

***Corresponding Author:** Hui Zhu
Sichuan Province Engineering Technology Research Center of Liquor-Making Grains, School of Food and Liquor Engineering, Sichuan University of Science and Engineering, Yibin 644000, China

Polyphenols are oxidatively polymerised by microbial polyphenol oxidases to form theabrownins, while the contents of bioactive components such as tea polysaccharides and theanine change, endowing the product with specific health benefits [8, 9]. In recent years, this technique has been extended to fruit and vegetable processing. Zhang *et al*., applied blackening to jujube processing and found that the contents of functional components such as total phenolics, flavonoids and cyclic adenosine monophosphate in blackened jujube were significantly increased, and the DPPH radical scavenging capacity was markedly enhanced [10]. These studies indicate that blackening technology has good application prospects for improving the nutritional quality and sensory properties of medicinal and edible plant materials.

Although blackening is well established in tea processing, its application in goji berry processing has not yet been reported. To date, no systematic study has investigated the effect of high-temperature blackening on the transformation of bioactive components and antioxidant activity of goji berries. Therefore, this study applied high-temperature blackening to goji berry processing for the first time. The effects of blackening temperature, time and water replenishment on total phenolic content, flavonoid content, reducing sugar content, DPPH radical scavenging capacity and sensory quality were systematically investigated, and the process parameters were optimised using response surface methodology. The aim is to provide a scientific basis and an industrially applicable technical solution for high-value processing of goji berries.

2. MATERIALS AND METHODS

2.1 Materials

Dried goji berries (*Lycium barbarum* L.) from Zhongning, Ningxia, China (moisture content 10–13 %, harvested in 2023) were purchased from the local market. Main reagents: Folin–Ciocalteu reagent, gallic acid standard, rutin standard, DPPH (1,1-diphenyl-2-picrylhydrazyl) were purchased from Beijing Solarbio Co., Ltd. (Beijing, China). Anhydrous ethanol, sodium hydroxide, sodium nitrite, aluminium nitrate and other analytical grade reagents were purchased from Chengdu Kelong Chemical Co., Ltd. (Chengdu, China).

2.2 Instruments

Main instruments included: UV-1800PC UV–Vis spectrophotometer (Shanghai Mapada Instruments Co., Ltd.), electric hot air drying oven (101-WA, Shanghai Yiheng), precision analytical balance (FA324C, Shanghai Lichen), refrigerated high-speed centrifuge (Microfuge20R, Beckman Coulter), and pH test pen (LC-PHS-4S, Shanghai Lichen).

2.3 Blackening Process Flow

Dried goji berries (moisture content 10–13 %), plump and free from mildew, rot and pests, were selected, cleaned, and supplemented with distilled water according to the designed water replenishment level. The berries were then placed in glass bottles with a one-way valve and subjected to blackening in an electric hot air drying oven under the set temperature and time conditions. After blackening, various physicochemical indices were determined.

2.4 Single-Factor Experiments

The effects of blackening temperature (60, 64, 68, 72, 76 °C; time 50 h; water replenishment 25 %), blackening time (40, 45, 50, 55, 60 h; temperature 70 °C; water replenishment 25 %) and water replenishment (25, 30, 35, 40, 45 %; temperature 70 °C; time 60 h) on the quality of blackened goji berries were examined. Total phenolic content, flavonoid content, reducing sugar content, DPPH radical scavenging capacity and sensory score were used as evaluation indices.

2.5 Response Surface Optimisation

Based on the single-factor experiments, a three-factor, three-level Box-Behnken design was performed using Design-Expert 13 software. The DPPH radical scavenging capacity was taken as the response value. The independent variables were blackening temperature (A: 68, 72, 76 °C), time (B: 45, 50, 55 h) and water replenishment (C: 30, 35, 40 %). A total of 17 experimental runs (including 5 centre points) were conducted.

2.6 Determination of Physicochemical Indices

Total phenolic content was determined by the Folin–Ciocalteu colorimetric method [11], and expressed as gallic acid equivalents (mg/100 g). Total flavonoid content was determined by the sodium nitrite-aluminium nitrate colorimetric method [12], and expressed as rutin equivalents (mg/100 g). Reducing sugar content was determined by the 3,5-dinitrosalicylic acid (DNS) colorimetric method. DPPH radical scavenging capacity was determined according to a published method [13], and expressed as μmol Trolox equivalents/mL.

2.7 Sensory Evaluation

Twelve food professionals conducted a blind sensory evaluation of the blackened goji berries from four aspects: colour (20 points), aroma (30 points), taste (40 points) and mouthfeel (10 points). The total sensory score was 100 points.

2.8 Data Processing

All experiments were performed independently in triplicate, and the data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed using SPSS 27.0, with $P < 0.05$ considered statistically significant. Response surface optimisation and plotting were performed using Design-Expert 13 and Origin 2024.

3. RESULTS AND DISCUSSION

3.1 Effect of Blackening Temperature on Goji Berry Quality

Table 1 shows the effect of different blackening temperatures on the physicochemical indices and sensory quality of goji berries. The reducing sugar content increased from 73.2 to 77.5 g/100 g with increasing temperature, suggesting that non-reducing sugars (e.g., sucrose) were hydrolysed to reducing sugars under the hydrothermal conditions. Total phenolic content increased with temperature from 60 to 72 °C, reaching a maximum of 367.2 mg/100 g at 72 °C, and then decreased at higher temperatures, possibly due to oxidative polymerisation or degradation of phenolic compounds at excessive temperatures. Flavonoid content remained at a relatively high level (221.4–234.3 mg/100 g) in the range of 64–72 °C. The DPPH radical scavenging capacity peaked at 21.4 µmol/mL at 72 °C and then slightly decreased. The sensory score was also highest at 72 °C (83.5 points). Based on these results, 68, 72 and 76 °C were selected as the levels for response surface optimisation.

Table 1: Effect of blackening temperature on goji berry quality

Temperature (°C)	Reducing sugar (g/100g)	Total phenolics (mg/100g)	Flavonoids (mg/100g)	DPPH scavenging (µmol/mL)	Sensory score
60	73.2±0.63^{ab}	312.4±4.5^{b}	213.8±4.5^{b}	18.3±0.31^{c}	73.3±3.1^{c}
64	75.1±0.15^{ab}	362.1±6.6^{a}	221.4±2.3^{ab}	19.0±0.25^{bc}	75.0±1.5^{bc}
68	74.2±0.93^{ab}	351.7±4.6^{ab}	234.3±4.4^{a}	20.4±0.30^{ab}	81.6±2.1^{ab}
72	75.9±0.92^{b}	367.2±7.8^{a}	232.7±2.9^{a}	21.4±0.30^{a}	83.5±1.8^{a}
76	77.5±2.10^{a}	332.1±3.7^{ab}	225.0±1.7^{ab}	20.5±0.31^{ab}	77.3±1.5^{bc}

3.2 Effect of Blackening Time on Goji Berry Quality

As blackening time increased (Table 2), reducing sugar content decreased from 40 to 50 h, reaching a minimum of 74.1 g/100 g at 50 h, and then increased afterwards. This suggests that reducing sugars are consumed by microbial metabolism in the early stage, while later hydrolysis of polysaccharides replenishes the reducing sugar content. Total phenolic content gradually increased with time and stabilised after 50 h. Flavonoid content peaked at 229.7 mg/100 g at 50 h. The DPPH radical scavenging capacity reached its highest value (20.5 µmol/mL) at 50 h and then significantly decreased at 60 h ($P < 0.05$), possibly due to excessive degradation of active components and the formation of off-flavours during prolonged blackening. The sensory score was also highest at 50 h (79.6 points). Therefore, 45, 50 and 55 h were selected as the levels for response surface optimisation.

Table 2: Effect of blackening time on goji berry quality

Time (h)	Reducing sugar (g/100g)	Total phenolics (mg/100g)	Flavonoids (mg/100g)	DPPH scavenging (µmol/mL)	Sensory score
40	71.6±0.25^{c}	216.4±4.2^{b}	174.2±5.7^{c}	17.9±0.35^{c}	73.7±5.6^{ab}
45	74.5±0.21^{b}	221.7±4.3^{b}	179.8±6.4^{c}	18.3±0.15^{c}	76.3±2.6^{ab}
50	74.1±0.12^{b}	230.8±8.1^{a}	229.7±6.4^{a}	20.5±0.31^{a}	79.6±3.5^{a}
55	74.1±0.40^{b}	232.1±3.9^{a}	224.7±5.3^{ab}	19.8±0.21^{b}	78.8±4.0^{a}
60	76.6±0.60^{a}	233.4±5.2^{a}	221.9±3.8^{b}	18.3±0.55^{c}	72.2±6.0^{b}

3.3 Effect of Water Replenishment on Blackened Goji Berry Quality

Water replenishment significantly affected goji berry quality (Table 3). Reducing sugar content showed little variation among the different water replenishment levels. Total phenolic content increased with water replenishment from 25 % to 35 %, reaching a maximum of 437.4 mg/100 g at 35 %, and then decreased significantly ($P < 0.05$), probably because excessive water diluted the material system and reduced the efficiency of enzymatic reactions. Flavonoid content reached its maximum (188.4 mg/100 g) at 35 % water replenishment. DPPH radical scavenging capacity was optimal in the range of 30–35 % water replenishment (21.8–22.1 µmol/mL) and then decreased significantly above 35 %. Therefore, 30, 35 and 40 % were selected as the levels for response surface optimisation.

Table 3: Effect of water replenishment on blackened goji berry quality

Water replenishment (%)	Reducing sugar (g/100g)	Total phenolics (mg/100g)	Flavonoids (mg/100g)	DPPH scavenging (µmol/mL)	Sensory score
25	64.7±0.52^{b}	346.7±8.3^{bc}	169.1±5.1^{b}	16.8±0.27^{c}	69.8±2.6^{c}
30	68.0±0.42^{a}	420.5±7.1^{a}	175.4±8.3^{ab}	22.1±0.16^{a}	75.1±0.8^{b}
35	67.7±0.50^{a}	437.4±7.7^{a}	188.4±3.6^{a}	21.8±0.32^{a}	77.6±2.0^{ab}
40	67.0±0.32^{a}	353.6±4.5^{b}	179.9±2.3^{ab}	17.6±0.10^{bc}	78.5±1.2^{a}
45	66.7±0.90^{ab}	307.8±6.9^{c}	176.8±4.8^{ab}	17.4±0.29^{bc}	72.4±2.5^{bc}

3.4 Response Surface Optimisation

Based on the single-factor results, a three-factor, three-level Box-Behnken design was performed with blackening temperature (A), time (B) and water replenishment (C) as independent variables and DPPH radical scavenging capacity (Y) as the response value. The experimental design and results are shown in Table 4.

Table 4: Response surface experimental design and results

Run	A: Temp (°C)	B: Time (h)	C: Water (%)	DPPH capacity (μmol/mL)
1	72	50	35	21.7±0.21
2	68	50	30	18.1±0.28
3	68	45	35	17.0±0.28
4	72	50	35	22.8±0.19
5	68	55	35	16.4±0.31
6	76	50	30	19.7±0.22
7	72	45	30	20.1±0.25
8	72	55	40	18.9±0.13
9	68	50	40	16.8±0.35
10	72	50	35	21.8±0.09
11	72	55	30	19.5±0.24
12	72	50	35	21.6±0.21
13	72	50	35	21.5±0.23
14	72	45	40	20.1±0.11
15	76	45	35	18.4±0.03
16	76	55	35	20.2±0.09
17	76	50	40	17.9±0.14

Using Design-Expert 13, the experimental data were fitted to obtain the following quadratic polynomial regression equation for DPPH radical scavenging capacity (Y):

$$Y = 21.88 + 0.8750A - 0.0750B - 0.3500C + 0.4750AB + 0.0750AC - 0.1750BC - 2.84A^2 - 1.15B^2 - 1.10C^2$$

The ANOVA for the regression model is presented in Table 5. The model was highly significant ($P = 0.0011 < 0.01$), and the lack-of-fit was not significant ($P = 0.2 > 0.05$), indicating that the model fitted the data well and could be used to predict the blackening process parameters. The coefficient of determination R^2 was 0.9473, and the adjusted R^2 was 0.8796. From the F-values, the order of influence strength of the factors on DPPH radical scavenging capacity was: blackening temperature (A) > water replenishment (C) > blackening time (B). The linear term A and the quadratic terms A^2, B^2 and C^2 had highly significant effects ($P < 0.01$), while the interaction terms AB, AC and BC were not significant ($P > 0.05$).

Table 5: ANOVA for the response surface quadratic model

Source	Sum of squares	df	Mean square	F-value	P-value
Model	57.06	9	6.34	13.99	0.0011
A-Temperature	6.13	1	6.13	13.51	0.0079
B-Time	0.045	1	0.045	0.0993	0.7619
C-Water	0.98	1	0.98	2.16	0.1849
AB	0.9025	1	0.9025	1.99	0.2011
AC	0.0225	1	0.0225	0.0496	0.8301
BC	0.1225	1	0.1225	0.2702	0.6192
A^2	34.26	1	34.26	75.58	<0.0001
B^2	5.59	1	5.59	12.34	0.0098
C^2	5.12	1	5.12	11.29	0.0121
Residual	3.17	7	0.4533		
Lack of fit	2.07	3	0.6883	2.48	0.2000

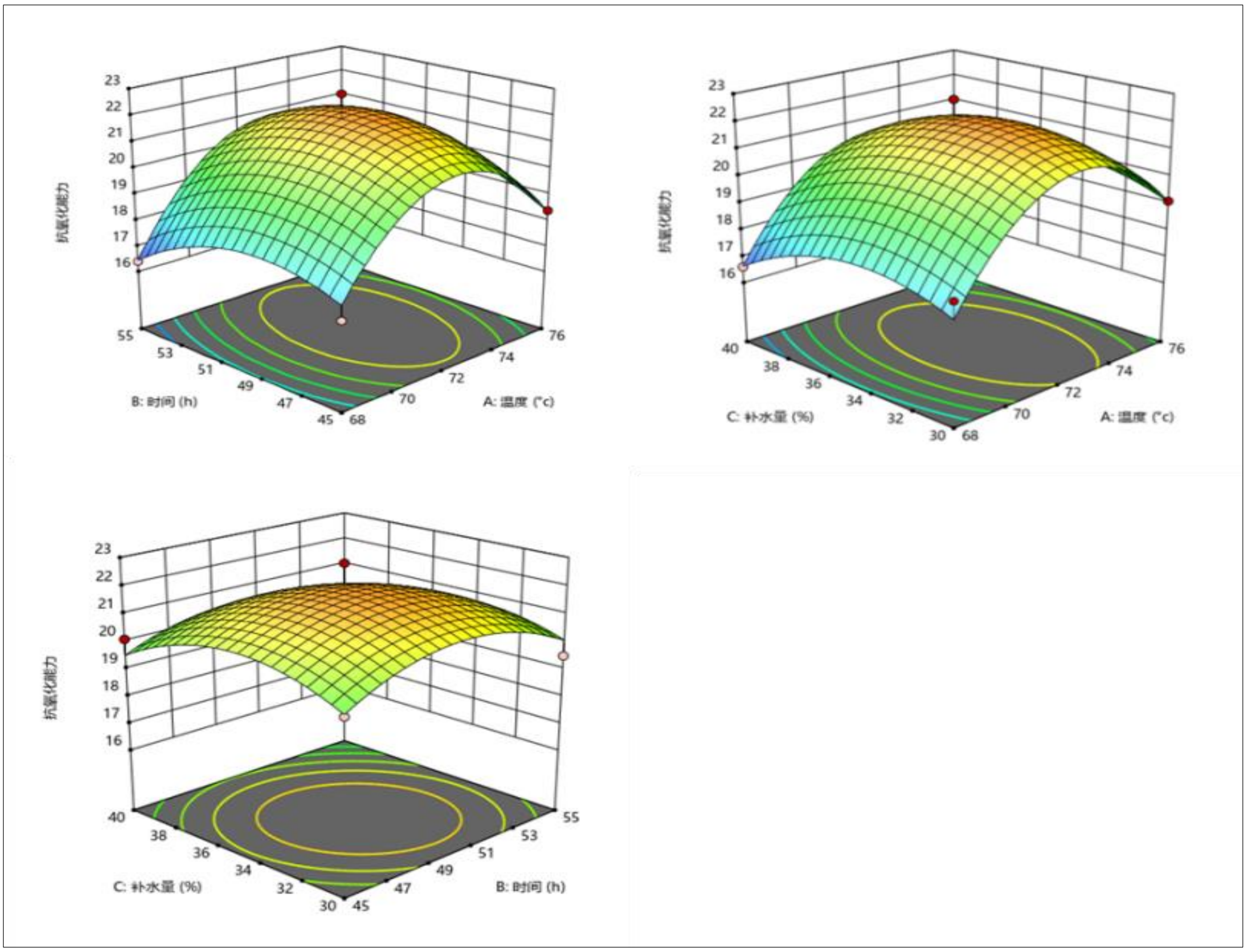

Figure 1: (to be inserted – original 3D response surface plots from the master's thesis)

Description:

Response surface plots (A: temperature vs time; B: temperature vs water replenishment; C: time vs water replenishment) showing the interactive effects on DPPH radical scavenging capacity. All surfaces are convex with a clear maximum point, contour lines approximately circular, indicating non-significant interactions. Optimal region: temperature ~72 °C, time ~50 h, water replenishment ~35 %.

3.5 Verification of the Optimal Process

Solving the regression model predicted the optimal blackening conditions to be: temperature 72.67 °C, time 50.58 h, water replenishment 34.28 %. For practical operability, the conditions were adjusted to: 72 °C, 50 h, water replenishment 35 %. Three parallel verification experiments under these conditions gave a DPPH radical scavenging capacity of 21.7 ± 0.25 μmol/mL, which was close to the predicted value (22.1 μmol/mL), with a relative error of 1.8 %. This indicates that the model had good predictive accuracy. Under the optimised process, total phenolic and flavonoid contents remained high, and the sensory score was excellent, demonstrating both functionality and good flavour.

3.6 Discussion

The "increase-then-decrease" pattern of blackening temperature revealed the internal mechanism of quality formation during goji berry blackening. At 72 °C, the high temperature promoted the release and transformation of phenolic compounds and may have activated microbial enzymes such as polyphenol oxidase and β-glucosidase, hydrolysing bound polyphenols to free aglycones and thereby improving their bioavailability and antioxidant activity [14]. However, when the temperature exceeded 75 °C, the degradation rate of active components exceeded their formation rate, leading to a decrease in total phenolic content and antioxidant activity. This temperature response is similar to that reported for blackened jujube. The blackening time of 50 h was a key turning point for quality. Before this time, flavonoid content and antioxidant activity gradually increased with time, peaking at 50 h; further blackening led to increased loss of active components and possibly the formation of undesirable compounds such as 5-hydroxymethylfurfural, which negatively affected product flavour quality [15]. The effect of water replenishment is related to water activity regulation: an appropriate water supplement (35 %) provides a suitable moisture environment for microbial growth and enzymatic reactions, promoting the degradation of macromolecular substances and the release of active components; excessive water dilutes the material system and changes the microenvironment osmotic pressure, which is unfavourable for normal enzyme metabolism.

From an application perspective, the blackened goji berries obtained under the optimal conditions exhibited superior colour, aroma and taste, with a notable reduction in raw astringency and enhanced sweet-sour balance. Furthermore, preliminary trials (based on subsequent fermentation experiments in our laboratory) indicated that blackened goji berries serve as an excellent raw material for compound fruit wine production, as they provide a richer precursor pool for esters and polyphenols, and show better compatibility with selected yeast strains (e.g., CVE-7). Thus, the established blackening process not only improves the nutritional and sensory quality of goji berries but also expands their potential for high-value applications in functional beverages and health products.

4. CONCLUSION

This study is the first to systematically investigate the effect of high-temperature blackening on the bioactive components and antioxidant activity of goji berries. Through single-factor experiments and response surface optimisation, the influence rules of the parameters and the optimal process conditions were determined. Single-factor experiments showed that temperature was the key factor affecting the quality of blackened goji berries. DPPH radical scavenging capacity and total phenolic content peaked at 72 °C. At a blackening time of 50 h, DPPH radical scavenging capacity (20.5 μmol/mL) and flavonoid content (229 mg/100 g) simultaneously reached their highest values. The optimal water replenishment was 35 %, which gave the best total phenolic content and DPPH radical scavenging capacity. The order of influence strength was: blackening temperature > water replenishment > blackening time.

The response surface optimisation determined the optimal process as follows: select dried goji berries with a moisture content of 10–13 %, supplement water to 35 %, and blacken at 72 °C for 50 h. Under these conditions, the DPPH radical scavenging capacity of the product reached 21.7 μmol/mL, total phenolic and flavonoid contents remained high, and the sensory score was excellent. The optimised process balances the retention of active components with production efficiency. This study provides a scientific basis and an industrially applicable technical solution for high-quality deep processing of goji berries, and can support the development of goji-based functional beverages and health products.

REFERENCES

1. Wang X, Zheng Y, Fu L, et al. Advances in the study of bioactive compounds and nutraceutical properties of goji berry (*Lycium barbarum* L.). Journal of Agricultural and Food Chemistry. 2023;71(12):4721-4735.
2. Li S, Liu Y, et al. On-line coupling pressurised liquid extraction with two-dimensional counter current chromatography for isolation of natural acetylcholinesterase inhibitors. Phytochemical Analysis. 2021;32(4):640-653.
3. He Y, Wang X, et al. *Lycium barbarum* polysaccharides improves cognitive functions in ICV-STZ-induced Alzheimer's disease mice model. Neuromolecular Medicine. 2024;26(1):15.
4. Wang S, Wang Q, Cheng X. Research progress on chemical components and pharmacological effects of *Lycium barbarum*. Chinese Journal of Pharmacology and Toxicology. 2023;37(S1):75.
5. Xing Z, Sha R, Xing Y, et al. Research status of jujube industry development in China. Agriculture and Technology. 2025;45(4):84-88.
6. Deng J, He Y, Chen J, et al. Changes in chemical components of Tibetan tea during blackening process. Food and Machinery. 2023;39(9):26-31.
7. Wang G, Wang Z, Liang S, et al. Research progress on microorganisms in pile fermentation of dark tea. China Tea. 2024;46(9):21-27.
8. Wei J, Nie K, Chen X, et al. Research progress on microbial diversity and functional activities in pile fermentation of dark tea. Science and Technology of Food Industry. 2025;46(9):424-434.
9. Luo L, Wu X, Deng Y, et al. Changes of biochemical components during pile fermentation of Yunnan Pu-erh tea and its relationship with quality formation. Journal of Tea Science. 1998;18(1):53-60.
10. Zhang X. Component changes during jujube blackening and preparation of polysaccharide-iron complex [Master’s thesis]. Shandong Agricultural University; 2025.
11. Zhang X, Sun Y, Hu Z, et al. Total phenolics, anthocyanin content and antioxidant activity of blueberry wine during brewing process. Journal of Northeast Agricultural University. 2010;41(3):120-124.
12. Li X, Yang D, Li Z. Study on extraction technology of flavonoids from wild jujube by ethanol reflux method. Shandong Chemical Industry. 2020;49(2):48-50.
13. Wang P, Guo J, Wang L, et al. Study on the antioxidant and hypoglycemic activity of coriander polysaccharides. China Food Additives. 2024;35(1):171-176.
14. Lv H, Zhang Y, et al. Phytochemical profiles and antioxidant activities of Chinese dark teas obtained by different processing technologies. Food Research International. 2017;100:486-493.
15. Song Y. Study on quality formation and antioxidant properties of blackened jujube [Master’s thesis]. Shandong Agricultural University; 2022.

Cite This Article: Yu Zhang, Muhammad Aamer Mehmood, Hui Zhu, Ning Wang, Hang Zhong, Heyue Li, Tao Xiong, Leilei Guo, Lingyu Zhao (2026). Optimization of Blackening Process for *Lycium barbarum* L. Using Response Surface Methodology and Its Impact on Bioactive Components and Antioxidant Activity. *EAS J Nutr Food Sci, 8*(2), 79-84.