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UPLC-QDA combined with pharmacology and chemometrics to analyze the correlation between the processing technology and color of Siegesbeckiae herba

Ajiao Hou,[a] Chao Yu,[a] Jinbo Li,[a] Liu Yang,[a] Haixue Kuang,[a] Yanyan Zhou*[b] and Hai Jiang*[a]

In the Chinese Pharmacopoeia (2025 Edition), it is reported that Siegesbeckiae herba (SH) can be processed *via* a single herb preparation, demonstrating significant economic value and development potential. However, the quality evaluation of its processed products mostly relies on personal experience, which severely restricts the trade and circulation of SH. This study aimed to explore the correlation between different processing methods and the appearance color of SH, simplify the quality evaluation process, and improve the accuracy of sensory evaluation. Firstly, a colorimeter was used to quantify the appearance color of SH processed by different methods, UPLC-QDA was employed to establish the fingerprint of SH under different processing methods, and pharmacodynamic experiments were conducted to determine its analgesic and anti-inflammatory activities. Subsequently, chemometrics was applied to analyze the correlation between different processing methods and the appearance color of SH, thereby optimizing the optimal processing technology of SH. Finally, osteoarthritis experiments were performed to further verify the results. The colorimeter, fingerprint analysis, and pharmacodynamic results all confirmed that the SH steamed repeatedly for nine times exhibited a shiny black color and the best efficacy. This study provides a reference for the color evaluation of the processing standard of SH and the standardization of the macroscopic characteristics of traditional Chinese medicine.

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1. Introduction

Siegesbeckiae herba (SH) is the dried aerial part of the plant *Siegesbeckia orientalis* L., *Siegesbeckia pubescens* Makino or *Siegesbeckia glabrescens* Makino, which belong to the Asteraceae family.[1] It tastes pungent and bitter, has a cold property, and enters the liver and kidney meridians, with the effects of dispelling wind-dampness, activating joints, and detoxifying.[2] It is commonly used to treat wind-damp arthralgia.[3] In the context of traditional Chinese medicine (TCM) clinical practice, where multi-herb formulas are mostly used for arthritis treatment, the Chinese Pharmacopoeia (2025 Edition) records a single herb preparation of SH, namely Xixian Pills.[1] This preparation is made from SH processed with honey and wine, which indicates that SH exerts significant therapeutic effects.[4] Furthermore, most of the SH currently available are wild varieties, and they possess considerable economic value and development potential in the TCM field. Moreover, after processing with honey and wine, the property of SH changes from cold to warm, its taste shifts from bitter to sweet, and its warming and nourishing effects are enhanced, thereby strengthening its function of tonifying the liver and kidneys. It is suitable for being made into health tea for long-term consumption by people with liver and kidney deficiency, as it benefits kidney Qi to promote blood production and invigorate the spleen.

The earliest record of SH requiring nine cycles of steaming and drying is found in Zheng Lei Ben Cao. Subsequently, most ancient literature specified that SH should be steamed and dried nine times with honey and wine, emphasizing that steaming fewer than nine times would result in poor therapeutic efficacy.[5] However, the current Chinese Pharmacopoeia only retains the wine-steamed processed variety of SH, requiring it to be steamed once until the appearance turns brownish-green or blackish-green. Therefore, this study investigates the changes in SH under different processing times to optimize the optimal processing method for SH.[6]

As an important appearance property of TCM, color has played a crucial role in the rapid quality evaluation of TCMs since ancient times. "Discriminating quality by appearance" was the main method used in ancient China to determine whether processed medicinal materials were qualified.

[a]*Key Laboratory of Basic and Application Research of Beiyao, Heilongjiang University of Chinese Medicine, Ministry of Education, Harbin, 150040, PR China. E-mail: jianghai_777@126.com*

[b]*Key Laboratory of Basic Theory of Chinese Medicine, Heilongjiang University of Chinese Medicine, Harbin, 150040, PR China. E-mail: 13339319259@163.com*

However, it requires extensive practical experience and may be inaccurate due to individual differences and environmental factors.[7–9] Therefore, establishing an objective and quantifiable color analysis method is particularly important for TCM quality identification. This study introduces colorimeter, which is a color analysis device that can conduct quantitative and digital measurements of the sensory characteristics of TCMs, turning the experience of "discriminating quality by appearance" into a precise, rapidly promotable, and highly operable technique.[10–12]

The appearance and characteristics of SH change after processing, which may be related to alterations in the types and contents of its chemical components, which are caused by high-temperature steaming and the addition of excipients. Fingerprint analysis is widely used in the identification and evaluation of TCMs, enabling more accurate and comprehensive assessment of TCM quality.[13–16] However, the establishment of the fingerprint can only reflect the types and quantities of compounds contained in the TCM, and cannot explain the efficacy of the TCM.

Therefore, this study focused on the SH processed by different processing methods. It uses a colorimeter to quantify the appearance color of processed SH, investigating the effect of processing on its appearance color from the perspective of physical properties. Meanwhile, combined with modern instrumental techniques, a fingerprint of SH is established to observe the fingerprint peaks of SH under different processing methods at the chemical level. Simultaneously, analgesic and anti-inflammatory activities are used as efficacy indicators to study the pharmacodynamic effects of SH. Chemometrics is applied to explore the correlations between the appearance color, fingerprint peaks, and pharmacodynamics of SH, and to optimize the optimal processing technology for SH. Finally, *in vivo* experiment using osteoarthritis rats are conducted for further verification. This study aims to provide a basis for the quality control, quality evaluation, and screening of the optimal processing method of SH.

2. Experimental

2.1 Chemicals and reagents

Methanol (HPLC grade) was supplied by Fisher Scientific. Formic acid (HPLC grade) was provided by Dikma Corporation. Wahaha purified water was supplied by Hangzhou Wahaha Group. Honey was supplied by Shanghai Guan Sheng Yuan Bee Products Co., Ltd. Formalin was supplied by Beijing Yili Fine Chemicals Co., Ltd. Carrageenan was supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. Wine was supplied by Xinhua Jin Group Shandong Jimo Yellow Rice Wine Factory Co., Ltd. The enzyme-linked immunosorbent assay (ELISA) kits for IL-1β, IL-6, and TNF-α was supplied by Beijing Biotopped Biotechnology Co., Ltd.

2.2 SH samples

SH samples were purchased from the medicinal materials market in Bozhou, Anhui province, were identified as the dried aerial parts of *Siegesbeckia pubescens* Makino. S0 was unprocessed raw SH, and SH was processed using different processing methods, S1–S9, C1–C9, and T1–T9, respectively. S1–S9: 100.00 g raw SH was mixed with 50.00 g honey and 24.00 g wine, single steaming process with durations set at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, and 18 h, respectively. C1–C9: excipients were added to 100.00 g raw SH in each steaming-sun-drying cycle, and the dosage per cycle same. The amount of excipients added in each cycle was calculated as: 1/9 of the total amount of excipients (5.55 g honey and 2.66 g wine). T1–T9: excipients were added to 100.00 g raw SH in each steaming-sun-drying cycle, but the dosage per cycle varied. The amount of excipients added in each cycle was calculated as: the total amount of excipients (50.00 g honey + 24.00 g wine) divided by the total number of steaming-sun-drying cycles (1–9). Finally, the processed SH samples were mixed back with the steaming liquor and dried to a constant weight at a constant temperature of 60 °C (SI material 1).

2.3 Determination of color values

SH processed by different methods was taken and crushed, passed through an 80-mesh sieve. The color of the SH powder under different processing methods was measured using the YS6010 desktop spectrophotometric colorimeter (Shenzhen 3nh Technology Co., Ltd). The L, a, b values were recorded, and the chromaticity values were calculated using formula $E = (a^2 + b^2 + L^2)^{1/2}$.

2.4 Establish fingerprint spectrum

2.4.1 Chromatographic conditions. The Waters Acquity™ ultra-high performance liquid chromatography (UPLC) system coupled with a single quadrupole mass spectrometry system (QDA). Thermo Hypersil GOLD column (100 mm × 2.1 mm, 1.9 μm), column temperature: 40 °C. Methanol (mobile phase A), 0.3% formic acid water (v/v, mobile phase B), 0–5 minutes, 35–55% (A); 5-9min, 55–57% (A); 9–16 min, 57–65% (A); 16–19 min, 65–82% (A); 19–30 minutes, 82–100% (A). Mass scan range m/z 50–800. Collision energy 30 V.

2.4.2 Preparation of sample solution. Powders of SH processed by different methods were taken and successive extraction with 95% ethanol, 50% ethanol, and water in sequence. The extract was concentrated to 0.10 g mL^{-1} (based on crude herb), centrifuged at 3000 rpm for 15 minutes, and passed through a 0.22 μm microfiltration membrane before being used for UPLC-QDA analysis.

2.4.3 Methodology validation and similarity evaluation. Randomly selected S0 samples were used to calculate the relative standard deviation (RSD) of relative retention time (RRT) and relative peak area (RPA) of common fingerprint peaks. Precision was evaluated by repeated measurement of the S0 sample for 6 times. Repeatability was assessed by determining 6 different S0 samples. Stability was investigated by measuring the sample at 0, 2, 4, 8, 12, 24, and 48 hours. The similarity evaluation of SH samples under different processing methods was performed using the TCM Chromatographic Fingerprint Similarity Evaluation System.

2.5 Pharmacodynamics

2.5.1 Animal. Specific pathogen-free (SPF) female ICR mice, weighing 18 ± 2 g (six weeks old), were supplied by Liaoning Changsheng Biotechnology Co., Ltd. The mice were housed in an SPF-level animal laboratory: with a standard room temperature of 25 ± 2 °C, relative humidity of 50 ± 15%, and a 12 hours light/dark cycle. All animal experiments were conducted in compliance with the Experimental Animal Ethics Guidelines of Heilongjiang University of Chinese Medicine (2025102809).

2.5.2 Hot plate test. 300 mice were randomly divided into 30 groups, including blank group, positive group, S0–S9 group, C1–C9 group, T1–T9 group. The blank group was given normal saline at 0.30 mL per day, the positive group was given aspirin at 60.00 mg kg^{-1} (6.25 mg mL^{-1}), and the remaining groups were given SH samples processed by different methods at 4.80 g kg^{-1} (crude herb 0.50 g mL^{-1}) respectively. Each group was given the drug for 7 days.

The constant temperature water bath pot was heated and the temperature was adjusted to 55 ± 0.5 °C. After last administration 1 h, the mice were individually placed on the hot plate and the time when they showed licking paws or jumping reactions was recorded.

2.5.3 Formalin test. 300 mice were grouped and administered drugs as described in "2.5.2". After last administration 20 min, 20 μL of 5% formalin was subcutaneously injected into the plantar region of the right forelimb of each mouse. The mice's paw responses were observed and graded for pain intensity as follows: 3 points for paw licking, biting, or shaking; 2 points for paw lifting; 1 point for limping while walking; and 0 points for normal movement. The pain reaction score was calculated as the total seconds of the above responses occurring within 10–25 minutes (delayed phase) multiplied by their corresponding scores.

2.5.4 Carrageenan-induced paw swelling test. The model group was given normal saline at 0.30 mL per day, the remaining groups were treated as "2.5.2", each group was given the drug for 14 days. Except for the blank group, all other groups were injected with 50 μL of 1% carrageenan. After injection 2 h, blood samples were collected, and centrifuged at 3000 rpm for 15 min. The ELISA kit was used to determine the concentration levels of IL-1β, IL-6 and TNF-α in the serum of each group of mice.

2.6 Pharmacological validation

2.6.1 Preparation of SH extract. 100.00 g raw SH was extracted by the continuous reflux method 2 hours and repeated for 3 times, combined filtrates and freeze-dried to obtain 18.90 g of raw SH freeze-dried powder. 100.00 g of raw SH was processed with T9 processing method, followed by continuous reflux extraction and freeze-drying, yielding 60.90 g of processed SH freeze-dried powder. Based on the guidelines by the Food and Drug Administration of the United States, the body surface areas of rats and humans were evaluated. The administration doses for rats were adjusted and converted according to the total extract contents of raw and processed SH. The doses administered to the raw and nine-processed SH group were 137.65 mg mL^{-1} and 443.55 mg mL^{-1}, respectively, which were equivalent to a raw SH dosage of 3.64 g kg^{-1}.

2.6.2 Animal. 50 SPF Sprague-Dawley rats, weighing 200 ± 20 g (two months old), were supplied by Liaoning Changsheng Biotechnology Co., Ltd. The rats were anesthetized with 2–4% isoflurane, and 1 mg/50 μL of sodium monoiodoacetate (MIA) solution was injected into the intercondylar fossa using a sterile insulin syringe to establish an osteoarthritis animal model. The blank group was injected with an equal volume of normal saline.

Then the rats were divided into the blank group (Blank), rats with successful modeling were randomly divided into the model group (Model), raw SH group (RSH), nine-processed SH group (WSH), and positive drug Duhuo Jisheng Pill group (Positive). The Blank group and model group were given normal saline, while the Positive group was given Duhuo Jisheng Pill at a concentration of 728.00 mg mL^{-1}, the RSH and WSH groups were given 137.65 mg mL^{-1} and 443.55 mg mL^{-1} respectively. The volume of administration was 5.00 mL kg^{-1}. Each group was given the drug for 4 weeks.

2.6.3 Index determination. After the last administration, the rats were anesthetized by intraperitoneal injection of 1.5% pentobarbital sodium (2.00 mL kg^{-1}), and blood samples were collected, and centrifugation at 3000 rpm for 15 minutes. The ELISA kit was used to determine the concentration levels of ALT, AST, CORT and TNF-α.

2.6.4 HE and S–O staining. Fix the complete knee joints of each group of rats in paraformaldehyde. Hematoxylin–Eosin (HE) staining and Safranin O and Fast Green (S–O) staining were performed to observe the degree of cartilage degeneration in the knee joints.

2.7 Chemometrics and data analysis

The TCM Chromatographic Fingerprint Similarity Evaluation System was used to generate chromatographic fingerprints of SH samples processed by different methods and conduct similarity evaluation. The SIMCA 14.1 software was used for chemometric analysis. GraphPad Prism 5.0 and Origin 2019b software were employed for graphing and data analysis.

3. Result

3.1 Determination of color values

The changes in appearance and color values of SH under different processing methods are shown in Fig. 1. The results indicate that with the extension of steaming time and the increase in steaming times, the color of SH gradually changes from yellow-green to brown-green, and finally to black-green.

3.2 Determination of fingerprint

3.2.1 Methodological validation. The fingerprint profiles of SH samples under different processing methods were established using the TCM Chromatographic Fingerprint Similarity Evaluation System, as shown in Fig. 2. A total of 36 common peaks were identified. A batch of S0 samples was randomly

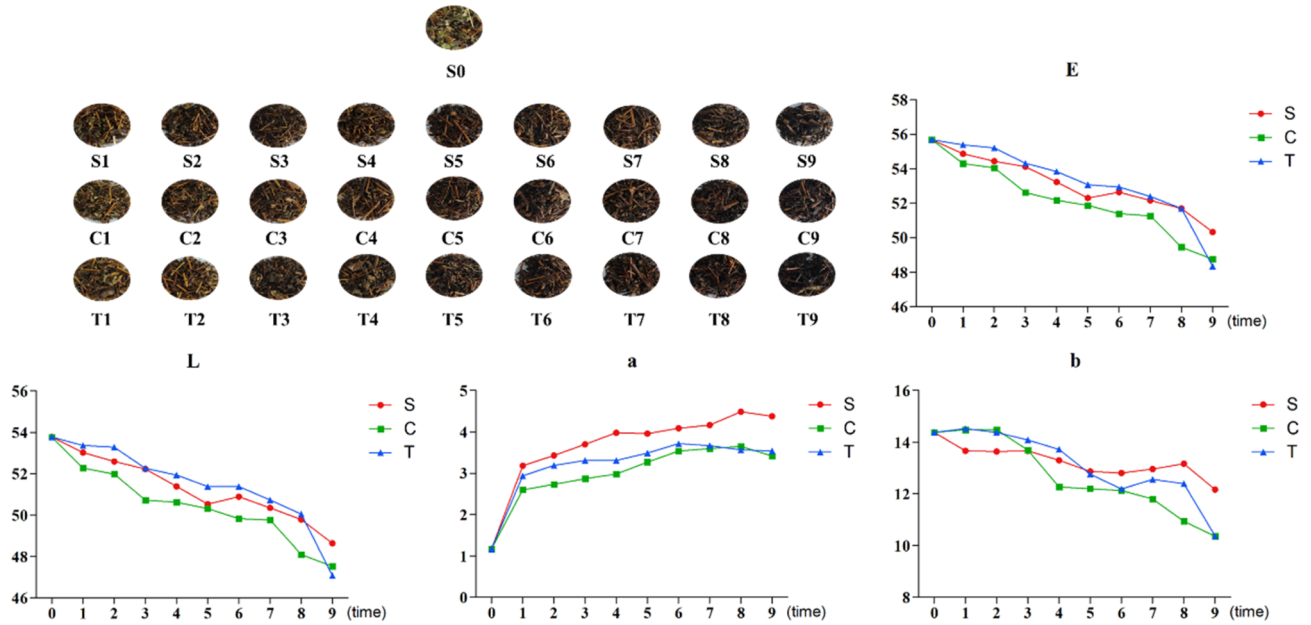

Fig. 1 Changes in appearance and color values of SH under different processing methods.

selected, and peak 7 (Kirenol) was used as the reference peak for methodological validation. The results shown in Table 1, indicated that the established method exhibited good precision, repeatability, and stability, meeting the technical requirements for fingerprint analysis.

3.2.2 Similarity analysis. The results of similarity evaluation of SH samples processed by different methods are shown in Table 2. The similarity among SH samples under different processing methods was all above 0.80, indicating that the fingerprint of SH samples from the same source but processed by different methods exhibit a consistent overall trend and good similarity. However, there were still differences among SH samples processed by different processing methods, suggesting that different processing methods have different effects on the intrinsic quality of SH.

3.3 Pharmacodynamic results

The results of the analgesic and anti-inflammatory test are shown in Fig. 3. All SH-administered groups exhibited a significant inhibitory effect on hot plate-induced pain, formalin-induced chronic inflammatory pain and carrageenan-induced inflammatory ($P < 0.05$, $P < 0.01$). Regardless of the processing method used, the analgesic effect of processed SH was superior to that of the raw SH ($P < 0.05$, $P < 0.01$). With the extension of

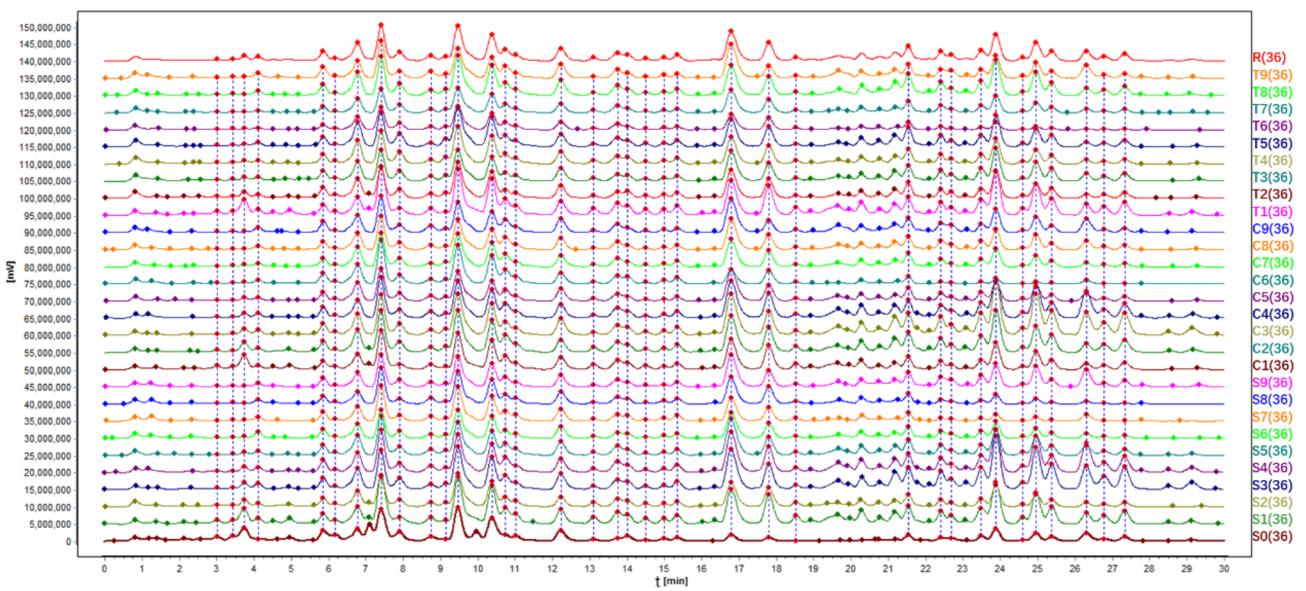

Fig. 2 Fingerprint of SH under different processing methods.

Table 1 The results of RRT and RPA of the precision, repeatability, and stability of SH samples

	Precision (RSD%)		Repeatability (RSD%)		Stability (RSD%)	
Peak no.	RRT	RPA	RRT	RPA	RRT	RPA
1	0.34	4.60	0.77	3.56	0.28	4.82
2	0.23	2.89	0.39	2.29	0.16	3.59
3	0.05	2.34	0.17	2.40	0.06	0.62
4	0.22	2.14	0.26	1.99	0.10	1.79
5	0.07	3.09	0.07	3.29	0.05	1.92
6	0.06	4.12	0.01	2.17	0.03	1.44
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.01	4.24	0.01	2.18	0.04	2.93
9	0.01	3.95	0.07	1.31	0.06	0.85
10	0.06	4.00	0.06	1.96	0.10	1.51
11	0.06	4.66	0.07	3.43	0.05	2.19
12	0.08	2.91	0.08	2.41	0.07	3.86
13	0.06	3.17	0.06	2.88	0.07	3.33
14	0.08	3.74	0.06	3.71	0.08	0.98
15	0.06	4.12	0.10	1.78	0.18	2.43
16	0.08	4.44	0.10	2.82	0.10	1.61
17	0.09	4.42	0.06	3.24	0.08	2.71
18	0.07	4.50	0.08	2.71	0.09	1.69
19	0.06	4.56	0.07	3.45	0.14	2.98
20	0.06	2.84	0.07	2.59	0.10	4.93
21	0.06	3.79	0.07	3.36	0.12	3.21
22	0.08	3.95	0.06	3.20	0.10	1.44
23	0.06	2.91	0.06	2.12	0.09	1.01
24	0.07	2.88	0.06	3.73	0.09	0.98
25	0.05	4.14	0.08	4.97	0.05	3.27
26	0.08	4.25	0.06	3.14	0.09	1.22
27	0.08	3.25	0.08	4.78	0.08	2.81
28	0.09	3.32	0.05	4.81	0.09	4.23
29	0.12	3.90	0.06	3.79	0.09	3.53
30	0.14	2.68	0.07	3.73	0.09	1.66
31	0.18	3.57	0.08	4.00	0.09	4.46
32	0.17	4.80	0.08	4.14	0.10	3.34
33	0.17	3.46	0.09	3.99	0.09	1.49
34	0.24	4.76	0.10	4.74	0.10	3.24
35	0.19	3.21	0.11	1.82	0.09	4.64
36	0.20	3.31	0.09	2.85	0.08	1.71

Table 2 Similarity of SH sample under different processing methods compared with S0 sample

No.	Similarity	No.	Similarity	No.	Similarity
S1	0.88	C1	0.89	T1	0.88
S2	0.90	C2	0.85	T2	0.89
S3	0.80	C3	0.80	T3	0.86
S4	0.81	C4	0.82	T4	0.90
S5	0.85	C5	0.85	T5	0.89
S6	0.88	C6	0.92	T6	0.89
S7	0.90	C7	0.88	T7	0.86
S8	0.87	C8	0.86	T8	0.87
S9	0.84	C9	0.85	T9	0.85

steaming time and the increase in steaming time, the analgesic and anti-inflammatory effect of processed SH was enhanced, and the analgesic and anti-inflammatory effect of T9 was the strongest.

3.4 Chemometrics analysis

3.4.1 Colorimeter analysis. The principal component analysis (PCA) of the appearance color of SH under different processing methods, as shown in Fig. 4A. When extracting the two principal components, $R^2X_1 = 0.779$, $R^2X_2 = 0.194$. Regardless of the processing method used, when SH was processed 4–5 times, it was located on both sides of the *Y*-axis, indicating that the color of SH changed. When processed 8–9 times, the SH samples significantly deviated from the central coordinate system, suggesting a marked change in their color. As shown in Fig. 4B, different excipients adding methods had a significant impact on the color change of SH.

3.4.2 Fingerprint analysis. The PCA of the fingerprint of SH under different processing methods, as shown in Fig. 4C and D. When extracting the two principal components, $R^2X_1 = 0.458$, $R^2X_2 = 0.212$. From Fig. 4C, it can be seen that regardless of the processing method used, SH samples deviated significantly from the central coordinate system when processed 7–9 times, indicating a marked change in their intrinsic quality. Compared with increasing processing time or processing time, different excipients adding methods had a smaller impact on the intrinsic quality of SH.

3.4.3 Pharmacodynamic analysis. The PCA of the pharmacodynamic data of SH under different processing methods, as shown in Fig. 4E and F. When extracting the two principal components, $R^2X_1 = 0.637$, $R^2X_2 = 0.204$. From the PCA results, regardless of the processing method used, SH samples were distributed on both sides of the *Y*-axis when processed 5–6 times, which suggested the analgesic and anti-inflammatory activities of SH were significantly enhanced. As shown in Fig. 4F, different excipients adding methods had a significant impact on the pharmacodynamic effects of the SH.

3.4.4 Colorimeter – pharmacodynamic analysis. Partial least squares regression (PLSR) analysis was performed with the *a*, *b*, *L* and *E* values as the independent variables (*X*), and the analgesic and anti-inflammatory inhibition rates as the dependent variable (*Y*). The correlation results are presented in Fig. 5A. Except for the hot plate test, the *a* value was positively correlated with the analgesic and anti-inflammatory effects, while the *b*, *L* and *E* values were negatively correlated with these effects. These findings indicate that the higher the *a* value and the lower the *b*, *L* and *E* values (the darker the color is), the stronger the analgesic and anti-inflammatory activities of SH. Among these color parameters, the *E* and *L* values exhibited higher contribution rates, as shown in Fig. 5B.

3.4.5 Fingerprint – pharmacodynamic analysis. PLSR analysis was conducted using 36 common peaks as the independent variables (*X*) and the analgesic and anti-inflammatory inhibition rates as the dependent variable (*Y*). The correlation results are illustrated in Fig. 5C. P1, P2, P3 and P6 were predominantly negatively correlated with the analgesic and anti-inflammatory effects during processing, whereas P4 was mainly positively correlated with these effects. These results suggest that material transformation occurs during the processing of SH. With the extension of steaming duration and the increase in steaming times, low-efficiency components are

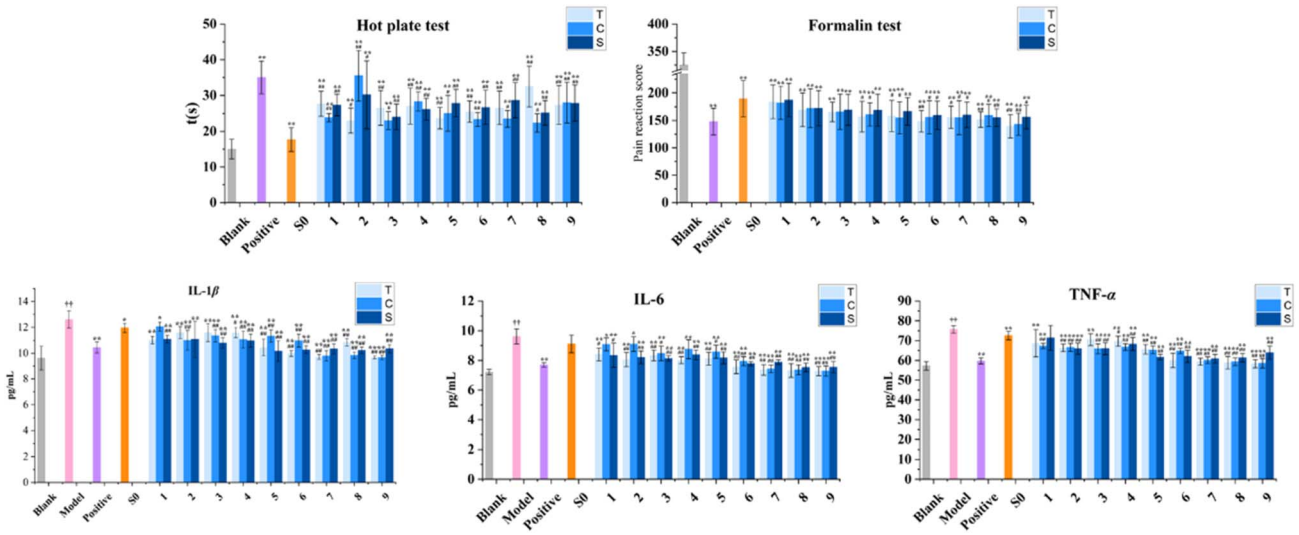

Fig. 3 The pharmacodynamic result of SH under different processing methods.

converted into high-efficiency components, thereby enhancing the analgesic and anti-inflammatory effects. Among these peaks, P1, P2, P3 and P6 showed higher contribution rates, as presented in Fig. 5D. This finding implies that future studies on the processing of SH could focus on the effects of processing on material transformation.

3.4.6 Fingerprint – colorimeter analysis. PLSR analysis was carried out with 36 common peaks as the independent variables (X) and the colorimeter indices as the dependent variables (Y). The correlation results are displayed in Fig. 5E. P1, P2, P3 and P6 were negatively correlated with the a value and positively correlated with the b, L and E values. These data demonstrate that the lower the contents of P1, P2, P3 and P6, the higher the a value and the lower the b, L and E values, which corresponds to stronger analgesic and anti-inflammatory activities of SH. Notably, P1, P2, P3 and P6 were also the common peaks with higher contribution rates, as shown in Fig. 5F.

3.5 Pharmacological results

3.5.1 Serum index detection. The concentration levels of ALT, AST, CORT, and TNF-α in the serum of rats in each group are shown in Fig. 6. Compared with the Blank group, the concentration levels of ALT and TNF-α in the model group increased significantly ($P < 0.01$), while the concentration level of AST showed no significant change ($P > 0.05$), and the concentration level of CORT decreased significantly ($P < 0.01$). Compared with the Model group, RSH could significantly reduce the concentration levels of CORT and TNF-α in the model group ($P < 0.05$), WSH could significantly reduce the concentration levels of ALT, CORT, and TNF-α in the model group ($P < 0.01$). Moreover, compared with RSH, WSH exerted a more significant reducing effect on the concentration levels of ALT and TNF-α ($P < 0.05$).

3.5.2 HE and S–O staining. The results of HE and S–O staining of rats in each group are shown in Fig. 6. In model group, accompanied by massive infiltration of inflammatory cells, and a few new blood vessels appeared, erosion was observed in multiple parts of the articular cartilage and meniscus. After administration of RSH and WSH, the synovial connective tissue showed mild hyperplasia, along with minimal infiltration of inflammatory cells, and a few new blood vessels appeared. Compared with the RSH group where local articular cartilage showed erosion, the articular cartilage surface in the WSH group was smooth, with no obvious bone erosion observed.

4. Discussion

This study systematically explored the correlation between the processing technology of SH and its appearance color and pharmacodynamic activity through multi-dimensional technical approaches. It integrated color difference quantification, fingerprint, pharmacodynamics and chemometrics, thereby providing a scientific basis for the standardized evaluation of the processing quality of SH for the first time.

The "discriminating quality by appearance" of TCMs relies on empirical judgment, which is relies highly subjective experience and is susceptible to environmental factors. In this study, a colorimeter was introduced to quantify color parameters of TCMs. It was found that with the extension of steaming time or increase in steaming time, the color of SH gradually changed from yellow-green to brown-green, and eventually turned black-green, the SH sample steamed nine times (T9) exhibited the characteristic of black and shiny, suggesting that color changes can serve as an intuitive indicator for evaluating the processing degree. Chemometric analysis showed that the SH samples significantly deviated from the central coordinate system after processed 8–9 times, indicating a qualitative change in the appearance properties. This change may be related to the transformation of chemical components caused by high-temperature steaming or the addition of excipients, such as the Maillard reaction between sugars in honey and amino acids in the herb. Additionally, the more excipients used, the larger

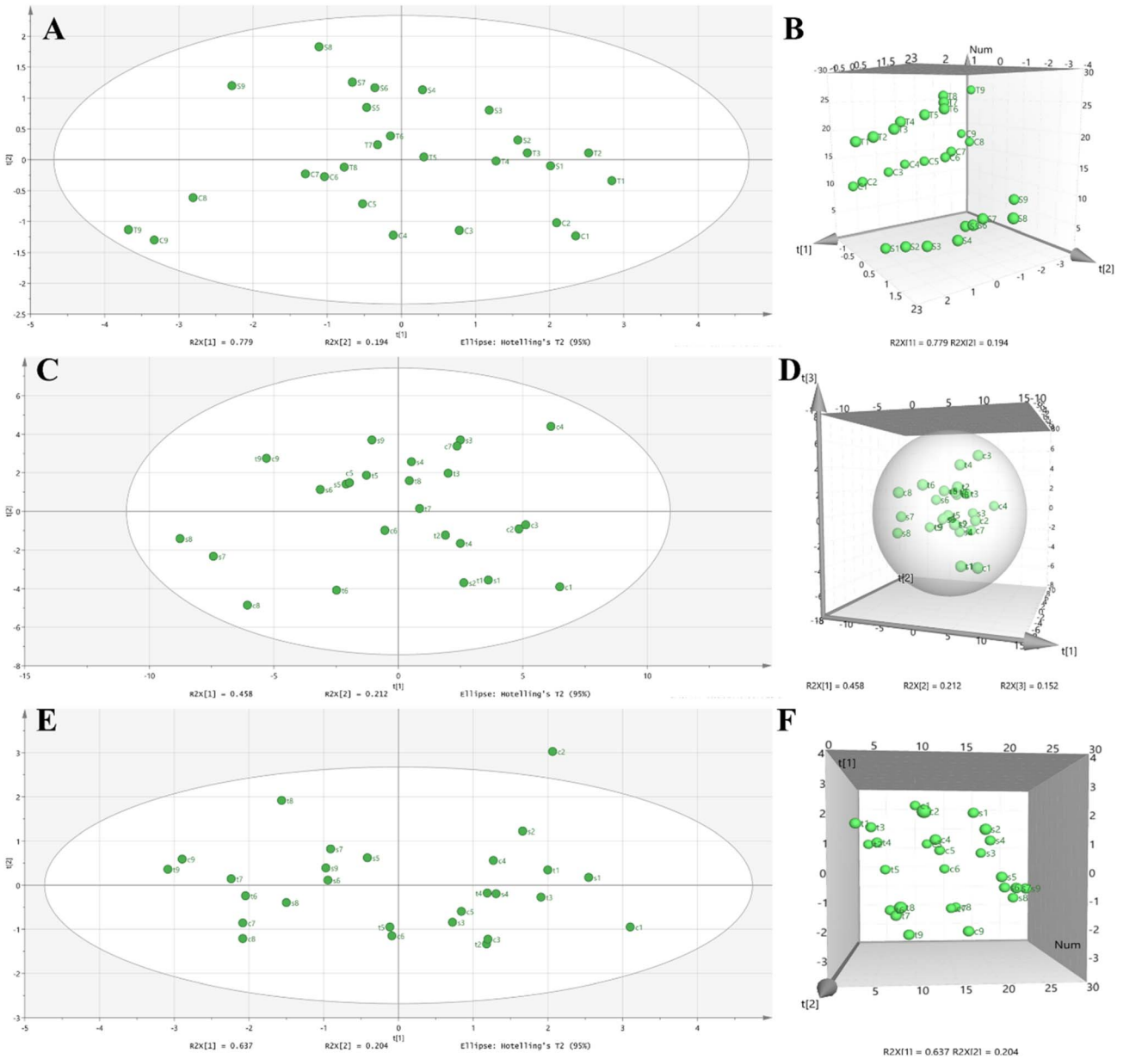

Fig. 4 PCA and 3D diagram of SH under different processing methods ((A), (C) and (E) represents PCA diagram; (B), (D) and (F) represents 3D diagram).

the *a*, *L*, and *E* values, and the brighter the color, which may be associated with the inherent colors of honey and wine, as well as the slight luster of honey. The quantification of color difference not only verifies the rationality of traditional experience but also converts color evaluation into repeatable and scalable digital indicators, laying a foundation for establishing the color evaluation standard of SH.

A total of 36 common peaks were identified in the UPLC-QDA fingerprint. Similarity analysis showed that the similarity of different processed SH samples was greater than 0.8, but there were still differences among SH samples processed by different processing methods, suggesting that different processing methods affect the quality of SH by altering its chemical composition. Pharmacodynamic results indicated that the analgesic and anti-inflammatory activities of processed SH samples superior to raw SH samples, and the activities enhanced with the increase in steaming time, among which the T9 sample exhibited the best efficacy. This is consistent with the record in ancient literature that insufficient nine times of steaming results in poor therapeutic effect, indicating the scientificity of traditional processing techniques. Combined with chemometric, the results indicated that the analgesic and anti-inflammatory effects of SH were significantly enhanced after 5–6 times of steaming. In addition, the combined results of colorimeter and fingerprint showed that the color of SH changed significantly after 4–5 times of steaming, and its intrinsic quality changed significantly after 5 times of steaming. Regardless of the processing method used, the appearance,

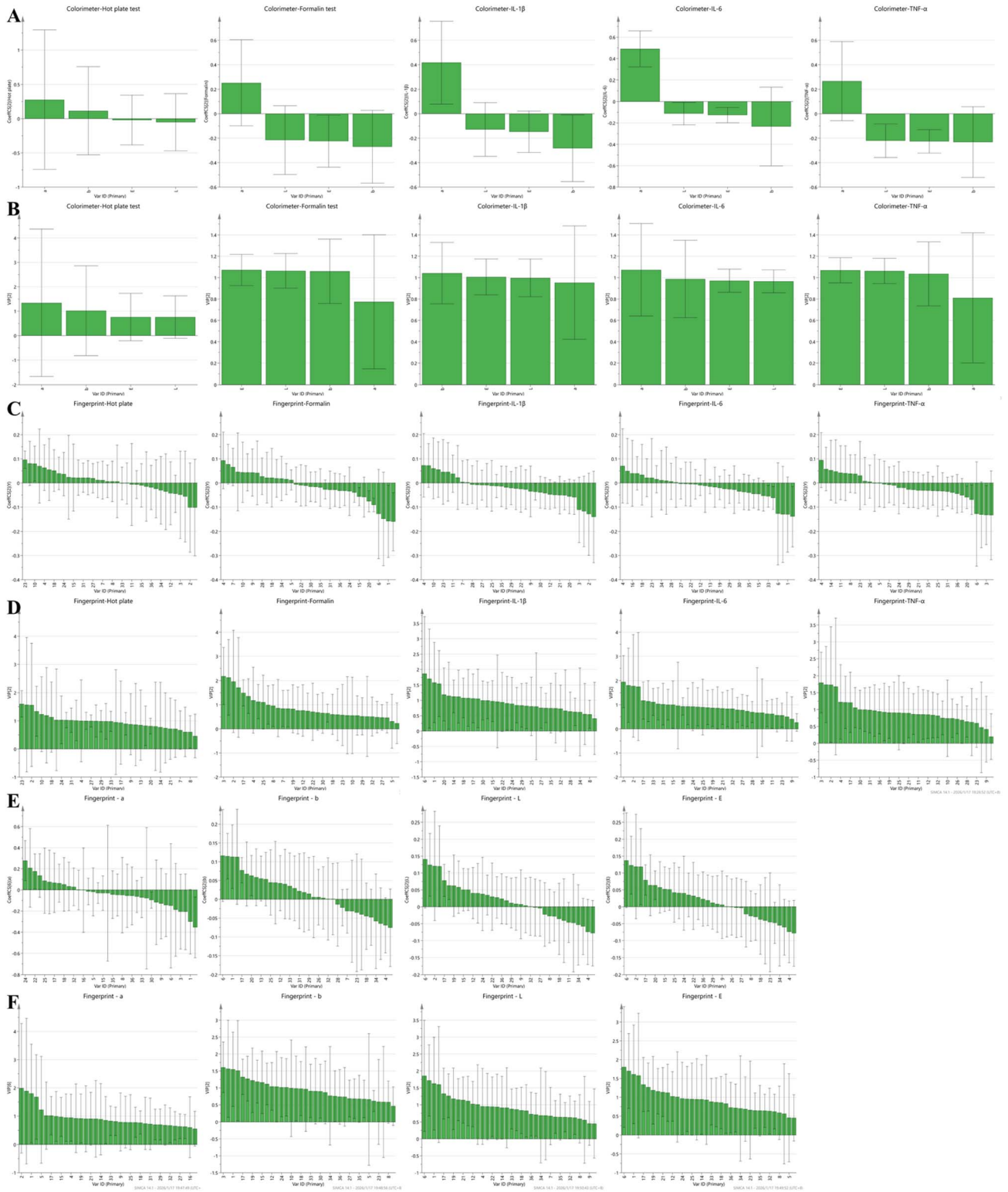

Fig. 5 Colorimeter-fingerprint-pharmacodynamic PLSR and VIP values analysis ((A) colorimeter-pharmacodynamic PLSR analysis; (B) colorimeter-pharmacodynamic VIP values analysis; (C) fingerprint-pharmacodynamic PLSR analysis; (D) fingerprint-pharmacodynamic VIP values analysis; (E) fingerprint-colorimeter PLSR analysis; (F) fingerprint-colorimeter VIP values analysis).

intrinsic quality, and therapeutic effects of SH remained relatively stable when steamed 6 times or 9 times. Pharmacodynamic results further demonstrated that the analgesic and anti-inflammatory effects of the sample steamed 9 times were significantly superior to those steamed 6 times, and the T9 sample showed the strongest efficacy (see SI material 2), suggesting that the cumulative changes in chemical components

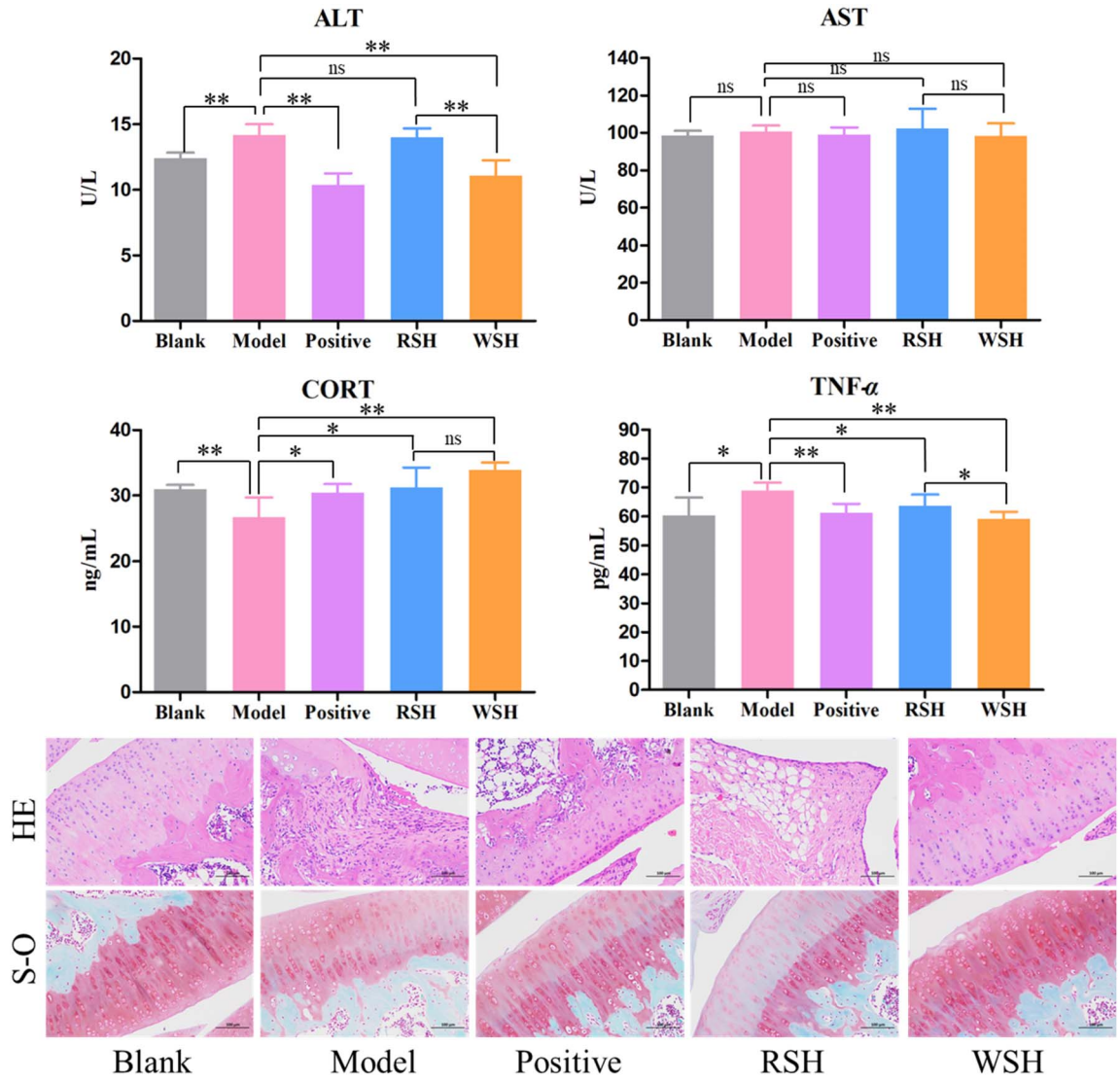

Fig. 6 The result of serum indicators and knee joint staining in osteoarthritis rats administration of RSH and WSH.

serve as the material basis for the improvement of pharmacodynamic effects of SH.

Pharmacological experiments further confirmed that WSH could significantly reduce the serum ALT level, increase the CORT level in osteoarthritis model rats, and alleviate knee joint synovial hyperplasia, inflammatory infiltration, and cartilage erosion. Its efficacy was superior to that of RSH and the positive medicine Duhuo Jisheng Pills. These indicates that nine-processed can enhance the effects of SH in tonifying the liver and kidney, as well as anti-inflammatory and analgesic effects, providing experimental evidence for its application as a health tea or single herb preparation.

This study is the first time to establish a correlation model between the color, components, and pharmacodynamic effects of SH with verification supported by pharmacological models. It standardized the traditional sensory evaluation and confirmed the scientificity of the nine-time repeating steaming and sun-drying processing technique for SH. In future research, a colourimetric card for SH under different processing methods can be developed with reference to Fig. 1. The color values of SH steamed 6 times ($L < 51.38$, $a > 3.54$, $b < 12.81$, $E < 52.94$) and 9 times ($L < 48.63$, $a > 3.42$, $b < 12.17$, $E < 50.32$) can be used as the indicator for its qualification and optimal processing method respectively. This provides a reliable reference for simplifying the evaluation of processing quality and facilitate industrial application.

5. Conclusion

Processed SH exhibits a good effect in nourishing the liver and kidney and can be made into a health tea for long-term consumption. In this study, a colorimeter was used to quantify the color values of SH samples under different processing methods, enabling the rapid identification and differentiation between raw and processed SH, simplified the process of evaluating the processing quality of SH, and improving the accuracy of sensory experience-based evaluation. Meanwhile, fingerprint, pharmacodynamic and pharmacological were used as

supplementary verification. Ultimately, an exclusive and objective optimal processing method for SH, one that is associated with the herb's properties and functional attributes was determined. This confirms the scientificity of the nine-time repeating steaming and sun-drying processing technique for SH, and provides important references for formulating the processing standards of SH and standardizing the macroscopic characteristics of TCMs.

Ethical statement

This article does not contain any studies with human participants, and all animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Heilongjiang University of Chinese Medicine and experiments were approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine (Approval number: 2025102809).

Author contributions

Conceptualization: Hai Jiang, Haixue Kuang, Liu Yang and Yanyan Zhou. Data curation: Ajiao Hou, Chao Yu, Jinbo Li. Writing – original draft: Ajiao Hou. Writing – review & editing: Ajiao Hou and Hai Jiang. Supervision, funding acquisition and resources: Hai Jiang.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this article.

Supplementary information (SI): the different processing methods of SH and the selection of the optimal processing method for SH. See DOI: https://doi.org/10.1039/d5ra09611a.

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