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A Study on the Processing Method of Fresh-Cut Processing of Yu Salvia Miltiorrhiza Drink Slices in Henan Province

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Abstract

To study the effects of traditional regional processing methods and modern drying methods on the ketone and phenolic acid components in Yu Salvia Miltiorrhiza produced in Fangcheng, Henan Province, as well as their effects on the characteristics of the final product of Salvia Miltiorrhiza drink slices, in order to determine the processing method for fresh-cut Yu Salvia Miltiorrhiza drink slices. Different regional processing methods, such as sun drying, shade drying, sweating, and drying, were selected. High-performance liquid chromatography (HPLC) was used to determine and analyze the content of ketone and phenolic acid components. Acetonitrile-0.02% phosphoric acid solution was used as the mobile phase for gradient elution; the detection wavelength was set at 270 nm, and the column temperature was maintained at 20°C. Fresh Yu Salvia Miltiorrhiza was washed, dried in a constant-temperature oven at 30-40°C until the moisture content reached 20%-30%, sliced into 3 mm thick pieces, and further dried in a constant-temperature oven at 30-40°C. The appearance, content of active ingredients, and overall quality of the Salvia Miltiorrhiza drink slices obtained by this processing method were superior to those obtained by traditional processing methods, improving production efficiency and reducing costs, and showing promising application prospects.

Keywords: Salvia Miltiorrhiza drink slices, fresh-cut processing, integrated regional processing and preparation, Salvia Miltiorrhiza ketone components, Salvia Miltiorrhiza phenolic acids.

1. Introduction

Danshen, listed as a top-grade herb in the "Shennong Ben Cao Jing," is known for its blood-activating, stasis-resolving, heart-clearing, and tranquilizing effects [1]. As a blood-activating and stasis-resolving medicine, it has been highly regarded by medical professionals throughout history and is often combined with other herbs, as seen in the formula "Yiwei Danshen Yin" (Danshen drink with other four herbs). Currently, there are many varieties of Danshen preparations in clinical use, playing an important role in traditional Chinese medicine formulations. A database search reveals that there are up to 40 Chinese medicinal preparations containing "Danshen" in their names. Among them, Tianlishi Group's Compound Danshen Droplets and Baiyunshan's Compound Danshen Tablets have annual sales exceeding billions of yuan. The Danshen Danshensuan Injection developed by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, is hailed as a milestone in the modernization of traditional Chinese medicine. According to surveys, the main production areas of Danshen in China include Shaanxi, Sichuan, Henan, Shanxi, Shandong, and others. However, variations in external environmental

conditions such as climate, altitude, soil moisture, and varieties can affect the quality of Danshen. Moreover, cultivation techniques and regional processing methods differ, leading to variations in yield and the content of active ingredients in Danshen. Studies in relevant literature have reported significant differences in yield and quality among Danshen from different regions, with some failing to meet the requirements of the Chinese Pharmacopoeia [1-5]. These observations indicate a chaotic quality situation in the Danshen herbal market, with some manufacturers still circulating poor-quality herbs as "legitimate medicinal materials" for the sake of profit, resulting in lower content of the main ingredient in formulations and reduced therapeutic effects. Additionally, differences in processing methods of raw medicinal materials can also lead to significant variations in content between different manufacturers and different batches from the same manufacturer.

The 2020 edition of the Chinese Pharmacopoeia describes the harvesting and processing of Danshen as "removing impurities and drying." The processing method for Danshen drink slices is described as "removing impurities and residual stems, washing, moistening, slicing, and drying." This method provides a detailed description of the processing steps from fresh herb to medicinal material and then to drink slices, separating regional processing from preparation, which belongs to the traditional processing method. However, Danshen primarily contains two types of components: liposoluble diterpenes, such as Tanshinone IIA, cryptotanshinone, and Tanshinone I, and water-soluble phenolic acids, such as Salvianolic Acid B, sodium Danshensu, and rosmarinic acid. Tanshinone IIA is prone to decomposition under light and high temperature conditions, and traditional sun-drying, with direct exposure to sunlight during natural drying, leads to significant loss of its content. Furthermore, traditional Danshen processing "avoids water washing" because prolonged and extensive water washing causes substantial loss of water-soluble phenolic acid components. Based on these considerations, the traditional Danshen processing method, which involves repeated washing, drying, moistening, and slicing steps, results in a loss of effective medicinal components, directly affecting the quality of Danshen drink slices. Therefore, studying the fresh-cut processing of Danshen drink slices in their production regions is of great significance in promoting an increase in yield and the content of active ingredients in Danshen, achieving standardized and maintaining the high-quality market for Chinese medicinal materials [6-10].

2. Experimental Materials

2.1 Reagents

The experimental water used in this study was double-distilled water. Specific information about the experimental reagents used is provided in Table 1.

Table 1 Reagent information table.

Serial No.	Reagent	Grade	Lot No.	Manufacturer
1	Phosphoric acid	Analytical grade	181112834	Tianjin chemieurope reagent co., ltd.
2	Methanol	Chemically pure grade	181112834	Tianjin chemieurope reagent co., ltd.
3	Acetonitrile	Chromatographic grade	180917256	Tianjin chemieurope reagent co., ltd.
4	Methanol	Chromatographic grade	180411245	Tianjin chemieurope reagent co., ltd.
5	Danshensu (salvianolic acid a)	Standard substance	110766-201721;⩾98%	China national institutes for food and drug control
6	Salvianic acid b	Standard substance	111562-201716;⩾98%	China national institutes for food and drug control

2.2 Instrument

The specific information of the experimental instruments used in the experiment is shown in Table 2.

Table 2 Instrument information table.

Number	Instrument	Model	Manufacturer
1	High-performance liquid chromatography (hplc) system	Lc-20a	Shimadzu (china) instrument co., ltd.
2	Electric constant temperature blast drying oven	Dhg9076a	Shanghai yiyao technology co., ltd.
3	Analytical balance with a precision of 1/10,000	Bs210s	Mettler-toledo instrument co., ltd.
4	Numerical control ultrasonic cleaner	Kq-500dv	Sian technology co., ltd.
5	High-speed pulverizer	Sc-1000y	Shanghai shangceng technology co., ltd.

2.3 Experimental samples

The samples of Salvia miltiorrhiza Bge., a dicotyledonous plant of the Labiatae family, were collected from the Yudan Ginseng Planting Base in Fangcheng County, Nanyang City, Henan Province. The samples consisted of dried roots and rhizomes.

3. Experimental Methods and Results

3.1 Different processing methods of salvia miltiorrhiza samples

Based on the traditional processing experience of Salvia miltiorrhiza and the processing methods documented in relevant literature [3-4] and the 2020 edition of the Chinese Pharmacopoeia, the research team investigated four different processing methods for Salvia miltiorrhiza samples: sun drying, shade drying, fresh cutting, and sweating, as well as the influence of different drying temperatures on the quality of the herbal material. Fresh Salvia miltiorrhiza samples were cleaned to remove impurities, and the adventitious roots and stems were trimmed. The samples were then divided into equal portions and processed according to the steps of sun drying, shade drying, fresh cutting, and sweating. The processed Salvia miltiorrhiza samples were pulverized and passed through a No. 3 sieve for further use. The information regarding the different processing methods is presented in Table 3.

Table 3 Information on the processing methods of salvia miltiorrhiza samples.

Origin Processing Methods		Operational Steps	Appearance Characteristics
Traditional Processing Methods	Sun drying	Randomly take approximately 200g of Salvia Miltiorrhiza sample, spread it evenly on a sample tray, and expose it to direct sunlight. Retrieve the sample during the night and dry it until it can be easily broken.	The herbal material is in the form of strips, with a light red color
	Shade drying	Randomly take approximately 200g of Salvia Miltiorrhiza sample, spread it evenly on a sample tray, and air-dry it in a cool and ventilated area until it loses enough moisture to easily break.	The herbal material is in the form of strips, with a light red color.
	Sweating drying	Take approximately 600g of fresh Salvia Miltiorrhiza herbs, partially air-dry them indoors until they are about 40-50% dry. When the roots become soft, seal and store them away from light. After 3 days, remove the herbs, air-dry them, and then "sweat" them for 2 days. Finally, take them out and air-dry them until completely dry.	The herbal material is in the form of strips, with a bright red color.
Drying methods	35°C drying	Randomly take approximately 200g of Salvia Miltiorrhiza sliced samples, spread them evenly on a sample tray, and place them in a 35°C electric constant-temperature convection drying oven. Dry the samples, flipping them at appropriate intervals to ensure even heating, until they can be easily broken.	The herbal material is in the form of strips, with a bright red color.
	40°C drying	Randomly take approximately 200g of Salvia Miltiorrhiza sliced samples, spread them evenly on a sample tray, and place them in a 40°C electric constant-temperature convection drying oven. Dry the samples, flipping them at appropriate intervals to ensure even heating, until they can be easily broken.	The herbal material is in the form of strips, with a bright red color.
	60°C drying	Randomly take approximately 200g of Salvia Miltiorrhiza sliced samples, spread them evenly on a sample tray, and place them in a 60°C electric constant-temperature convection drying oven. Dry the samples, flipping them at appropriate intervals to ensure even heating, until they can be easily broken.	The herbal material is in the form of strips, with a bright red color.
	80°C drying	Randomly take approximately 200g of Salvia Miltiorrhiza sliced samples, spread them evenly on a sample tray, and place them in an 80°C electric constant-temperature convection drying oven. Dry the samples, flipping them at appropriate intervals to ensure even heating, until they can be easily broken.	The herbal material is in the form of strips, with a bright red color.

3.2 Effect of different processing methods on salvia miltiorrhiza ketone components [11]

3.2.1 Chromatographic conditions

The chromatographic conditions were as follows: the stationary phase was an octadecylsilane bonded silica gel; acetonitrile was used as mobile phase A, and a 0.02% phosphoric acid solution was used as mobile phase B, following the gradient elution described in Table 4; the column temperature was set at 20°C; and the detection wavelength was 270 nm.

Table 4 Chromatographic conditions for salvia miltiorrhiza ketone IIA.

Time (minutes)	Mobile Phase A (%)	Mobile Phase B (%)
0~6	61	39
6~20	61→90	39→10
20~20.5	90→61	10→39
20.5~25	61	39

3.2.2 Preparation of reference solution

An appropriate amount of Salvia Miltiorrhiza Ketone IIA reference substance was accurately weighed and placed in a brown volumetric flask. Methanol was added to prepare a solution containing 20 μg/mL per 1 mL.

3.2.3 Preparation of test solution

Approximately 0.3 g of each sample powder (passed through a No. 3 sieve) was accurately weighed and placed in a stoppered conical flask. Precisely 50 mL of methanol was added, and the flask was tightly sealed. The weight was determined, and the flask was subjected to ultrasonic treatment (at a power of 140 W and a frequency of 42 kHz) for 30 minutes. After cooling, the weight was determined again. The lost weight was replenished with methanol, and the solution was mixed thoroughly. The mixture was filtered, and the filtrate was collected.

3.2.4 Analytical method

Precisely 10 μL of the reference solution and test solution were separately taken and injected into the high-performance liquid chromatography system for automatic injection and analysis. Using Salvia Miltiorrhiza Ketone IIA reference substance as the reference, the corresponding peak was designated as the S peak, and the relative retention times of Cryptotanshinone and Danshensu I were calculated. The relative retention time should be within ±5% of the specified value. The relative retention times and correction factors are shown in Table 5.

The peak areas of Cryptotanshinone, Danshensu I, and Salvia Miltiorrhiza Ketone IIA were multiplied by their respective correction factors to calculate their contents. According to the "Chinese Pharmacopoeia" (2020 edition), the content of Salvia Miltiorrhiza Ketone IIA, Cryptotanshinone, and Danshensu I, calculated based on the dry product, should not be less than 0.25%.

Table 5 Relative retention times and correction factors.

Component (peak)	Relative Retention Time	Correction Factor
Cryptotanshinone	0.75	1.18
Danshensu I	0.79	1.31
Salvia miltiorrhiza ketone IIA	1.00	1.00

3.2.5 Preparation of the standard curve

Precisely pipette 4 μl, 6 μl, 8 μl, 10 μl, 12 μl, and 14 μl of the above-mentioned prepared reference solution into the automatic injection system of the high-performance liquid chromatography (HPLC) instrument and measure the peak areas. Plotting the peak area (Y) against the injection volume (μl) (X), a regression analysis was performed, and the regression equation was found to be $Y = 114825X - 59067$, with an R^2 value of 0.9993. The results indicate a good linear relationship for Salvia Miltiorrhiza Ketone IIA within the range of 4-14 μl.

3.2.6 Stability test

Precisely pipette 10 μl of the test solution into the automatic injection system of the HPLC instrument at 0, 2, 4, 8, and 16 hours and measure the peak area automatically. The relative standard deviation (RSD) was found to be 0.92%, indicating the stability of the test solution within 16 hours.

3.2.7 Reproducibility test

Take 5 aliquots of the same batch of samples and prepare solutions following the aforementioned preparation method. Inject 10 μl of the solution into the automatic injection system of the HPLC instrument. The RSD was found to be 1.02%, indicating good reproducibility.

3.2.8 Intermediate precision test

Measure the samples at different times and by different analysts using 10 μl injection volume into the automatic injection system. The RSD was found to be 0.74%, indicating good reproducibility.

3.2.9 Determination of content

Determine the content of Salvia Miltiorrhiza Ketone IIA in the test solutions of different samples using the chromatographic conditions. Then calculate the content of Cryptotanshinone ($C_{19}H_{20}O_3$) and Danshensu I ($C_{18}H_{12}O_3$) based on the calibration factors, and calculate the total content of Salvia Miltiorrhiza Ketone IIA ($C_{19}H_{20}O_3$), Cryptotanshinone ($C_{19}H_{20}O_3$), and Danshensu I ($C_{18}H_{12}O_3$). The results are shown in Table 6.

3.3 Influence of different origins and processing methods on salvianic acid b content [12-13]

3.3.1 Chromatographic conditions

Using octadecylsilane-bonded silica gel as the stationary phase, the mobile phase consisted of acetonitrile-0.1% phosphoric acid solution (22:78). The column temperature was maintained at 20°C, and the flow rate was set at 1.2 ml/min. Detection was performed at a wavelength of 286 nm. The theoretical plate number for Salvianic Acid B peak should not be less than 6000.

3.3.2 Preparation of the reference solution

Take an appropriate amount of Salvianic Acid B reference substance, accurately weigh it, and dissolve it in a mixture of methanol and water (8:2) to obtain a solution containing 0.10 mg per 1 ml.

3.3.3 Preparation of the test solution

Take about 0.15 g of the sample powder (passed through a No. 3 sieve), accurately weigh it, place it in a stoppered conical flask, and add 50 ml of methanol-water (8:2) mixture. Seal the flask, weigh it, perform ultrasound treatment (140W power, 42kHz frequency) for 30 minutes, let it cool, weigh it again, make up for the weight loss with methanol-water (8:2) mixture, shake well, filter, precisely take 5 ml of the filtrate, transfer it to a 10 ml volumetric flask, dilute with methanol-water (8:2) mixture up to the mark, shake well, filter, and collect the filtrate.

3.3.4 Analytical method

Precisely pipette 10 μl of the reference solution and the test solution, respectively, into the liquid chromatograph for analysis.

3.3.5 Preparation of the standard curve

Precisely pipette 5 μl, 10 μl, 15 μl, 20 μl, 25 μl, and 30 μl of the prepared reference solution into the automatic injection system of the high-performance liquid chromatography (HPLC) instrument, sequentially inject, and measure the peak areas. Plotting the peak area (Y) against the injection volume (μl) (X), a regression analysis was performed, and the regression equation was found to be $Y = 33166X - 24675$, with an R^2 value of 0.998. The results indicate a good linear relationship for Salvianic Acid B within the range of 5-30 μl.

3.3.6 Stability test

Precisely pipette 10 μl of the test solution into the automatic injection system of the HPLC instrument at 0, 2, 4, 8, and 16 hours and measure the peak area automatically. The relative standard deviation (RSD) was found to be 0.48%, indicating the stability of the test solution within 16 hours.

3.3.7 Reproducibility test

Take 5 aliquots of the same batch of samples and prepare solutions following the aforementioned preparation method. Inject 10 μl of the solution into the automatic injection system of the HPLC instrument. The RSD was found to be 1.15%, indicating good reproducibility.

3.3.8 Intermediate precision test

Measure the samples at different times and by different analysts using 10 μl injection volume into the automatic injection system. The RSD was found to be 0.74%, indicating good reproducibility.

3.3.9 Content determination

Determine the content of Salvianic Acid B in the test solutions of different samples using the chromatographic conditions. The results are shown in Table 6.

Table 6 Effects of different processing methods and origins on the content of tanshinone components and danshensu B in Salvia miltiorrhiza.

Method	Tanshinone IIA/%	Cryptotanshinone/%	Tanshinone I/%	Total Content/%	Average Content/%	Danshensu b Content/%	Average Content/%
Sun-dried-1	0.3225	0.3805	0.4225	1.1255		12.0289	
Sun-dried-2	0.3233	0.3815	0.4235	1.1283	1.1286	12.2132	12.1040
Sun-dried-3	0.3244	0.3828	0.4249	1.1321		12.0698	
Shade-dried-1	0.4055	0.4785	0.5312	1.4153		11.6787	
Shade-dried-2	0.4073	0.4806	0.5336	1.4215	1.4195	11.6209	11.6323
Shade-dried-3	0.4074	0.4807	0.5336	1.4217		11.5973	
Sweating-dried-1	0.2970	0.3505	0.3891	1.0366		10.3135	
Sweating-dried-2	0.2961	0.3494	0.3879	1.0335	1.0362	10.4457	10.3628
Sweating-dried-3	0.2976	0.3512	0.3898	1.0386		10.3294	
35℃-1	0.3172	0.3743	0.4155	1.1070		11.2551	
35℃-2	0.3191	0.3765	0.4180	1.1136	1.1104	11.2760	11.1856
35℃-3	0.3182	0.3755	0.4168	1.1105		11.0258	
40℃-1	0.3576	0.4220	0.4685	1.2481		13.1395	
40℃-2	0.3609	0.4259	0.4728	1.2595	1.2538	13.0719	13.0610
40℃-3	0.3592	0.4238	0.4705	1.2536		12.9715	
60℃-1	0.3193	0.3767	0.4183	1.1143		11.6879	
60℃-2	0.3113	0.3673	0.4078	1.0865	1.1046	11.8436	11.6961
60℃-3	0.3189	0.3763	0.4178	1.1130		11.5567	
80℃-1	0.2856	0.3370	0.3741	0.9966		10.7390	
80℃-2	0.2842	0.3354	0.3723	0.9919	0.9939	10.7020	10.7082
80℃-3	0.2846	0.3358	0.3728	0.9931		10.6836	

3.4 Results analysis

2.4.1 In the historical records of ancient literature, various processing methods for Salvia miltiorrhiza, such as removing the stalks, soaking in alcohol, roasting in alcohol, sun-drying, shade-drying, and sweating, have been documented. These traditional methods have significant guiding significance for modern processing techniques. Modern processing of Salvia miltiorrhiza generally follows the ancient methods, while actively exploring and innovating new approaches. For example, advanced scientific instruments like temperature-controlled drying

ovens, high-performance liquid chromatography, and others have been utilized to quantitatively analyze the processing techniques and the content of active components in Salvia miltiorrhiza. This enables us to maximize the exploration of the potential utilization value of Salvia miltiorrhiza [14,15].

2.4.2 Based on the above results analysis, the content of tanshinone components and danshensu B in Salvia miltiorrhiza processed by shade-drying, fresh cutting, and direct sweating methods are similar. However, the content of danshensu B is highest in the shade-drying method. This is because the shade-drying method is less affected by temperature, resulting in a smaller impact on the active components. Therefore, it is suitable to use the shade-drying method for processing Salvia miltiorrhiza, although it requires a longer processing time [16].

2.4.3 The content of tanshinone IIA is higher under drying conditions of 35°C, 40°C, 60°C, and 80°C. This is because tanshinone IIA is chemically unstable and its content is related to the loss of internal moisture in the herb. However, the content of tanshinone IIA is lowest under the drying condition of 80°C, as tanshinone IIA's thermal stability decreases, and 80°C is its critical temperature. The content of danshensu B decreases in the drying conditions of 35°C, 40°C, 60°C, and 80°C. This is because the phenolic acid components in Salvia miltiorrhiza are unstable under heat, leading to the decomposition of danshensu B. It is recommended to use the drying method, preferably at low temperatures such as 35°C or 40°C, for processing Salvia miltiorrhiza [17-20].

4. Optimization of Fresh Cutting Process for Danshen Tablets Using Orthogonal Design Method

4.1 Effects of different drying levels on the appearance of danshen tablets

Fresh Danshen herbs were cleaned to remove dirt and washed roots and stems. The herbs were then mixed and divided into equal portions for low-temperature drying at 35°C. The fresh weight of the roots was measured at different time intervals, followed by measuring the dry weight. The drying ratio was calculated based on the fresh weight and dry weight to examine the effects of different drying levels on the appearance of Danshen tablets. The results are shown in Table 7.

Table 7 Effects of different drying levels on the appearance of danshen tablets.

Serial No	Drying Time /h	Drying Ratio/%	Appearance
1	0	76.2	Easy to cut, with a red color on the cut surface.
2	4	71.8	Easy to cut, with a red color on the cut surface.
3	8	67.4	Easy to cut, with a red color on the cut surface.
4	13	65.3	Easy to cut, with a red color on the cut surface.
5	18	56.7	Easy to cut, with a red color on the cut surface.
6	23	53.0	Easy to cut, with a red color on the cut surface.
7	28	50.2	Easy to cut, with a red color on the cut surface.
8	31	48.6	Easy to cut, with a red color on the cut surface.
9	34	45.2	Easy to cut, with a red color on the cut surface.
10	37	40.3	Easy to cut, with a red color on the cut surface.
11	40	30.8	Relatively easy to cut, with a yellow-white color on the cut surface.
12	43	23.2	Difficult to cut, with a yellow-white color on the cut surface.

The experimental data above indicates that when Danshen herbs are dried at 35°C to a moisture content of 23.2% to 30.8%, Danshen tablets are relatively easy to cut, and the cut surface appears yellow-white. Through content determination, the content of Danshensu B can reach 10.24%.

4.2 Orthogonal experimental design

Based on the above experimental results, when the moisture content is between 20% and 30% and the drying temperature is between 30°C and 40°C, the appearance evaluation of the tablets is better. According to the principles of orthogonal combination, the moisture content is set at three levels: 20%, 25%, and 30%, and the drying temperature is set at three levels: 30°C, 40°C, and 50°C. Based on the thickness requirements for Danshen tablets in the "Chinese Pharmacopoeia" (2020 edition), the thickness of the slices is set at three levels: 2mm, 3mm,

and 4mm. An L9 (34) orthogonal experimental table is established. A total of 9 batches of tablets are prepared. The factors and levels of the experiment are shown in Table 8.

Table 8 Factors and levels of orthogonal design.

Level	A (Moisture Content / %)	B (Drying Temperature / °C)	C (Slice Thickness / mm)
1	20	30	2
2	25	40	3
3	30	50	4

The description of Danshen tablets in the Chinese Pharmacopoeia 2020 edition states that "the product is a thick tablet with a round or elliptical shape. The external surface is brownish-red or dark brownish-red, rough, and has longitudinal wrinkles. The cut surface may have cracks or be slightly flat and compact, some showing a horny texture. The outer bark is brownish-red, the wood is grayish-yellow or purplish-brown, and there are yellow-white radial textures." The evaluation of the appearance of Danshen tablets in this experiment is based on the following criteria: color of the cut surface, degree of warping, and texture solidity. The scoring criteria for evaluating the appearance characteristics of the tablets are shown in Table 9.

Table 9 Scoring criteria for appearance evaluation.

Characteristics	Evaluation Criteria	Score
Color of the cut surface	Dark brown	0.4
	Reddish-brown	0.6
	Orange-yellow	0.8
	Yellow-white	1.0
Color of the cut surface	Easy warping (approximately 70%)	0.4
	Slightly warped (approximately 50%)	0.6
	Very little warping (approximately 30%)	0.8
	No warping	1.0
Color of the cut surface	Bark and wood separation, with debris	0.5
	Bark and wood tightly connected, no debris	1.0

4.3 Comprehensive weighted scoring criteria

The investigation is conducted based on the criteria of appearance, water-soluble extractives, alcohol-soluble extractives, total content of salvianolic acid B and salvianone compounds. The weight coefficients for appearance and active ingredients are 0.2 and 0.8, respectively. The weight coefficients for water-soluble extractives, alcohol-soluble extractives, salvianolic acid B, and salvianone compounds are 0.1, 0.1, 0.3, and 0.3, respectively. The appearance score is calculated using Formula (1), water-soluble extractives score using Formula (2), alcohol-soluble extractives score using Formula (3), salvianolic acid B score using Formula (4), salvianone compounds score using Formula (5), and the comprehensive score is calculated using Formula (6). A higher comprehensive score indicates a better result.

Formula (1): Appearance Score = (0.2/W1max) × W1

Formula (2): Water-soluble Extractives Score = (0.1/W2max) × W2

Formula (3): Alcohol-soluble Extractives Score = (0.1/W3max) × W3

Formula (4): Salvianolic Acid B Score = (0.3/W4max) × W4

Formula (5): Salvianone Compounds Score = (0.3/W5max) × W5

Formula (6): Comprehensive Score (OD) = (Appearance Score + Water-soluble Extractives Score + Alcohol-soluble Extractives Score + Salvianolic Acid B Score + Salvianone Compounds Score) × 100

Where, Wmax is the highest appearance score among the 9 assessments, W is the score obtained in a particular assessment, and Wmax refers to the highest value among the 9 measurements.

4.4 Orthogonal experimental design and results

Based on the experimental sample preparation, control sample preparation, and chromatographic conditions mentioned above, the content of samples at different levels and factors is determined, and the weighted scores are evaluated for the Salvia Miltiorrhiza slices. The final results are presented in Table 10.

Table 10 Orthogonal experimental design and results for fresh-cut processing of salvia miltiorrhiza slices.

Serial Number	A (Moisture Content / %)	B (Drying Temperature / °C)	C (Slice Thickness / mm)	Appearance	Water-Soluble Extractives	Alcohol-Soluble Extractives	Salvianolic Acid B	Total Salvianone Compounds	Comprehensive Score
1	30	50	4	2.6	35.45	15.54	3.17	0.30	94.46
2	25	30	4	2.4	35.08	15.50	3.63	0.38	94.59
3	30	30	3	2.6	35.75	15.79	3.61	0.40	95.55
4	25	50	2	2.4	35.74	15.46	3.21	0.34	94.75
5	20	40	4	2.8	35.07	15.71	3.64	0.39	94.81
6	30	40	2	2.6	35.62	15.91	3.69	0.40	95.61
7	25	40	3	3.0	36.10	16.34	3.76	0.39	96.59
8	20	50	3	2.8	35.87	15.16	3.20	0.35	94.58
9	20	30	2	2.6	36.07	15.98	3.46	0.39	95.90

Based on the intuitive analysis from the table, it can be observed that A2B2C2 has the highest comprehensive score, indicating the optimal process parameters. A2B2C1 has similar content of active ingredients to A2B2C2. Therefore, A2B2C2 and A2B2C1 are selected as the best optimized processes, which involve washing the fresh Salvia miltiorrhiza, drying it in a constant temperature oven at 30-40°C until the moisture content reaches 20%-30%, and slicing it into 3mm thick pieces, followed by drying in a constant temperature oven at 30-40°C.

5. Conclusion

In this experiment, different processing methods were applied to Salvia miltiorrhiza, including sun drying, shade drying, fresh cutting, and direct sweating, to investigate their effects on the content of active ingredients. The Salvia miltiorrhiza used in the experiment was cultivated in Nanyang City, Henan Province, China. It has undergone multiple selections of high-quality strains and is one of the main varieties of Salvia miltiorrhiza available on the market. Based on the experimental data, the following conclusions were drawn: the content of salvianone IIA is relatively low in the sun drying process. Shade drying, fresh cutting, and direct sweating without exposure to light resulted in higher content, as salvianone IIA is chemically unstable and easily decomposes under light. The optimal processing method for Salvia miltiorrhiza was found to be shade drying, although it has the drawback of longer processing time. It is recommended to use low-temperature drying instead of traditional sun drying, as the latter leads to significant loss of active ingredient content.

Rinsing fresh Salvia miltiorrhiza with water can effectively remove surface soil, ensuring the cleanliness of the herbal material and reducing the contamination of harmful heavy metals. Water rinsing has minimal impact on the content of salvianone compounds but can slightly decrease the content of water-soluble components such as phenolic acids. Some literature commonly advises against using water during the processing of Salvia miltiorrhiza to remove soil. The Chinese Pharmacopoeia does not specify how soil should be removed. Based on years of experience in production areas, the commonly adopted method is to dry the herbal material and shake off the soil. Some methods involve piling up the herbal material and using water to wash away the surface soil, but this method clearly affects the quality and can even lead to mold and deterioration. Further research is needed to explore alternative methods for fresh cutting, processing in the production areas, and soil removal. For large-scale processing, a low-temperature drying machine can be used, with the temperature controlled at 30-40°C. Using a drying machine not only speeds up the drying process but also ensures temperature control and uniformity, thus achieving better quality control compared to conventional drying rooms, where temperature control is more susceptible to human factors. Considering various factors, processing fresh Salvia miltiorrhiza into slices with a moisture content of 20%-30% after water rinsing, followed by low-temperature drying, can guarantee excellent appearance and quality of the slices.

In conclusion, the integrated production and processing method obtained from this experiment, as well as the processed Salvia miltiorrhiza slices, demonstrated superior appearance, content of active ingredients, and overall quality compared to traditional processing methods. It not only improves production efficiency but also reduces costs, making it highly promising for practical application.

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References

[1] National Pharmacopoeia Committee. Pharmacopoeia of the People's Republic of China, Part I. Beijing: China Medical Science Press, 2020:76.

[2] Song Yan, Zhao Zhigang, Gao Shurui. Evaluation of the quality of cultivated and wild salvia miltiorrhiza herbal materials from different production areas. Chinese Journal of Traditional Chinese Medicine, 2014, 36(05): 1026-1029.

[3] Li Yuekai. Study on the differences in yield and effective components of salvia miltiorrhiza in different ecological regions of Shaanxi Province. Northwest A&F University, 2014.

[4] Liu Qian, Zhang Yuan, Li Anping, Zhao Jianbang, Chen Chen, Wu Yanlin, Gao Hua. Determination of active ingredients in salvia miltiorrhiza injection and comparison of dose-effect relationships among different manufacturers. Journal of Pharmaceutical Analysis, 2014, 34(03): 500-504.

[5] Zhao Jun, Cheng Yuefa, Zhang Aibing, Pei Lu, Liu Yingshuo. Comparative analysis of four active components in salvia miltiorrhiza tablets and capsules from different manufacturers by HPLC. Journal of Chinese Medicinal Materials, 2012, 23(11): 2790-2791.

[6] Yang Junjie, Li Lin, Ji De, Mao Chunqin, Wu Qinan, Lu Tulin. Historical evolution and modern research on integrated processing of traditional chinese medicinal materials from production areas. Chinese Traditional and Herbal Drugs, 2016, 47(15): 2751-2757.

[7] Yue Linwei, Qin Kunming, Zhu Yanhui, Cai Hao, Li Weidong, Cai Baochang. Research status and prospect of production area processing of chinese medicinal materials. China Journal of Chinese Materia Medica, 2015, 40(04): 602-606.

[8] Zhao Zhigang, Gao Shurui, Hou Junling, Wang Wenquan, Xu Zhenguang, Song Yan, Zhang Xianming, Li Jun. Effects of different production areas and processing methods on the quality of shandong salvia miltiorrhiza herbal materials. China Journal of Chinese Materia Medica, 2014, 39(08): 1396-1400.

[9] Duan Jin'ao, Su Shulan, Lü JieLi, Yan Hui, Ding Anwei. Traditional experience and modern scientific understanding of production area processing of medicinal materials. China Journal of Chinese Materia Medica, 2009, 34(24): 3151-3157.

[10] Yang Junjie, Zhang Zhenling. Discussion on the integrated processing of medicinal materials from production areas and the preparation of chinese medicinal pieces. Journal of Chinese Medicinal Materials, 2005(09): 817-818.

[11] Rastegarnejad Fahimeh, Mirjalili Mohammad Hossein, Bakhtiar Ziba. Enhanced production of tanshinone and phenolic compounds in hairy roots culture of Salvia miltiorrhiza Bunge by elicitation. Plant Cell, Tissue and Organ Culture (PCTOC). 2023, 156 (01).

[12] Mónica L. Pérez Ochoa, Araceli M. Vera Guzmán, Demetria M. Mondragón Chaparro, Sadoth Sandoval Torres, José C. Carrillo Rodríguez, et al. Effects of annual growth conditions on phenolic compounds and antioxidant activity in the roots of eryngium montanum. Plants. 2023, 12(18).

[13] Ewa Kochan, Halina Wysokinska, Aleksander Chmiel, Barbara Grabias. Rosmarinic acid and other phenolic acids in hairy roots of hyssopus officinalis. Zeitschrift für Naturforschung C.2015, 54(1-2):11-16.

[14] Ayvazyan Arpine, Deutsch Lenard, Zidorn Christian, Kircher Brigitte, Çiçek Serhat S. Cytotoxic diterpenoids from Salvia glutinosa and comparison with the tanshinone profile of danshen (Salvia miltiorrhiza). Frontiers in Plant Science. 2023,14:1269710-1269710.

[15] Hwang Cho Hyun, Jang Eungyeong, Lee JangHoon. Pharmacological Benefits and Underlying Mechanisms of Salvia miltiorrhiza against Molecular Pathology of Various Liver Diseases: A Review. The American journal of Chinese medicine. 2023, 51(07):31-35.

[16] Karalija Erna, Dahija Sabina, Tarkowski Petr, Zeljković Sanja Ćavar. Influence of climate-related environmental stresses on economically important essential oils of mediterranean salvia sp. Frontiers in Plant Science. 2022, 13: 864807-864807.

[17] Ko Geon, Kim Jinho, Jeon YeongJae, Lee Donghun, Baek HyeonMan et al. Salvia miltiorrhiza alleviates memory deficit induced by ischemic brain injury in a transient mcao mouse model by inhibiting ferroptosis. Antioxidants (Basel, Switzerland). 2023, 12 (4).

[18] Jeon Julie H, Kaiser Erin E, Waters Elizabeth S, Yang Xueyuan, Lourenco Jeferson M et al. Tanshinone IIA-loaded nanoparticles and neural stem cell combination therapy improves gut homeostasis and recovery in a pig ischemic stroke model. Scientific reports. 2023, 13(01):2520-2520.

[19] Bryson Abigail E, Lanier Emily R, Lau Kin H, Hamilton John P, Vaillancourt Brieanne et al. Uncovering a miltiradiene biosynthetic gene cluster in the Lamiaceae reveals a dynamic evolutionary trajectory. Nature communications. 2023, 14(01):343-343.

[20] Szymczyk Piotr, Kuźma Łukasz, Jeleń Agnieszka, Balcerczak Ewa, Majewska Małgorzata. Isolation of salvia miltiorrhiza kaurene synthase-like (KSL) gene promoter and its regulation by ethephon and yeast extract. Genes. 2022, 14(01):54-54.