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Effects of Post-Harvest Processing Methods on the Quality of *Radix et Rhizoma Salviae Miltiorrhizae*

Huyen Thu Nguyen[1], Hang Thi Cu[1], My Thi Chu[1], Nguyen Thi Yen Chi[2], Quach Anh Kiên[3] & Nguyen Minh Nhat[4]

[1] National Institute of Medicinal Material, Vietnam

[2] Hanoi-Amsterdam Highschool for the Gifted, Vietnam

[3] Phan Dinh Phung Highschool, Vietnam

[4] Quang An Secondaryschool, Vietnam

Correspondence: Huyen Thu Nguyen, National Institute of Medicinal Material, Km12.9 Ngochoi, Hanoi, Vietnam. Tel: 84-975-668-945. E-mail: nhatduchuyen@gmail.com

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Abstract

The effect of pretreatment, drying and storage methods on maintaining the quality of *Radix et Rhizoma Salviae mitiorrhzae* postharvest was studied. The experiments used organic acids as acid citric and sodium bisulfate solution to evaluate the pretreatment of *Radix et Rhizoma Salviae mitiorrhzae*. Rhizome and roots of *Salvia miltiorrhiza* Bunge were pretreated and dried by convection drier and heat pump drier and were preserved by low pressure. The results showed that dipping acid citric solution pH 2.5-3 for 30 minutes restricted the developing of total microorganisms. Using heat pump drier were good sensory quality and retaining tanshinone IIA, salvianolic acid content in *Radix et Rhizoma Salviae mitiorrhzae*. The method of preserving *Radix et Rhizoma Salviae mitiorrhzae* by the low pressure 200 mmHg, and 400 mmHg after 9 months at ambient temperature (25-30 ^{o}C), humidity (80-85%) maintained better quality than the control. Beside, the moisture's medicine, tanshinone IIA, salvianolic acid content, and total microorganisms in the experiment samples changed slowly during the storage period.

Keywords: *Radix et Rhizoma Salviae mitiorrhzae*, citric acid, sodium bisulfate, convective drier, low pressure

1. Introduction

Salvia miltiorrhiza Bunge belongs to the mint family (*Lamiaceae*) and in mountainous provinces of Vietnam. *Radix et Rhizoma Salviae mitiorrhzae* (Danshen), is a precious medicinal plant, the part used is the root and rhizome. *Radix et Rhizoma Salviae mitiorrhzae* is a perennial herb. The rhizome are small, long, cylindrical, 0.5-1.5 cm in diameter, red-brown in color. The tree is 40 to 80 cm tall, has 4 sides and has soft light yellow hairs. The dried roots and rhizomes of *S. miltiorrhiza* promote blood circulation, relieve dysmenorrhea and reduce swelling (Loi, 2006). Danshen has a distinctive red exterior, bitter taste, slightly welding properties, enters the heart and liver meridians, has the effect of expelling blood stasis, activating blood, draining pus, improving the skin, regulating menstruation, and enhancing health. In traditional medicine (https://www.medigoapp.com/hoat-chat/djan-sam), *Radix et Rhizoma Salviae mitiorrhzae* is a valuable medicinal plant. Danshen has the effect of dilating coronary arteries, causing blood flow of coronary arteries to increase significantly, improving heart function, limiting myocardial infarction, and having a hypotensive effect, antibacterial effect, sedative effect, irregular menstruation, menstrual blockage, menstrual cramps, blood clots, angina pectoris; insomnia, mental disturbances (Medical Publishing House, 2018). *Radix et Rhizoma Salviae mitiorrhzae* contains tanshinone, which is the common name for the group of lipophilic diterpenoid active ingredients found in *Radix et Rhizoma Salviae mitiorrhzae* (tanshinone II A, cryptotanshinone, tanshinon I, dihydro-tanshinone...) (Medical Publishing House, 2018). Preliminarily preparing and preserving Danshen has been done for a long time. However, the processing method is simple, manual and based on experience: Every year, in the spring or fall, *Radix et Rhizoma Salviae mitiorrhzae* are dug up, washed, the secondary roots are cut off, dried and used as medicine. When harvesting, choose tubers with red skin color, approximately 1 centimeter in diameter, not scratched, rotten, bruised and stored in a cool, dry place, away from termites (Hoat, 2005). Tan (2015) evaluated some methods of preliminary processing of medicinal herbs *Radix et Rhizoma Salviae mitiorrhzae* in Hanoi, concluded that drying at a

temperature of 50-60 ^{o}C and SO_2 fumigation with 1.5 kg sulfur/100 kg and compared with the control which is naturally dried in the sun. The results showed that the criteria of extractable substances in water and substances extracted in ethanol all met according to Vietnam Pharmacopoeia IV.

The purpose of study on preliminary treatment and storage of *Radix et Rhizoma Salviae mitiorrhzae* is to reduce the effect of fungi, maintaining the quality of medicinal plant after storage time, limiting the increasing of moisture content, keeping a good sensory quality by using organic acid in combination with low pressure.

2. Materials and Methods

2.1 Materials

Salvia miltiorrhiza Bunge grown in Hoa Binh is harvested in December 2022 after a growing period of about 280 to 310 days from planting when there was enough growing time and high active elements.

2.2 Methodology

After being harvested, *Salvia miltiorrhiza* Bunge is transported to the Hanoi Research Centre for Cultivation and Processing of Medicinal Plants, National Institute of Medicinal Materials, Vietnam. Root and rhizome of *Salvia miltiorrhiza* Bunge was preliminarily classified according to size from 20 cm to 30 cm long, 2 cm to 4 cm in diameter, rotten roots that did not meet the requirements are removed, and then the sandy soil is washed with clean water. Randomly arrange the experiments as follows: (i) Experiment on the effects of preliminary treatment on the quality of medicinal herbs, the formula was as follows: Formula 1 (F1): Treatment of citric acid pH 2.5-3 in 30 minutes; Formula 2 (F2): Treat sodium bisulfate solution 2 g/liter for 30 minutes; Formula 3 (F3): untreated control sample; after treatment, the *Radix et Rhizoma Salviae mitiorrhzae* are dried by convective drier at temperature 55±2 ^{o}C until < 12% moisture content and evaluated. (ii) Experimentally after treatment of citric acid pH 2.5-3 in 30 minutes then evaluate the effects of drying methods on the quality of medicinal herbs and formulas as follows: Formula 4 (F4): sun drying (4-5 days until reaching < 12% moisture content) as control sample; Formula 5 (F5): heat pump drier at 40 ^{o}C (until < 12% moisture content); Formula 6 (F6): convective heat drier at 55 ^{o}C (until < 12% moisture content); (iii) Experiment on the effects of low pressure on the quality of medicinal herbs in preservation; *Radix et Rhizoma Salviae mitiorrhzae* are treated citric acid pH 2.5-3 in 30 minutes, dried by heat pump drier, packaged in 0.4 mm thick polyvinyl chloride (PVC) packaging, sealed and then smoked with the following formulas: 8 (F8): pressure 200 mmHg; formulas: 9 (F9): pressure 400 mmHg; and sample formulas 7 (F7) was the control sample untreated and packaged in 0.1 mm thick polyethylene (PE) packaging. Take evaluation samples at 0, 3, 6 and 9 months of storage at was the control sample at ambient temperature (25-30 ^{o}C), humidity (80-85%). For each experiment, choose the best formula to serve as the basis for processing materials for the following experiments. Each formula in the experiments was repeated 3 times.

2.3 Methods

Sensory assessment: evaluate sensory quality according to TCVN 3215-79 and Vietnam Pharmacopoeia V standards.

Active ingredients: *Sample assay*. *Radix et Rhizoma Salviae mitiorrhzae* weighed (5 g), grinded powders (80 mesh) of samples were extracted twice with 70% aM (20 mL) in an ultrasonic bath at room temperature for 20 min. The supernatants after centrifugation (3000 rpm for 10 min) were combined and diluted with 70% aM to 50 mL. The solutions were filtered through a 0.45 μm nylon syringe filter (Millex-HN, Massachusetts, USA) before tanshinone IIA and salvianolic acid B analysis by High-performance liquid chromatography (HPLC-MS/MS) analysis. According to Vietnam Pharmacopoeia V: the quantitative requirement of tanshinone IIA is not less than 0.2% and salvianolic acid B is not less than 3.0% calculated based on dried medicinal herbs.

Total microorganisms: Determine the number of total microbial cells according to TCVN 6404:2016.

Moisture: According to Vietnam Pharmacopoeia V.

Statistical analysis was carried out using SPSS v.17.0 software (SPSS Inc., IL, USA). Duncan's multiple range test to analyze the significant differences ($P \leq 0.05$) between samples.

3. Result and Discussions

3.1 The Effect of Pretreatment on the Quality of Radix et Rhizoma Salviae mitiorrhzae

The results of research on the effects of pretreatment on the quality of *Radix et Rhizoma Salviae mitiorrhzae* are shown in Table 1:

Table 1. The effects of preliminary treatment on the quality of *Radix et Rhizoma Salviae mitiorrhzae*

Sample	Tanshinone IIA (%)	Salvianolic acid B (%)	Sensory assessment (score)	Moisture (%)	Total microorganisms (CFU/g)
F1	0.93a	5.53a	19.27a	11.65a	$9.66.10^{2b}$
F2	0.70b	3.77b	18.63a	11.03a	$8.73.10^{2b}$
F3	0.90a	4.88ab	18.40a	11.56a	$1.28.10^{4a}$

Note. In the same column, numbers with the same letter are not significantly different at the $\alpha = 0.05$.

Results from Table 1 show that:

Preliminary treatments affect the total number of aerobic bacteria on the product surface, with significant differences between the formulas at the $\alpha = 0.05$ level. The surface treatment samples of *Radix et Rhizoma Salviae mitiorrhzae* had limited growth of total aerobic bacteria to lower than the control samples. The results are consistent with some studies showing that using citric acid and sodium bisulfate solutions was effective in killing total microorganisms on product surfaces. The results showed that the author Popova (2019), said that enterobacteriaceae species and total microorganisms were also affected to the maximum extent, even when stored for 7 months, they still retained their antibacterial activity.

Tanshinone IIA and salvianolic acid B were the main active ingredients of *Radix et Rhizoma Salviae mitiorrhzae*, so it is used to evaluate the quality of Danshen. In this experiment, pretreatment methods affected the content of tanshinone IIA and salvianolic acid B in the product. The *Radix et Rhizoma Salviae mitiorrhzae* sample treated with citric acid had the highest salvianolic acid content of 4.53%, with a significant difference at the 0.05 level compared to the control sample of 3.88%; and all higher than the standards of Vietnam Pharmacopoeia V.

The results showed that *Radix et Rhizoma Salviae mitiorrhzae* were treated with citric acid solution pH 2.5-3 for 30 minutes and indicated the best formula in limiting surface microorganisms, high sensory perception without affecting the active ingredients.

3.2 The Effect of Drying Method on the Quality of Radix et Rhizoma Salviae mitiorrhzae

The active ingredient many pharmacological studied about the water-soluble components from Danshen have reported, especially salvianolic acids. It turned out that salvianolic acids showed strong anti-lipid peroxidation and anti-thrombic activities, and among them, SalAA and SalAB were the most potent (Guanhua, 2020). This review focused on the achievements in research of salvianolic acids regarding their bioactivities and temperature effects. During the drying process, temperature, humidity and time all had a certain impact on product quality, so choosing the appropriate drying method was of great significance in ensuring the quality of medicinal herbs (Difeng, 2002).

The results of research on the effects of drying methods on the quality of *Radix et Rhizoma Salviae mitiorrhzae* medicinal herbs are shown in Table 2:

Table 2. Effect of drying method on the quality of *Radix et Rhizoma Salviae mitiorrhzae* medicinal herbs

Sample	Tanshinone IIA (%)	Salvianolic Acid B (%)	Sensory assessment (score)	Moisture (%)	Total microorganisms (CFU/g)
F4	0,80a	4.96b	18.07a	11.15a	$1.15.10^{4a}$
F5	0.89a	6.05a	19.43a	11.01a	$9.87.10^{2b}$
F6	0.79a	5.05b	18.83a	11.08a	$1.03.10^{3b}$

Note. In the same column, numbers with the same letter are not significantly different at the $\alpha = 0.05$.

Results from Table 2 show that:

Different drying methods impacted on the Salvianolic Acid B content in *Radix et Rhizoma Salviae mitiorrhzae*, high temperature heat, fast drying speed and short drying time, accelerates the transpiration of medicinal herbs, reduced the rate of internal oxidation reactions, thereby reducing the decomposition of Salvianolic Acid B in control with a significant difference at the 0.05 level between other formulas. Zhi-Gang (2014) shown that the content of active ingredients in *Radix et Rhizoma Salviae Miltiorrhizae* processing costing etc., shade-drying or oven dry under low temperature (40-60 ^{o}C) should be the most suitable original processing method.

From the above results, it shows that dried *Radix et Rhizoma Salviae mitiorrhzae* using a heat pump dryer gave the best quality of medicinal herbs in terms of Salvianolic Acid B content, organoleptic and total microorganisms on

the surface of medicinal herbs. Besides, this drying method also reduced drying time and improves medicinal production efficiency.

3.3 The Effect of Low Pressure Preservation on the Quality of Radix et Rhizoma Salviae mitiorrhzae

Storage under low pressure conditions was to reduce the amount of air in the product batch and seal it. This preservation increased product storage time by inhibiting microbial growth and reducing the risk of cross-contamination. This method also ensured sensory quality and prevents high moisture of the product.

The results of research on the effects of low pressure preservation on the quality of *Radix et Rhizoma Salviae mitiorrhzae* medicinal herbs are shown in Table 3 and Figures 1 and 2:

Table 3. Effects of low pressure preservation on the quality of *Radix et Rhizoma Salviae mitiorrhzae* medicinal herbs

Time storage	**Sample**		
	F7	**F8**	**F9**
0 months (initial)			
Total microorganisms (CFU/g)	$1.30.10^{4b}$	$9.40.10^{2a}$	$9.73.10^{2a}$
Moisture (%)	11.11a	11.13a	11.14a
Sensory assessment (score)	18.83a	19.20a	19.30a
3 months			
Total microorganisms (CFU/g)	$3.02.10^{4b}$	$9.87.10^{2a}$	$9.93.10^{2a}$
Moisture(%)	12.94b	11.30a	11.32a
Sensory assessment (score)	18.20a	19.00a	18.90a
6 months			
Total microorganism (CFU/g)	$4.03.10^{4b}$	$1.43.10^{3a}$	$1.22.10^{3a}$
Moisture (%)	13.23a	11.49b	11.38b
Sensory assessment (score)	17.00b	18.53a	18.37a
9 months			
Total microorganisms (CFU/g)	$2.43.10^{5b}$	$3.63.10^{3a}$	$3.34.10^{3a}$
Moisture (%)	13.62a	11.56b	11.49b
Sensory assessment (score)	15.90b	17.87a	17.93a

Note. In the same row, numbers with the same letter are not significantly different at the $\alpha = 0.05$.

During 9 months of storage, the total number of aerobic bacteria increased in the samples and there was a significant difference at the $\alpha = 0.05$ level between the experimental samples and the control samples. Specifically, the highest total number of aerobic bacteria in the control sample was $2.43.10^5$ CFU/g, and the lowest in the 200 mgHg and 400 mgHg experiments were $3.63.10^3$ and $3.34.10^3$ cfu/g, respectively. Standards according to Vietnam pharmacopoeia V total aerobic bacteria count $\leq 5.10^4$ (cfu/g). There were significant fluctuations during the storage of the active ingredient Tanshinon IIA. Specifically, after 9 months of storage, the active ingredient Tanshinon IIA decreased in all experimental samples and there was a significant difference at $\alpha = 0.05$ with the control formula and the decrease was lower than the Vietnam Pharmacopoeia V after 9 months of storage at ambient temperature (25-30 ^oC), humidity (80-85%). The results shown that storage in a low-pressure environment is effective in maintaining active medicinal ingredients. Zhang et al. (2017) reported that as moisture content decreased over the drying process, the contents of salvianolic acid B and total tanshinones changed (increased or decreased) and the shade-drying method might be the best drying method for preserving the main active ingredients.

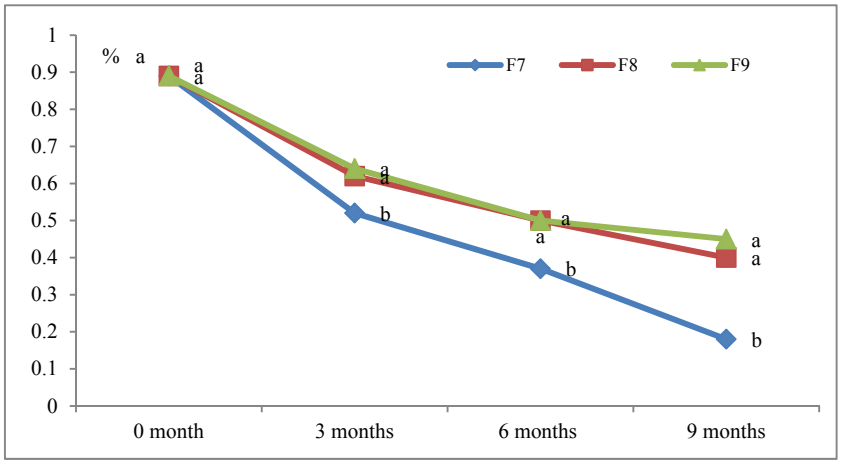


Figure 1. The influence of Tanshinon IIA (%) on the storage time of *Radix et Rhizoma Salviae mitiorrhzae*

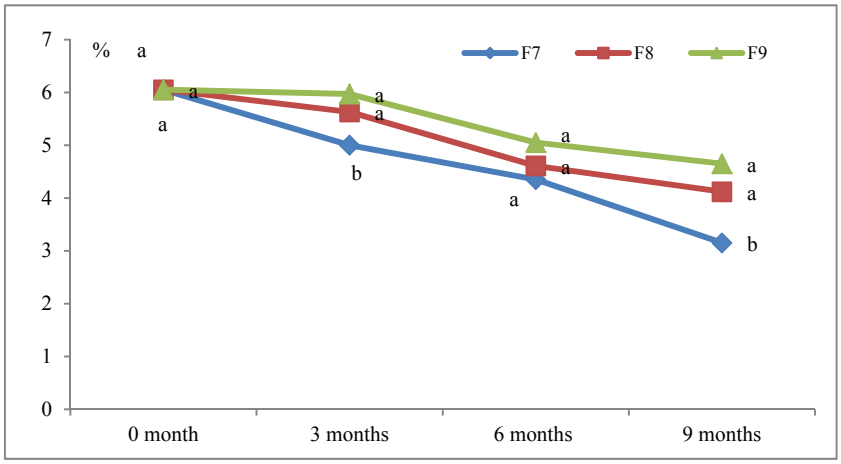


Figure 2. The influence of salvianolic acid B (%) on the storage time of *Radix et Rhizoma Salviae mitiorrhzae*

During storage, moisture in raw materials was one of the important factors affecting storage time. Results in Table 3 shown that after 9 months of storage, the tested sample had the highest moisture content of 16.51% compared to the initial 11.11%, while the tested humidity increased slowly, after 9 months of storage reaching 11.49%. This reflects one of the advantages of low pressure packaging which was to effectively control the moisture content of the product. The control sample at ambient temperature (25-30 ^{o}C), humidity (80-85%) packaged in 0.1 mm thick polyethylene (PE) packaging-poor water resistance so not suitable preservation long time after 9 months moisture higher than other sample.

The results were consistent with some studies showing that using pH 2.5-3 citric acid solution and low-pressure packaging effectively reduced the number of bacteria initially and during storage, the total number of bacteria reduced to 99.5 % and completely eliminates yeast and mold and did not affect the quality of *Radix Angelicae acutilobae* (Hang, 2022). However, Meena et al. (2017) shown that although low-pressure packaging can be used to extend shelf life and maintain product quality, spoilage due to aerobic microorganisms can still occur in packaged products, depending on the oxygen level in the space in the bag.

From the above results, it shown that preserving *Radix et Rhizoma Salviae mitiorrhzae* at a pressure of 400 mmHg provided good sensory quality, moisture, reduces the growth of microorganisms and prevents insect damage, maintaining active ingredients in medicine.



F1 **F2** **F3**

Figure 3. *Radix et Rhizoma Salviae mitiorrhzae* after preliminary process



F7 **F8** **F9**

Figure 4. *Radix et Rhizoma Salviae mitiorrhzae* after 9 months preservation

4. Conclusions

Radix et Rhizoma Salviae mitiorrhzae were used that pre-treatment of citric acid pH 2.5-3 for 30 minutes, is effective in limiting surface microorganisms, maintaining sensory quality and preserving the content of active ingredients in medicinal herbs. Then, the heat pump drier was a suitable method, ensuring the quality of *Radix et Rhizoma Salviae mitiorrhzae*. Low pressure preservation at 400 mmHg combined with 0.4 mm thick PVC film after 9 months of storage at ambient temperature (25-30 ^{o}C), humidity (80-85%) kept good medicinal quality, slow increased in moisture and total number of microorganisms, the content of Tanshinone IIA, Salvianolic acid kept after storage, still met Vietnam Pharmacopoeia V standards, compare with control sample high increased in moisture and total number of microorganisms, reduced medicinal quality, the content of Tanshinone IIA, Salvianolic acid un-maintained after storage 9 months.

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Data Sharing Statement

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