Optimization of Pretreatment Process for Northern Mugwort Leaf Volatile Oil and Research on Its Commercial Value and Application Scenarios

Xinyi An[1], Fei Meng[2], Likun Ren[1,*]

[1]*College of Food Science and Engineering, Bohai University, Jinzhou ,113001,China*
[2]*Liaoning Zhongke Zhongheng Testing Technology Co., Ltd., Shenyang,110170,China*
[*]*Corresponding author：likun931006@163.com*

Abstract: *To address the issues of low extraction yield and insufficient retention of active components in the volatile oil of Northern Artemisia argyi leaves, and to clarify the commercial suitability of the process, this study investigated the effects of six pretreatment methods on the volatile oil of Artemisia argyi leaves grown in Liaoning for 75 days. The results showed that freeze-drying (-50 ° C, 200 Pa, 12 h), shade-drying (5 ° C ± 2 ° C, 50% ± 5% humidity, 7 d), and bio-enzyme pretreatment (cellulase 0.4%, pH 4, 50 ° C, 4 h) were optimal, with a volatile oil extraction yield of 1.87% to 1.91% (nearly double that of fresh Artemisia argyi leaves), caryophyllene content of 2.89% to 3.05%, and phytol content of 8.51% to 8.95%, exhibiting excellent properties. In contrast, micro-thermal explosion, steam explosion, and acid-base pretreatment resulted in loss of active components and low commercial cost-effectiveness. Commercially, freeze-dried products are suitable for pharmaceutical-grade raw materials (800-1200 RMB/kg), shade-dried products for food preservation-grade raw materials (300-500 RMB/kg), and bio-enzyme pretreated products for cosmetic-grade raw materials (600-800 RMB/kg). This study provides support for the industrialization and high-value applications of volatile oil from Northern Artemisia argyi leaves.*

Keywords: *Northern Artemisia argyi leaves; volatile oil; pretreatment process; active components; commercial value; application scenarios*

Introduction

Volatile oil from Artemisia argyi Levl. et Vant. leaves contains active components such as caryophyllene (antibacterial) and phytol (antioxidant), leading to its wide application in pharmaceuticals, food preservation, and cosmetics[1]. Due to the climate characterized by short summers and significant diurnal temperature variations in northern China, the volatile oil composition of Northern Artemisia argyi leaves differs significantly from that of southern varieties. However, current research primarily focuses on optimizing technical parameters, often overlooking their commercial value and applicability in different scenarios. Traditional pretreatment methods, such as oven-drying, are time-consuming and result in substantial loss of active components, making it difficult for Northern Artemisia argyi volatile oil to meet commercial demands across various fields.

Pretreatment is a critical step that determines the extraction efficiency, quality, and commercial value of volatile oil. This study used Artemisia argyi leaves from Liaoning as raw material to compare the technical effects of six pretreatment methods. By integrating market demands, the commercial positioning of each process was clarified. The aim is to provide a basis for translating laboratory research on Northern Artemisia argyi volatile oil into industrial commercialization and to promote the high-value utilization of regional Artemisia argyi resources.

1. Experimental Section

1.1 Reagents and Instruments

Raw Material: Artemisia argyi leaves were collected from Fushun City, Liaoning Province. They were identified as Artemisia argyi Levl. et Vant. of the genus Artemisia in the Asteraceae family. Fresh leaves grown for 75 days (the period of higher oil content) were selected and processed on the day of collection.

Reagents: Cellulase, absolute ethanol, dichloromethane (chromatographically pure), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), all of analytical grade; nutrient broth medium and potato dextrose agar medium were purchased from Beijing Baocang Biotechnology Co., Ltd.

Instruments: Freeze Dryer (Model: SCIENTZ-10N, Ningbo Scientz Biotechnology Co., Ltd.), Micro-Thermal Explosion Pretreatment Device, Steam Explosion Device (custom-made for the laboratory), Rotary Evaporator (Model: RE-52AA, Shanghai Yarong Biochemical Instrument Factory), Gas Chromatograph-Mass Spectrometer (Model: Agilent 5975C, Agilent Technologies Inc.), Visible Spectrophotometer (Model: 722S, Shanghai Lengguang Technology Co., Ltd.).

1.2 Experimental Methods

1.2.1 Pretreatment Method Design

Eight treatment groups (including one control group) were set up, with each experiment conducted in triplicate; the results are presented as the mean value:

(1) Fresh Artemisia argyi leaves (Control): Fresh leaves were chopped and extracted directly without any pretreatment.

(2) Shade-drying treatment: 10 g of fresh Artemisia argyi leaves were placed in a ventilated, shaded area (temperature 5°C ± 2°C, humidity 50% ± 5%) and left to air-dry completely (approximately 7 days, moisture content < 10%). The dried leaves were then crushed and passed through a 40-mesh sieve for later use[2].

(3) Freeze-drying treatment: 10 g of fresh Artemisia argyi leaves were placed in a freeze dryer and lyophilized at -50°C and 200 Pa for 12 hours until completely dehydrated (moisture content < 5%). The dried material was then crushed and passed through a 40-mesh sieve for later use.

(4) Micro-thermal explosion pretreatment: After washing and air-drying, fresh Artemisia argyi leaves were evenly spread in the micro-thermal explosion device. SO_3 gas (generated from the reaction of P_2O_5 and concentrated H_2SO_4) was introduced, and the leaves were treated at 45°C under atmospheric pressure for 30 minutes. After treatment, the leaves were taken out, crushed, and passed through a 40-mesh sieve for later use.

(5) Steam explosion pretreatment: After washing and air-drying, fresh Artemisia argyi leaves were placed in the steam explosion device. The vessel was sealed, and high-pressure steam was introduced until the pressure reached 0.4 MPa. The pressure was maintained for 40 seconds before being rapidly released to complete the explosion. The treated material was then crushed and passed through a 40-mesh sieve for later use[3].

(6) Acid pretreatment: Fresh Artemisia argyi leaves were soaked in a 2.0% (v/v) H_2SO_4 solution for 30 minutes, with stirring every 10 minutes during the soaking period. After soaking, the leaves were rinsed with deionized water until neutral pH was achieved, then air-dried, crushed, and passed through a 40-mesh sieve for later use.

(7) Alkali pretreatment: Fresh Artemisia argyi leaves were soaked in a 2.0% (v/v) NaOH solution for 30 minutes, following the same procedure as the acid pretreatment. After rinsing to neutral pH, the leaves were air-dried, crushed, and prepared for later use.

(8) Bio-enzyme pretreatment: Fresh Artemisia argyi leaves were submerged in deionized water. Cellulase was added at 0.4% (based on the dry weight of the leaves), the pH was adjusted to 4, and the mixture was placed in a constant temperature water bath at 50°C for enzymatic hydrolysis for 4 hours. After hydrolysis, the mixture was immediately heated to 80°C to deactivate the enzyme for 10 minutes. The treated material was then air-dried, crushed, and passed through a 40-mesh sieve for later use[4].

1.2.2 Volatile Oil Extraction Method

The volatile oil from all pretreatment groups was extracted using the reduced-pressure steam distillation method. The specific steps are as follows: 1000 g of the pretreated Artemisia argyi leaves were weighed and placed into a 1 L round-bottom flask; 800 mL of deionized water was added. A vertical condenser was connected, and a vacuum pump was activated (vacuum degree: 0.08 MPa). The mixture was distilled for 3 hours, and the distillate was collected. The distillate was extracted three times with an equal volume of dichloromethane; the extracts were combined, and anhydrous sodium sulfate was added for drying over 24 hours. After filtration, the dichloromethane was recovered using a rotary evaporator (50° C, 0.08 MPa) to obtain the volatile oil from the Artemisia argyi leaves[5].

The volatile oil extraction yield was calculated using the following formula:

$$\text{Extraction Rate}=\frac{\text{Volume of Volatile Oil from Artemisia Leaves (mL)}}{\text{Mass of Artemisia Leaves}}\times 100\%$$

1.2.3 Chemical Composition Analysis

The extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The primary components and their contents in the Artemisia argyi leaf volatile oil were determined by referencing the qualitative data from GC-MS analysis and the industry standard GB/T 22179-2008.

GC Conditions: The initial oven temperature was set at 40° C and held for 3 minutes; it was then raised to 280° C at a rate of 4° C per minute and maintained for 10 minutes. The carrier gas (He) flow rate was constant at 1.0 mL/min, and the injection port temperature was set at 250° C. For solid-phase microextraction fibers, desorption was conducted at 250° C for 3 minutes prior to injection; liquid samples were injected directly.

MS Conditions: The mass spectrometric analysis utilized an EI ion source. The interface temperature was 280° C, and the ion source temperature was 200° C. The emission current was 34.6 μA, the electron energy was 70 eV, and the detector voltage was 1000 V. The scan mass range was m/z 35-500.

2. Results and Discussion

2.1 Effects of Different Pretreatments on the Volatile Oil of Artemisia argyi Leaves

2.1.1 Effect on Extraction Yield

Figure 1 shows that the pretreatment method significantly affected the extraction yield. Freeze-drying, shade-drying, and bio-enzyme pretreatment yielded the highest extraction rates (1.87% – 1.91%), representing an increase of 94% – 110% compared with fresh leaves, with no significant differences observed among these three methods. Micro-thermal explosion (1.60%) and acid-base pretreatment (1.45% – 1.50%) resulted in moderate extraction yields. Steam explosion (1.40%) and fresh leaves (0.91%) yielded the lowest extraction rates.

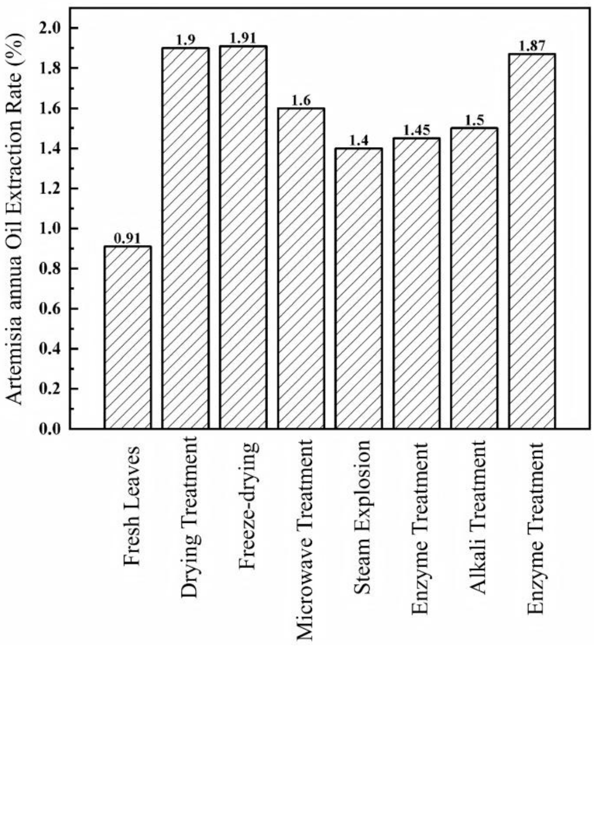

Fig 1 effect of different pretreatment methods on the extraction rate of Artemisia vulgaris essential oil

2.1.2 Effect on Properties

Table 1 effects of different pretreatment methods on the properties of Artemisia vulgaris volatile oil

=No.	Pretreatment Method	Color	Odor	Transparency	Yield (%)
1	Drying Treatment	Dark Green	Rich Aroma	Transparent	1.90
2	Freeze-drying	Dark Green	Rich Fresh Aroma	Transparent	1.91
3	Microwave Treatment	Green	Fresh Aroma	Transparent	1.60
4	Steam Explosion	Green	Light Aroma	Transparent	1.40
5	Enzyme Treatment	Light Green	Fresh Aroma	Transparent	1.45
6	Alkali Treatment	Green	Fresh Aroma	Transparent	1.50
7	Enzyme Treatment	Green	Rich Fresh Aroma	Transparent	1.87

As shown in Table 1, the pretreatment methods directly affect the appearance and odor of the volatile oil:

Color: The volatile oils from freeze-drying, shade-drying, and bio-enzyme pretreatment exhibit dark green or green hues with uniform coloration; whereas those from micro-thermal explosion, steam explosion, and acid-base pretreatment appear light green, which is presumably due to chlorophyll degradation under high temperatures or chemical oxidation.

Odor: The volatile oils from freeze-drying and bio-enzyme pretreatment possess a "rich and fresh fragrance," while shade-drying yields a "rich aroma." The remaining pretreatment groups produce oils with a "fresh fragrance" or "light fragrance."

Transparency: All groups demonstrate clear transparency without introduction of impurities, indicating that the pretreatment methods do not compromise the purity of the volatile oil.

In summary, the pretreatment methods significantly influence both the color and odor of the Artemisia argyi volatile oil, which may further affect its extraction efficiency and quality. Therefore, when selecting a pretreatment method for Artemisia argyi leaves, its impact on the properties of the volatile oil should be thoroughly considered to optimize both the extraction process and the final product quality.

2.1.3 Effect on Composition

This experiment conducted qualitative and quantitative analysis of the volatile oil from Artemisia argyi leaves using GC-MS. The contents of caryophyllene and phytol were determined, and the results are shown in Table 2.

Table 2 effects of different pretreatment methods on the Components of Volatile Oil from Artemisia vulgaris

Pretreatment Method	Caryophyllene	Phytol
Fresh Leaves	1.47±0.05	5.32±0.12
Drying Treatment	2.89±0.08	8.51±0.15
Freeze-drying	3.05±0.06	8.72±0.13
Microwave Treatment	2.33±0.07	6.89±0.11
Steam Explosion	2.15±0.06	6.53±0.14
Enzyme Treatment	2.03±0.05	6.32±0.12

Pretreatment Method	Caryophyllene	Phytol
Alkali Treatment	2.11±0.04	6.78±0.10
Enzyme Treatment	3.02±0.07	8.95±0.16

As shown in Table 2, the treatment methods (freeze-drying, shade-drying, and bio-enzyme pretreatment) effectively preserved the active components:

Caryophyllene (core antibacterial component): Freeze-drying yielded the highest content (3.05%), followed by bio-enzyme pretreatment (3.02%) and shade-drying (2.89%). These levels represent an increase of 96% to 108% compared to fresh Artemisia argyi leaves (1.47%). In contrast, steam explosion (2.15%) and acid-base pretreatment (2.03%–2.11%) showed significantly lower contents.

Phytol (core antioxidant component): Bio-enzyme pretreatment resulted in the highest content (8.95%), followed by freeze-drying (8.72%) and shade-drying (8.51%). These values represent an increase of 60% to 68% compared to fresh leaves (5.32%). Acid pretreatment yielded the lowest content (6.32%), which is attributed to oxidative degradation.

2.2 Commercial Value and Application Scenarios of Different Pretreatment Processes

2.2.1 Freeze-drying Pretreatment: High-value Pharmaceutical-grade Raw Material

Freeze-dried products demonstrate the highest retention rate of active components (caryophyllene 3.05%, phytol 8.72%) and exhibit excellent antibacterial and anti-inflammatory properties. These characteristics fully meet the stringent requirements of the pharmaceutical industry for raw material purity and efficacy, making them suitable as core ingredients for antibacterial ointments and respiratory anti-inflammatory preparations. The current terminal price of pharmaceutical-grade Artemisia argyi volatile oil reaches 800-1200 RMB/kg. Furthermore, pharmaceutical enterprises maintain stable demand for highly active raw materials. Although the initial investment in freeze-drying equipment is relatively high, the high added value enables cost recovery within 1.5-2 years, with a gross profit margin exceeding 60%[6].

2.2.2 Shade-drying Treatment: Low-cost Food Preservation-grade Raw Material

The shade-dried product achieves an extraction yield of 1.90% and requires no complex equipment. Its unit pretreatment cost is only 0.8 RMB/kg of leaves, which is significantly lower than other processes. With its natural antibacterial properties (producing an inhibition zone diameter exceeding 25 mm against Aspergillus niger), it can replace chemical preservatives such as sodium benzoate. It is suitable for preserving baked goods, nuts, fruits, and vegetables. The terminal price ranges from 300 to 500 RMB/kg, meeting the bulk procurement needs of small and medium-sized food enterprises. Furthermore, Artemisia argyi-flavored preservative additives (e.g., preservatives for Artemisia argyi-flavored mooncakes) can be developed, thereby enhancing product added value and expanding food application scenarios.

2.2.3 Bio-enzyme Pretreatment: Green Cosmetic-grade Raw Material

The bio-enzyme pretreated product contains no residual chemical reagents throughout the entire process. Its environmentally friendly attributes align with the current "natural and organic" trend in cosmetics. Furthermore, it possesses the highest phytol content (8.95%) and demonstrates excellent antioxidant activity (DPPH free radical scavenging IC_{50} = 1.21 mg/mL). It can serve as an antioxidant in skincare products (delaying skin aging) and as a fragrance component in aromatherapy products. The current terminal price of cosmetic-grade Artemisia argyi volatile oil ranges from 600 to 800 RMB/kg. It can supply mid-to-high-end cosmetic brands (e.g., developed into Artemisia argyi essential oil face masks or aromatherapy candles). Moreover, stability can be enhanced through microencapsulation technology, extending its application to personal care products and expanding market capacity[7].

2.2.4 Inefficient Processes: Low-value Auxiliary Applications

The volatile oils obtained from micro-thermal explosion, steam explosion, and acid-base pretreatment exhibit significant losses of active components and inferior quality. Consequently, they are only suitable for developing low-cost products, such as botanical pesticides (repelling aphids and spider mites) and household cleaners (inhibiting E. coli). The terminal price ranges from 150 to 200 RMB/kg, primarily meeting the demands of the agricultural market and general household cleaning needs. With low commercial added value, these processes serve only as supplementary approaches for the resource utilization of raw materials.

3. Summary and Discussion

The optimal pretreatment processes for volatile oil from Northern Artemisia argyi leaves are freeze-drying (-50°C, 200 Pa, 12 h), shade-drying (5°C ± 2°C, 50% ± 5% humidity, 7 d), and bio-enzyme pretreatment (cellulase 0.4%, pH 4, 50°C, 4 h). These methods achieve an extraction yield of 1.87%–1.91%, with high retention of active components and excellent properties of the volatile oil.

The commercial value varies significantly among different processes: freeze-dried products are suitable for high-value pharmaceutical-grade scenarios, shade-dried products for low-cost food preservation applications, and bio-enzyme pretreated products for green cosmetic applications. Inefficient processes are only appropriate for low-value auxiliary applications.

This study clarifies the alignment logic between pretreatment processes and commercial application scenarios. It provides technical support for the industrial production and high-value commercial implementation of volatile oil from Northern Artemisia argyi leaves, promoting the upgrade of regional Artemisia argyi resources from primary processing to specialized, high-value applications.

References

[1] JIAN Li-na, SONG Xue-li, GUO Jiang-tao, et al. Chemical Composition and Clinical Application of Artemisia argyi . Chemical Engineer, 2021, 35(7): 58-62.

[2] REN Ling-li, LI Hao, QIAN Xue, et al. Chemical Composition of Volatile Oil from Fresh and Aged Artemisia argyi Leaves . Chinese Wild Plant Resources, 2022, 41(11): 39-42, 55.

[3] GUI Chao, ZUO Wen, CHEN Yu-bao, et al. Research Progress on Steam Explosion Pretreatment of Solanaceous Plants . Agriculture and Technology, 2023, 43(11): 27-30.

[4] HUANG Yu-mei, GUO Jian-ye, YUAN Yu-feng, et al. Semi-bionic Enzymatic Extraction of Volatile Oil from Artemisia argyi Leaves and Preparation of Microcapsules . Journal of Nanchang University (Engineering & Technology), 2022, 44(2): 140-145.

[5] SUN Wu-qian, ZHANG Xiu-fang, XIN Xiao-dong, et al. Comparative Study on Steam Distillation and Extraction-Azeotropic Distillation Coupling Technology for Extracting Volatile Oil from Artemisia argyi Leaves . Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai, 2022, 36(1): 27-32.

[6] HONG Zong-guo, YANG Guang-zhong, MEI Zhi-nan, et al. Study on Active Substance Basis and Application Development of Qiai and Moxibustion [R]. South-Central Minzu University, 2012.

[7] QI Qiao-ming, LONG Xu, LUO Feng, et al. Optimization of Extraction Process and Antioxidant Activity of Volatile Oil from Artemisia argyi Leaves by Response Surface Methodology . Chemistry and Bioengineering, 2020, 37(5): 22-26.