HEAT-INDUCED DEGRADATION OF MAGNOLOL AND HONOKIOL IN SUPERCRITICAL FLUID CO_2 EXTRACTION OF CORTEX *MAGNOLIA OFFICINALIS* (HOUPO)

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ABSTRACT: **AIM** To elucidate the factors that affect the stability of magnolol and honokiol in Cortex *Magnolia officinalis* (Houpo) in the process of extraction . **METHODS** The contents of magnolol and honokiol in Houpo SFE-CO_2 extract , treated on different conditions , were determined by HPLC . The shelf lives of magnolol and honokiol were also estimated . **RESULTS** The shelf lives of magnolol and honokiol were found to be 387 days and 476 days respectively at 25 ℃ . **CONCLUSION** Temperature is the main factor that affects the stability of magnolol and honokiol . The degradation is in accord with the first order rate of reaction . High temperature should be avoided during the extraction , figuration and storage of the products .

KEY WORDS: *Magnolia officinalis* ; magnolol ; honokiol ; SFE-CO_2 ; stability

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Cortex *Magnolia officinalis* (Houpo , 厚朴) is derived from the dried stalk bark , root bark and branch bark of *Magnolia officinalis* Rehd . *et* Wils . , *Magnolia officinalis* var . *biloba* Rehd . *et* Wils . and *Magnolia obovata* Thunberg . Houpo has been used in traditional Chinese medicine for more than 2 000 years for promoting the circulation of *Qi* (indigestion and stagnation of vital energy) , removing dampness , eliminating food stagnancy and relieving asthma[1] .

Many patent medicines containing extracts of Houpo have been developed for stomach coordination and intestinal disorder , such as Huoxiang Zhengqi pill (藿香正气丸) and Baoji pill (保济丸) . Various ingredients have been isolated from Houpo and identified . They are α-eudesmol , α- and β-pinenes and bornyl acetate as essential oils ; magnolol and honokiol as diphenyl compounds ; and magnocurarine and magnoflorine as alkaloids[2] . Houpo contains a high concentration of magnolol and honokiol , normally ranging from 3 % to 7 %[2] . Chinese Pharmacopoeia has specified that Cortex *Magnolia officinalis* contains not less than 3 % of magnolol and honokiol[1] . Recent studies show that magnolol and honokiol have a broad spectrum of anti-bacteria[3] and anti-fungi activities[4] , relaxing striated muscles as a cholesterol acetyltransferase inhibitor[5] , anti-arrhythmic effect[6,7] , anxiolytic agent effect[8,9] , and anti-oxidativing[10,11] . Several methods have been developed to extract and quantify magnolol and honokiol in raw material as well as in its derived patent medicines[1,2] . However , the extraction efficiency of magnolol and honokiol from Houpo was not stable[12] , and in most cases , exposure to high temperature can not be avoided .

Unfortunately , the rate of degradation of magnolol and honokiol has never been a concern . The supercritical fluid extraction-carbon dioxide (SFE-CO_2) method provides an effective method for the extraction of magnolol and honokiol[12~16] . Here , about 74 % of enrichment of magnolol and honokiol was achieved from Cortex *Magnolia officinalis* by SFE-CO_2 method as determined by high performance liquid chromatography (HPLC) . In addition , the chemical stability of magnolol and honokiol was calibrated , and high temperature was shown to be a critical factor for the degradation of the diphenyl compounds .

MATERIALS AND METHODS

Acquisition of plant materials The bark of *M. officinalis* var . *biloba* was collected from Ninggang

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County , Jiangxi Province , China , during August to September , 1999 . The corresponding voucher specimens were deposited in the Herbarium of Guangzhou University of Traditional Chinese Medicine , Guangzhou , China . The materials for extraction were finely powdered and kept dry before being used .

Supercritical fluid carbon dioxide extraction In the optimization of the condition for extraction , SFE-CO_2 was performed in HA-9508 SFE-CO_2 apparatus (Hua-An SFE-CO_2 Apparatus Co . , Jiangsu Province , China) by using 60 mesh powder of Houpo . The flow rate of liquid CO_2 was 96 liter in an hour . The pressure and temperature during extraction were at 22 MPa and 35 ℃ respectively , while the resolution was at 7.0 MPa , and 40 ℃ .

HPLC quantitative analysis Reference standards of magnolol and honokiol were purchased from the National Institute for the Control of Pharmaceutical & Biological Products , Beijing . Analytical reagent and HPLC grade reagents were purchased from Sigma (St . Louis , MO , USA) . Double distilled water was used . The reference standards were weighed and dissolved in 1 mL methanol to give serial concentrations . The standard curves were calibrated by using the linear least-squares regression equation derived from the peak area . The concentrations of these compounds in the samples were calculated according to the regression parameters derived from standard curves . HPLC was performed with a NOVA-PAK C_{18} column with particle size of 4 μm , 3.9 mm × 300 mm , Waters™ PC 800 Integrator , Waters™ 486 tunable absorbance detector and Waters™ 600 Pump . The mobile phase was a step gradient of acetonitrile and 0.1 % acetic acid , which was 40 % from 1 to 19 minute , 60 % from 20 to 40 minute and 100 % from 41 to 80 minute . The flow rate was 1.0 mL·min^{-1} at 20 ℃ and the detection wavelength was 294 nm .

Analysis of degradation For degradation determination , 621 mg of SFE-CO_2 extract from Houpo was dissolved in 50 mL ethyl acetate . One hundred μL ethyl acetate extract was evaporated to dryness in 2 mL glass vials at 83 ℃ dry-bath (Type 17600 , Thermolyne , USA) in open or closed vial for 12 hours with no solvent , or 1 mL of water , or 1 mL of ethanol . The samples with solvent (water or ethanol) were dried for 30 minutes . For the variation of temperature , 100 μL of the ethyl acetate extract was evaporated to dryness in 2 mL glass vials at 83 ℃ , or 93 ℃ , or 103 ℃ in open vial . For determination of kinetics , the time course at different temperature was studied . One hundred μL of ethyl acetate extract solution was evaporated to dryness in 2 mL glass vials separately at 83 ℃ , or 93 ℃ , or 103 ℃ in open vials for different time intervals (1 hour for 103 ℃ , 3 hours for 93 ℃ and 5 hours for 83 ℃) . For all determinations , the residues were dissolved in 1 mL of acetonitrile and filtered through a syringe filter unit (0.2 μm , Millipore , USA) before loading to HPLC column . The amount of magnolol and honokiol were used as an index of kinetics . The time course at different temperatures was analyzed by first order reaction rate equation , and the shelf life at room temperature (20 ℃ , 25 ℃ and 30 ℃) was calculated by Arrhenius equation .

RESULTS AND DISCUSSION

1 Extraction of magnolol and honokiol

The optimized condition for SFE-CO_2 extraction was at 22 MPa , 35 ℃ and resolution at 7.0 MPa , 40 ℃ for 3 hours . Under the optimized condition , (63.3 ± 1.7) g·kg^{-1} of total extract , (20.4 ± 1.4) g·kg^{-1} of magnolol and (27 ± 4) g·kg^{-1} of honokiol were isolated from the crude drug , where $n = 8$. During the extraction , about 74 % enrichment of magnolol and honokiol was achieved . These materials were used for degradation analysis .

In the calibration curves performed with HPLC , magnolol and honokiol exhibited good linearity in the range of 100 ~ 750 μg·mL^{-1} . The repeatability was good , and the RSD was within the range from 2 % to 3 % . The recoveries of magnolol and honokiol were 97 % and 98 % respectively . Figure 1 shows typical HPLC profiles of SFE-CO_2 extracts which contain primarily magnolol and honokiol .

2 Degradation of magnolol and honokiol

The degradation of diphenyl compounds is a major problem in the process of extraction , which reduces its pharmacological activities . The degradation is mainly due to the oxidation of magnolol and honokiol . Figure 2A shows the color change of the extracts from yellowish to dark after degradation under high temperature . Except the color change , the HPLC peak areas corresponding to magnolol and honokiol were also significantly reduced after degradation (Figure 2B) . Unfortunately , the degradation of the products was not revealed in the HPLC profiles . Different parameters usually encountered in the process of classical extraction and purification were tested to determine the degradation on magnolol and honokiol .

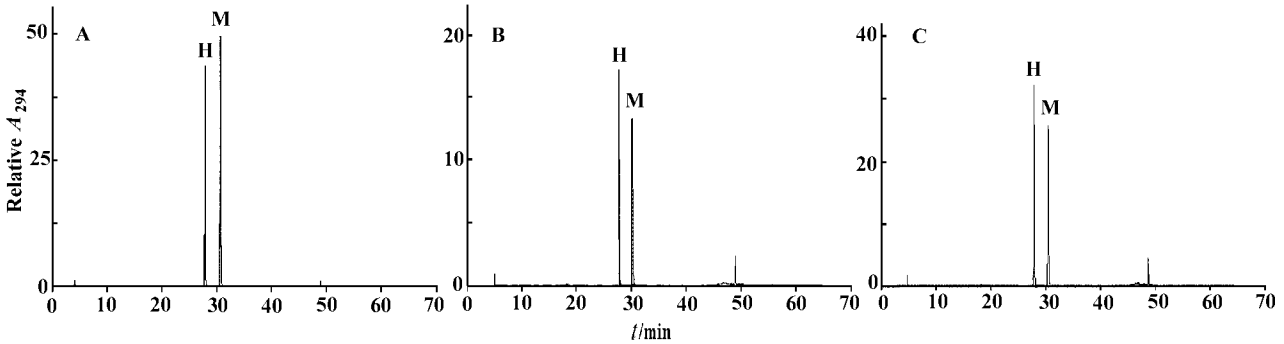

Figure 1 HPLC chromatograms of SFE-CO_2 extracts from Houpo

Magnolol (M) and honokiol (H) migrated as distinct peaks in (A) 3 μg each of standards , (B) 5 μg of SFE-CO_2 extract , and (C) 5 μg of SFE-CO_2 extract spiked with 1 . 5 μg each of standards . The X-axis is the migration time in minutes

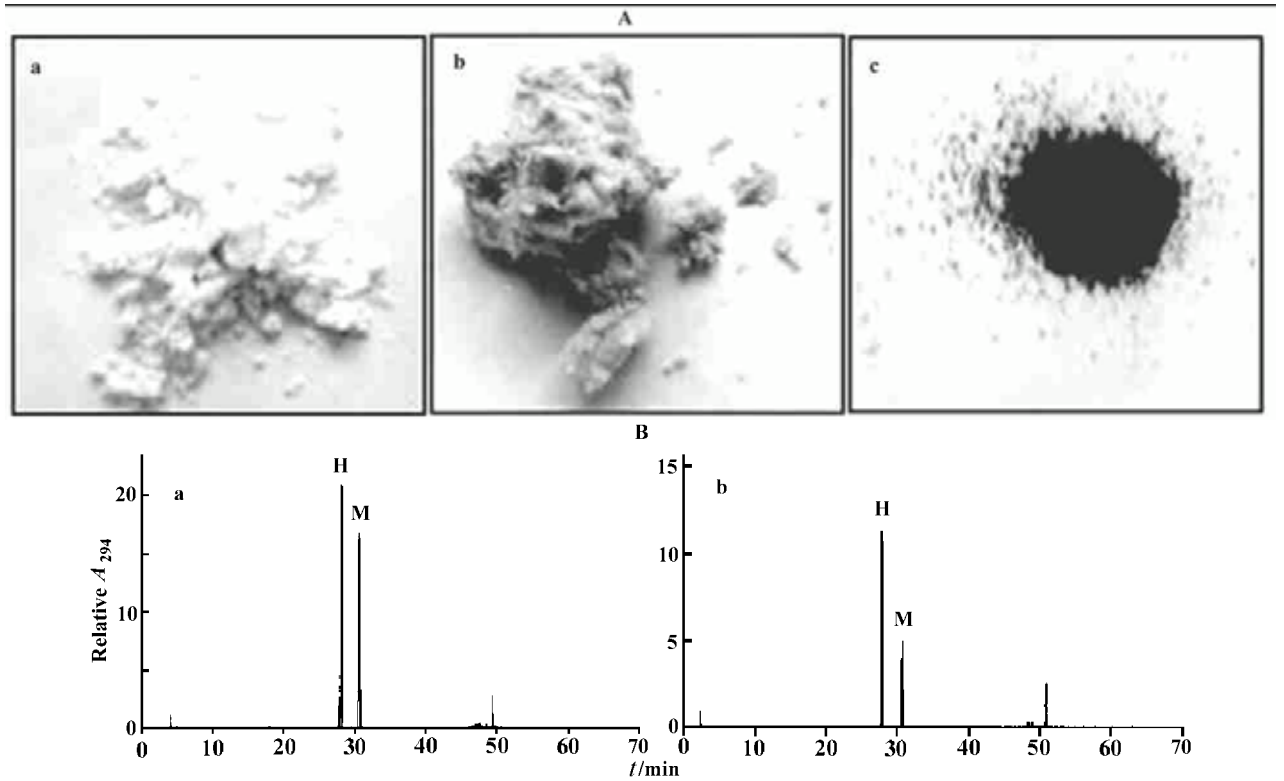

Figure 2 Color appearance at different stages of extraction from Houpo

A: Different stages of SFE-CO_2 extraction show variation of color (a) fresh residue after SFE-CO_2 extraction , (b) SFE-CO_2 extract after a year of storage at room temperature and (c) degraded SFE-CO_2 extract after 100 ℃ for 24 hours . B: HPLC chromatograms of degraded SFE-CO_2 extract (a) before heating and (b) after heating at 100 ℃ for 24 hours . H: Honokiol ; M: Magnolol

The degradation rate was significantly reduced at low temperature. The degradation rate of magnolol and honokiol was temperature dependent (Figure 3A) . Higher temperature such as 103 ℃ showed over 60 % increase of degradation comparing to 40 ℃ . In addition , the heat-induced degradation was increased significantly in the first hour , and thereafter , the degradation reached a plateau (Figure 3B) . Both magnolol and honokiol showed a very similar profile of degradation according to temperature and time . The other physical parameters such as different solvents , opening and closing of the vial , did not show strong induction effects (Figure 3C) .

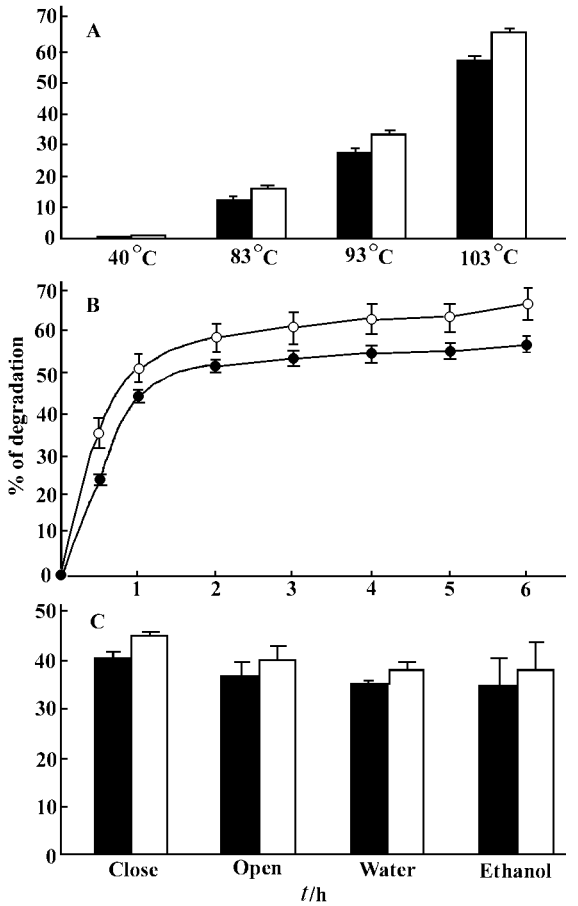

Figure 3 Degradation of magnolol and honokiol at different conditions

A: The SFE-CO_2 extract was exposed to different temperature for 6 hours; B: The SFE-CO_2 extract was exposed to 103 ℃ at different times; C: The SFE-CO_2 extract was exposed to closed environment, open air, water and ethanol for 12 hours at 83 ℃. Values are expressed in $\bar{x} \pm s$, $n = 4$. 1.2 mg of SFE-CO_2 extract was used to test the degradation of magnolol and honokiol. ■ Honokiol; □ Magnolol; ○—○ Magnolol; •—• Honokiol

Figure 4 shows that the degradation kinetics of magnolol and honokiol is in accord with the first order rate reaction, which markedly depends on temperature. The shelf life (90 % of ingredients remain active, $t_{0.9}$) of magnolol and honokiol at high temperature, such as 83 ~ 103 ℃, was only from 0.4 ~ 6.5 hours (Figure 4 and Table 1), which could hardly be accepted by the industry. The shelf life at room temperature was calculated by Arrhenius equation (Figure 4). The results of chemical kinetic parameters at room temperature were calculated (Table 1), which showed the shelf life ($t_{0.9}$) of honokiol and magnolol at various temperatures. For example, the shelf life ($t_{0.9}$) at 25 ℃ was 476 days for honokiol and 387 days for magnolol while the shelf life ($t_{0.9}$) at 30 ℃ was 218 days for honokiol and 177 days for magnolol.

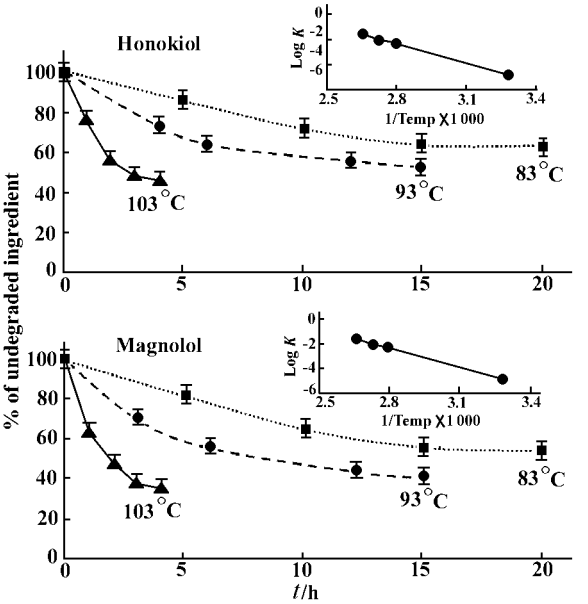

Figure 4 The temperature-induced degradation of magnolol and honokiol is in accord with first order rate reaction

The amount of undegraded magnolol and honokiol was determined by HPLC analysis before and after the induction effects. Three different high temperatures, 83 ℃, 93 ℃ and 103 ℃ were indicated. The inserts show a plot of log K (the first order rate constant) against 1/temperature, which are derived from degradation curves of honokiol and magnolol by Arrhenius equation

Table 1 Calculated degradation parameter of magnolol and honokiol in SFE-CO_2 extract

Temperature	Magnolol			Honokiol		
/ ℃	K^*	$t_{0.9}$ **	$t_{0.5}$ ***	K^*	$t_{0.9}$ **	$t_{0.5}$ ***
83	0.0169	6.25 h	41.10 h	0.0162	6.50 h	42.737 h
93	0.0506	2.08 h	13.70 h	0.0411	2.56 h	16.86 h
103	0.2470	0.43 h	2.81 h	0.2075	0.51 h	3.34 h
20	5.07×10^{-6}	865.7 d	5692.1 d	4.11×10^{-6}	1067.7 d	7020.2 d
25	1.14×10^{-5}	386.7 d	2542.5 d	9.22×10^{-6}	476.1 d	3130.1 d
30	2.48×10^{-5}	177.4 d	1166.3 d	2.01×10^{-5}	218.0 d	1433.5 d

* K: The first-order rate constant; ** $t_{0.9}$: The shelf life, remaining 90 % honokiol or magnolol; *** $t_{0.5}$: The half life, remaining 50 % honokiol or magnolol

3 The advantage of SFE-CO_2 extract

Houpo is an important traditional Chinese medicine, which is commonly used today either in herbal medicine or as a major constituent in patent medicine. On the market, the pharmacological efficacy of Houpo depends on several parameters. (1) The content of magnolol and

honokiol is frequently used as a marker for quality control. *Magnolia officinalis* and *M. officinalis* var. *biloba* in China are the main sources of Cortex *Magnolia officinalis* (Houpo) which may contain magnolol and honokiol up to 7% by weight. In general, many extraction methods can achieve good yield of diphenyl compounds. (2) The extraction method is also a crucial factor for consideration. Although water, alcohol and alkali water have been used for the extraction of magnolol and honokiol, none of them is comparable to the extraction by SFE-CO_2 method that provides about 74% enrichment of magnolol and honokiol as described here. (3) High temperature should be avoided in the process of extraction and storage. The traditional liquid extraction always requires heat treatment such as solvent evaporation and sample drying. For example, the hydro-alcohol extraction requires high temperature up to 100 ℃ for 10 ~ 30 hours in industry, which definitely degraded most of the active magnolol and honokiol. SFE-CO_2 extraction can be performed at relatively-low temperature and shorter time. This is important for the extraction of heat sensitive chemicals such as magnolol and honokiol. Moreover, the time required for liquid extraction is normally more than 60 hours that is extremely long as compared to the 3-hour extraction by SFE-CO_2 method.

Although magnolol and honokiol are the predominant components (over 74% by weight) in the SFE-CO_2 extract, there are still at least 13 other components in the extract as determined by GC-MS analysis[13]. These components are mainly volatile oils such as α-eudesmol, and alkaloids, which are part of the active ingredients of Houpo[12]. Their extraction efficiency has to be considered too. Indeed, the extraction efficiency of these volatile oils is being calibrated in our laboratory.

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高温致厚朴 SFE-CO_2 萃取物中厚朴酚、和厚朴酚降解

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摘要 : 目的　了解影响厚朴中有效成分厚朴酚、和厚朴酚的不稳定因素。方法　用 HPLC 法测定在不同实验条件下 ,厚朴 SFE-CO_2 萃取物中厚朴酚、和厚朴酚的含量 ;用加速实验测定有效期。结果　厚朴酚、和厚朴酚的有效期(25 ℃) 分别为 387 d 和 476 d。结论　温度是影响厚朴酚、和厚朴酚有效期的最主要因素 , 降解符合一级速度反应。

关键词 : 厚朴 ; 厚朴酚 ; 和厚朴酚 ; SFE-CO_2 ; 稳定性