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# The Effects of Chinese Traditional Processing Method on Components in Semen Sinapis Albae

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**Abstract:** Techniques of infrared (IR) spectrophotometry, High Performance Liquid Chromatography (HPLC) and HPLC-mass spectrometry are employed in this study to reveal the changes of the components in Semen sinapis Albae during the Chinese traditional processing. While the degradation of proteins and polysaccarides can be observed, sinapine in this drug can be known to decompose immediately after 15 mins during processing. A new substance, which is produced, has been studied and identified as p-hydroxybenzoic acid.

**Key words:** Semen sinapis albae, processing mechanism, IR, HPLC, determination

## INTRODUCTION

Recently, great importance has been attached to the side effect of Chinese herbal medicine on clinical application. Usually, there are three reasons to cause the side effect. One is the existence of noxious substance in some crude drugs. For example, aristolochic acids, which are often contained in plants from Aristolochiae species[1,2,3,4], can cause Chinese herbs nephropathy (CHN). Safrole in Herba Asari has strong carcinogenesis and teratogenesis[5,6,7]. Another is the wrong dispensing in the use of some drugs, which are specified as incompatibility by doctors of traditional Chinese medicine, just as "Antagonism in the eighteen medicinal herbs" and "incompatibility in the nineteen medicinal herbs"[8,9]. Finally, wrong processing methods of crude drugs will also result in the increase of toxic substance and the decrease of active substance[10,11]. Therefore, the understanding of the components` change in processing will be beneficial to the decrease of side effect of Chinese herbal medicine.

Semen sinapis Albae (White musturd seed) is the seed of Sinapis alba *L.*, a cruciferous plant. The components in this drug have been reported as sinalbin, myrosinase, sinapine, sinapic acid and so on, which have wide biological activities[12,13,14,15]. It has been used as a drug since the ancient year in China. It is often used to treat the articulation and neuropathic pain, especially the chronic bronchitis desease. Because of the strong stimulus on skin and mucosa, it will cause edema and blistering on the skin and make the patiens feel pain and vomit if the crude drug is taken orally. So the Pharmacopoeia of the People's Republic of China has specified that Semen sinapis Albae should be processed by traditional stir-baked method before use[18].

We have analyzed the changes of the components in this drug during its processing by using the techniques of Infrared Spectrophotometry (IR), High Performance Liquid Chromatography (HPLC) and HPLC-mass spectrometry. The study will also be beneficial to the safe use of this drug.

## MATERIALS AND METHODS

**Apparatus and chemicals:** The HPLC and

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HPLC-mass system utilized in this work was Agilent 1100 series HPLC and LC/MSD Trap (Agilent, USA). Data analysis was carried out by Agilent ChemStation. The IR spectrum was performed with Perkin Elmer Spectrum GX FT-infrared spectrometer detector and DTGS detector (USA). Data analysis was carried out by the 2D-correlation IR analysis soft researched by Tsinghua University.

$KH_2PO_4$ and HAC (both in Analytical Grade) were purchased from Shanghai No.2 Chemical Reagent Factory and Nanjing Chemical Reagent Factory respectively. Methanol and acetonitrile

(Chromatographic Grade) was purchased from Tedia company, inc. (2880 Symmes Road, Fairfield, USA). Reference substance (sinapine thiocyanate) was isolated from Semen sinapis Albae by author and the purity is more than 99%(by HPLC). The sample (Semen sinapis Albae) was purchased from Henan Provincial Drug Distribution Company in Zhengzhou, China and was identified as the seed of Sinapis alba L. by author. The stir-baked Semen sinapis Albae was processed according to Pharmacopoeia of the People's Republic of China (Edition 2000,Volume I) and taken out at 5,10,20,25,30 min respectively.

**IR analysis:** The powder of crude and stir-baked Semen sinapis Albae was obtained through the sieve of No.4 (average internal diameter of aperture is 250±9.9μm). We prepared the potassium bromide tableting containing 1~2%(w/w) of each sample and the resolution we used was $4cm^{-1}$. The infrared spectrograms were obtained by scanning and cumulating for 16 times.

**HPLC**

**Chromatographic system:** The column we used was Alltima phenyl 5u, 250 × 4.6mm eluted with 0.08mol/L monopotassium phosphate solution and acetonitrile solution (25:75) at a flow rate of 0.5ml/min. The detection wavelength was 326nm and the column temperature was 25°C.

**Sample preparation:** 1.0g Powder of Semen sinapis Albae (through the sieve of No. 4) was weighed accurately in a 100 mL conical flask and ultrasonicated for 20 minutes after adding 50 mL of methanol. The residue was solved in 50 mL methanol and re-extracted for 2 times. The filtrate was mixed and evaporated the solvent. The residue was dissolved by the mobile phase and transferred into a 50 ml volumetric flask, diluted to the volume and mixed well, then the sample solution was finished. After being filtered through a membrane filter with the aperture of 0.45 μm, the successive filtrate was collected for HPLC analysis.

**HPLC-mass:** HPLC: The column we used was Zorbax $C_{18}$, 150×4.6 mm eluted with 0.05% acetate acid solution and methanol solution (12:88) at a flow rate of 1.0ml/min. The detection wavelength was 281 nm and the column temperature was 25°C.

**Mass:** Negative ion mode; capillary voltage: 3500V; nebulier: 35.0psi; dry gas: 10.0 L/min; Target: 30000; Scan

**range:** 100~200 m/z; Average: 5

## RESULTS AND DISCUSSION

**Infrared spectrogram:** According to the variation of the superposition of absorbance at definite range of wavelength from the total group of the components containing in the testing Chinese herb medicine, the IR spectrometry can reveal the changes of the chemical component in this drug during the processing. The powder of the sample is directly mixed with potassium bromide to prepare the tablet, which is determined by the instrument to obtain an infrared spectrogram. The drug at different processing stage will show its special character of IR spectrogram because of its difference in confirmation and proportion of their components[19,20, 21,22,23]. In this work, the IR fingerprint spectrogram and the 2D-correlation IR analysis have been carried out in analysis of the changes of the components in Semen sinapis Albae. The results are shown in Fig.1.

It can be known that the peak at $1056cm^{-1}$ will soon disappear after processing for 5 mins, accordingly the 2D-correlation IR spectrogram shows a positive correlation (red), which indicates the decomposition of polyoses in seed coat. On the other hand, proteins and enzymes will also degrade within 15mins because the peaks at 1656 $cm^{-1}$ and 1547 $cm^{-1}$ are strikingly reduced during these periods. The 2D-correlation IR spectrogram can also reveal this result. Therefore the occurrence of 4-hydroxybenzyl isothiocyanate, the strong stimulus substance, has been prevented by the inactivation of the enzymes such as myrosinase. Thus, the side effect of the drug can be avoided. No change of oils and fats in the drug can be detected, because the peak at 1746 $cm^{-1}$ shows good stability during 30mins processing and 2D-correlation IR spectrogram shows a negative correlation (blue).

**HPLC:** In order to test the changes of the components in the drug in details, HPLC has been employed in this work and sinapine thiocyanate has taken as the example.

Sinapine thiocyanate by HPLC has been examined and a calibration graph is obtained over the range from 21.90mg/L to 219.0mg/L. The linear relationship (Y=155.0X+130.2) between the peak areas and the injected quantities is pretty good with the correlation coefficient of 0.9995.The reproducibility has also been examined with five replicate injections of one sample.

Peak areas are measured and the relative standard deviation is calculated as 0.43% (n=5). The stability study has performed by injecting 10µl of the same reference solution six times during 6 h. The relative standard deviation of peak area is 2.2%. The result indicates that this component is very stable in our

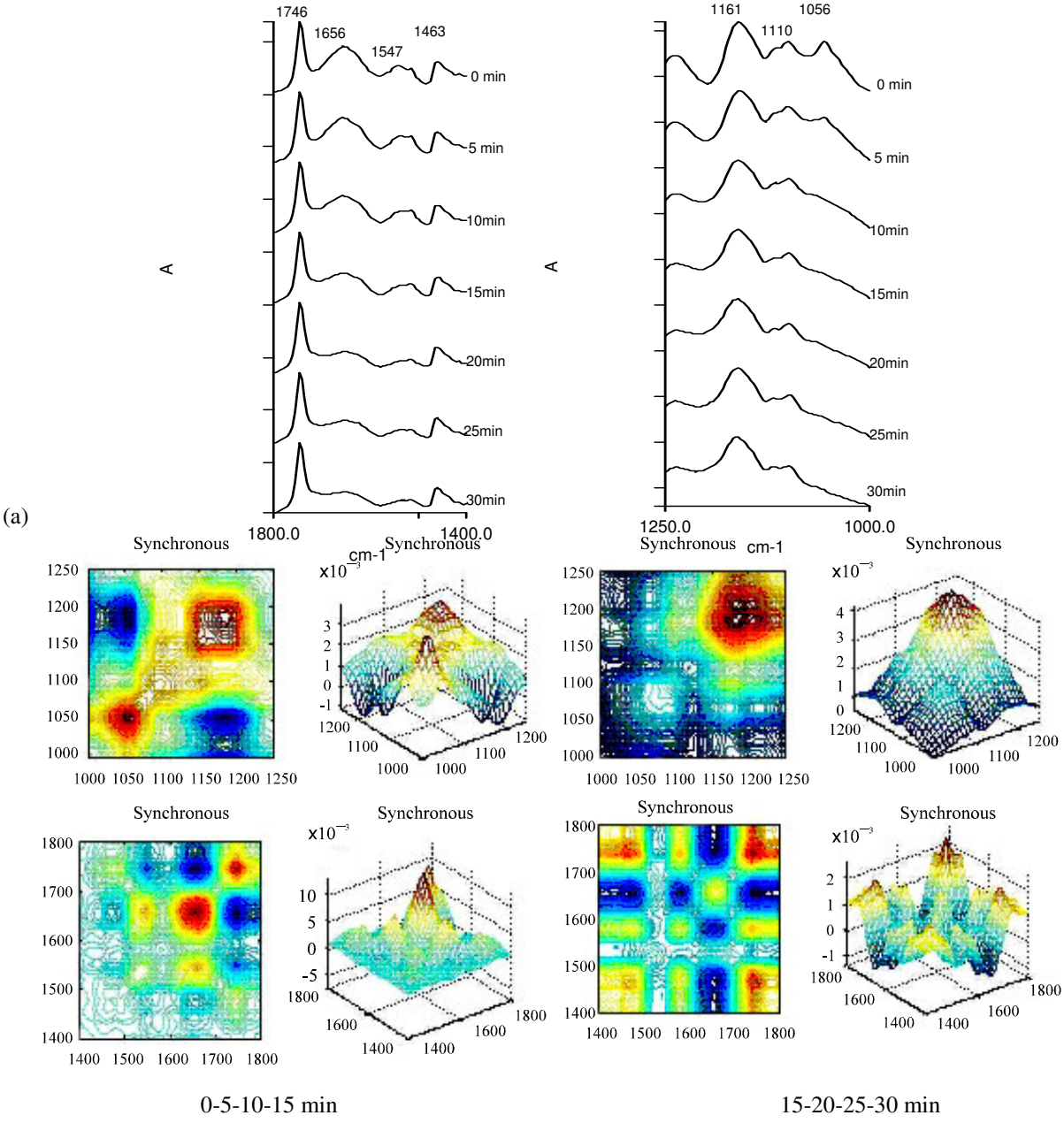


Fig. 1: Infrared spectrogram of Semen sinapis Albae crude drug and its processing sample from different time (a) infrared spectrogram; (b) 2D-correlation infrared spectrogram

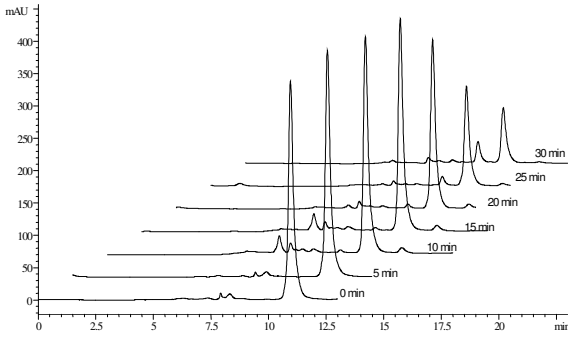


Fig. 2: Chromatogram of Semen sinapis albae crude drug and its processing sample from different time. Elution conditions: acetonitrile - 0.08M monopotassium phosphate solution (25:75), flow rate: 0.5 mL/min

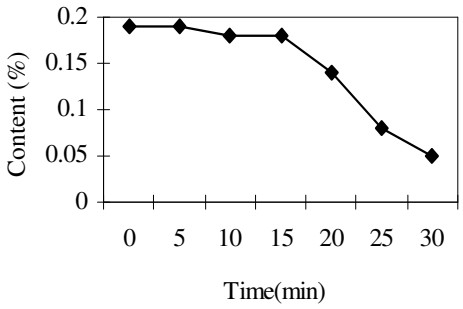


Fig. 3: Chromatogram and total ion of semen sinapis albase (processed for 30 min). Elution condition: 0. 05% accurate acid solution-methanol solution (12:88), flow rate: 1.0ml/min

experimental conditions. The detection limit is 0.044mg/L, which is calculated as three times of the average background noise level. A spike of 20mg/L sinapine thiocyanate in the sample evaluated the accuracy. After the pretreatment described previouslyand chromatographic detection, the recovery is achieved as 98.1%, (0.7% RSD, n=5).

Inject 10µl of sample solution into the column accurately. The result can be educed by calculating the content of the sample according to the calibration curve. The results are shown in Fig. 2 and Fig. 3.

It can be known that sinapine, a main component in Semen sinapis Albae has been changed during the processing, especially after 15mins. Meanwhile, Fig.2 shows that another substance has been produced. The retention time of this substance in this chromatography system is 10.1min. Obviously, a new substance, which cannot be found before processing, was produced.

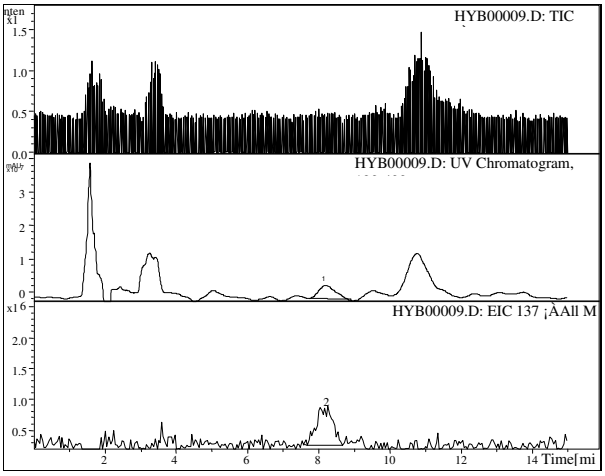


Fig. 4: Chromatogram and total ion current of Semen sinapis albae (processed for 30 mins). Elution conditions: 0.05% acetate acid Solution - methanol solution (12:88), flow rate: 1.0ml/min

**HPLC-mass:** We have used the technique of HPLC-mass spectrum to identify the structure of the substance produced from sinapine. Another chromatography system should be utilized to analyze it because non-volatile salts cannot be allowed to use in HPLC-mass. The results are shown in Fig. 4.

The retention time of this substance in this system has been known to be 8.2min. It can be well separatedwith sinapine through the $C_{18}$ column. The peak of 8.2min has been determined by mass spectrography (ion trap). ESI-MS (1) m/z (negative mode) is 137.1 $(M-H)^+$ and ESI-MS (2) m/z is 92.9$(M-COOH)^+$. The results suggest that the molecular formula should be HO-Ar-COOH. Meanwhile, the peak of this new product is well correspondent with the peak of p-hydroxybenzoic acid standard solution in above-mentioned two chromatography systems. So the product is p-hydroxybenzoic acid, the precursor of p-hydroxybenzoylcholine, which proves that the new path we proposed in our previous study is reasonable[24]. However, The degradation of sinapine in Semen sinapis Albae would influence the clinical effects of this drug because of its important biological activity[25,26]. According to this study, we suggest that the time of stir-baked processing should not be more than 15min.

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