# Detection of Moldy *Gastrodia Elata* Based on Mid Infrared Spectroscopy

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**Abstract: In order to solve the problem that *Gastrodia elata* is easy to mildew and produce mycotoxins during storage, and overcome the limitations of traditional detection methods, such as strong subjectivity and low efficiency, the spectral characteristics of normal and laboratory mildew samples were systematically analyzed by Fourier transform infrared spectroscopy combined with potassium bromide compression method. The results showed that although there were differences in the background spectra of *Gastrodia elata* in different production areas, the mildew samples showed consistent changes in the three key spectral areas of 3400 $cm^{-1}$, 1630 $cm^{-1}$ and 1050 $cm^{-1}$, which revealed the chemical nature of microbial biomass and water increase, protein degradation and transformation, and polysaccharide system reconstruction, respectively, providing a reliable spectral basis for mildew identification. This study confirmed that mid infrared spectroscopy technology can surpass the differences of production areas, and achieve rapid and nondestructive identification of moldy *Gastrodia elata* by capturing common chemical changes, which provides an effective means for quality and safety control.**

**Keywords: Mid Infrared Spectroscopy; *Gastrodia Elata*; Mildew Detection; Rapid Identification; Changes in Chemical Composition; Cross Region Analysis**

## 1. Introduction

*Gastrodia elata*, as a valuable traditional Chinese medicine in China, has excellent effects of calming wind and spasm, calming liver Yang, removing wind and unblocking collaterals, and is widely used in the field of traditional Chinese medicine and health care [1]. Its main production areas are concentrated in Yunnan, Guizhou, Shaanxi, Hubei and other provinces. However, the differences in soil, climate and other ecological environments in different production areas lead to significant differences in the chemical composition and quality of *Gastrodia elata* [2]. *Gastrodia elata* is rich in polysaccharides, proteins and other nutrients. In the process of harvesting, processing and storage, if the environmental temperature and humidity are not properly controlled, it is very easy to cause mildew due to moisture absorption. Mildew not only leads to the degradation of the appearance and effective components of *Gastrodia elata*, but also the mycotoxins (such as aflatoxin, ochratoxin, etc.) produced by the metabolism of mold will pose a serious threat to the safety of drug use, and may even cause liver injury, cancer and other risks. Therefore, the establishment of a rapid, accurate and efficient detection method of mildew *Gastrodia elata* is of great significance to ensure the quality of traditional Chinese medicine, maintain the health of consumers and promote the high-quality development of *Gastrodia elata* industry.

At present, the detection of *Gastrodia elata* mildew still relies on traditional methods. Although sensory identification (such as observing mycelium and smelling mildew) is simple and fast, it relies too much on the personal experience of inspectors and has strong subjectivity, so it is difficult to detect early or slight internal mildew. Although the results of microbiological culture counting method are relatively accurate, the operation process is cumbersome and time-consuming (usually several days), which can not meet the rapid screening needs of large quantities of samples in the circulation link. Although the mycotoxin detection method based on high performance

 

liquid chromatography (HPLC) or mass spectrometry (MS) is accurate and reliable, and is regarded as the gold standard, its sample pretreatment is complex, the equipment is expensive, the chemical reagent is consumed, and the professional requirements for operators are high, so it is difficult to popularize in the production area. To sum up, the existing technologies have their limitations, and the industry urgently calls for a new technology that can realize in-situ, rapid and nondestructive testing.

Mid infrared (MIR) spectroscopy technology (usually referred to as 4000-400 $cm^{-1}$ band) has shown great application potential in the field of food and drug quality and safety detection due to its outstanding advantages of fast, non-destructive, high throughput, low cost and green environmental protection. The theoretical basis of this technology is molecular vibrational spectroscopy. When the sample is irradiated by mid infrared light, the chemical bonds in the molecule (such as C-H, O-H, N-H, C=O, etc.) will change in dipole moment, absorb photons of a specific frequency, and generate vibrational energy level transitions [3]. These absorption frequencies are closely related to the type of molecular groups and their chemical environment, thus forming a unique "molecular fingerprint" [4]. When the medicinal materials are mildewed, the growth and reproduction of mold will decompose and utilize the inherent nutritional components of *Gastrodia elata* (such as polysaccharides and proteins), and produce new metabolites (such as mycotoxins, organic acids, etc.). This series of biochemical changes will inevitably lead to the change of the overall chemical composition, and then cause the characteristic changes of the mid infrared spectrum. By mining and analyzing these subtle or significant spectral differences through chemometrics methods, qualitative discrimination and quantitative prediction models can be established to accurately identify and evaluate the degree of mildew *Gastrodia elata* [5].

This study aims to break through the bottleneck of traditional detection methods and explore the use of mid infrared spectroscopy combined with chemometrics to achieve rapid and nondestructive identification of moldy *Gastrodia elata* [6]. Normal and moldy *Gastrodia elata* samples from Yunnan, Guizhou, Shaanxi and Hubei were carefully selected to cover the diversity of *Gastrodia elata* major producing areas and enhance the universality and robustness of the model. By systematically collecting the mid infrared spectra of all samples, and deeply analyzing the differences of spectral characteristics between normal *Gastrodia elata* and moldy *Gastrodia elata*, this study will build an efficient discrimination model, in order to provide a new technical means and solid theoretical basis for the quality control and safety monitoring of *Gastrodia elata* and other valuable Chinese medicinal materials.

## 2. Experimental Materials and Methods

The experimental samples were *Gastrodia elata* from Yunnan, Guizhou, Shaanxi and Hubei. Each production area contains normal *Gastrodia elata* and moldy *Gastrodia elata* samples confirmed by microbiological methods. All samples are cleaned and sliced, then manually ground, sealed and stored in a dryer for standby. Potassium bromide (KBr) powder used in the experiment was spectral pure (Sinopharm Chemical Reagent Co., Ltd., CAS No.: 7758-02-3). To eliminate moisture interference, KBr powder is dried in a 120 ℃ vacuum drying oven (Shanghai Jinghong dzf-6050) for 4 hours before use, and transferred to a dryer for cooling [7,8].

The ftir-850 Fourier transform infrared spectrometer of Tianjin Gangdong was used for spectrum acquisition, which was equipped with potassium bromide tablet transmission module. The spectrometer setting parameters are: the spectral scanning range is 4000 400$cm^{-1}$, the resolution is 1$cm^{-1}$, and the cumulative number of scans is 32 to eliminate the interference of background noise. The auxiliary equipment includes: yp-24a tablet press (Tianjin Gangdong, pressure range 025t), dzf-6050 vacuum drying oven (Shanghai Jinghong, temperature control accuracy ± 1℃) and an analytical balance with an accuracy of 0.001 G.

Standard KBr tablet method was used for sample preparation. Accurately weigh 1.5 mg of dried *Gastrodia elata* sample powder and 200 mg of dried KBr powder, fully mix them in an agate mortar and grind them evenly. Take an appropriate amount of mixed powder and put it into the tablet pressing die, keep it for 1 minute under the pressure of 15 tons, and press it into a thin sheet with uniform thickness and transparent for testing. Repeat the preparation of each sample and scan it for 3 times to obtain

 

highly representative and reproducible spectral data. Before scanning, perform background scanning with blank KBr film to deduct background interference [9].

Firstly, omnic software (Thermo Fisher Scientific) is used for baseline correction, smoothing and other pretreatment of the collected original spectral data. Subsequently, the data were exported to SPSS for statistical analysis and the establishment of chemometrics models (such as principal component analysis, PCA, discriminant analysis, etc.) [10]. The Origin software was used to draw and visualize the map.

## 3. Analysis of Mid Infrared Spectral Characteristics

### 3.1 Spectral Analysis of *Gastrodia Elata* in Xiaocaoba, Zhaotong, Yunnan

As shown in Figure 1, the mid infrared spectrum analysis of Xiaocaoba *Gastrodia elata* in Zhaotong, Yunnan clearly revealed the significant difference between normal and moldy samples. The spectrum of normal *Gastrodia elata* shows the typical characteristics of natural organic matter: the broad and strong absorption peak at 3400 $cm^{-1}$ is attributed to the O-H stretching vibration in polysaccharide and water, indicating that it contains rich hydrophilic components and normal water content; The absorption peaks at 2925 $cm^{-1}$ and 2850 $cm^{-1}$ come from the C-H stretching vibration of lipids; The strong absorption peak at 1630 $cm^{-1}$ was the C=O stretching vibration of protein amide I band, which was an important sign of its nutritional composition; The strong broad absorption peak at 1050 $cm^{-1}$ was due to the vibration of C-O and glycosidic bond (C-O-C) in the polysaccharide, which directly reflected the content of *Gastrodia elata* polysaccharide, its core active component. These clear and well shaped characteristic peaks jointly indicate that the effective components of the *Gastrodia elata* sample are well preserved and of high quality [11].

In sharp contrast, the spectrum of moldy *Gastrodia elata* has undergone fundamental changes, mainly reflected in the significant changes in chemical composition. The most intuitive difference appeared in the 3400 $cm^{-1}$ region, and its absorption peak intensity increased sharply and widened, which was mainly due to the large amount of metabolic water produced by the reproduction and metabolism of mold and the hydroxyl of microbial polysaccharides (such as chitin and dextran) contained in the mycelium itself, which was a strong indication of mold. At the same time, the protein characteristic peak at 1630 $cm^{-1}$ also changed significantly, and the peak shape may widen or shift, suggesting that the inherent protein of *Gastrodia elata* has been degraded into small molecular peptides or amino acids by protease secreted by mold, and microbial metabolism may also synthesize new amide substances. In the polysaccharide region near 1050 $cm^{-1}$, the relative intensity and peak shape of absorption also changed, which reflected a complex dynamic process: the intrinsic polysaccharide of *Gastrodia elata* was consumed by mold as a carbon source, and mold synthesized its own new polysaccharide, which changed the spectral characteristics of this region. In addition, the weak absorption peak (if any) that may appear near 1740 $cm^{-1}$ is the signal of c=o vibration of esters or carboxylic acids, which is due to the organic acids produced by mold metabolism (such as citric acid and oxalic acid), leading to the rancidity of *Gastrodia elata* [12].

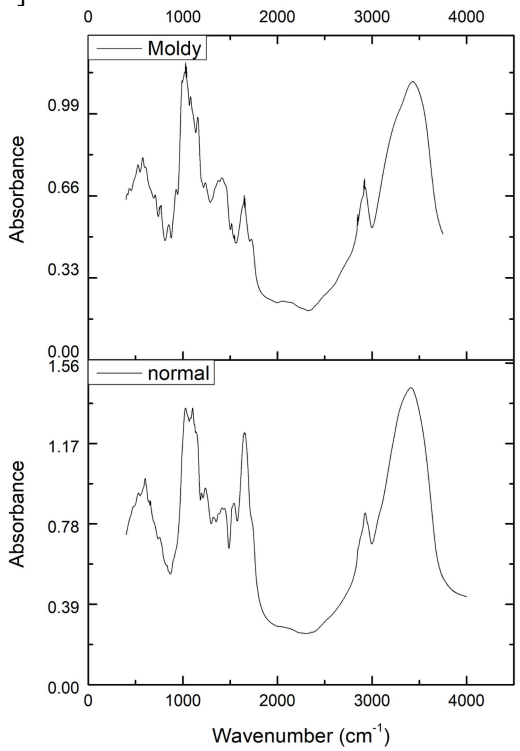


**Figure 1. Infrared Spectrum of *Gastrodia elata* in Xiaocaoba, Zhaotong, Yunnan**

### 3.2 Spectral Analysis of *Gastrodia Elata* in Dafang County, Guizhou Province

As shown in Figure 2, the mid infrared spectrum

of *Gastrodia elata* in Dafang County, Guizhou Province shows the overall outline of organic compounds similar to the sample in Zhaotong, Yunnan Province, but there are discernible differences in absorption intensity and local characteristics, reflecting the chemical composition basis and mildew response characteristics of *Gastrodia elata* in different production areas. The normal *Gastrodia elata* spectrum showed relatively gentle O-H stretching vibration absorption near 3400 $cm^{-1}$, suggesting that the initial water content or free hydroxyl content of this sample may be lower than that of Yunnan sample; The C-H stretching vibration absorption near 2925 $cm^{-1}$ and 2850 $cm^{-1}$ was clear, indicating that the lipid composition was well preserved; The absorption of amide I band at 1630 $cm^{-1}$ showed that the protein structure was complete, while the strong absorption of C-O-C and C-O vibration near 1050 $cm^{-1}$ confirmed the existence of *Gastrodia* polysaccharide as the main component again.

The spectrum of moldy samples deviated from the normal trajectory with multi-dimensional characteristics. The most significant change occurred in the 3400 $cm^{-1}$ region, where the absorption intensity increased and the half height width significantly expanded, suggesting that the mold proliferation introduced a large number of bacterial polysaccharides and metabolic water, which changed the overall hydrogen bond network structure of the sample. At 1630 $cm^{-1}$, the absorption peak of amide I band was significantly deformed and enhanced, which could be attributed to two parallel processes: the intrinsic protein of *Gastrodia elata* was degraded into polypeptides or amino acids by microbial enzymes, while the fungal protein and metabolites (such as amide toxins) accumulated, which jointly reshaped the profile of the band. It is worth noting that there are absorption disturbances around 1550 $cm^{-1}$ (amide II band) and 1400 $cm^{-1}$, which may involve protein N-H deformation vibration and organic acid radical ion vibration, further supporting the inference of microbial metabolic acid production and transformation of nitrogen-containing substances. Although there was no peak position shift in polysaccharide related absorption in 1050 $cm^{-1}$ region, the changes of absorption intensity and peak symmetry indicated the superposition effect of partial degradation and consumption of *Gastrodia elata* polysaccharides and the new components of microbial extracellular polysaccharides.

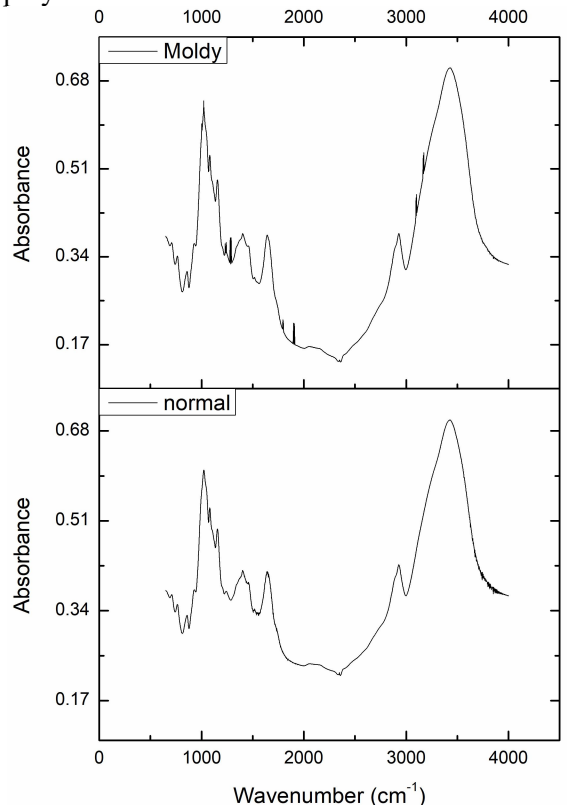


**Figure 2. Infrared Spectrum of *Gastrodia Elata* in Dafang County, Guizhou Province**

### 3.3 Spectral Analysis of *Gastrodia Elata* in Yichang, Hubei Province

As shown in Figure 3, the spectrum of normal samples of *Gastrodia elata* Bl. in Yichang, Hubei Province shows a typical and moderate intensity O-H stretching vibration wide absorption band near 3400 $cm^{-1}$, suggesting that its initial hydration state is slightly different from that of Yunnan or Guizhou samples; The C-H stretching vibration at 2925 $cm^{-1}$ and 2850 $cm^{-1}$ was clearly absorbed, indicating that the lipid composition was well preserved; The significant absorption of amide I band at about 1630 $cm^{-1}$ once again confirmed the integrity of protein composition; The strong and wide absorption peaks caused by C-O and glycosidic bond vibrations near 1050 $cm^{-1}$ are the signs of the core components of *Gastrodia elata* polysaccharide.

The mildew process induced significant changes in spectral characteristics, and the disturbance mode had clear chemical directivity. The most prominent change occurred in the 3400 $cm^{-1}$ area, and its absorption intensity increased sharply, and the peak shape widened significantly, which was strongly attributed to the increase of

 

mycelial biomass (rich in chitin and other polysaccharides) and the large production of metabolic water. In the amide I band near 1630 $cm^{-1}$, the absorption peak not only changed in intensity, but also widened or appeared shoulder peaks, which revealed the transformation of protein components: the endogenous proteins of *Gastrodia elata* were degraded by microbial protease, and the fungi metabolized and synthesized new substances containing amide or carbonyl (possibly including some mycotoxins), which together reshaped the profile of the band. It is worth noting that if there is a recognizable absorption shoulder near 1740 $cm^{-1}$, it can be used as direct spectral evidence of the outflow of fungal metabolites (such as fatty acid esters or organic acids), indicating that the sample has been rancidized. In addition, although the peak position of polysaccharide characteristic region near 1050 $cm^{-1}$ was stable, its absorption intensity and shape changed, revealing the complex biochemical process of *Gastrodia elata* intrinsic polysaccharide consumed by microbial enzymatic hydrolysis and microbial extracellular polysaccharide regeneration.

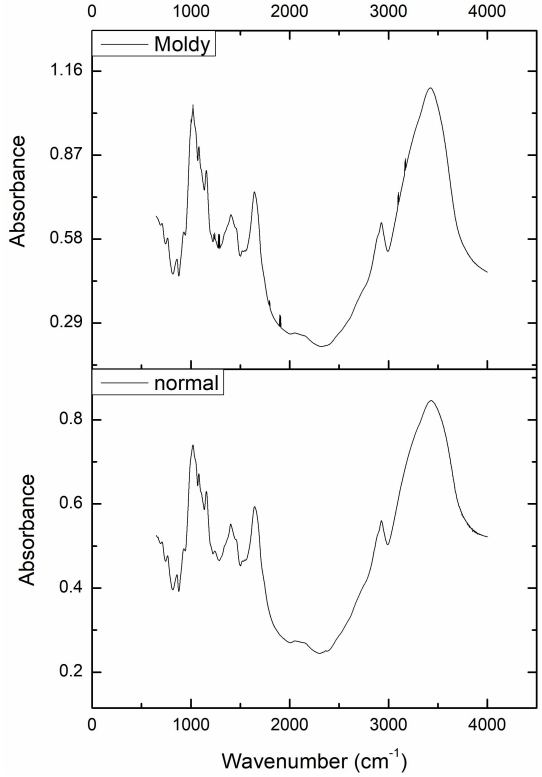


**Figure 3. Infrared Spectrum of *Gastrodia Elata* in Yichang, Hubei Province**

### 3.4 Spectral Analysis of *Gastrodia Elata* Powder in Hanzhong, Shaanxi

As shown in Figure 4, the mid infrared spectrum of *Gastrodia elata* in Hanzhong, Shaanxi Province shows similar and slightly different spectral characteristics compared with the samples from other production areas. The spectrum of the normal sample shows a relatively wide o-h stretching vibration absorption near 3400 $cm^{-1}$, with moderate intensity, reflecting the vibration characteristics of the sample's fixed water and polysaccharide hydroxyl; The C-H stretching vibration absorption at 2925 $cm^{-1}$ and 2850 $cm^{-1}$ was clear, indicating that the lipid components were well preserved; The absorption of amide I band near 1630 $cm^{-1}$ was still prominent, which confirmed the complete existence of protein components; The strong absorption near 1050 $cm^{-1}$ caused by C-O and glycosidic bond vibration fully reflects the composition characteristics of *Gastrodia elata* polysaccharide as the main active ingredient.

The mildew process caused significant structural changes in the spectrum of *Gastrodia elata* in Hanzhong, and its disturbance mode showed a systematic response. The most prominent change is still reflected in the 3400 $cm^{-1}$ region, where the absorption intensity increased significantly and the peak shape widened significantly, which is mainly due to the introduction of mycelial biomass by mold proliferation and the large amount of water produced in the process of metabolism, which changed the overall hydrogen bonding environment of the sample. At the amide I band near 1630 $cm^{-1}$, the absorption peak was significantly deformed and enhanced, revealing the transformation path of protein composition: the endogenous protein of *Gastrodia elata*. was degraded to small molecular peptides or amino acids under the action of microbial enzymes, while the fungi metabolized and synthesized new amide substances, which together led to the remodeling of the absorption profile of the region. If weak absorption can be identified near 1740 $cm^{-1}$, it can be used as spectral evidence for the production of esters or organic acids by mold metabolism, indicating that the sample has undergone oxidative rancidity. In addition, the absorption of polysaccharide characteristic region near 1050 $cm^{-1}$ also showed changes in intensity and peak shape, reflecting the dynamic biochemical process of *Gastrodia elata* intrinsic carbohydrate decomposition and utilization by microorganisms and microbial polysaccharide synthesis.

 

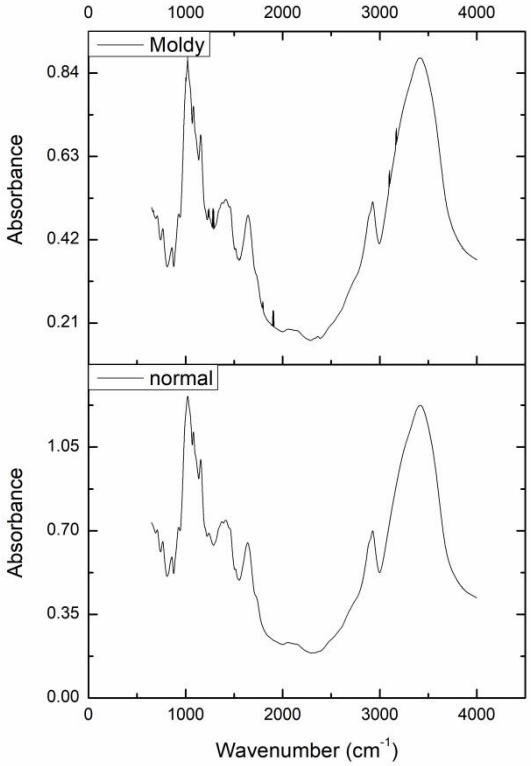


**Figure 4. Infrared Spectrum of *Gastrodia Elata* in Hanzhong, Shaanxi**

## 4. Comprehensive Identification and Analysis of Mildew *Gastrodia Elata* in Cross Production Areas

Through the systematic comparison and analysis of the mid infrared spectra of normal and moldy samples from four major *Gastrodia elata* production areas in Zhaotong, Yunnan, Dafang, Guizhou, Yichang, Hubei and Hanzhong, Shaanxi, it was found that although *Gastrodia elata* from different regions had subtle differences in spectral baseline, absorption intensity and other background characteristics due to differences in soil, climate and other growth environments, the spectral response induced by mildew showed highly consistent and repeatable regular changes, which fully proved the feasibility and universality of mid infrared spectroscopy technology in the identification of moldy *Gastrodia elata* across production areas. The essence of mildew process is the decomposition and transformation of inherent chemical components of *Gastrodia elata* by microbial activities, accompanied by the accumulation of microbial metabolites and biomass. This series of biochemical reactions are clearly reflected in the specific fingerprint area of mid infrared spectrum. The core and most sensitive criterion is located in the O-H stretching vibration region near 3400 $cm^{-1}$, where mildew samples from all production areas show the common characteristics of significantly enhanced absorption intensity and significantly broadened peak shape. This is mainly due to the introduction of bacterial polysaccharides (such as chitin and glucan) and the water produced by their metabolism, which changes the overall hydrogen bonding environment of the sample. This phenomenon is independent of the production area and is the direct spectral evidence of microbial pollution; At the same time, the amide I band (protein C=O stretching vibration) near 1630 $cm^{-1}$ generally observed the deformation, broadening or shoulder peak of the absorption peak, revealing the reconstruction process of *Gastrodia elata* intrinsic protein degraded into small molecular peptides or amino acids by microbial protease and acting together with new proteins synthesized by microorganisms and amide metabolites (such as potential mycotoxins); Although the peak position of polysaccharide characteristic region (C-O and glycosidic bond vibration) near 1050 $cm^{-1}$ is relatively stable, the change of absorption intensity and peak shape reflects the dual dynamic process of the decomposition and consumption of intrinsic active polysaccharides from *Gastrodia elata* and the coexistence of new components of extracellular polysaccharides from microorganisms. In addition, if there is a recognizable absorption shoulder peak near 1740 $cm^{-1}$, it can be regarded as a sign of the metabolism of fungi to produce esters or organic acids (such as fatty acids, oxalic acid, etc.), indicating that the sample is rancid. These systematic and cross verifiable spectral features together constitute a reliable basis for the identification of mildew. It shows that the mid infrared spectroscopy technology can effectively surpass the inherent background differences of *Gastrodia elata* in different production areas and accurately capture the common chemical changes caused by mildew. It can not only be used for the qualitative discrimination of "whether it is mildew", but also reveal "what kind of deterioration has occurred" from the molecular level. It provides a fast, nondestructive and efficient advanced means for the quality control of *Gastrodia elata* and other valuable Chinese medicinal materials in the aspects of storage, circulation and market supervision, and has important value for ensuring the safety of traditional Chinese

 

medicine and the high-quality development of the industry.

## 5. Conclusions and Prospects

This study showed that the mid infrared spectroscopy technology could effectively capture the systematic chemical changes of *Gastrodia elata* in the process of mildew in Yunnan, Guizhou, Hubei and Shaanxi, showing a good ability of cross production identification. It was found that in the key characteristic spectral regions of 3400 $cm^{-1}$, 1630 $cm^{-1}$ and 1050 $cm^{-1}$, mildew *Gastrodia elata* showed regular changes such as enhanced absorption intensity, peak broadening or deformation. These changes were directly related to the biochemical processes such as biomass increase, metabolic water accumulation, protein degradation and transformation, and polysaccharide system reconstruction caused by microbial proliferation. It proved that this technology could not only realize the qualitative discrimination of "whether mildew or not", but also reveal the chemical nature of mildew from the molecular level. However, it must be pointed out that there is an obvious limitation in this study: the mildew samples used in the experiment are all artificially induced by normal *Gastrodia elata* under laboratory controlled conditions. Although this ensures the consistency of the experimental conditions, there may be differences in species diversity, degree of mildew and complexity of metabolites between the mildew samples naturally occurring in the field or actually produced in the storage process, which makes the external validity and reliability of the discrimination model established in this study still need to be further verified in practical application.

Looking forward to the future, this study laid a solid foundation for the application of mid infrared spectroscopy technology in *Gastrodia elata* quality monitoring, but the follow-up work should focus on the following aspects: first, we must expand the source of samples, and widely collect the actual samples with clear mildew background produced in different production areas under natural conditions, including samples with different degrees of mildew and different types of mildew (such as Aspergillus, Penicillium and other different strains), in order to verify and improve the accuracy and robustness of the existing discriminant model in the actual scene; Secondly, efforts should be made to build a standard database of *Gastrodia elata* mid infrared spectra covering the main production areas and different mildew States, and develop an intelligent diagnosis system that can automatically identify the degree of mildew and even potential mildew strains by combining chemometrics and machine learning algorithms; More importantly, it is necessary to analyze the correlation between spectral data and mycotoxin content measured by classical chromatographic analysis methods (such as HPLC-MS/MS), and explore the establishment of rapid prediction model of toxin content based on spectral technology, so as to realize the leap from quality identification to safety risk assessment; Finally, the technology will be promoted from laboratory to industrial application. By developing portable or online detection equipment, it can be embedded in the storage, processing and circulation of *Gastrodia elata*, providing key technical support for the construction of the whole chain quality monitoring system of Chinese medicinal materials, and truly ensuring the safety and quality of Chinese medicinal materials from the source.

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