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Discrimination of Fritillary according to geographical origin with Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy

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Abstract

Fritillaria is a traditional Chinese herbal medicine for eliminating phlegm and relieving a cough with a long history in China and some other Asian countries. The objective of this study is to develop a nondestructive and accurate method to discriminate *Fritillaria* of different geographical origins, which is a troublesome work by existing analytical methods. We conducted a systematic study on five kinds of *Fritillaria* by Fourier transform infrared spectroscopy, second derivative infrared spectroscopy, and two-dimensional (2D) correlation infrared spectroscopy under thermal perturbation. Because *Fritillaria* consist of a large amount of starch, the conventional IR spectra of different *Fritillaria* only have very limited spectral feature differences. Based on these differences, we can separate different *Fritillaria* to a limited extent, but this method was deemed not very practical. The second derivative IR spectra of *Fritillaria* could enhance spectrum resolution, amplify the differences between the IR spectra of different *Fritillaria*, and provide some dissimilarity in their starch content, when compared with the spectrum of pure starch. Finally, we applied thermal perturbation to *Fritillaria* and analyzed the resulting spectra by the 2D correlation method to distinguish different *Fritillaria* easily and clearly. The distinction of very similar *Fritillaria* was possible because the spectral resolution was greatly enhanced by the 2D correlation spectroscopy. In addition, with the dynamic information of molecular structure provided by 2D correlation IR spectra, we studied the differences in the stability of active components of *Fritillaria*. The differences embodied mainly on the intensity ratio of the auto-peak at 985 cm^{-1} and other auto-peaks. The 2D correlation IR spectroscopy (2D IR) of *Fritillaria* can be a new and powerful method to discriminate *Fritillaria*.

Keywords: *Fritillaria*; Fourier transform infrared spectroscopy; Two-dimensional correlation infrared spectroscopy; Alkaloid; Saponin

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1. Introduction

Fritillaria, the bulb of several kinds of vegetables of *Fritillaria* category *Liliaceae* section, has a long history of use in Asian countries, such as China and Korea, and is now classified as a traditional Chinese herbal medicine in common use. *Fritillaria* has been claimed to have remarkable curative effects in clearing heat, eliminating phlegm, moistening the lungs and relieving a cough. The effective components are mainly alkaloid and saponin [1]. But due to the different climate and soil regime of different regions, the differences in composition and properties among different *Fritillaria* are observed. Different *Fritillaria* have discrepancies in flavor and property, as well as in their function [2]. China has a vast territory and many *Fritillaria* growing areas. And for this reason, *Fritillaria* of different geographical origins have often been confused, and the authenticity of *Fritillaria*-based medicine is compromised. There are also some difficulties in selecting specific *Fritillaria* for curing diseases. And even worse, there might be a potential for drug poisoning when *Fritillaria* is misused. So it is very important to establish a reliable and convenient method to distinguish *Fritillaria* from different areas.

Currently, the main method for identifying various *Fritillaria* is chromatography. Since there are tens of major components, which are slightly different according to growing conditions, such as geographical origin, we cannot select only a limited number of specific components as essential screening criteria. Indeed there have been some contradicting results concerning the contents of some components of *Fritillaria* in the documents [3,4]. We must thus conclude the *Fritillaria* cannot be discriminated and identified very well at this moment using only a conventional method.

Two-dimensional correlation infrared (2D IR) spectroscopy was proposed by Noda in 1986 [5]. The construction of 2D correlation IR spectra is based on the detection of dynamic changes of a system under an external perturbation. Such a perturbation induces selective changes in molecular constituents associated with individual normal modes of vibration in the system. The correlation analysis is applied to a set of spectra taken during the perturbation, so as to yield 2D correlation IR spectra. There has been a rapid progress in the technique of 2D correlation spectral analysis. The external perturbations used to produce 2D correlation IR spectra were sinusoids in early days [6]. Nowadays, however, arbitrary wave forms with non-periodical perturbations are commonly used [7]. The applied perturbations can be light, heat, electricity, magnetism, chemistry, concentration changes, or mechanical force [8–10]. The 2D correlation technique has been wildly used in traditional spectroscopy, such as IR, Raman, UV and fluorescence. There have been studies on many aspects, including biologically important systems like *N*-methylacetamide (NMA) and polyhydroxyalkanoates [10,11]. We have successfully used the 2D correlation IR analysis to discriminate traditional Chinese medicines and analyze the deteriorative process of medicines [12,13].

In the holistic theory of Chinese pharmacy, medicinal materials take effects in curing diseases as a whole. So any method or experiment, if it destroys the wholeness of traditional Chinese medicines, will not be fundamentally acceptable. IR spectroscopy, a nondestructive, fast and integrity-emphasized method, has important practical utility in identifying and distinguishing the Chinese medicines according to geographical origin. In this study, we discriminated five kinds of common *Fritillaria* of different geographical origins: *Fritillaria walujewii*, *Fritillaria hupehensis*, *Fritillaria thunbergii*, *Fritillaria ussuriensis* and *Fritillaria cirrhosa*, by FT-IR, second derivative IR, and 2D correlation IR spectroscopy (2D IR) By studying the different behavior of their components under thermal perturbation with 2D correlation analysis, we analyzed the differences of the stability of different *Fritillaria* components in the thermal process. The objective of this study is to search for a new method to effectively discriminate *Fritillaria* according geographical origin.

2. Experiment

2.1. Apparatus

Spectrum 2000 GX FT-IR spectrometer (Perkin Elmer), equipped with a DTGS detector, in the 800–4000 cm^{-1} range with a resolution of 4 cm^{-1}. Spectra were calculated from a total of 32 scans.

Love Control Corporation's Portable programmable temperature Controller (Model 50-886). Range: 50–90 °C.

2.2. Samples

All *Fritillaria* were identified and provided by National Institute for the Control of Pharmaceutical and Biological Products.

2.3. Procedure

First, all the *Fritillaria* were purified, comminuted, and desiccated. Then each sample of *Fritillaria* powder was blended with KBr powder, ground again, and pressed into a tablet.

Put the sample tablet in the sample pool of temperature controller. A pre-established program controlled the whole process of increasing temperature. During the process of increasing temperature from 50 to 90 °C, the spectra were collected at intervals of 5 °C. The full temperature scan took a total time of 30 min.

All the second derivative IR spectra were 13-point second derivative IR spectra after 13-point smoothing of the original IR spectra taken at room temperature.

2D IR correlation spectra were obtained by analyzing the series of temperature-dependent dynamic spectra with a 2D IR correlation analysis software programmed by our group.

3. Results and discussion

3.1. Assignments and comparison of the IR spectra

IR spectra, taken at room temperature, of the five *Fritillaria* samples are shown in Fig. 1. The IR spectra belong to *F. walujewii*, *F. hupehensis*, *F. thunbergii*, *F. ussuriensis* and *F. cirrhosa*, respectively, arranged from the top down.

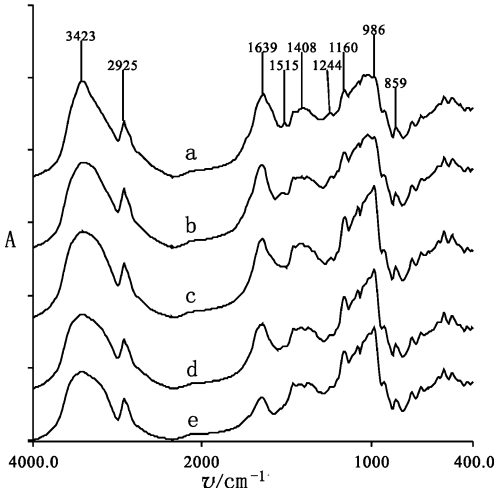

Fig. 1. IR spectra of five *Fritillaria* at room temperature. (a) *F. walujewii*, (b) *F. hupehensis*, (c) *F. thunbergii*, (d) *F. ussuriensis*, (e) *F. cirrhosa*.

The IR spectra of five *Fritillaria* are rather similar, except that some peaks at a given wavenumber have slight differences in shape or intensity. The peak at 1515 cm^{-1} may be an example. *F. walujewii* has a sharp peak, *F. hupehensis* has a wider one, and others have no noticeable peak. The peak at 1244 cm^{-1} is most obvious in the

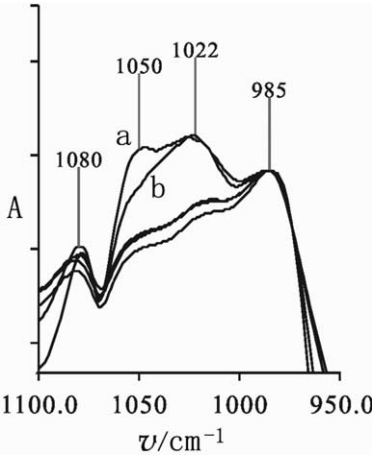

Fig. 2. Comparison of the IR spectra of five *Fritillaria* of 950–1100 cm^{-1} (auto scaled at 985 cm^{-1}). (a) *F. walujewii*, (b) *F. hupehensis*, (c) *F. thunbergii*, (d) *F. ussuriensis*, (e) *F. cirrhosa*.

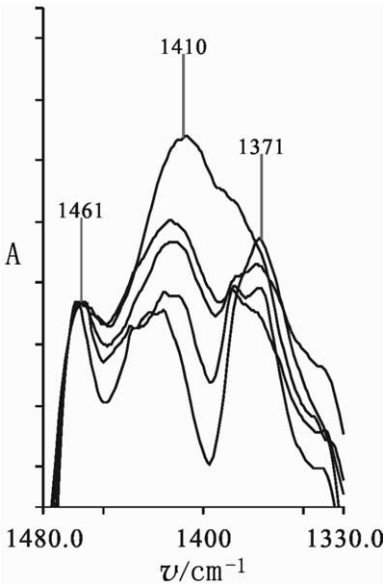

Fig. 3. Comparison of the IR spectra of five *Fritillaria* of 1330–1480 cm^{-1} (auto scaled at 1461 cm^{-1}). (a) *F. walujewii*, (b) *F. hupehensis*, (c) *F. thunbergii*, (d) *F. ussuriensis* and (e) *F. cirrhosa*.

spectrum of *F. walujewii*, followed by the spectra of *F. thunbergii* and *F. ussuriensis*, but becomes illegible in the spectra of *F. hupehensis* and *F. cirrhosa* due to overlapping with other peaks. There are also apparent differences in the IR spectra of 950–1100 and 1330–1480 cm^{-1} regions, which are enlarged, respectively, in Figs. 2 and 3.

In Fig. 2, the five spectra are auto scaled at the peaks near 985 cm^{-1} to make it clear to observe the differences among them. When spectra are auto scaled at a wavenumber, the absorbance of each spectrum at this wavenumber will be the same so that the differences at other wavenumbers will be more clearly to observe. All the five *Fritillaria* have two apparent peaks at 985 and 1080 cm^{-1}, which match together, respectively, in Fig. 2 because the two peaks of each *Fritillaria* have the similar intensity ratio. *F. walujewii* also has two relatively strong peaks at 1022 and 1050 cm^{-1} of the same intensity. *F. hupehensis* has an noticeable peak at 1022 cm^{-1}, which overlaps with the weak peak at 1050 cm^{-1}. The other three *Fritillaria* have little difference in Fig. 2.

The five spectra are auto scaled at the peaks near 1461 cm^{-1} in Fig. 3. The five *Fritillaria* have three noticeable peaks at 1461, ~1414 and ~1370 cm^{-1}. But the intensity ratios of these peaks of different *Fritillaria* are not the same. For example, *F. walujewii* has the strongest peak around 1414 cm^{-1} relatively to the peak at 1461 cm^{-1}, and *F. cirrhosa* has the weakest. *F. walujewii* and *F. thunbergii* have no observable peaks around 1370 cm^{-1}. *F. hupehensis* and *F. thunbergii* have peaks at 1384 cm^{-1}. The other three *Fritillaria* with peaks at 1370 cm^{-1} also have differences in the intensity and shape of these peaks. In addition, each of *F. cirrhosa* and *F. hupehensis* has an obvious valley around 1398 cm^{-1}, but *F. walujewii* does not.

The noticeable difference in the IR spectra of five *Fritillaria* is found for the different intensity ratio of the peak at 1640 to the peak at 1407 cm^{-1}. Although the peak at 1640 cm^{-1} may be interfered by the presence of moisture, the well-controlled experimental conditions and operative process and the fine reproducibility of each *Fritillaria* spectrum strongly suggest that the difference is due to the *Fritillaria* themselves. The intensity ratios of peaks at 1460 and 985 cm^{-1} also have some disparities. The exact ratios are shown in Table 1.

The peak at 3413 cm^{-1} is due to the overlapping bands of hydroxyl contributions, and the peak at 2926 cm^{-1} is assigned to $-CH_2-$ groups. The peak at 1640 cm^{-1} is largely the peak of C(O stretching, and the peak at 1407 cm^{-1} is assigned to the bending of t-OH groups.

Methyl and methylene have special absorption peaks at 1460 cm^{-1}, and many –C–O–C– have peaks between 950 and 1100 cm^{-1}. The alkaloid and saponin, the effective components of *Fritillaria*, both consist of C(O groups. Furthermore, saponin also has many –C–O–C– groups. So the data in Table 1 also offer some valuable information about the content of effective components in *Fritillaria*.

According to the data of Table 1, the five *Fritillaria* can be sorted into three classes: *F. walujewii* and *F. hupehensis* belong to one class, *F. thunbergii* and *F. ussuriensis* belong to another class, *F. cirrhosa* alone belongs to the third class. And when the differences of the IR peaks in Figs. 2

Table 1
The intensity ratios of some IR peaks of different *Fritillaria*

Fritillaria	*F. walujewii*	*F. hupehensis*	*F. thunbergii*	*F. ussuriensis*	*F. cirrhosa*
Intensity ratio of peaks at 1640–1407 cm^{-1}	1.3653	1.3484	1.1524	1.1622	0.7989
Intensity ratio of peaks at 985–1460 cm^{-1}	1.4979	1.5301	1.8091	1.9669	1.8993

and 3 are taken into consideration, all the five *Fritillaria* can be roughly separated.

3.2. Comparison and discussion of second derivative IR spectra

Fritillaria consist largely of starch, so *Fritillaria* and pure starch have similar second derivative IR spectra, which are illustrated in Fig. 4. We can know the differences in the starch content of different *Fritillaria* by comparing the dissimilarity of the second derivative IR spectra of the five *Fritillaria* and starch. The more similar the second derivative IR spectrum of *Fritillaria* is to pure starch, the more starch it consists of. In other words, the *Fritillaria* has relatively lower content of the active components.

We now compare the dissimilarity among the five *Fritillaria* and between *Fritillaria* and starch in the second derivative spectra more closely. *F. walujewii* has a stronger peak at 1515 cm^{-1} than other *Fritillaria*. *F. hupehensis* has a weaker peak at this wavenumber. And the other *Fritillaria* and starch have no such peak. The result is the same with that from conventional FT-IR spectra. *F. thunbergii*, *F. ussuriensis* and *F. cirrhosa* have more similar features with starch in the second derivative spectra, like the sharp peak at 985 cm^{-1}. The enlarged second derivative spectra between 1100 and 900 cm^{-1} of starch and five *Fritillaria* are shown in Fig. 5. *F. walujewii* and *F.*

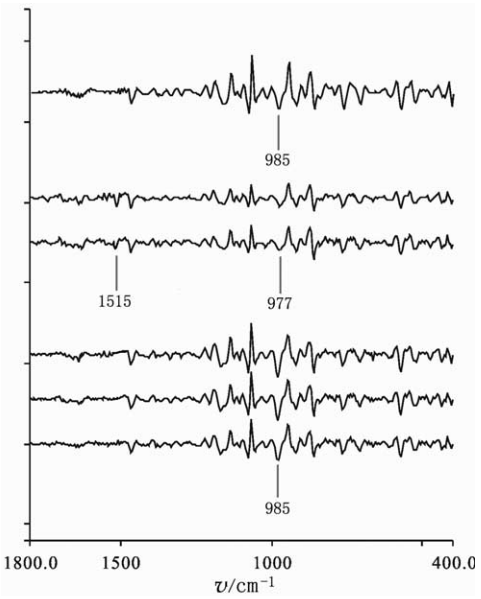

Fig. 4. Comparison of second derivative IR spectra of starch and five *Fritillaria* (room temperature, 400–1800 cm^{-1}). (a) Starch, (b) *F. walujewii*, (c) *F. hupehensis*, (d) *F. thunbergii*, (e) *F. ussuriensis*, (f) *F. cirrhosa*.

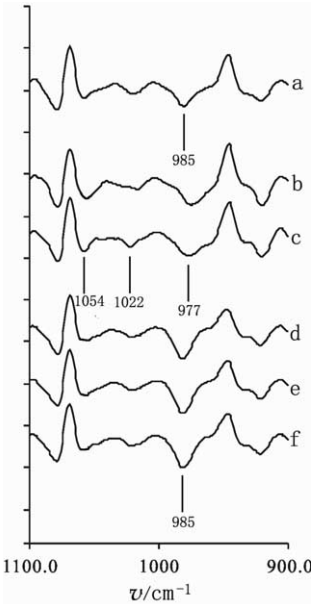

Fig. 5. The second derivative spectra of starch and five *Fritillaria* of 900–1100 cm^{-1}. (a) Starch, (b) *F. walujewii*, (c) *F. hupehensis*, (d) *F. thunbergii*, (e) *F. ussuriensis* and (f) *F. cirrhosa*.

hupehensis have no peak at 985 cm^{-1} but have broader peaks at 977 cm^{-1}. The peak at 985 cm^{-1} is mainly attributed to the stretching of –C–O–C–. Thus, saponin and other similar substances, consisting of many –C–O–C– groups, will affect the IR spectra of this range greatly. So we can deduce that *F. walujewii* and *F. hupehensis* may have a higher content of saponin and other similar substances.

We can see some additional information from Fig. 5. *F. walujewii* and *F. hupehensis* each has a peak at 1054 cm^{-1}, and the peak of the former is more noticeable. *F. hupehensis* also has a fairly obvious peak at 1022 cm^{-1}. The two peaks are not featured by the other three *Fritillaria* and starch. The results correspond well to the previous conclusion drawn from the analysis of Fig. 2.

Figs. 6 and 7 show the second derivative IR spectra of five *Fritillaria* and starch in the range of 1500–1800 and 1360–1480 cm^{-1}, respectively.

In Fig. 6, starch has a noticeable peak at 1462 cm^{-1}. The peak does not exist in the spectra of *F. walujewii* and *Fritillaria hupehensi*. And the peaks

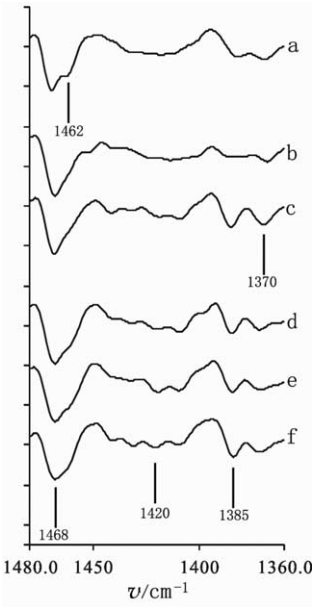

Fig. 6. The second derivative spectra of starch and five *Fritillaria* of 1360–1480 cm^{-1}. (a) Starch, (b) *F. walujewii*, (c) *F. hupehensis*, (d) *F. thunbergii*, (e) *F. ussuriensis* and (f) *F. cirrhosa*.

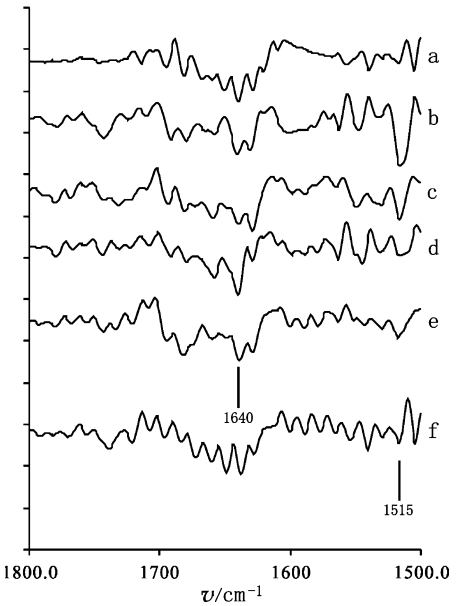

Fig. 7. The second derivative spectra of starch and five *Fritillaria* of 1500–1800 cm^{-1}. (a) Starch, (b) *F. walujewii*, (c) *F. hupehensis*, (d) *F. thunbergii*, (e) *F. ussuriensis* and (f) *F. cirrhosa*.

of other three *Fritillaria* at 1462 cm^{-1} are not very clear for overlapping. While *F. ussuriensis* and *F. cirrhosa* have peaks at 1420 cm^{-1}, although they are weak, the other *Fritillaria* and starch have not. Expect for starch and *F. walujewii*, other *Fritillaria* all have the peak at 1385 cm^{-1}. And *F. hupehensis* features the unique noticeable peak at 1370 cm^{-1}.

Fig. 7 shows the peak at 1640 cm^{-1} is different for each *Fritillaria* and starch. The peak of *F. walujewii* at 1640 cm^{-1} is fairly broad, the peak of *F. thunbergii* is strong and sharp, the peak of *F. hupehensis* is weak, and *F. cirrhosa* has no peak at 1640 cm^{-1} but adjacent wavenumber. The peak at 1515 cm^{-1} is also different. For the intensity of this peak, *F. walujewii* occupies the first place, *F. hupehensis* comes second. The peak intensities at 1515 cm^{-1} of other *Fritillaria* are rather weak. Starch has no such peak.

The above results show that the second derivative spectra of *F. walujewii* and *F. hupehensis* have the least similarities with that of starch Thus, the two *Fritillaria* have relatively lower content of starch than the other three *Fritillaria* and, by inference, may have better curative effects.

In brief, the second derivative spectra can illustrate the features of the IR spectra of *Fritillaria* more clearly, and provide some information about the content of starch of *Fritillaria*.

3.3. Results and discussion of 2D correlation IR spectra

The effective components of *Fritillaria* are alkaloid and saponin, which mainly contain C(O, $-CH_2-$, $-C-O-C-$ and some rings containing N. These groups are well suited for the 2D correlation analysis of IR spectra of the corresponding wavenumber. Due to the noises produced by the tiny but non-negligible changes of moisture in the light path during the experiment, especially when the perturbation applied on *Fritillaria* tablet was very small, we could not use the spectra around 1640 cm^{-1} to make the reliable 2D correlation spectra. We selected instead the wavenumber range of 950–1350 and 1350–1600 cm^{-1} to take the full advantage of the 2D correlation analysis.

Temperature-dependent FTIR spectra of the five *Fritillaria* in the region of 1800–400 cm^{-1} were obtained from 50 to 90 °C, with an increment of 5 °C. The full temperature scan took a total time of 30 min. Fig. 8 shows the temperature-dependent FTIR spectra of *F. walujewii*. And to overcome the baseline shifts, we made baseline auto-correction of all these spectra and calculated them so that the lowest points of them were at zero. All the main peaks of *F. walujewii* decreased with the temperature increasing.

Fig. 9(a–e) are the 2D correlation IR synchronous spectra of the five *Fritillaria* in the region of 1350–950 and 1600–1350 cm^{-1}, respectively.

The peaks in the synchronous spectra present the consistency of intensity variations of the related IR vibrations with the temperature as a variable [7,14]. The diagonal peak is called an autopeak, which is the result of the autocorrelation of perturbation-induced dynamic fluctuations of IR signals. They indicate the susceptibility of corresponding absorbance bands to a given external perturbation. More specifically, if the dynamic variation of the IR spectrum reflects the population change of system constituents, autopeaks in a synchronous 2D IR spectrum represent the ease of constituent transformation, and consequently the thermal susceptibility, of chemical groups contributing to the molecular vibration [7,14]. Aside from the diagonal autopeaks, there

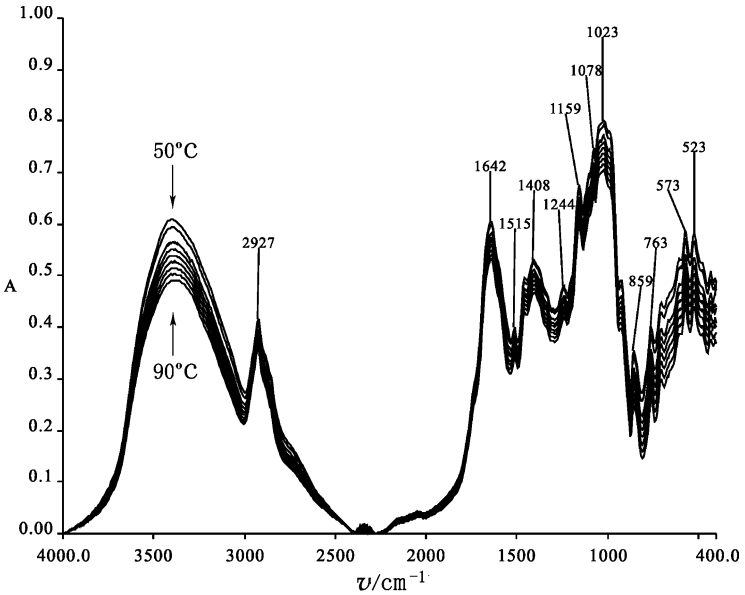

Fig. 8. Temperature-dependent FTIR spectra of *F. walujewii* in the region of 1800–400 cm^{-1} over a temperature range from 50 to 90 °C.

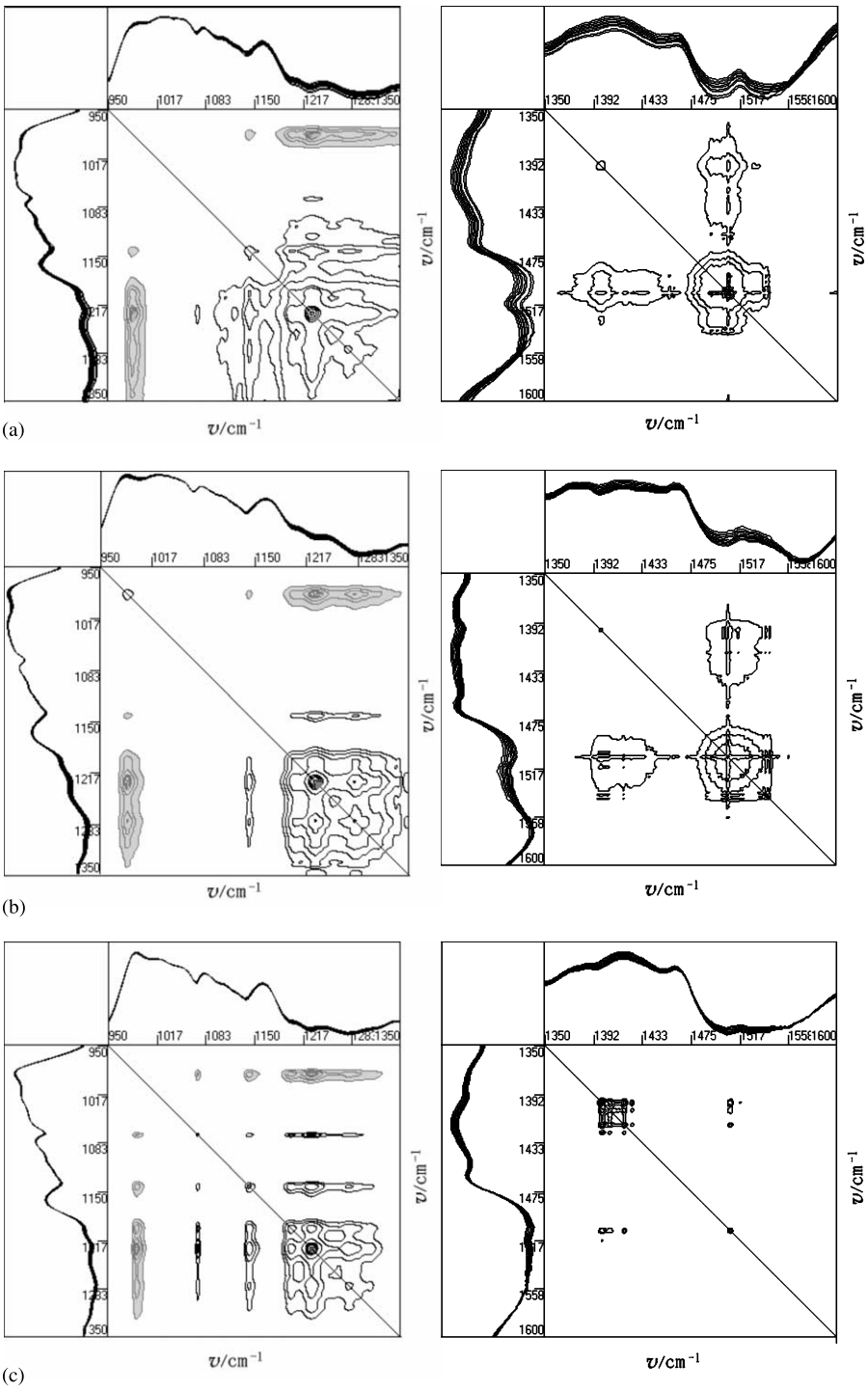

Fig. 9. (a) 2D correlation IR synchronous spectra of *F. walujewii*. (b) 2D correlation IR synchronous spectra of *F. hupehensis*. (c) 2D correlation IR synchronous spectra of *F. thunbergii*. (d) 2D correlation IR synchronous spectra of *F. ussuriensis*. (e) 2D correlation IR synchronous spectra of *F. cirrhosa*.

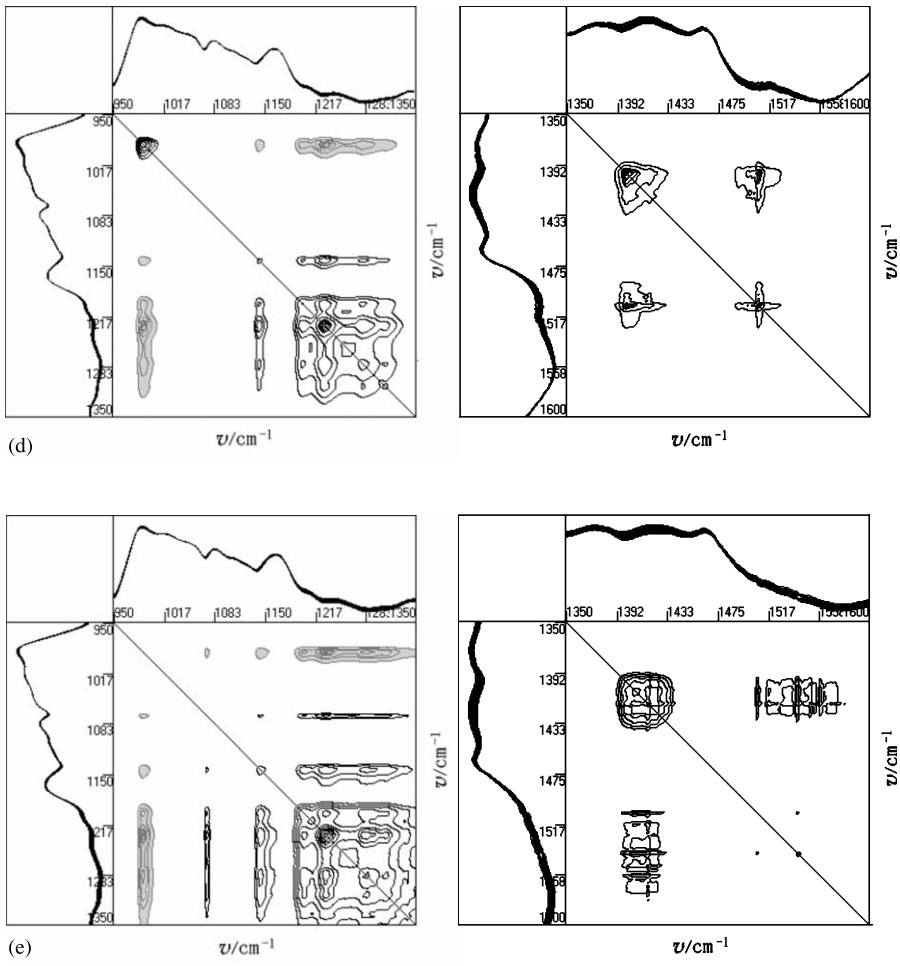

Fig. 9 (*Continued*)

are off-diagonal peaks called cross peaks. Cross peaks appear when the dynamic variations of the IR spectrum at two different wavenumbers are correlated or anticorrelated to each other. For a synchronous spectrum, this occurs when the two IR signals are fluctuating in phase with each other. The synchronized variation of IR absorbance intensities can result from the simultaneous intensity changes of a pair of absorption bands. A positive cross peak represents the consistency of the population changes, either simultaneous increase or decrease, of different groups under an external perturbation. The more coordinated the intensity changes, the stronger the cross peak is. In contrast, a negative cross peak represents the coordinated changes of band intensities in the opposite directions. In other words, the intensity of one band increases, while the other decreases simultaneously. No cross peak at the crossed position of two bands means changes of band intensities are not coordinated at all [7,14].

Based on the 2D-IR correlation principle above, we study Fig. 8 and in particular take Fig. 8a as a representative example. There are three obvious autopeaks in the synchronous spectra between 950 and 1350 cm^{-1}. One is at 1142 cm^{-1}, the other two are at 1228 and 1277 cm^{-1}, respectively. The first peak corresponds to the stretching vibration of 3° –OH groups in *Fritillaria* and is very weak. The latter two correspond to the vibrations of (C–

Table 2
The differences of the auto peak intensities in the 2D correlation spectra of five *Fritillaria*

	–C–O–C– of saponin etc. ($\cong$985 cm^{-1})	3° –OH ($\cong$1400 cm^{-1}, 1150 cm^{-1})	–C–O–C– (1200–1300 cm^{-1})	N-ring (1500–1550 cm^{-1})
F. walujewii	Invisible	Very weak	Middle	Strong
F. hupehensis	Middle	Very weak	Middle	Very strong
F. thunbergii	Weak	Very strong	Very strong	Very strong
F. ussuriensis	Very strong	Very strong	Very strong	Very strong
F. cirrhosa	weak	Middle	Middle	Middle

O–C– groups of *Fritillaria* constituents. The sign of cross peaks among them are positive, suggesting their intensity changes during the heating processes are in the same directions. The autopeak at 985 cm^{-1} is mainly due to the changes of the functional groups consisting of –C–O–C–, and it has close relationship with the components of saponin. *F. walujewii* does not have such an autopeak. There are two noticeable autopeaks at 1402 and 1506 cm^{-1} in the synchronous spectra between 1350 and 1600 cm^{-1}. The former is weak and assigned to the bending of 3° –OH. The latter is rather strong and assigned to the functional groups of N-ring. The positive sign cross peaks suggests the simultaneous decrease of band intensities during the heating process. The 2D-IR spectra of other *Fritillaria* can be analyzed in a similar manner.

Because the auto-peaks in 2D synchronous spectra have close relation with the intensity of corresponding IR peaks, the 2D correlation spectra and FT-IR spectra can be compared and studied together. In the FT-IR spectra, *F. walujewii* and *F. hupehensis* each has a distinct peak at 1515 cm^{-1}, but other *Fritillaria* do not. The discrepancy can be illustrated more clearly and directly in the 2D correlation spectra between 1350 and 1600 cm^{-1}, where the intensity ratio between the auto peaks near 1510 and 1400 cm^{-1} are different. The ratios of *F. walujewii* and *F. hupehensis* are much greater than one. And the ratios of *F. thunbergii* and *F. ussuriensis* are approximately equal to one. But *F. cirrhosa* has an autopeak not near 1510 cm^{-1} (or it is so weak to be seen), but at 1541 cm^{-1} instead, and its intensity is much weaker than the peak at 1417 cm^{-1}.

We also noted that the intensity ratios of auto peaks in 2D IR spectra were not always consistent with the intensity ratios of peaks in FT-IR spectra. The results can explain the differences in the component content of *Fritillaria*, which result in the differences of thermal sensitivity.

F. thunbergii and *F. ussuriensis*, which have almost the same FT-IR spectra, have distinct 2D IR spectra in the range of 950–1350 cm^{-1}. The autopeak at 985 cm^{-1} of *F. thunbergii*, which is assigned to saponin and related substances is relatively weak in its 2D spectrum. On the other hand, such a peak of *F. ussuriensis* is clearly the strongest. The comparison shows the differences in the composition and properties of saponin in the two *Fritillaria.* The saponin and related substances in *F. ussuriensis* apparently have poorer thermal stability. *F. hupehensis*, like *F. walujewii*, has a relatively weak peak at 985 cm^{-1} in FT-IR spectrum. But it has a distinct peak at 985 cm^{-1} in the 2D spectrum, while *F. walujewii* has almost no peak. The result shows the difference in the composition and properties of saponin in the two *Fritillaria*. The saponin and related substances in *F. hupehensis* have a poorer thermal stability.

It can be concluded from the above discussion that the 2D correlation IR spectra not only enlarge the differences in the FT-IR spectra, but also provide useful information for studying the stability of effective components of *Fritillaria*. We can see rather obvious differences in the 2D spectra of different *Fritillaria* and 2D correlation IR spectra have good reproducibility. So the 2D correlation

spectra can be used as characteristic spectra of *Fritillaria*.

Table 2 summarized the differences of the auto peak intensities in the 2D correlation spectra of different *Fritillaria* between 950 and 1600 cm^{-1}.

As mentioned above, the alkaloid and saponin are the main active components of *Fritillaria*, so the stability of the two classes of substances directly determines the medicinal value of *Fritillaria*. These two substances have the similar effects in eliminating phlegm and relieving the cough, which has studied by some research [15,16]. The results of this experiment showed that the alkaloids of different *Fritillaria* have almost the same thermal stability, but saponins do not. *F. hupehensis* is poorer than *F. walujewii* and *F. ussuriensis* is poorer than *F. thunbergii* in thermal stability. So the differences of various *Fritillaria* should be considered in the processing, maintenance, and transportation in order to keep them from deterioration.

4. Conclusions

This study showed the dissimilarities between different *Fritillaria* of geographical origins. Based on the peak shapes and intensities of Fritillaria in FT-IR spectra, we can roughly separate the five Fritillaria. With the help of second derivative spectra, which enlarged the differences of *Fritillaria*, the information about the saponin contents of different *Fritillaria* can be obtained. 2D correlation analysis was applied to the IR spectra of *Fritillaria* observed under different temperatures. The dissimilarities between *Fritillaria* are most clearly seen in the 2D correlation spectra. We combined the information of peaks in FT-IR spectra and autopeaks in 2D spectra, and determined the stability differences of active components in *Fritillaria*. The results provided theoretical basis for preserving *Fritillaria* in the processing, maintenance, and transportation.

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