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Use of X-ray Diffractometry for Identification of Different *Fritillaria* Traditional Chinese Medicine

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Abstract: Comparative studies of different *Fritillaria* Chinese medicinal materials were carried out using X-ray powder diffraction. Due to existence of plenty of starches in *Fritillaria*, which is a very good semicrystalline macromolecule, the X-ray diffraction pattern of *Fritillaria* powders mainly showed the crystal properties of starch. However, the crystal properties of starch contained in *Fritillaria* were highly different due to the differences of species. These differences in crystal properties of starch resulted in the differences in the diffraction pattern. In addition, the differences in other chemical constituents contained in *Fritillaria* also resulted in the differences in the diffraction patterns. All of these differences could be utilized for the discrimination and identification of different *Fritillaria* Traditional Chinese Medicine. In the present research, we conducted a systematic study on eight kinds of *Fritillaria* traditional Chinese medicines by X-ray power diffraction. X-ray diffraction is proved to be a new and powerful method to discriminate *Fritillaria* traditional Chinese medicines from different geographical origins.

Key words: *Fritillaria*, starch, X-ray, crystal type, degree of crystallinity

Introduction

Fritillaria (Chinese name Beimu), the bulbs of various species of the genus *Fritillaria* (Liliaceae), is a very useful Traditional Chinese Medicine (TCM) with antitussive and expectorant functions (Cheetham *et al*., 1998; Chen and Liu, 2004; Gao and Li, 1994; Gao *et al*., 1997). Officially, herbal Beimu is derived from the bulbs of five *Fritillaria* species documented in China pharmacopoeia (Hua *et al*., 2003). These species include *Fritillaria thunbergii* Miq., *Fritillaria ussurensis* Maxim., *Fritillaria pallidifloca* Schrenk, *Fritillaria cirrhosa* D. Don and *Fritillaria hupehensis* Hsiao et K.C. Hsia which are all quality goods. Moreover, *F. cirrhosa* consists of *F. cirrhosa* D. Don, *F. unibracteata* Hsiao et K.C. Hsia, *F. przewalskii* Maxim., *F. delavayi* Franch. The former three are usually named as Song Beimu and Qing Beimu, whereas the later one is named as Lu Beimu according to their different characters. China has a wide range of *Fritillaria* resources which are distributed extensively. The *Fritillaria* Chinese medicinal materials are very easy to confuse, in addition, the size of *Fritillaria* bulb is much relevant to the growing conditions (Li *et al*., 1999). All of these result in the difficulty in original identification of *Fritillaria*.

By far, chromatographic method is mainly utilized for the identification of different *Fritillaria* Chinese medicinal materials. Since there are tens of major bioactive components, which are slightly different due to different growing conditions and geographical origins, we can not select only a limited number of specific constituents as essential evaluative criteria. Indeed, there are some contradicting results concerning the contents of some ingredients contained in the *Fritillaria* in the

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literature (Li *et al*., 2000; 2001). In the holistic theory of traditional Chinese medicine, the medicinal materials take effects in curing diseases as a whole. Any method or technique which destroys the wholeness of the traditional Chinese medicine will not be primarily accepted. Recently, there are some methods which keep the integrity of traditional Chinese medicine for discrimination of *Fritillaria* such as Fourier Transform Infrared spectroscopy (FTIR), differential thermal analysis (DTA) and X-ray diffraction analysis (Lin *et al*., 2001; Lu *et al*., 1997; Song and Zhao, 1997).

X-ray diffraction has the advantages of good fingerprint character and non-destruction. As we all know, the main component in the bulbs of *Fritillaria* is starch which occupied approximately 80% content in the total biomass (Wang *et al*., 2005). Starches separated form different *Fritillaria* showed different physicochemical, morphological, thermal and crystal properties in our previous studies (Wang *et al*., 2006a, b; Xiao, 2002). Starch is a good semicrystalline polymer which shows obvious diffraction peaks in the X-ray diffraction patterns. So, diffraction peaks of starch predominated in the diffraction pattern of *Fritillaria* powder. In order to discriminate the *Fritillaria* Traditional Chinese Medicine, in this study, different *Fritillaria* were studied by using X-ray powder diffraction. By analyzing the differences in the X-ray diffraction data of the *Fritillaria*, we could easily discriminate the *Fritillaria* Chinese medicinal materials.

Materials and Methods

Apparatus

X-ray powder diffraction measurements were done using Panalytical X'Pert Pro diffractometer (PANalytical, Holand).

Samples

Fritillaria thunbergii Miq., *Fritillaria ussurensis* Maxim., *Fritillaria pallidifloca* Schrenk, *Fritillaria cirrhosa* D.Don, *Fritillaria hupehensis* Hsiao et K.C., Song Beimu, Qing Beimu, Lu Beimu were identified and provided by National Institutes for the control of Pharmaceutical and Biological Products.

Procedure

Firstly, all the eight *Fritillaria* bulbs were purified, comminuted to powders which were sieved with 160 mesh sifter and then kept in a desiccator. The eight *Fritillaria* powders were placed in the air for two weeks (in order to balance the water content of *Fritillaria* powder) and then would be tested. The dried *Fritillaria* powders were extracted with 95% ethanol by cold immersion method for 24 h. The supernatant was removed and the settled solid layer was resuspended in 95% ethanol and the cold immersion lasted for 24 h once again. The resulting suspension was filtrated with a G4 type anti-acid filter. The residue was washed with 95% alcohol for several times, allowed to dry at room temperature. The dried residue powder was resuspended with distilled water again and again till the supernatant was transparent. The starch was then collected and dried at room temperature for further use.

Secondly, each sample of *Fritillaria* powder and *Fritillaria* starch was packed tightly in a rectangular glass cell and put the glass cell in the diffractometer. The operating conditions included: the $C_oK|\alpha$tube operated at a fixed power source(40KV, 40mA), Scanning range of [4-60°] for *Fritillaria* powders or [4-40°] for *Fritillaria* starch, $\lambda = 1.78901$, step size of 0.0330, scan step time 30.8451. All X-ray diffraction measurements were done in air, at room temperature. The same measurements were made at room temperature for three times.

Thirdly, the degree of crystallinity of samples was quantitatively. A smooth curve which connected peak baselines was computer-plotted on the diffractograms (Fig. 1). The area above the smooth curve was taken as the crystalline portion and the lower area between smooth curve and the

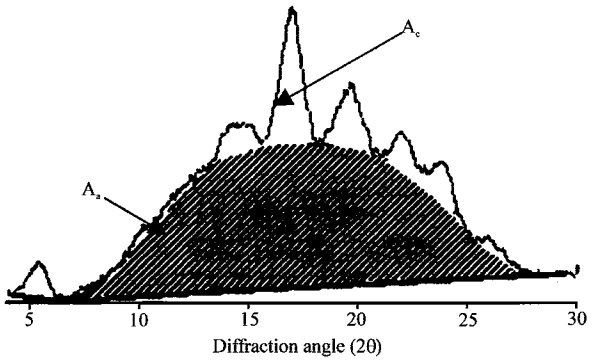

Fig. 1: Calculation of the relative degree of the crystallinity

linear baseline which connected the two points of the intensity 2θ of 30° and 10° in the samples was taken as the amorphous section. The upper diffraction peak area and the total diffraction area over the diffraction angle 10-30°2θ were integrated using Smadchrom software (Morgan and Kennedy Research, Australia). The ratio of upper area to total diffraction was taken as the degree of crystallinity. For this evaluation, we utilized the powders which had almost identical moisture contents (~12%) in order to minimize the effect of different moisture contents on crystallinity.

The Equation of the degree of crystallinity is as follows:

$$X_c = A_c / (A_c + A_a)$$

Where:

X_c refers to the degree of crystallinity; A_c refers to the crystallized area on the X-ray diffractogram; A_a refers to the amorphous area on the X-ray diffractogram.

Lastly, the data reported in all the tables are average of triplicate observations. Statistical comparison of means was conducted using the Student's t-test in a General Linear Model (GLM) procedure on an SAS system (release 8.2, SAS Institute, Cary, NC).

Results and Discussion

X-ray Diffraction Patterns of Fritillaria Powders

X-ray diffraction is an important method to study phase and crystal structure of substance. With the development of X-ray diffraction technology, it is now used for the identification of the Traditional Chinese Medicine (TCM) and TCM preparation. Spectra obtained by this method possess the characteristics of much information, strong fingerprint and stability and reliability. Some of them were utilized to analyze qualitatively according to a set of powder diffraction data cards published by international powder X-ray association board.

The X-ray powder diffractograms of *F. thunbergii*, *F. ussurensis*, *F. palldifloca*, *F. cirrhosa* and *F. hupehensis* were presented in Fig. 2.

All the five *Fritillaria* powders gave the strongest diffraction peak at 20.0°2θ and a few small peaks at around 2θ values of 6.6°, 16.6°, 23.1°, 26.1°, 28.3°, 30.1°. These diffraction peaks were mainly attributed to the semicrystalline starch contained in the *Fritillaria*. This result revealed that crystal type of starches contained in the five *Fritillaria* was a characteristic B-type. As for the *F. thunbergii*, the intensity of diffraction peak at 6.7°2θ was weaker than that of the other *Fritillaria*.

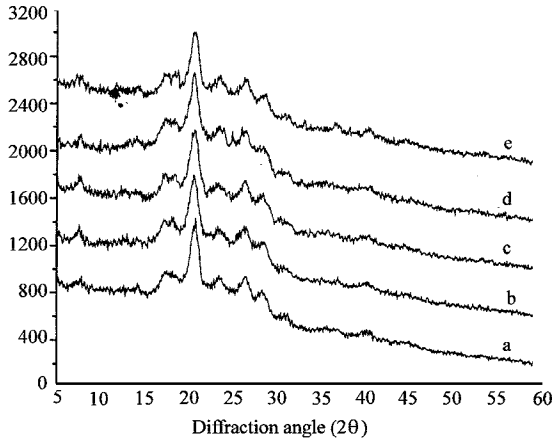

Fig. 2: X-ray diffraction patterns of five *Fritillaria* powders. a: *F. thunbergii*; b: *F. ussurensis*; c: *F. pallidifloca*; d: *F. cirrhosa* and e: *F. hupehensis*

Table 1: Degree of crystallinity of the five *Fritillaria* powders

Samples	Degree of crystallinity (%)
F. thunbergii	49.0b
F. ussurensis	45.2c
F. pallidifloca	54.5a
F. cirrhosa	43.3c
F. hupehensis	55.2a

Mean values with the same letters are not significantly different

F. thunbergii and *F. cirrhosa* only showed a small single-diffraction peak at 16.8°2θ, whereas the single-diffraction peak at 16.8°2θ was divided into two diffraction peaks at 16.4° and 17.3° 2θ for *F. ussurensis*, *F. pallidifloca* and *F. hupehensis*. *F. hupehensis* showed two additional diffraction peaks at around 2θ values of 36.4°and 40.4° while it was only one diffraction peak at about 40.4°2θ for the other *Fritillaria*.

According to the above analysis, the five *Fritillaria* can be sorted into three classes: *F. ussurensis* and *F. pallidifloca* belong to one class, *F. thunbergii* and *F. cirrhosa* belongs to another class, *F. hupehensis* belongs to the third class.

The degree of crystallinity of five kinds of *Fritillaria* powders calculated from the above Fig. 2 were shown in Table 1.

In the light of the degree of crystallinity in Table 1, the five *Fritillaria* can also be divided into three classes: *F. thunbergii* belongs to one class, *F. ussurensis* and *F. cirrhosa* belong to another class, *F. pallidifloca* and *F. hupehensis* belong to the third class. If the differences in characteristic diffraction peaks of *Fritillaria* powders were considered, the five *Fritillaria* could clearly be discriminated.

In order to discriminate the different *F. cirrhosa* better, they were studied by X-ray diffraction analysis. The X-ray diffraction patterns of three *F. cirrhosa* were shown in Fig. 3.

Three *F. cirrhosa* also gave the strongest diffraction peak at 20.1°2θ and a few small peaks at around 2θ values of 6.6°, 16.6°, 23.1°, 26.1°, 28.3°, 30.1°. As for Song Beimu, it showed two obvious diffraction peaks at 16.6°2θ while there was not obvious double peaks for Qing Beimu and Lu Beimu in the X-ray diffraction patterns. The degree of crystallinity of three *F. cirrhosa* calculated from Fig. 3 was shown in Table 2.

The degree of crystallinity of the three *F. cirrhosa* differed significantly (Table 2), Song Beimu showed the highest degree of crystallinity, whereas Qing Beimu showed the lowest degree of crystallinity. According to the difference in the degree of crystallnity of three *F. cirrhosa,* they can be separated into three classes clearly.

Table 2: Degree of crystallinity of the three *F. cirrhosa* powders

Samples	Degree of crystallinity (%)
Qing Beimu	43.0c
Song Beimu	51.4a
Lu Beimu	45.3b

Mean values with the same letters are not significantly different

Table 3: X-ray diffraction data of starches from different *Fritillaria*

	Diffraction peaks at 2θ values (° angle)							
Samples	6°	17°	20°	23°	26°	28°	30°	Degree of crystallinity (%)
F. thunbergii	6.61 (15.52 Å)	16.47 (6.25 Å)	20.08 (5.13 Å)	23.26 (4.44 Å)	26.18 (3.95Å)	28.25 (3.67 Å)	30.93 (3.35 Å)	45.9c
F. ussurensis	6.68 (15.34 Å)	16.81 (6.12 Å)	20.15 (5.11 Å)	22.84 (4.52 Å)	26.06 (3.97 Å)	28.17 (3.68 Å)	30.82 (3.37 Å)	44.7cd
F. pallidifloca	6.76 (15.17 Å)	16.58 (6.20 Å)	20.19 (5.10 Å)	23.18 (4.45 Å)	25.87 (4.00 Å)	28.40 (3.65 Å)	30.78 (3.37 Å)	49.4b
F. cirrhosa	6.65 (15.43 Å)	16.89 (6.09 Å)	20.08 (5.13 Å)	23.11 (4.47 Å)	26.06 (3.97 Å)	28.09 (3.69 Å)	30.78 (3.37 Å)	43.8d
F. hupehensis	6.61 (15.52 Å)	16.47 (6.25 Å)	20.00 (5.15 Å)	22.99 (4.49 Å)	25.91 (3.99 Å)	28.29 (3.66 Å)	30.70 (3.38 Å)	52.3a

[a] Means with the same letter are not significantly different ($p<0.05$)

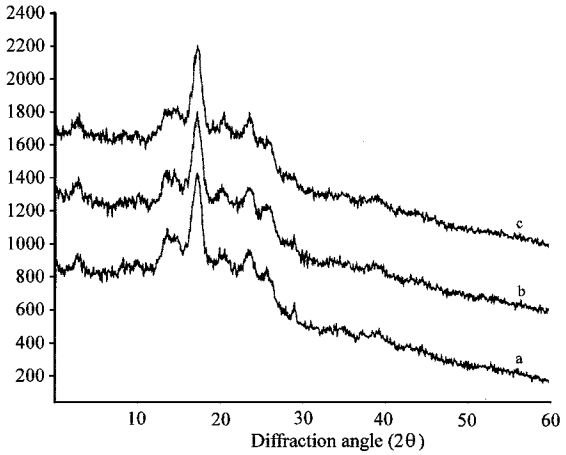

Fig. 3: X-ray diffraction patterns of different *F. cirrhos*a powders. a: Qing Beimu; b: Song Beimu; c: Lu Beimu

X-ray Diffraction Measurements of Fritillaria Starches

In order to further discriminate the different *Fritillaria*, the X-ray diffraction patterns of starch contained in the *Fritillaria* was obtained (Fig. 4).

The corresponding X-ray diffraction parameters and crystallinity level calculated from the ration of diffraction peak area and total diffraction area were given in Table 3. The scattering angle, at which the diffraction intensities can be observed was 2θ and the d spacing was used to discriminate the planes of different sites. The five *Fritillaria* starches showed the highly similar X-ray diffraction patterns. They gave the strongest diffraction peaks at 6.6°and 20°2θ and a few small peaks at around 2θ values of 17°, 23°, 26°, 28° and 30°. These diffraction patterns were basically the same as that of potato starch. This analysis indicated that the crystal type of starches separated from the five *Fritillaria* was a characteristic B-type.

Due to the different origins of *Fritillaria,* starch contained in the bulb of *Fritillaria* had the different crystal properties such as diffraction angle, d spacing and degree of crystallinity. For example, although the crystal type of all the five *Fritillaria* starches was characteristic B-type, diffraction angles

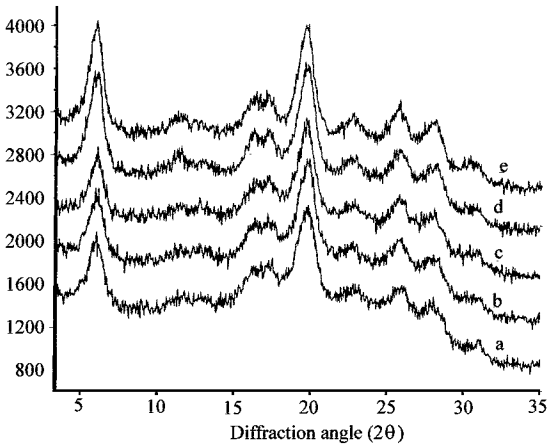

Fig. 4: X-ray diffraction spectra of the five *Fritillaria* starches. a: *F. thubergii*; b: *F. ussurensis*; c: *F. crrhosa*; d: *F. pallidifloca;* e: *F. huphensis*

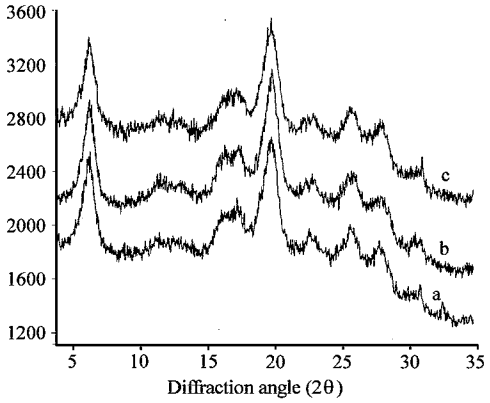

Fig. 5: X-ray diffraction spectra of the three *Fritillaria* starches, a: Qing Beimu., b: Song Beimu, c: Lu Beimu

Table 4: X-ray diffraction data of starches from different *F. cirrhosa*

	Diffraction peaks at 2θ values (° angle)							
Samples	6°	17°	20°	23°	26°	28°	30°	Degree of crystallinity (%)
Qing Beimu	6.65 (15.43Å)	17.50 (5.88 Å)	20.31 (5.07 Å)	22.99 (4.49 Å)	25.95 (3.98 Å)	28.06 (3.69 Å)	30.82 (3.37 Å)	42.6b
Song Beimu	6.61 (15.52Å)	17.66 (5.83 Å)	20.08 (5.13 Å)	22.99 (4.49 Å)	26.14 (3.96Å)	28.21 (3.67Å)	30.78 (3.37Å)	38.4c
Lu Beimu	6.57 (15.61Å)	17.50 (5.88 Å)	19.92 (5.17 Å)	23.07 (4.47 Å)	25.83 (4.00 Å)	28.21 (3.67 Å)	31.05 (3.34 Å)	47.5a

[a] Means with the same letter are not are not significantly different ($p<0.05$)

at 6°, 20° 2θ and corresponding d spacing were different from each other. The crystallinity level also differed significantly each other. According to the above detailed diffraction parameters and crystallinity level of starch from different *Fritillaria*, the five *Fritillaria* could be easily separated.

Although three *Cirrhosa F.* starches showed the highly similar X-ray diffraction patterns (Fig. 5), they still showed the different X-ray diffraction parameters and crystallinity level (Table 4). For example, d spacing at 20° 2θ of Qing Beimu starch was 5.07 Å, whereas they were 5.13 Å and

5.17 Å for Song Beimu and Lu Beimu starches. Further more, degree of crystallinity of starches from different *F. cirrhosa* differed significantly. All of these could provide us information to discriminate different *F. cirrhosa*.

Conclusions

According to the above analysis, we could conclude that different *Fritillaria* from different geographical origins could be identified by X-ray powder diffraction patterns. Due to the differences in types and content of chemical constituents in different *Fritillaria,* they showed the characteristic X-ray diffraction patterns and parameters which could help us discriminated the different *Fritillaria* from different geographical origins. In addition, starch from different *Fritillaria* also showed the different parameters and degree of crystallinity. These differences in the crystalline properties of starch also could provide us the information to discriminate different *Fritillaria.* Because this technology is very advanced and simple, it could be recommended for the discrimination and identification of other Chinese medicine which were very difficult to identify.

References

Cheetham, N.W.A.H. *et al*., 1998. Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. Carbohydrate Polymer, 36: 277-284.

Chen, C.G. and X.H. Liu, 2004. Study on the identification of *Fritillaria* Chinese medicinal materials DTA. Chin. Tradit. Herb Drugs, 35: 210-212.

Gao, W.Y. and Z.L. Li, 1994. The anatomic study of *Fritillaria* Thunbergii in the senescent process. Guihaia, 14: 65-69.

Gao, W.Y. *et al*., 1999. Ultrastructure of amyloplasts and intrecellular transport of old and new scales in *Fritillaria ussuriensis*. J. Plant Biol., 42: 117-123.

Hua, R. *et al*., 2003. Discrimination of Fritillary according to geographical origin with Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. J. Pharm. Biom. Anal., 33: 199-209.

Li, S.L. *et al*., 1999. Pre-column derivatization and gas chromatographic determination of alkaloids in bulbs of *Fritillaria*. J. Chromatogr. A, 859: 183-192.

Li, S.L. *et al*., 2000. Simultaneous determination of seven major isosteroidal alkaloids in bulb of *Fritillaria* by gas chromatography. J. Chromatogr. A, 873: 221-228.

Li, S.L. *et al*., 2001. Determination of the major isosteroidal in bulbs of *Fritillaria* by high-performance liquid chromatography coupled with evaporative light scattering detection. J. Chromatogr. A, 909: 207-214.

Lin, G. *et al*., 2001. Chromatographic analysis of *Fritillaria* isosteroidal alkaloids, the active ingredients of Beimu, the antitussive traditional Chinese medicinal herb. J. Chromatogr. A, 935: 321-338.

Lu, Y. *et al*., 1997. Studies on X-ray Diffraction pattern of traditional Chinese medicinal material. Acta Pharmaceutica Sinica, 92: 193-198.

Pharmacopoeia of the People's Republic of China, Vol. 1, Ministry of Public Health of the People's Republic of China, 2000.

Song, F.K. and Q. Zhao, 1997. Comparison of total alkaloids content of Fritillaria from different origin. J. Huaxi Med., 12: 259-261.

Wang, S.J. *et al*., 2005. New starches from *Fritillaria* species medicinal plants. Carbohyd. Polym., 61: 111-114.

Wang, S.J. *et al*., 2006. Crystallography, morphology and thermal properties of starches from four different medicinal plants of *Fritillaria* species. Food Chem., 96: 591-596.

Wang, S.J. *et al*., 2006. Studies on the morphological, thermal and crystalline properties of starches separated from medicinal plants. J. Food Eng., (In Press).

Xiao, P.G., 2002. Modern Chinese Materia Medica, Chemistry Industry Press, pp: 114.