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Crystallography, morphology, and thermal properties of starch in *Fritillaria thunbergii* and *F. ussurensis* as well as comparison with potato starch

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Abstract: Objective To fully understand the medicinal plants of *Fritillaria* L., the physicochemical properties of starch in two species of *Fritillaria* L., *F. thunbergii* and *F. ussurensis*. were investigated by means of various analytical methods. **Methods** The properties of starch in the two different species of *Fritillaria* L. were compared by X-ray diffraction, scanning electron microscope (SEM) and thermogravimetric analysis (TGA). **Results** The crystal type of starch in the two species of *Fritillaria* L. was the characteristic B-type which was in consistent with that of potato starch. The degrees of crystallinity of *F. thunbergii* starch and *F. ussurensis* starch were about 29.9% and 20.1%, respectively. However, the degree of crystallinity of the potato starch was 44.9%. From the crystallinity degree of the starch in two species of *Fritillaria* L., it could be concluded that the content of amylose in *F. ussurensis* starch was higher than that in *F. thunbergii* starch. The granule size of the starch in two species of *Fritillaria* L. ranged from 5 to 40 μm, which were all smaller than that of the potato. The starch granule in two species of *Fritillaria* L. was in cycloidal or elliptic-shape. It could be concluded that the thermal stability of the starch in two species of *Fritillaria* L. was different due to the different structures of different starch in various plants by TGA. **Conclusion** The physicochemical properties of starch in two different species of *Fritillaria* L. differ a lot due to their geographical origin.

Key words: *Fritillaria* L.; starch; crystallography; morphology; thermal stability

浙贝母和平贝母中淀粉的结晶学、形态学和热性质研究及与马铃薯淀粉的比较

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摘 要：目的 为了能够更加充分了解贝母属药用植物，通过各种分析方法对两种贝母——浙贝母和平贝母中所包含的淀粉的物理化学性质进行了研究。**方法** 采用X射线衍射，扫描电子显微镜(SEM)以及热分析(TGA)的方法对两种贝母中淀粉的性质进行了比较。**结果** 通过研究发现，两种贝母淀粉的晶体类型都为典型的B型，这与马铃薯淀粉的晶体类型是一致的。浙贝母和平贝母淀粉的结晶度分别为29.9%和20.1%，而马铃薯淀粉的结晶度为44.9%。从两种贝母淀粉的结晶度可以看出，平贝母淀粉中直链淀粉的量要高于浙贝母淀粉中直链淀粉的量。两种贝母淀粉的颗粒尺寸为5～40 μm，而且他们都小于马铃薯淀粉的颗粒尺寸。两种贝母淀粉颗粒的形状是圆形的和椭圆形的。热稳定性表明由于植物来源的不同导致淀粉颗粒结构不同，从而热稳定性存在明显的差异。**结论** 两种贝母淀粉由于来源不同，物理化学性质存在明显的差异。

关键词：贝母属；淀粉；结晶学；形态学；热稳定性

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1 Introduction

The bulbs of species of *Fritillaria* L., Beimu, have been used as one of the most important antitussive, expectorant, and antihypertensive drugs in traditional Chinese medicine (TCM) for thousands of years. Various chemical and pharmacological studies on Beimu have demonstrated that the major bioactive ingredients to relieve cough are alkaloids in the bulbs with their types and contents varying in different species of *Fritillaria* L. Up to now, tens of alkaloids in bulbs of *Fritillaria* L. have been investigated and eight isosteroidal alkaloids including epeiedine, ebeiedinone, isovertivine, vertivine, verticinone, hupehenine, ebeienine, and imperialine are established as bioactive components[1~7]. In addition, some non-alkaloid constituents containing saponin, terpenoids, steroids, succinic acid, thymidine, and adenisine in different species of *Fritillaria* L. have also been identified[8].

However, little research has been carried out on the starch in the bulbs of *Fritillaria* L. As reported earlier, the main component in the bulbs of species of *Fritillaria* L. is starch occupying approximately 80% content in the total biomass[9]. This medicinal plant starch, however, has always been ignored and disposed of resulting in the waste of biomass in resources of *Fritillaria* L.

Starch is an important polysaccharide reserved in higher plants. It consists of two main components, amylose and amylopectin. Native starch plays an important role in the food and plastic industry as an inexpensive, renewable, and biodegradable natural resource[10~12]. Different types of starch derived from different plants have different properties. In order to make full use of the medicinal plant resources of *Fritillaria* L., the physicochemical properties of starch in the two species of *Fritillaria* L. were studied comprehensively.

2 Materials and methods

2.1 Plant resources of *Fritillaria* L. and pretreatment: *F. thunbergii* Miq. and *F. ussurensis* Maxim. were provided by Meiwei TCM Company (Anguo, Hebei Province, China) and were identified by Professor Gao Wen-yuan, Tianjin University, China. The native potato starch (16% moisture) containing 30% amylose and 70% amylopectin, was obtained from Xuanwei Runkai Starch Company (Xuanwei, Yunnan Province, China).

The two species of *Fritillaria* L. were cleaned, comminuted to powder, and then dried in an oven and kept in a desiccator. The dried powder was extracted with 85% ethanol at 45 ℃ for 48 h. The sediment was washed with 85% alcohol for several times, and then desiccated at ambient temperature for further use.

2.2 Fourier transform infrared (FT-IR) spectroscopy: IR spectra were obtained with BIO—RAD FTS3000 IR Spectrum Scanner (BIO—RAD, USA). The starch and the powders of *Fritillaria* L. (including those extracted with 85% alcohol for 48 h) were blended with KBr powder, respectively, and pressed into tablets before measurement.

2.3 X-ray powder diffraction measurements: Monochromatic Cu-K_α radiation (wavelength = 0.154 2 nm) was produced by a BDX3300 X-ray Powder Diffractometer (Beijing University Equipment Manufacturer, China). The species of *Fritillaria* L. and starch powder were packed tightly in a rectangular aluminum cell. The samples were exposed to the X-ray beam from an X-ray generator running at 36 kV and 20 mA. The scanning regions of the diffraction angle 2 θ were 10°— 30°, which covered most of the significant diffraction peaks of the starch crystallites. Duplicated measurements were made at ambient temperature. Radiation was detected with a proportional detector.

2.4 Determination of the crystallinity degree: The crystallinity degree of samples was quantitatively estimated following the method of Nara and Komiya (1983)[13]. 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 linear baseline which connected the two points of intensity 2 θ 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° 2 θ were integrated using Smadchrom Software (Morgan and Kennedy Research, Australia). The ratio of upper area to total diffraction was taken as the crystallinity degree.

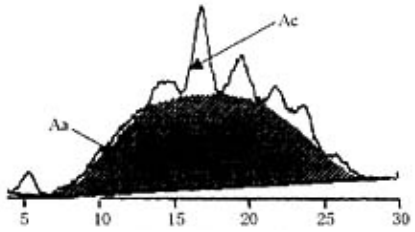


Fig. 1 Calculation of relative crystallinity degree

The equation of crystallinity degree is as follows: $X_c = A_c/(A_c + A_a)$

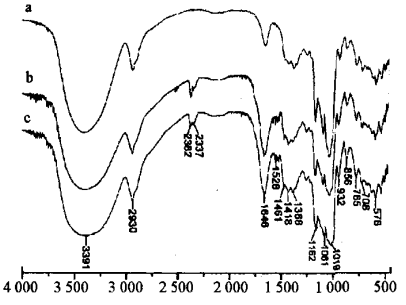
Where: X_c refers to crystallinity degree; A_c refers to crystallized area on X-ray diffractogram; A_a refers to amorphous area on X-ray diffractogram

2.5 Scanning electron microscope (SEM): Analysis of scanning electron micrographs was performed with an environmental scanning electron microscope (ESEM, Philips XL−3). Starch samples were suspended in acetone to obtain a suspension. One drop of the starch-acetone suspension was dropped on a glass slide. The starch was coated with gold powder to avoid electronic charge under the electron beam after the acetone volatilized. An accelerating potential of 30 kV was used during micrography.

2.6 Themogravimetric analysis (TGA): The thermal properties of the samples were measured with a ZTY−ZP type Thermal Analyzer (Beijing University Equipment Manufacture, China). The weight of samples varied from 4−6 mg. The samples were heated from room temperature to 500 ℃ at a rate of 15 ℃/min. The derivatives of TGA thermograms were obtained using origin 6.0 analysis software.

3 Results and discussions

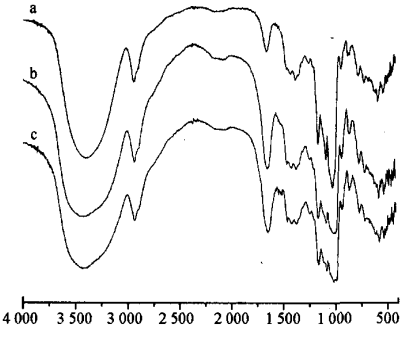
3.1 FT-IR spectroscopy: As shown in Fig. 2, the FT-IR spectra of the two powders of *Fritillaria* L. are similar with each other and that of potato starch. The noticeable differences between the FT-IR spectra of the two powders of *Fritillaria* L. and the potato starch were peaks at 2 362 cm^{-1}, 2 337 cm^{-1} and 1 528 cm^{-1}, which did not appear on the FT-IR spectrum of potato starch. The peaks at 2 362 cm^{-1}, 2 337 cm^{-1} mrght be due to the noise peaks of CO_2 in the test. However, the peak at 1 528 cm^{-1} might be resulted from the small molecule constituents in the two species of *Fritillaria* L. There were three characteristic peaks of starch between 1 019 cm^{-1} and 1 156 cm^{-1}, attributed to C-O bond stretching[14]. The peak near 1 019 cm^{-1} was ascribed to the C-O stretch of C-O-C in starch, and the peaks near 1 081 and 1 156 cm^{-1} were mainly attributed to C-O stretch of C-O-H in starch. There were also three strong peaks at 1 162, 1 081, and 1 019 cm^{-1} in the spectra of the two powders of *Fritillaria* L. indicating that the major components of the two powders of *Fritillaria* L. were starch.



a-potato starch b-*F. thunbergii* powder c-*F. ussurensis* powder

Fig. 2 FT-IR spectra of two powders of *Fritillaria* L. and potato starch

The spectra of potato starch and the two powders of *Fritillaria* L. extracted with 85% ethanol at 45 ℃ for 48 h were presented in Fig. 3.



a-potato starch b-*F. ussurensis* starch c-*F. thunbergii* starch

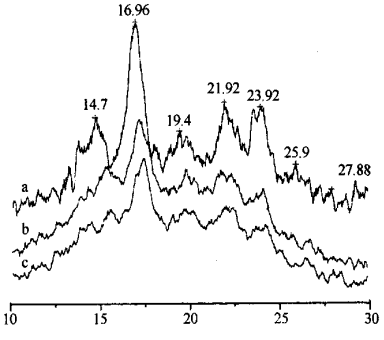
Fig. 3 FT-IR spectra of potato starch and two powders of *Fritillaria* L. extracted with 85 % ethanol at 45 ℃ for 48 h

As illustrated by Fig. 3, the two powders of *Fritillaria* L. extracted with 85% ethanol resulted in the same FT-IR spectra as the potato starch. The peak at 1 528 cm^{-1} in FT-IR spectra (Fig. 2) of the two powders of *Fritillaria* L. disappeared, indicating that some of the small-molecular chemicals in the powders of *Fritillaria* L. were removed

by the ethanol extraction. In other words, the residues of the powders of *Fritillaria* L. after extraction were basically starch.

3.2 X-Ray diffraction analysis: Much of the information about starch crystalline properties was acquired from X-ray powder diffraction studies. Starch can be classified to A, B, and C forms. In the native granular forms, the starch form A was associated mainly with cereal starch, such as maize starch and wheat starch. The X-ray patterns of these kinds of starch gave the stronger diffraction peaks at around 15°, 17°, 18°, and 23°. The starch form B was usually obtained from tuber starch, such as potato starch and canna starch. The strongest diffraction peak of the X-ray diffraction pattern appeared at 17° 2 θ. There were also a few small peaks at 2 θ values of 20°, 22°, and 24°. The starch form C was a mixture of both A and B types, such as smooth-seeded pea starch and various bean starch[14].

The X-ray powder diffractograms for the two starches of *Fritillaria* L. and potato starch were shown in Fig. 4.



a-potato starch b-*F. ussurensis* starch c-*F. thunbergii* starch

Fig. 4 X-Ray diffraction spectra of two starches of *Fritillaria* L. and potato starch

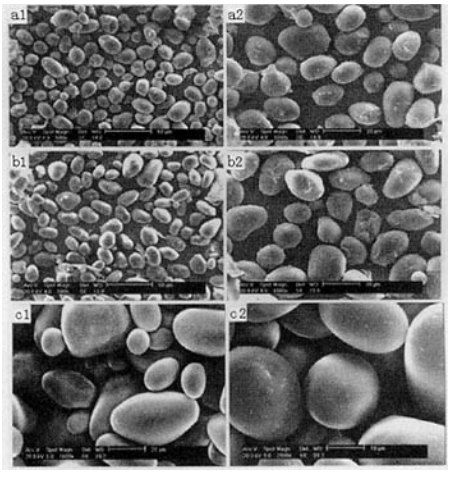
The two starches of *Fritillaria* L. showed the highly similar X-ray diffraction spectra with that of potato starch. As well known, potato starch showed the characteristic B-type pattern. The two starches of *Fritillaria* L. gave the strongest diffraction peak at around 17° 2 θ and a few small peaks at around 2 θ values of 15°, 20°, 22°, and 24°. This result demonstrated that the two starches of *Fritillaria* L. were also characteristic B-type starch.

The crystallinity degree of three kinds of starch calculated from the above figures was shown in Table 1. For this evaluation, starch with the same moisture contents at approximately 10% was used in order to minimize the error between different samples. The crystallinity degree of the potato starch was about 44.9%, and *F. thunbergii* starch and *F. ussurensis* starch were about 29.9% and 20.1%, respectively. Generally, the higher the content of the amylose, the lower the crystallinity degree of the starch. Hence, the content of the amylose in the *F. ussurensis* starch was higher than that in the *F. thunbergii* starch, and the content of amylose in the potato starch was the lowest in the three starches.

Table 1 X-Ray diffraction data of two starches of *Fritillaria* L. and potato starch

Samples	Crystallinity degree/%	Crystal pattern
F. thunbergii starch	29.9	B
F. ussurensis starch	20.1	B
potato starch	44.9	B

3.3 SEM Analysis: Morphology of starches from different plant sources varied with the genotype and cultural practices, depending on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant[15]. The granular structures of potato, *F. thunbergii*, and *F. ussurensis* starch showed significant variations in size and shape when viewed by SEM. Scanning electron micrographs of the starch granules from two species of *Fritillaria* L. and potato were illustrated in Fig. 5.

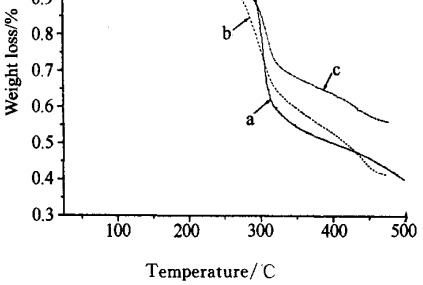


a-*F. thunbergii* starch b-*F. ussurensis* starch c-potato starch

Fig. 5 SEM of *F. thunbergii*, *F. ussurensis*, and potato starch

As shown in Fig. 5, the granule size of the *F. thunbergii* starch ranged from 5 to 40 μm. The average particle size ranged from 5 to 20 μm for small and 20 to 40 μm for large *F. thunbergii* starch granules. The average particle size of individual *F. ussurensis* starch granules was similar to that of the *F. thunbergii*. The average size of potato starch granules, however, was significantly greater than the former two starch granules of *Fritillaria* L., ranging from 25 to 40 μm for small and 40 to 100 μm for large granules. Potato starch granules appeared to be oval and irregular or cuboidal in shape. However, the starch granules were in cycloidal or elliptic-shape for two species of *Fritillaria* L. According to the X-ray diffraction analysis, the crystal pattern of the starch in the two species of *Fritillaria* L. was characteristic B-type. B-Type starch granules were roughly spherical or polygonal in shape. Physicochemical properties, such as light transmittability, amylose content, water-absorption capacity and swelling power of the starch granules varied, depending on the granule size and starch origin of *Fritillaria* L.

3.4 Thermal properties: The thermograms of the two species of *Fritillaria* L. and potato starch were presented in Fig. 6.



a-potato starch b-*F. ussurensis* starch c-*F. thunbergii* starch

Fig. 6 TGA thermograms of two species starch of *Fritillaria* L. and potato

Two well-defined shifts were observed in the TGA curves. The first shift, at around 100 ℃, was produced by water evaporation in the three kinds of starch; the second shift started at 200 ℃, resulting from the thermal degradation of starch occurred. The process continued gradually up to 400 ℃.

The thermal stability of the two species starch of *Fritillaria* L. and potato was different from each other, due to the different structures of starch from various plants. Comparatively, the thermal stability of the *F. ussurensis* starch was the best among the three kinds of starch. Further research on the inner structure of starch of *Fritillaria* L. will be carried out in the near future.

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