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Food Chemistry 96 (2006) 591–596
Food Chemistry
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Crystallography, morphology and thermal properties of starches from four different medicinal plants of *Fritillaria* species

Wang Shujun [a], Gao Wenyuan [a,*], Jia Wei [b], Xiao Peigen [c]

[a] College of Pharmaceuticals and Biotechnology, Tianjin University, Tianjin 300072, China
[b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200030, China
[c] Institute of Medicinal Plant, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, China

Received 27 September 2004; received in revised form 31 January 2005; accepted 7 March 2005

Abstract

To fully understand the medicinal plant, *Fritillaria*, and its species, we investigated the physical properties of starch contained in four *Fritillaria* species, *Fritillaria thunbergii* Miq., *Fritillaria ussurensis* Maxim., *Fritillaria pallidifloca* Schrenk and *Fritillaria cirrhosa* D.Don, by means of various analytical methods. The crystal type of the former three kinds of *Fritillaria* starches was in characteristic B-type, which was in agreement with the crystal type of potato starch. However, the *cirrhosa F.* starch showed a typical C_B-type pattern. The degrees of crystallining of the four *Fritillaria* starches were about 43.2%, 40.5%, 44.8% and 41.8%, corresponding to *thunbergii F.* starch, *ussurensis F.* starch, *pallidifloca F.* starch and *cirrhosa F.* starch. The granule sizes of the former two *Fritillaria* starches ranged from 5 to 40 μm, and were cycloidal or elliptic-shaped. However, the latter two *Fritillaria* starch granules had granule sizes ranging from 5 to 50 μm, and the granule shape varied from oval to irregular or cuboidal. From the thermogravimetric analysis, it was concluded that the thermal stabilities of the four kinds of starch differed from each other, due to their different structures.

Keywords: *Fritillaria*; Starch; Crystallography; Crystallinity; Morphology; Thermal stability

1. Introduction

Fritillaria (Chinese name Beimu), the bulbs of various species of the genus *Fritillaria* (Liliaceae), have been used as anti-tussive and expectorant herbs using the Chinese name "Beimu" in traditional Chinese medicine (TCM) for more than 2000 years (Li et al., 1999, Li, Li, Lin, Chan, & Ho, 2000, 2001; Lin, Li, Li, & Chan, 2001).The chemical constituents of Beimu have been extensively investigated, including alkaloids, saponin, terpenoids, steroids, succinic acid, thymidine, and adenosine (Li, Li, & Lin, 1999; Ruan, Zhang, & Wu, 2002). However, the starch contained in the bulb of *Fritillaria* has hardly been studied and always wasted. As reported earlier, the main component in the bulbs of *Fritillaria* species is starch constituting approximately 80% of the total biomass (Gao, Fan, & Paek, 1999). This plant starch, however, has always been ignored and disposed of, resulting in waste of biomass of *Fritillaria* resources.

Starches from different plant sources, such as corn, rice, wheat and potato, have been studied for several centuries. The physical properties of the native starch, such as crystallinity, morphology and thermal stability are very different from each other, due to the inner structure of the starch granule. Starch is an important polysaccharide reserve in higher plants. It consists of two main components, amylose and amylopectin. Amylose is an α-(1 → 4)-D-glucopyranosyl polymer, with linear or lightly branched structures or a mixture of both. The residues in amylopectin are α-(1 → 4)-D-glucopyranose units with α-(1 → 6)-linkages at intervals of

* Corresponding author. Tel.: +86 022 87401895.
E-mail address: edwinwa@hotmail.com (W. Gao).

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doi:10.1016/j.foodchem.2005.03.014

approximately 20 units, depending on plant sources (Atichokudomchai & Varavinit, 2003; Meera & Sun-ja, 2002; Navdeep & Narpinder, 2003). In order to make full use of the *Fritillaria* medicinal-plant resources and provide more information for the taxonomic research in *Fritillaria* species, we investigated the four *Fritillaria* starch properties by modern analytical methods.

2. Materials and methods

2.1. Sources and pretreatment of the four Fritillaria samples

Fritillaria thunbergii Miq., *Fritillaria ussurensis* Maxim., *Fritillaria pallidifloca* Schrenk and *Fritillaria cirrhosa* D.Don were provided by Meiwei TCM company (Anguo, Hebei province, China) and were identified by Professor Gao Wenyuan, Tianjin University, China.

The four *Fritillaria* were cleaned, comminuted to powders, which were then dried in an oven, and kept in a desiccator. The dried powders were refined with 85% alcohol at 25 °C for 48 h. The sediment was rinsed with 85% alcohol several times and then desiccated at ambient temperature for further use.

2.2. Fourier transform infrared (FT-IR) spectroscopy

The IR spectra were obtained with a BIO-RAD FTS3000 IR Spectrum Scanner (BIO-RAD, USA). The starch and the two *Fritillaria* powders (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 = 1.542 Å) was produced by a BDX3300 X-ray powder diffractometer (Beijing University Equipment Manufacturer, China). The *Fritillaria* and starch powders were packed tightly in a rectangular aluminium cell. The samples were exposed to the X-ray beam from a X-ray generator running at 36 V 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. Other operation conditions included: step interval 0.02, scan rate 2°/min, Sollet and divergence slit, 1°, receiving slit, 1°, and scattering slit, 0.15°. Duplicate measurements were made at ambient temperature. Radiation was detected with a proportional detector.

2.4. Determination of the degree of crystallinity

The degree of crystallinity of samples was quantitatively estimated, following the method of Nara and Komiy (1983). 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 linear baseline, which connected the two points of the 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 degree of crystallinity.

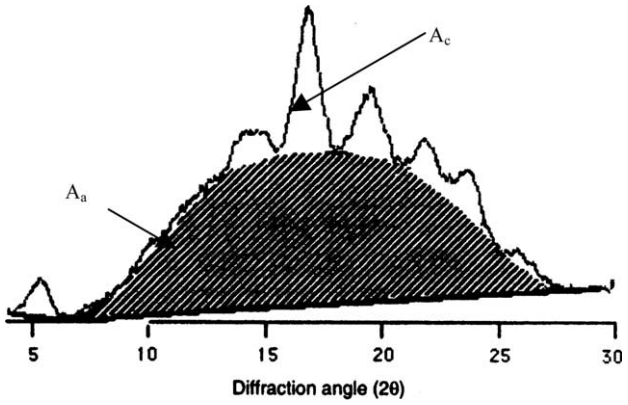

Fig. 1. Calculation of the relative degree of the crystallinity.

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.

2.5. Scanning electron microscope (SEM)

Analysis with scanning electron micrographs (SEM) 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 charging under the electron beam after the acetone volatilized. An accelerating potential of 30 V was used during micrography.

2.6. Thermogravimetric 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 to 5 mg. The samples were heated from ambient temperature to 500 °C at a rate of 15 °C/min. The derivatives of TGA thermograms were obtained using origin 6.0 analysis software.

3. Results and discussion

3.1. Fourier transform infrared (FT-IR) spectroscopy

As shown in Fig. 2, the FTIR spectra of the four *Fritillaria* powders are similar. There were three characteristic peaks of starch between 1019 and 1156 cm^{-1}, attributed to C–O bond stretching (Fang, Fowler, Tomkinson, & Hill, 2002). The peak near 1019 cm^{-1} was ascribed to the C–O stretch of C–O–C in starch, and the peaks near 1081 and 1156 cm^{-1} were mainly attributed to C–O stretch of C–O–H in starch. In Fig. 2, there were also three noticeable and strong peaks at 1162, 1081 and 1019 cm^{-1} in the spectra of the four *Fritillaria* powders. However, the characteristic absorption peaks of the small-molecule chemical ingredients in the *Fritillaria* were hardly displayed in the FTIR spectra of *Fritillaria*. These results revealed that the main component in four *Fritillaria* powders was starch.

The spectra of the four *Fritillaria* powders extracted with 85% alcohol at 25 °C for 48 h are presented in Fig. 3. The residue after extraction was called *Fritillaria* starch.

The four *Fritillaria* powders extracted with 85% alcohol showed very similar FTIR spectra. The two peaks at 2362 and 2337 cm^{-1} in the FTIR spectra (Fig. 2) of the four *Fritillaria* powders disappeared. These two peaks were due to the noise peaks of CO_2 in the test. What is more, the absorption peak at 1528 cm^{-1} in the FTIR spectra of the *F. thunbergii* and *F. ussurensis* powders also vanished. The result indicated that some of the small-molecule chemical components contained in the *Fritillaria* powder were removed with the alcohol extraction. In other words, the residue of the *Fritillaria* powders after extraction was mainly starch.

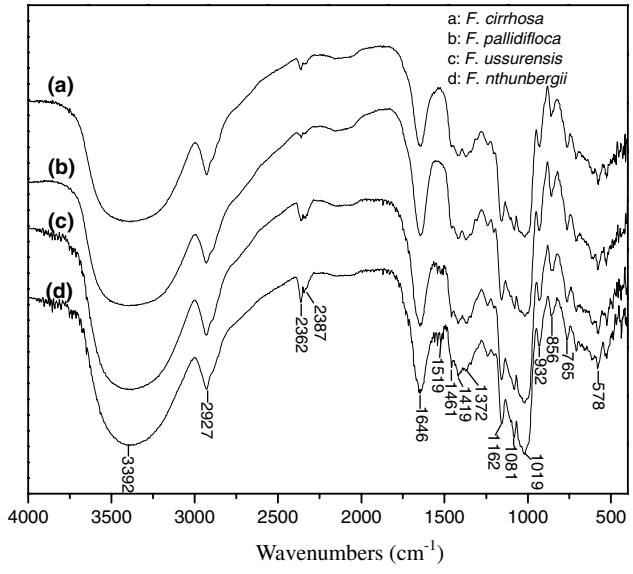

Fig. 2. FTIR spectra of the four *Fritillaria* powders.

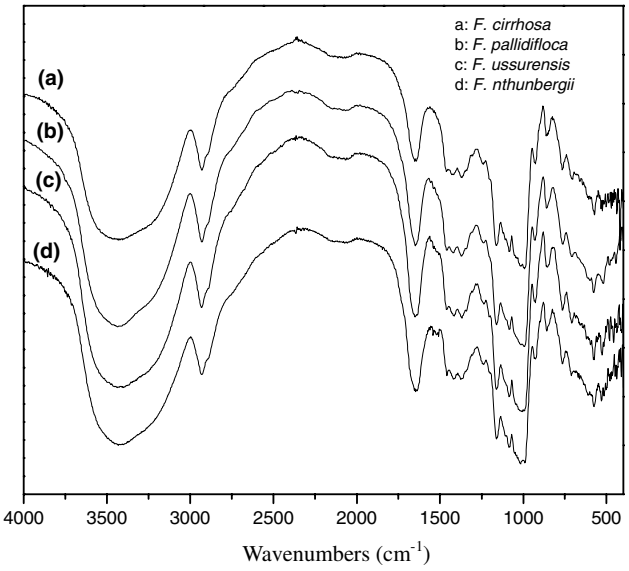

Fig. 3. FTIR spectra of the four *Fritillaria* starches.

3.2. Crystal properties of four Fritillaria starches

The X-ray diffractograms of the four *Fritillaria* powders are presented in Fig. 4. The three *F. thunbergii*, *F. ussurensis* and *F. pallidifloca* powders showed the strongest diffraction peak at 17.2°, 2θ, and a few small peaks at around 2θ values of around 15.3°, 19.6°, 22.2°, 24.4° and 26.4°. This result indicated that the crystal type of starches contained in the three *Fritillaria* powders is a characteristic B-type. The other small peaks were mainly caused by the small-molecule crystal components contained in *Fritillaria* (including alkaloid, saponin and polysaccharides). However, the X-ray diffraction spectrum of the *F. cirrhosa* powder

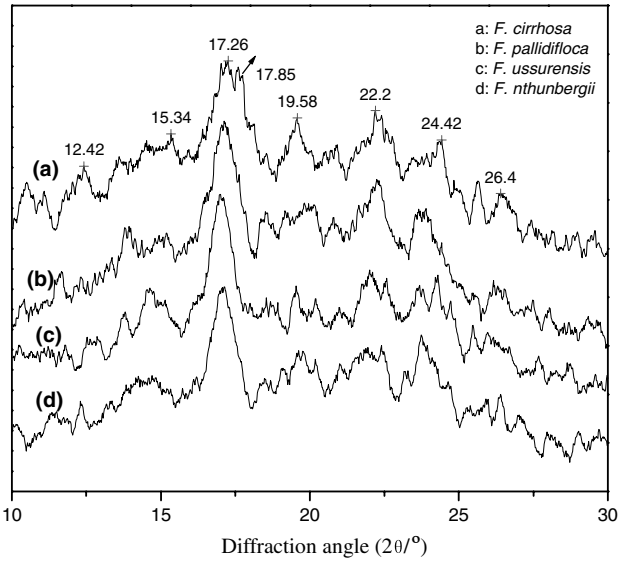

Fig. 4. X-ray diffraction spectra of the four *Fritillaria* powders.

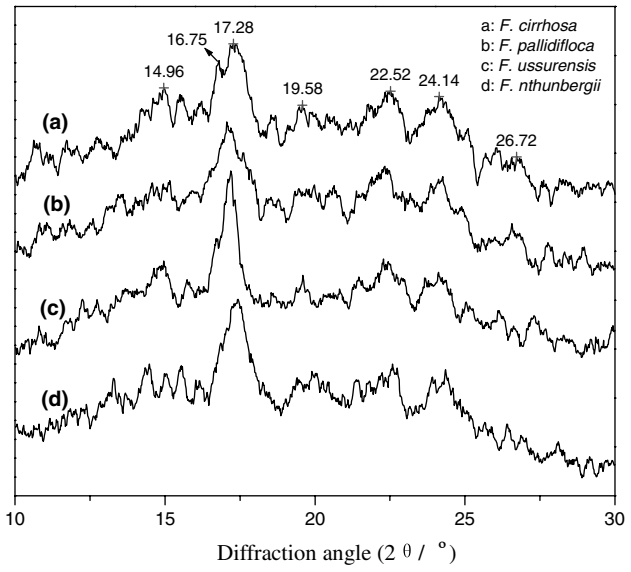

Fig. 5. X-ray diffraction spectra of the four *Fritillaria* starches.

was somewhat inconsistent with that of the other three *Fritillaria* powders. For example, the strongest diffraction peak at 17.2°, 2θ, in the other three *Fritillaria* powders was converted into two small peaks at 17.2° and 17.8°. This was indicative of A-type, while the other diffraction peaks at 19.6°, 22.2°, 24.4° and 26.4° are characteristic of B-pattern. This analysis indicated that the crystal type of starches contained in *F. cirrhosa* was classed as C_B-type, a mixture of both A and B types.

In Fig. 5, X-ray diffraction spectra of the four *Fritillaria* powders extracted with 85% alcohol are displayed.

Consistent with the above analysis, the X-ray diffraction spectra of the *F. thunbergii*, *F. ussurensis* and *F. pallidifloca* starches show the characteristic B-type pattern. The peak at 17.0°, 2 θ, was stronger than the other peaks at 15.0°, 19.6°, 22.5° and 24.1°, 2 θ, in the X-ray diffraction spectra of the three *Fritillaria* starches. For *F. cirrhosa* starch, there are also two small peaks at 16.8° and 17.3°, 2θ, which is also an indicative of A-type. The peaks at 15.0°, 19.6°, 22.5° and 24.1°, 2 θ are the characteristic peaks of B-type starch. So, the starch contained in *F. cirrhosa* was classified as C_B-type.

The degree of crystallinity of four kinds of starches, calculated from the above figure are shown in Table 1.

Table 1
X-ray diffraction data of the four *Fritillatia* starches

Samples	Degree of crystallinity (%)	Crystal pattern
F. thunbergii	43.2	B
F. ussurensis	40.5	B
F. pallidifloca	44.8	B
F. cirrhosa	41.4	C_B

For this evaluation, we utilized the powders which had almost identical moisture contents (~15%) in order to minimize the effect of different moisture contents on crystallinity.

3.3. Morphological properties of four Fritillaria starches

The granular structures of four *Fritilllaria* starches show significant variations in size and shape when viewed by SEM. Scanning electron micrographs of the starch granules from different medicinal plants of *Fritillaria* species are illustrated in Fig. 6.

The granule sizes of the *F. thunbergii* and *F. usssurensis* starch were variable and ranged from 5 to 40 μm. The average granule size ranged from 5 to 20 μm for small and 25 to 40μm for large *F. thunbergii* starch granules. The granule surface appeared smooth, cycloidal or elliptic-shaped for *F. thunbergii* and *F. ussurensis* starches. These two *Fritillaria* starches showed the presence of a fairly large number of large-sized, elliptic-shaped granules. However, the granule size of the *pallidifloca F.* starch was very variable and ranged from 5 to 50 μm. The average granule size of starch ranged between 5 and 15 μm for small and 20–50 μm for large starch granules. The surface of the starch granule was somewhat unsmoothed. Quiet differently from the above two *Fritillaria* starch granules, the *F. pallidifloca* starch had the small-sized granule in much larger numbers. The morphology of the *F. cirrhosa* starch granule was very different from the former three *Fritillaria*. The shape of the *F. cirrhosa* starch granule was variable, elliptic, oval, bread-shaped and stone-shaped. When observed under a scanning electron microscope, the surface of the granules from the *F. cirrhosa* appeared to be less smooth than the former three *Fritillaria* starch granules.

The difference in the granule morphology may be attributed to the biological origin, biochemistry of the amyloplast and physiology of the plant (Badenhuizen, 1969; Svegmark & Hermansson, 1993). Physicochemical properties, such as percent light transmittance, amylose content, swelling powder and water-binding capacity, were significantly correlated, with the average granule size of the starches different from other medicinal plants of *Fritillaria* sources.

3.4. Thermal stability

The TG thermograms of the four *Fritillaria* starches are presented in Fig. 7.

As shown in Fig. 7, two well-defined shifts were observed in the TG curves. The first shift, at around 100 °C, was produced by water evaporation in the four *Fritillaria* starches; the second shift started at 250 °C, and, as the thermal degradation of starch occurred, the process continued gradually up to 500 °C.

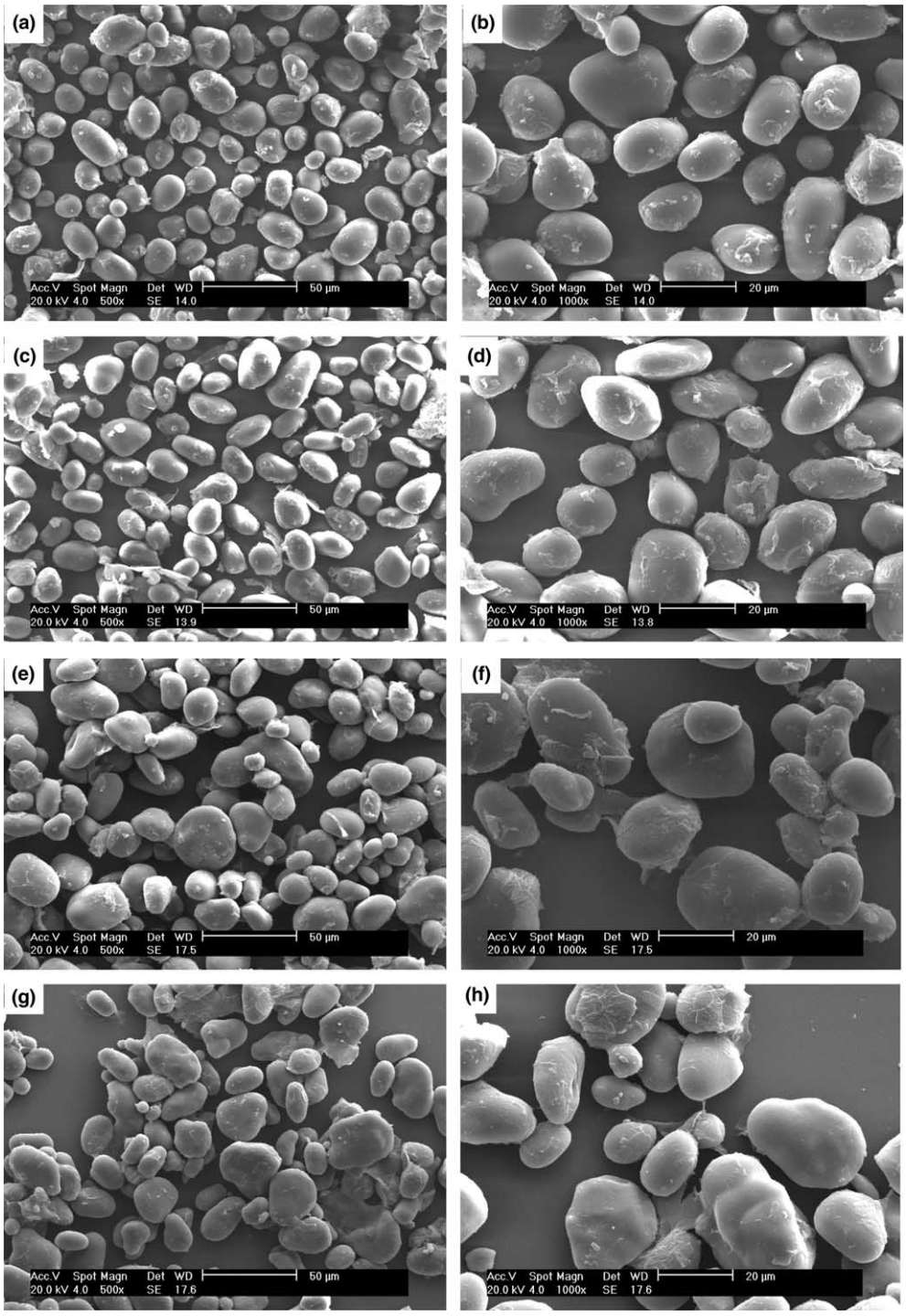

Fig. 6. SEM of *F. thunbergii*, *F. ussurensis*, *F. pallidifloca* and *F. cirrhosa* starches. (a) *F. thunbergii* 500×; (b) *F. thunbergii* 1000×; (c) *F. ussurensis* 500×; (d) *F. ussurensis* 1000×; (e) *F. pallidifloca* 500×; (f) *F. pallidifloca* 1000×; (g) *F. cirrhosa* 500×; (h) *F. cirrhosa* 1000×.

The thermal stabilities of the four *Fritillaria* starches were different. Overall, the thermal stability of *thunbergii F.* starch was the highest of all four *Fritillaria* starches; the others, in sequence, were: *F. pallidifloca*, *F. cirrhosa*and *F. ussurensis* starch. The discrepancy in thermal stability of the four *Fritillaria* starches was mainly due to difference in the inner starch structure. Further research on the inner structure of *Fritillaria* starch will be carried out in the near future.

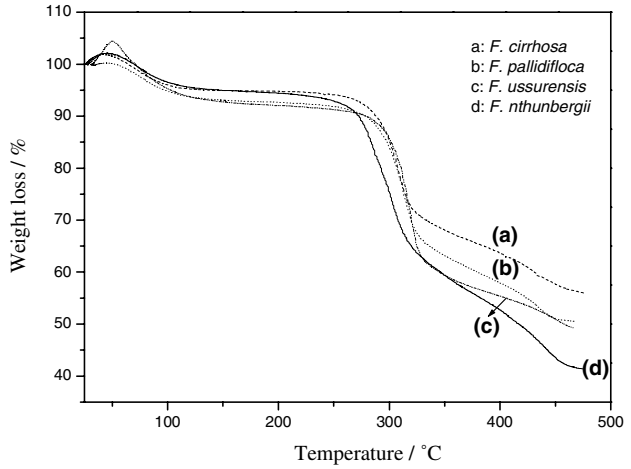

Fig. 7. TG thermograms of four *Fritillaria* starches.

4. Conclusion

The four *Fritillaria* starches differed significantly in morphological, thermal and crystal properties. The granule sizes of starches separated from *F. thunbergii* and *F. ussurensis* were smaller than those of the other two *Fritillaria* starches. The shape of the starch granules varied from round and oval to irregular and cuboidal. The crystal type of the *F. thunbergii*, *F. ussurensis* and *F. pallidifloca* starches were typical B-type, while *F. cirrhosa* starch was C_B-type . The thermal stabilities of the four *Fritillaria* starches differed significantly.

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