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Preparation and physicochemical properties of carboxymethyl *Fritillaria ussuriensis* Maxim. starches

Xia Li [a], Wen-yuan Gao [a,*], Li-jing Huang [a,b], Yan-li Wang [c], Lu-qi Huang [d], Chang-xiao Liu [c]

[a] School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China
[b] School of Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
[c] Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China
[d] Institute of Chinese Materia Medica, China Academy of Chinese Medicinal Sciences, Beijing 100200, China

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ABSTRACT

Starch isolated from an under-utilized Liliaceae plant (*Fritillaria ussuriensis* Maxim.) was carboxymethylated. Influences of reaction parameters were investigated on the degree of substitution (DS). The optimal molar ratio of NaOH/AGU and MCA/AGU is 2.43 and 1.03, respectively. The highest values of the DS obtained when the carboxymethylation was performed at 60 °C for 2.5 h. Scanning electron microscopy (SEM) showed that after carboxymethylation, the granular appearance of the native starch was distorted and many alveolate holes were seen. The new bands at 1608 cm^{-1} and 1426 cm^{-1} in Fourier transform infrared (FT-IR) indicated that the starch granules were substituted. Wide-angle X-ray diffractometry revealed that crystallinity was reduced significantly after carboxymethylation. Differential scanning calorimetry (DSC) suggests loss of crystallinity after carboxymethylation. The thermal behavior of the starches was investigated using thermogravimetric analysis (TGA). The results revealed that the decomposition temperature of the starch decreased from 316 °C to 252 °C and at a low decomposition speed.

1. Introduction

Fritillaria (Chinese name Beimu), the bulbs of various species of the genus *Fritillaria* L. (Liliaceae), have been used as one of the most important anti-tussive, expectorant, and antihypertensive herbs in traditional Chinese medicine (TCM) for more than 2000 years. In the 2005 edition of *Chinese Pharmacopoeia*, *F. ussuriensis* Maxim. (Ping-Beimu) was officially recorded. Steroidal alkaloids, saponins, flavonoids, adenosine, thymine deoxyriboside and other chemical ingredients were established to be the major biologically active components in various *Fritillaria* (Li, Lin, Chan, & Li, 2001; Lin, Li, Li, & Chan, 2001; Ruan, Zhang, & Wu, 2002).

However, the main component in the bulbs of *Fritillaria* L. species is starch occupying approximately 80% content in the total biomass (Gao, Fan, & Paek, 1999). Starch separated from different medicinal plants *Fritillaria* has been investigated for physicochemical (e.g. amylose content, swelling power, solubility, water binding capacity, and turbidity), morphological (including shape and size), thermal, and crystalline properties (Wang, Gao, Chen, & Xiao, 2006a; Wang, Gao, Jia, & Xiao, 2006b).

Unfortunately, using starch in its native form is often limited by certain undesirable characteristics such as poor solubility, low mechanical properties and instability at high temperature, pH and shear during processing. Hence it is always reasonable to modify it to suit specific industrial process. Chemical modification of starch involves reaction of the hydroxyl groups on the anhydroglucose unit (AGU) and these have been used to produce starch derivatives based on oxidation (Wang & Wang, 2003), acetylation (Amparo, Julie, Ben, David, & Elliot, 2009), hydroxypropylation (Olayide, 2009), carboxymethylation (Lawal, Lechner, & Kulicke, 2008a, 2008b) and cross-linking (Luo, Huang, Fu, Zhang, & Yu, 2009).

Modified starches possess some unique properties such as the solubility in unheated water, specific changes in rheological profiles, lower gelatinization temperature, less retrogradation, pH stability, etc. (Debasis, Rekha, & Pushpa, 1995; Lawal, Lechner, Hartmann, & Kulicke, 2007). Among starch derivatives, carboxymethyl starches (CMS) have attracted a lot of attention in recent years. They are usually prepared by a reaction between native starch and chloroacetic acid in an alkaline condition (Qiu & He, 1999; Stojanovic, Jeremic, & Jovanovic, 2000).

The sodium salt of CMS, also called CMS, is mainly used as a thickening agent, with applications in both the food and non-food industry (Roberts, 1967). CMS is used as a disintegrant, called sodium starch glycolate, in the pharmaceutical industry (Bolhuis, Zuurman, & Wierik, 1997), and as a sizing and printing agent in the textile industry (Ragheb, EI-Sayied, & Hebeish, 1997). The objectives of this work were to prepare carboxymethyl *F. ussuriensis* starches with different degree of substitution and to evaluate

* Corresponding author. Tel./fax: +86 22 8740 1895.
E-mail address: biochemgao@hotmail.com (W.-y. Gao).

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their physicochemical properties as potential functional materials for functional food and pharmaceutical industries.

2. Material and methods

2.1. Materials

Fritillaria ussuriensis Maxim. was provided by Xiangfeng TCM company (Yichun, Heilongjiang Province, China) and was identified by Professor Wenyuan Gao, Tianjin University, China. NaOH, monochloroacetic acid, ethanol were purchased from Guangfu Chemical Company (Tianjin, China).

2.2. Isolation of starches

The *F. ussuriensis* Maxim. was cleaned, comminuted to powders with a plant micro-muller, which were sieved with 160 mesh sifter and then kept in a desiccator. The dried powders were extracted with 95% ethanol by cold immersion method for 6–8 h. The supernatant was removed and the settled solid layer was resuspended in 95% ethanol and recentrifuged 3–5 times. After depositing, the supernatant was removed by suction and the settled starch layer was resuspended in distilled water. After seven or eight cycles of depositing and resuspension repeatedly, the slurry containing starch was centrifuged at 3000 rpm for 20 min. The supernatant was discarded and the upper non-white layer was removed. The white layer was resuspended in distilled water and recentrifuged 3–5 times. The starch suspension obtained was dried in a convection oven at 50 °C until weight constancy (Wang et al., 2008).

2.3. Preparation of carboxymethyl starch

For the preparation of carboxymethyl *F. ussuriensis* starch, NaOH and monochloroacetic acid (MCA) of different quantities (3–8 g) were added to 80 mL 80% (v/v) ethanol separately in a 500 mL three-necked round-bottomed flask equipped with motor driven stirrer and the mixture was stirred at 50 rpm. The temperature was raised to between 35 and 65 °C. Starch (10 g dry wt) was added to the mixture. After hydrolysis for some time, acetic acid was added to the slurry to reduce the pH to 6.0–7.0, finally the starch was isolated by filtration. The slurry obtained was dried in an oven at 40 °C for 48 h (Lin, Gao, Chen, & Cai, 2005).

2.4. Determination of degree of substitution

Titrimetry was used for the determination of the DS. CMS (5 g) was dispersed in acetone (150 mL) and 5 M HCl (15 mL) was added to the dispersion which was stirred for 30 min. During this process, the sodium form CMS was converted to the H-CMS (carboxymethyl starch in hydrogen form). H-CMS was washed four times with 80% (v/v) methanol until the solution became neutral with pH test. The neutral dispersion was filtered again, suspended in acetone and it was stirred for another 15 min, following which it was filtered and dried for 24 h in a desiccator over silica gel. 2 g of H-CMS was dissolved in 1% (w/v) NaCl solution and it was titrated with 1 M NaOH. The DS was determined as follows:

$$\mathrm{DS} = \frac{n_{\mathrm{NaOH}} \times M_0}{m_c - n_{\mathrm{NaOH}} \times M_R}$$

$$m_c = m_p - \left[\frac{m_p \times F}{100}\right]$$

where M_0 = molar mass of the anhydroglucose unit = 162 g/mol; M_R = molar mass of carboxymethyl residue = 58 g/mol; n_{NaOH} = quantity of sodium hydroxide used (mol); m_p = weight of polymer taken (g); m_c = corrected weight of polymer (g); F = moisture (%).

2.5. Morphological properties

For SEM analysis, starch samples were suspended in acetone to obtain a 1% suspension. One drop of the starch–acetone suspension was applied on a glass slide. After the acetone volatilized, the samples were then coated with gold powder to avoid charging under the electron beam and analyzed for starch granule shape and size using an environmental scanning electron microscope (ESEM, Philips XL-30). An accelerating potential of 20 kV was used during micrography.

2.6. Fourier transform infrared (FT-IR) spectroscopy

The IR spectra were obtained with a BIO-RAD FTS3000 IR Spectrum Scanner (Bio-Rad, USA). The native starch and CMS of different DS were blended with KBr powder, respectively, and pressed into tablets before measurement.

2.7. X-ray diffractometry

Crystallography of native *F. ussuriensis* starch and CMS were studied by a Rigaku D/max 2500 X-ray powder diffractometer (Rigaku, Tokyo, Japan) with Nickel filtered Cu-Ka radiation (λ = 1.54056 Å) at a voltage of 40 kV and current of 200 mA. The scattered radiation was detected in the angular range of 3–40° (2θ), with a scanning speed of 8° (2θ)/min and step size of 0.06° (2θ).

2.8. Differential scanning calorimetry (DSC)

The thermal analyses of starch samples were carried out using a differential scanning calorimeter (Pyris/Diamond DSC-7, American). About 10 mg of the dried, ground sample was heated from 20 to 300 °C at a rate of 10 °C/min in a nitrogen atmosphere. The glass transition temperature (T_g) and melting temperature (T_m) were recorded as the inflection point of the increment of specific heat capacity in the DSC curves.

2.9. Thermogravimetry analysis (TGA)

The thermogravimetric measurements were performed with a TG apparatus (Diamond TG-DTA, American). Samples of about 10 mg were heated from 20 to 500 °C at a rate of 10 °C/min and a flow rate of 25 ml/min in a nitrogen atmosphere.

2.10. Statistical analysis

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).

3. Results and discussion

3.1. Effect of various molar ratios of NaOH to starch

The effect of various molar ratios of NaOH to starch is presented in Fig. 1A. As observed, the initial increase in the molar ratio of NaOH/AGU favorably increased the DS until it reached 2.43. Thereafter, reductions were observed in both the DS. During the carboxymethylation process, NaOH is used in the formation of starch alkoxide and it also facilitates the swelling of starch for enhanced larger surface area for the etherification process. This accounts for the increase in the DS. However, there is a tendency

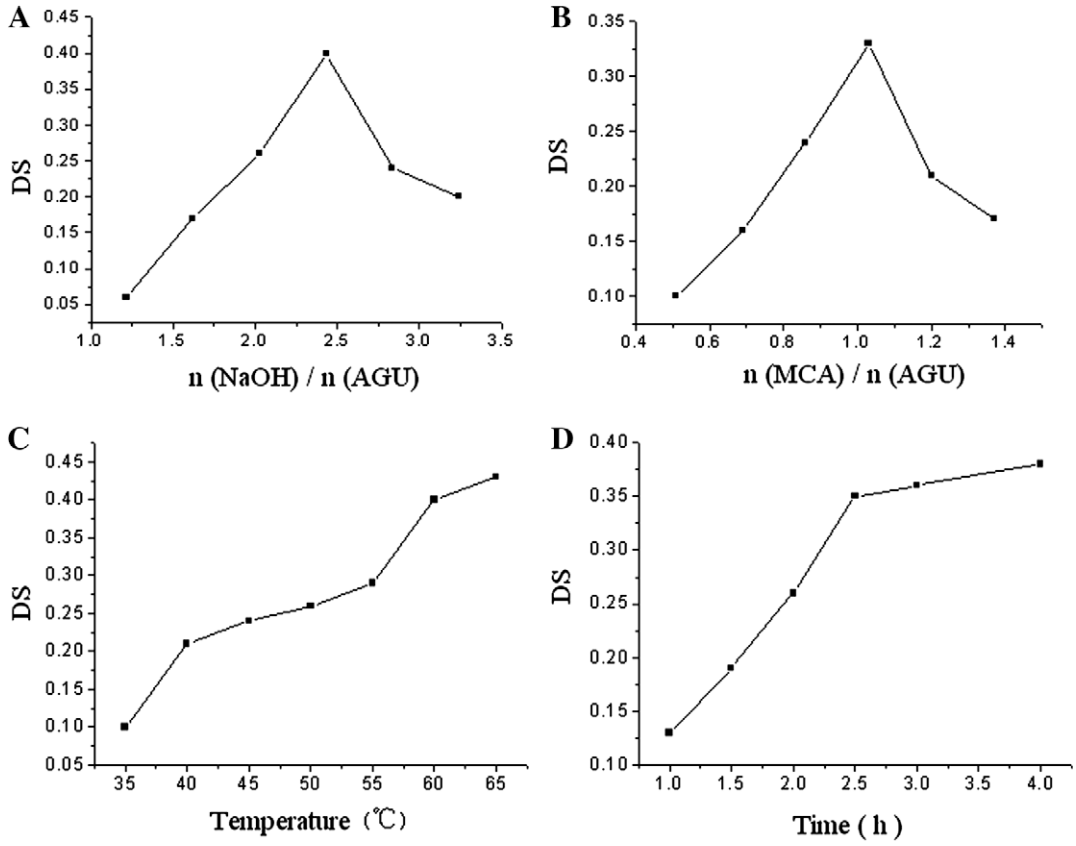

Fig. 1. The effect of reaction parameters on the DS for the carboxymethylation of *F. ussuriensis* starch. (A) Molar ratios of NaOH to starch (AGU); (B) molar ratios of MCA to starch (AGU); (C) different reaction temperatures (35–65 °C); (D) different reaction times (1.0–4.0 h). n_{NaOH}, moles of sodium hydroxide; n_{MCA}, moles of sodium hydroxide; n_{AGU}, moles of anhydroglucose unit; DS, degree of substitution.

for alkaline gelatinization as the amount of NaOH in the reaction mixture increases. This means inhibition of contact between starch etherifying agents within the reaction mixture, this development could account for the reduction in the DS at higher ratio of NaOH to starch. Similar observation was reported for corn and amaranth starches (Bhattacharyya, Singhal, & Kulkarni, 1995).

3.2. Effect of various molar ratios of MCA to starch

Keeping other parameters constant, studies showed progressive increases in the DS as the amount of MCA added to the reaction mixture increased (Fig. 1B). Contrarily, the reaction efficiency declined with increase in molar ratio of MCA to starch. Increase in the DS could reasonably be attributed to increased contact between the starch molecules and the etherifying agent as the concentration of MCA increased. However at higher MCA/AGU ratio >1.03), the DS decreased and it is the maximal value recorded within the range studied (Khalil, Hashem, & Hebeish, 1990).

3.3. Effect of temperature

The influence of different reaction temperature (35–65 °C) on DS was shown in Fig. 1C. The DS values went through a maximum when the etherification temperature was enhanced. Higher temperature increases the solubility and diffusion of the etherifying reagents and swelling of starch molecules. The proportion of molecules with higher energy than the activation energy increased as the temperature increased and this also contributes to higher rate of reaction. An attempt was made to investigate preparation of CMS at higher temperatures (60 °C). However, gelatinization occurred, stirring was difficult and it was also difficult to remove the product. The influence of carboxymethylation, particularly in alkaline medium, caused the gelatinization at lower temperature. No dramatic increases in the DS were observed above 60 °C (Kittipongpatana, Sirithunyalug, and Laenger, 2006).

3.4. Effect of duration of reaction

The effect of reaction time on DS is presented in Fig. 1D. The study revealed that the DS increased with increase in reaction time. Longer duration of reaction enhanced dissolution and diffusion of the reagents hence carboxymethylation was enhanced. Also, it is reasonable that swelling of starch molecules increased with time of reaction because of longer stay in reaction medium. However, no remarkable further increases were observed in DS after 2.5 h of reaction. It may be reasonable to keep the reaction at 2.5 h to safe time and the cost of production.

3.5. Scanning electron microscope (SEM)

The scanning electron microscope images of native *F. ussuriensis* starch and CMS were presented in Fig. 2. The granules of unmodified *F. ussuriensis* starch surface appeared smooth, round or elliptic-shaped, which suggests that the method of extraction and drying did not cause significant damage to the starch. However, after carboxymethylation, the granular appearance of the native starch was distorted. Most of the CMS granules appeared to be distorted and wrinkled. Some big groups were formed during the carboxymeth-

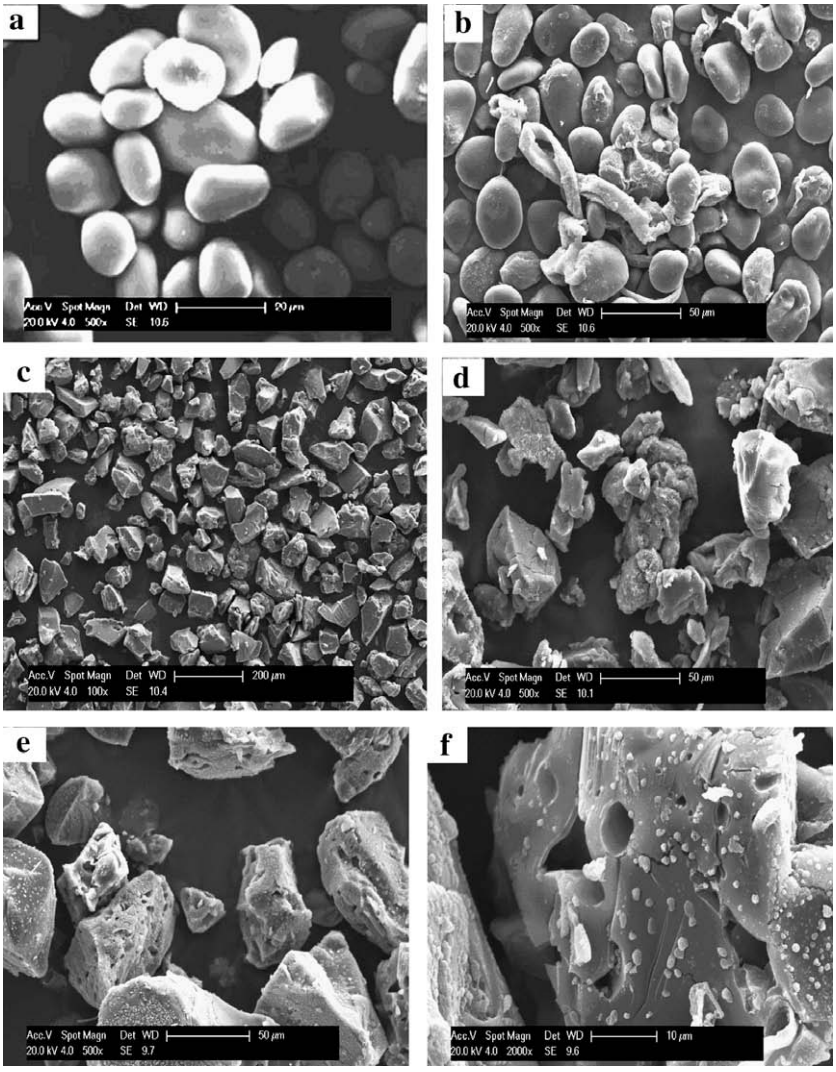

Fig. 2. The SEM images of native *F. ussuriensis* starch and CMS: (a) Native *F. ussuriensis* starch; (b) DS = 0.13; (c) DS = 0.25; (d–f) DS = 0.42.

ylation process, and a number of holes were appeared on the granule surface. This observation suggests that carboxymethylation affects the structural arrangement of the starch. It is reasonable that the strong alkaline condition used for the synthesis caused granular disintegration. Similar observations have been reported for cassava carboxymethyl starches in the literature (Bolhuis et al., 1997; Ragheb et al., 1997).

3.6. Fourier transform infrared (FT-IR) spectroscopy

The infrared spectra of native *F. ussuriensis* starch (DS = 0.00) and a representative carboxymethyl starch (DS = 0.06, 0.42) are presented in Fig. 3. The band stretch around 3500 cm^{-1} is attributed to hydrogen-bonded hydroxyls on the starch molecules. The band at $\nu = 2927\ cm^{-1}$ is attributed to CH_2 symmetrical stretching vibrations. The spectral pattern in the region 970 cm^{-1} and 1200 cm^{-1}, typical of starch (Battacharyya, Singhal, & Kulkarni, 1995), is preserved in all the CMS derivatives. In the native starch, the band at $\nu = 1658\ cm^{-1}$ is assigned to scissoring of two O–H bonds of absorbed water molecules. In the carboxymethyl starch, the intense band at $\nu = 1608\ cm^{-1}$ (DS = 0.42) and 1598 cm^{-1} (DS = 0.06) are assigned to carbonyl functional group. The new band confirms that carboxymethylation took place on the starch molecules. Similar observations were reported for carboxymethylated water yam (Lawal et al., 2008a, 2008b).

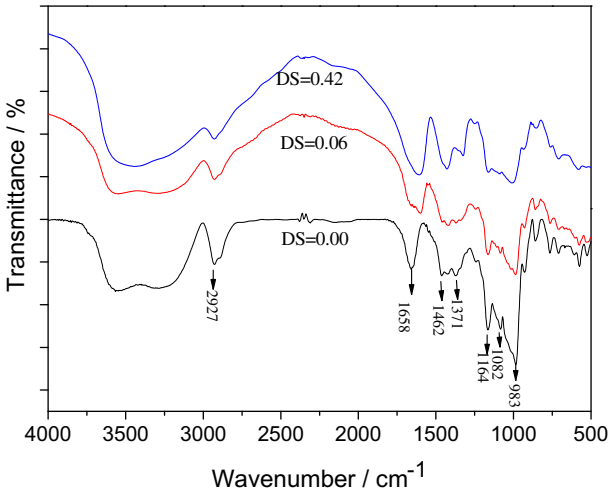

Fig. 3. The FT-IR spectra if native *F. ussuriensis* starch and CMS.

3.7. X-ray diffractometry (XRD)

The wide-angle X-ray diffractograms obtained for both native *F. ussuriensis* starch and the representative carboxymethylated derivative (DS = 0.13, 0.33, 0.42) are presented in Fig. 4. The native *F. ussuriensis* starch 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°. This analysis indicated that the crystal type of *F. ussuriensis* starch was a characteristic B-type (Wang et al., 2007). After carboxymethylation, a pronounced reduction in crystallinity was observed. This observation corroborates the changes in the granule morphology revealed by the SEM. This loss in crystallinity could be attributed to the effect of the alkaline environment during the modification (Kittipongpatana et al., 2006). The results also suggested that the loss of crystallinity could be due to the rupture of starch granules, which in the presence of water together with heat treatment caused the breakage of chemical bonds in starch molecules. Compared with other carboxymethylated starches, a loss in crystallinity of mungbean starch after carboxymethylation has been reported (Debasis et al., 1995). The loss of crystallinity would mean enhanced ability for carboxymethyl starch or other polymer products made from it to absorb water. This could be harnessed in the production of products such as super-absorbent hydrogels.

3.8. Differential scanning calorimetry (DSC)

The differential scanning calorimetry thermograms of *F. ussuriensis* starch and a representative CMS are presented in Fig. 5. T_g and T_m of the native starch were changed by the carboxymethylation process. T_g of the native starch was more than 250 °C and that of the CMS was 224 °C, which is lower than the native *F. ussuriensis* starch. This change can be explained by the fact that the intermolecular hydrogen bonds, which stiffen the macromolecular chain, decreased with the partial replacement of hydroxyl groups by carboxymethyl group. What's more, the increase in the free volume within the molecules due to the introduction of bulk groups that allows more molecular mobility also contributes to the reduction in T_g of starch with carboxymethylation. The decrease in the T_g can increase the starch thermoplasticity, which limited the utilization of native *F. ussuriensis* starch.

3.9. Thermogravimetry analysis (TGA)

The TG curves for the native *F. ussuriensis* starch and CMS are shown in Fig. 6. Thermo-physical parameters provide vital information about the thermal stability of polymeric materials. Such information is needed for reasonable industrial applications of polymers (Menard, 2000, chap. 8). The native *F. ussuriensis* starch showed a two-stage weight loss below 500 °C, with the first minor one corresponding to the loss of intramolecular water around

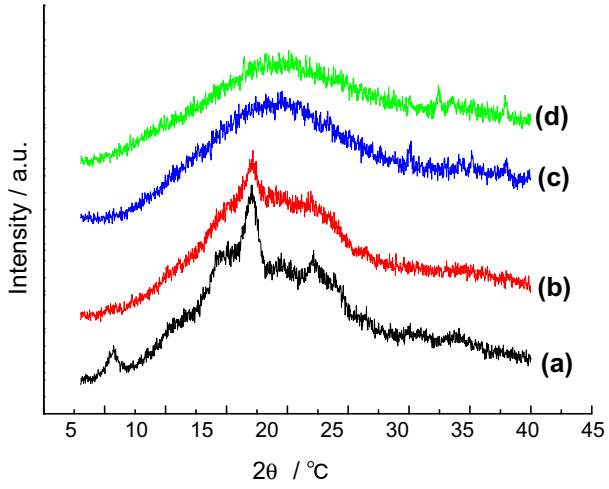

Fig. 4. The XRD graph of native *F. ussuriensis* starch and CMS: (a) Native *F. ussuriensis* starch; (b) DS = 0.13; (c) DS = 0.33; (d) DS = 0.42.

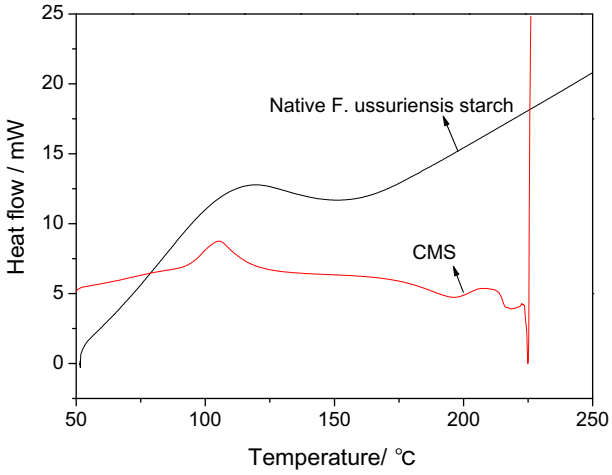

Fig. 5. The DSC curves of native *F. ussuriensis* starch and CMS.

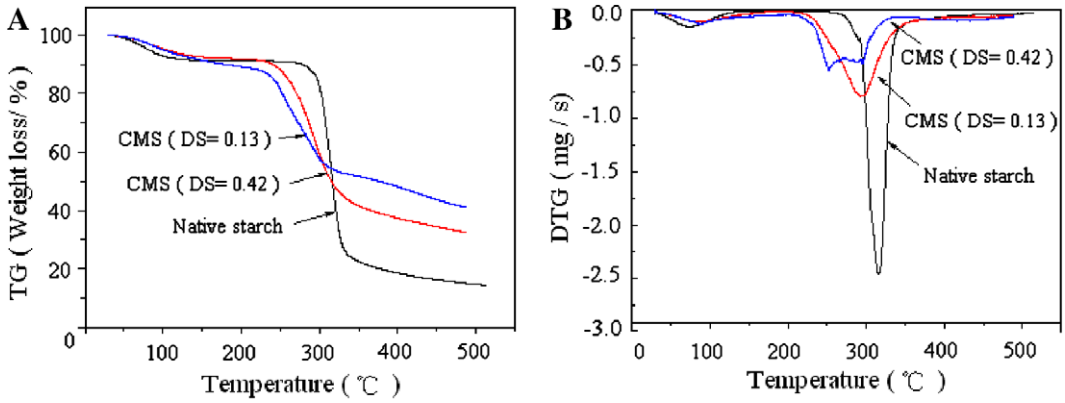

Fig. 6. The TG–DTG curves of native *F. ussuriensis* starch and CMS.

60–120 °C, and the second one corresponding to the starch material decomposition. Water is the main product of decomposition at temperatures below 320 °C. Further heating up to 500 °C resulted in carbonization and ash formation. The native *F. ussuriensis* starch began to decompose at 310 °C, and underwent 58% weight loss at 362 °C. The studies indicate that the maximum degradation occurred within the range 252–378 °C and the peak as indicated by the DTG (DTG_{max}) was at 316 °C. Also, the representative carboxymethyl starch showed a two-stage decomposition process of 12.3% and 44.5%. It is instructive that initial decomposition temperature (IDT) of carboxymethyl starch is lower than the IDT of the native. The reason for this development is the substitution of the hydroxyl groups on the native starch with carboxymethyl group after carboxymethylation. The main decomposition mechanism of starch is the dehydration reaction between starch hydroxyls; this suggests that the smaller the amount of hydroxyl group left on the starch, the more the stable it is. This position was also corroborated in the higher thermal stability of methylcellulose compared with the unmodified cellulose (Filho et al., 2007). From the DTG curves we can observe that the range of representative CMS (DS = 0.13, 0.42) maximum decomposition were within 210–389 °C and 216–392 °C (DTG_{max} = 252, 293 °C), respectively. The results also indicate that the CMS decomposed earlier but more slowly than native starch. This phenomenon can be attributed to the honeycomb-like holes on the surface of CMS granule, but the granule size was bigger than that of the native starch, so it took a long time to decompose the internal part.

4. Conclusion

The first preparation of carboxymethyl derivative of *F. ussuriensis* starch is reported in this work. The DS values of CMS could be controlled by the additional parameters such as temperature, reaction time, amount of NaOH and monochloroacetic acid and so on. Structure modification resulted in significant changes of the physicochemical properties of the starch. The SEM revealed that carboxymethylation affected the structural arrangement of the starch and many alveolate holes were seen on the granule surface. Thermal stability increased after modification and a tendency for increased water absorption was also observed because of enhanced amorphous component. For technical applications, such as the preparation of super-absorbent hydrogels, preparation of biopolymer based flocculants, drag-reduction biomaterials, drug-release and other applications where bio-based polymers are relevant, carboxymethyl *F. ussuriensis* starch could be strategic because the source material is the ignored component in the bulbs of *Fritillaria* species. This investigation should provide relevant information to biopolymer-based industries and the research of practical applications of modified starch is a hot topic in our future work.

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