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Carboxymethyl Chinese yam starch: synthesis, characterization, and influence of reaction parameters

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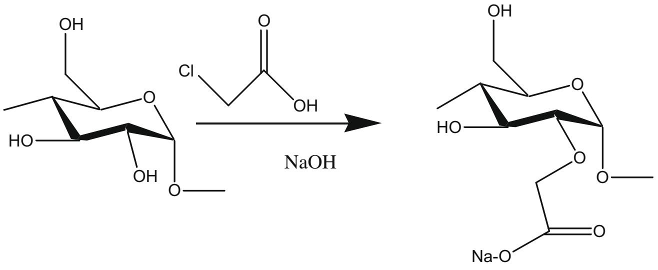
ABSTRACT

Carboxymethyl starch (CMS) was obtained as a product of the reaction of starch and monochloroacetic acid (MCA) in the presence of sodium hydroxide. The influence of the molar ratio of NaOH/AGU, the molar ratio of MCA/AGU, the reaction time, reaction temperature, and the water content on the degree of substitution (DS) was studied. The optimal molar ratio of NaOH/AGU and MCA/AGU is 2.4 and 1.0, respectively. Increase of the ratio of NaOH/AGU or MCA/AGU leads to an increase in DS, but only to certain extent. The highest values of the DS were obtained when the carboxymethylation was performed at 60 °C for 2.5 h. The water content in the reaction media ethanol was optimal at 20% (v/v). The scanning electron micrographs (SEMs) revealed that the carboxymethylation affected the structural arrangement of the starch and caused granular disintegration. The particle size distribution (PSD) also displayed that the average particle diameter increased greatly after modification from 37.37 μm to 72.88 μm. Wide angle X-ray diffractometry (XRD) revealed that starch crystallinity was obviously reduced after carboxymethylation. The new bands at 1600 cm^{-1} and 1426 cm^{-1} in FT-IR indicated that the starch granules were substituted.

1. Introduction

Starch is one of the major polysaccharides in plants and is in the form of granules that exist naturally within the plant cells. Starch separated from different cultivars of Chinese Yam (*Dioscorea opposita* Thunb.) has been investigated for physico-chemical (e.g., amylose content, swelling power, solubility, water-binding capacity, and turbidity), morphological (including shape and size), thermal, and crystalline properties in the previous study.[1,2] 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. Starch modification can overcome these shortcomings by means of altering the structure and affecting the hydrogen bonding of amylose and amylopectin in a controllable manner to enhance and extend starch application.[3] Among starch derivatives, carboxymethyl starches (CMS) have attracted a lot of attention in recent years.

CMS are usually synthesized by the reaction of starch with monochloroacetic acid or its sodium salt after activation of the polymer with aqueous NaOH in an aqueous organic solvent, in most cases ethanol. This method is based on Williamson's ether synthesis:



The reaction is usually carried out in the presence of strong bases to increase the nucleophilicity of the hydroxyl group and to aid the swelling of the starch particles. However, a side reaction could also occur with sodium hydroxide, producing sodium glycolate at the expense of starch glycolate.

$$NaOH + Cl{-}CH_2COONa \rightarrow HO{-}CH_2COONa + NaCl$$

The DS is an indication of the amount of carboxymethyl group formed on the starch molecule. Technically, the DS is defined as the average number of substituent per anhydroglucose unit (AGU). In my experiment, titrimetry was used for the determination of the DS. The functional properties of CMS are dependent on the DS. Such properties include the viscosity of the solution,

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film-forming properties, interaction with cations, formation of supramolecular aggregates, and rheological properties. In the literature, it was reported that the industrially applied carboxymethylation of starch in ethanol produces substitution in the order C-2, C-6, C-3.[4]

As an anionic polymer it has many unique properties. Carboxymethyl starches of corn, potato, wheat, sweet potato and so on have been produced.[5–10] However the authors are not aware of any previous publication on synthesis and characterization of carboxymethyl yam starch (CMS). Therefore, we studied the carboxymethylation process of yam starch. The main reaction parameters that are expected to affect the process of chemical modification were investigated in a systematic manner. These included reaction temperature, ratio of organic solvent to water, reaction time, and molar ratio of sodium hydroxide and monochloroacetic acid to AGU ($n_{\mathrm{NaOH}}/n_{\mathrm{AGU}}$ and $n_{\mathrm{MCA}}/n_{\mathrm{AGU}}$). The investigations explored the influence of these parameters on the DS of CMS by changing one of the parameters while others are kept constant. Consequently, the aim of the present work is not only to prepare carboxymethyl yam starch of higher DS with optimal carboxymethylation conditions, but also to study characterization of yam carboxymethyl starch as well as morphological (including shape and size) and crystalline properties. We are convinced that the information we present here would be relevant for various industrial applications of this biomaterial.

2. Results and discussion

2.1. Influence of various molar ratios of NaOH to starch (AGU) on the DS

The influence of various molar ratios of NaOH to starch on the DS is presented in Figure 1. Different quantities of NaOH (the molar ratios of NaOH to AGU increased from 1.2 to 3.2) were added to 80 mL of 80% (v/v) ethanol for each case, while other reaction conditions were kept constant. The temperature was kept at 45 °C, starch (10 g, dry weight) was added to the mixture and it was stirred until well distributed. Then MCA (5 g) was added to the mixture and the reaction mixture was kept for 2 h. As observed, the initial increase in the molar ratio of NaOH/AGU favorably increased the DS until it reached 2.4. During the carboxymethylation process, the NaOH provides the alkaline environment for the reaction as well as serves as the swelling agent to facilitate diffusion and penetration of the etherifying agent to the starch granular structure. Further increase in NaOH dosage caused an inactivation of MCA and hence it is consumed in the side reaction.[11] This observation is consistent with previous reports on carboxymethyl corn and amaranth starch.[12]

2.2. Influence of various molar ratios of MCA to starch on the DS

The influence of molar ratio of MCA to starch on DS is presented in Figure 2. NaOH (5 g) was added to 80 mL of 80% (v/v) ethanol for each case, while other reaction conditions were kept constant. The temperature was kept at 45 °C, starch (10 g, dry weight) was added to the mixture. Then different quantities of MCA (the molar ratios of MCA to AGU increased from 0.4 to 1.4) were used and the reaction mixture was kept for 2 h. The DS increased with increasing MCA/AGU ratio, suggesting that formation of sodium glycolate is favored within that range. However at higher MCA/AGU ratio (>1.0), the DS decreased and it is the maximal value recorded within the range studied.[13] This is because the increasing MCA/AGU ratio could lead to usage of NaOH, while under the reaction condition, the NaOH dosage is changeless. The more the MCA is, the less the NaOH can react with AGU, so higher MCA/AGU ratio leads to lower DS.

2.3. Influence of various ethanol concentrations on DS

The influence of water content in the reaction mixture was monitored by varying the ratio of water to ethanol (Fig. 3). The water content in the reaction mixture was varied between 5% and 50%. NaOH (5 g) was added to 80 mL ethanol (v/v), the temperature was raised to 45 °C, starch (10 g, dry weight) was added to the mixture, and it was stirred until well distributed. Then MCA (5 g) was added to the mixture and the reaction mixture was kept for 2 h. Water plays the significant role of aiding dissociation, diffusion, and adsorption of etherifying agents as well as facilitation of starch swelling during carboxymethylation process. After the critical ratio where the DS is maximal, further increase or decrease in water content reduces the DS. Both phenomena reduced contact of the etherifying agent with starch molecules. In addition the granular nature of the starch was lost.[12]

2.4. Influence of various reaction temperatures on DS

The influence of different reaction temperatures (35–65 °C) on DS is shown in Figure 4. NaOH (5 g) was added to 80 mL of 80% (v/v) ethanol, starch (10 g, dry weight) was added to the mixture and it was stirred until well distributed. Then MCA (5 g) was added to the mixture and the reaction mixture was kept for 2 h. The DS values increased with the increase in the etherifica-

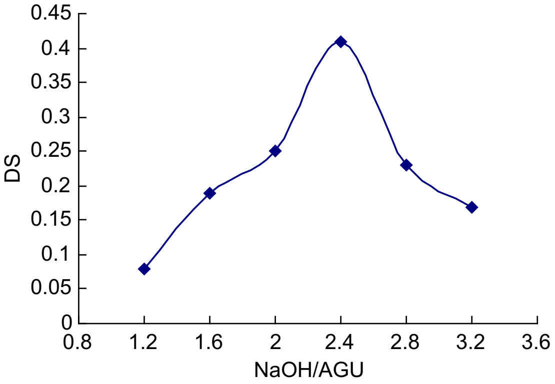

Figure 1. Effect of various molar ratios of NaOH to starch (1.2–3.2) on the DS (5 g MCA, 80 mL of 80% ethanol, 50 °C reaction temperature and 2 h reaction time).

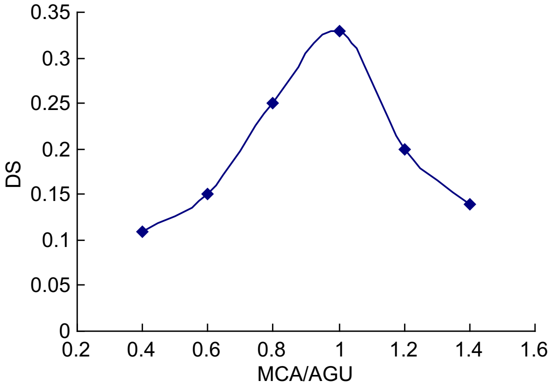

Figure 2. Effect of various molar ratios of MCA to starch (0.4–1.4) on the DS (5 g NaOH, 80 mL of 80% ethanol, 50 °C reaction temperature and 2 h reaction time).

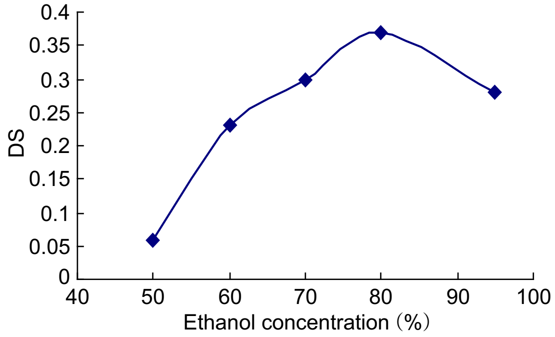

Figure 3. Effect of different ethanol concentrations (50–95%) on DS (5 g NaOH, 5 g MCA, 50 °C reaction temperature and 2 h reaction time).

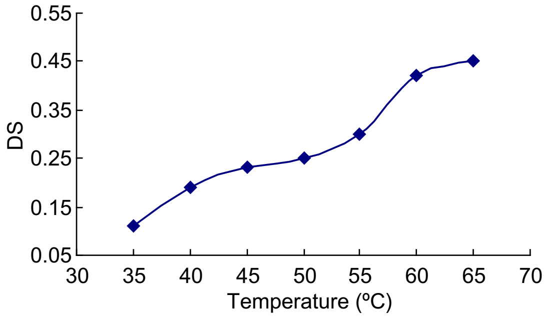

Figure 4. Effect of different reaction temperatures (35–65 °C) on DS (5 g NaOH, 5 g MCA, 80 mL of 80% ethanol and 2 h reaction time).

tion temperature from 35 °C to 65 °C. An increase in temperature enhanced solubility of the etherifying agents and it also facilitated both the swelling of the starch molecules and the diffusion of the reactants (Fig. 4). Increasing the temperature increased the proportion of molecules with higher energy than the activation energy, and consequently the rate of reaction increased and thus DS. An attempt was made to investigate the preparation of CMS at higher temperatures (60 °C). However, gelatinization occurred, stirring was difficult, and it was also difficult to remove the product. So the DS increased more slowly when the temperature was higher than 60 °C. The influence of carboxymethylation, particularly in alkaline medium, caused the gelatinization at lower temperature. In a previous study, the gelatinization temperature of native yam starch was found to be 80 °C.[13] From the above analysis, we can come to the conclusion that the optimal temperature was 60 °C.[14]

2.5. Influence of various duration of reaction on DS

The effect of reaction time on DS is presented in Figure 5. NaOH (5 g) was added to 80 mL of 80% (v/v) ethanol. The temperature was raised to 45 °C. Starch (10 g, dry weight) was added to the mixture and it was stirred until well distributed. Then MCA (5 g) was added to the mixture and the reaction time was varied between 0.5 h and 4 h. The DS increased with increase in reaction time. The increase in the DS with time is a result of enhanced period of contact of the etherifying reagent and the starch molecules. It is also reasonable that longer reaction time enhanced starch swelling and ultimately improved homogeneity of the reactants. However, no remarkable further increases were observed in DS after 2.5 h of reaction. This is because with the proceeding of the reaction, the reactants were used and led to lower DS.

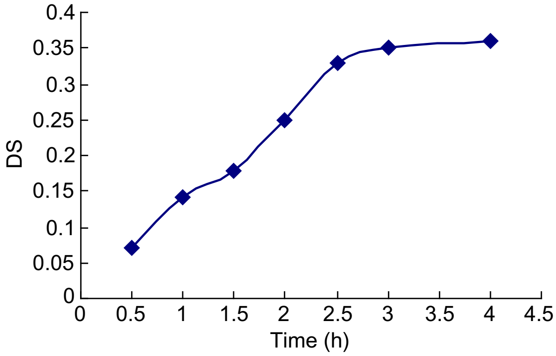

Figure 5. Effect of different reaction durations (0.5–4 h) on DS (6 g NaOH, 6 g MCA, 80 mL of 80% ethanol and 50 °C reaction temperature).

2.6. Starch granule morphology

As shown in Figure 6a, granules of unmodified yam starch were round and polygonal in shape.[15] However, after starch modification, as indicated in Figure 6b, the granular structure of starch changed. From the SEM of the 0.52 substituted starches, we can see many alveolate holes on the surface of granules. This change is attributed to the strong alkaline environment the starches are exposed to during carboxymethylation. We can come to the conclusion that the granular appearance of the native starch was distorted after carboxymethylation.[14] This suggested that carboxymethylation affected the structural arrangement of the starch. It is reasonable that the strong alkaline condition caused granular disintegration. The scanning electron microscope images of carboxymethyl cassava starch and sodium carboxymethyl mungbean starches also showed the same results.[6,16]

2.7. X-ray powder diffraction

X-ray diffractogram of native yam starch and CMS is presented in Figure 7. The diffractogram of native yam starch exhibits a typical C-type crystallinity pattern.[18] After carboxymethylation, the crystallinity is dramatically reduced. This loss in crystallinity could be attributed to the effect of the alkaline environment during the modification.[6] This opens up a potential utilization of carboxymethyl starches as superabsorbent because amorphous granules would enhance water absorption. Compared with other carboxymethylated starches, a loss in crystallinity of mungbean starch after carboxymethylation has been reported.[6] The results 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.[17]

2.8. FT-IR spectrum

The introduction of the carboxy group can be evidenced by means of FT-IR spectroscopy, displaying the spectra of the native yam starch and CMS derivative (Fig. 8). The spectral pattern in the region 970 cm^{-1} and 1200 cm^{-1}, typical of starch,[13] is preserved in all the CMS derivatives. The starting CMS contains carboxymethyl groups, which are partially protonated (–COOH) giving a very weak C=O band at, 1735 cm^{-1} and a strong band of the carboxylate form (–COO–) at 1604 cm^{-1}. Simultaneously, the

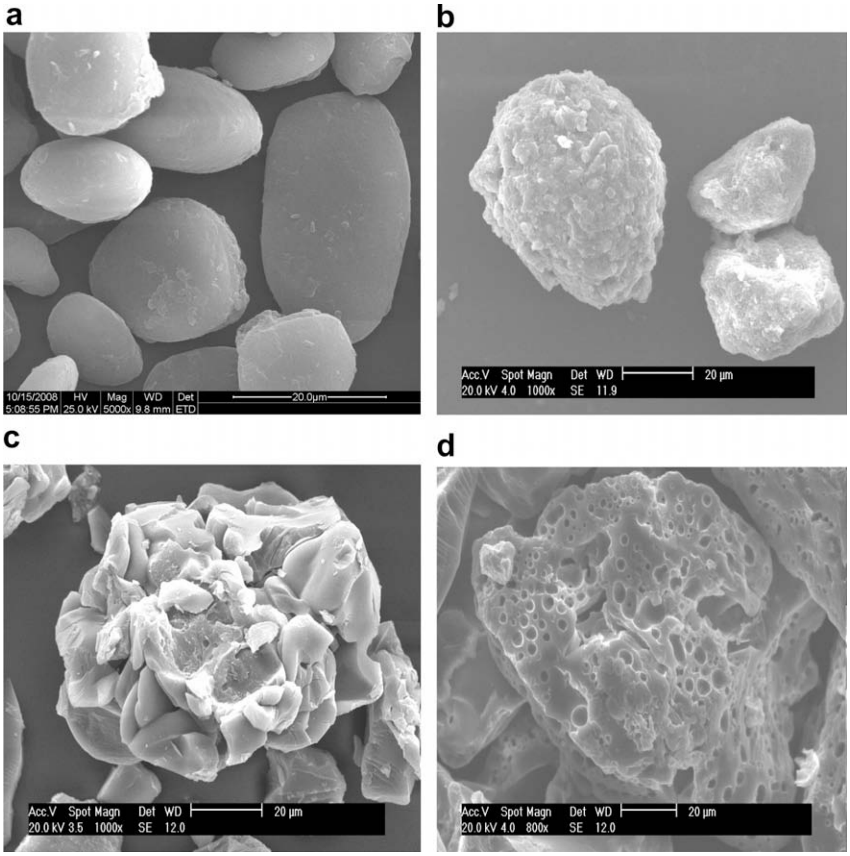

Figure 6. SEM photographs of native starch and carboxymethyl starch with different DS values: (a) native starch, (b) CMS DS 0.08, (c) CMS DS 0.23, and (d) CMS DS 0.52.

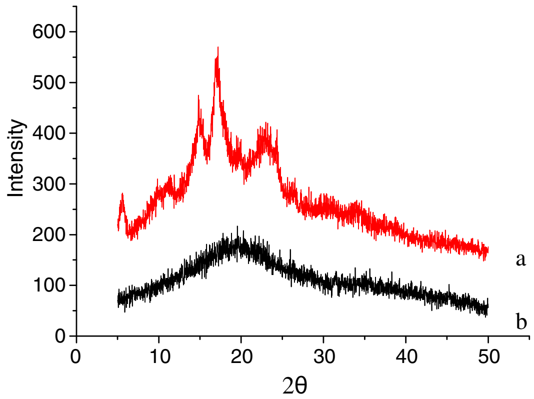

Figure 7. XRD profile of native starch and carboxymethyl starch: (a) native starch and (b) CMS.

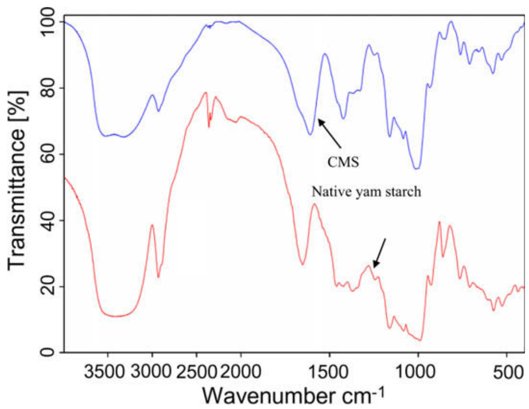

Figure 8. FT-IR spectroscopy of native yam starch and CMS.

intensity of the CH_2 stretch of the octenyl chain at 2929 cm^{-1} increases. In accordance with the increasing extent of esterification the relative proportion of the carboxylate band decreases. By esterification of OH groups, the strong band at 3350 cm^{-1} (hydrogen-bonded OH groups) in CMS decreases and is shifted toward 3420 cm^{-1} indicating disrupture of the supramolecular structure due to ester bond formation.

2.9. Particle size distribution

The average particle diameter of native yam starch was 37.37 µm; while after carboxymethylation, the average particle diameter of the granule increased to 72.88 µm. This increase in size was due to the big group formed in the carboxymethylation process. This result also proved that some granules get together and form big groups during the carboxymethylation, which was consistent with the results of SEM (Fig. 9).

3. Experimental

3.1. Materials

The Chinese yam (*D. opposita* Thunb.) flour was provided by Henan WanXi Pharmaceutical Company. NaOH, monochloroacetic acid, and ethanol were purchased from Guangfu Chemical Company (Tianjin, China).

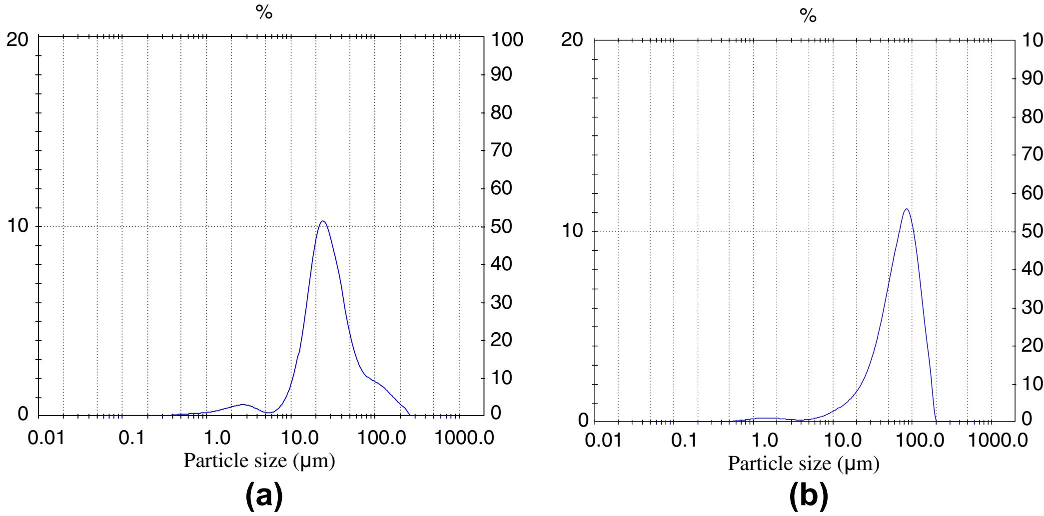

Figure 9. PSD of native starch and CMS: (a) native starch and (b) CMS.

3.2. Starch isolation

The *D. opposite* Thunb. was washed, cut into small pieces, and ground with a plant micro-muller, which was sieved with 160 mesh sifter. After having been sieved, the yam flour was steeped in ethanol to remove non-polar small constitutes. After depositing, the supernatant was removed by suction and the settled starch layer was re-suspended in distilled water. After seven or eight cycles of re-suspending repeatedly, the slurry was centrifuged at 3000 rpm for 20 min. The white layer was re-suspended in distilled water and re-centrifuged 3–5 times. The starch suspension obtained was dried in a convection oven at 50 °C.[18]

3.3. Preparation of carboxymethyl starch

Carboxymethylation of yam starch was carried out in aqueous–organic liquid media. Organic solvent used in this experiment was ethanol. The reactions were carried out in a 250-mL three-necked round-bottomed flask, equipped with motor-driven stirrer. NaOH of different quantities (3–8 g) was added to 80 mL ethanol. The temperature was raised to between 30 °C and 60 °C, the mixture was stirred at 250 rpm until complete dissolution of sodium hydroxide was observed. The water content in the reaction mixture was varied between 5% and 40%. Starch (10 g, dry weight) was added to the mixture and it was stirred at 400 rpm. After stirring, SMCA (3–8 g) was added to the mixture and the reaction time was varied between 0.5 and 4 h. 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 filtered, and suspended in methanol. Following filtration, the slurry was dispersed again in 80% ethanol and it was washed several times with 80% ethanol until the silver nitrate test for chloride of the filtrate was negative. The slurry obtained was suspended in acetone, stirred for 20 min, and dried in an oven at 40 °C for 48 h.[19]

3.4. Characterization

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 and 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 several 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. Two grams of H-CMS were dissolved in 1% (w/v) NaCl solution and it was titrated with 1 M NaOH. The DS was determined as follows.

$$\mathrm{DS} = \frac{n_{\mathrm{NaOH}} \times M_0}{m_\mathrm{c} - n_{\mathrm{NaOH}} \times M_\mathrm{R}}$$

$$m_\mathrm{c} = m_\mathrm{p} - \left[\frac{m_\mathrm{p} \times F}{100}\right]$$

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 (%).

Scanning electron micrographs (SEMs) were obtained with an environmental scanning electron microscope (ESEM, Philips XL-3). Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of the suspension was applied on an aluminum stub using double-sided adhesive tape and the starch was coated with gold powder to avoid charging under the electron beam after the acetone volatilized. An accelerating potential of 30 kV was used during micrography.

X-ray diffraction patterns of native yam starch and CMS were analyzed using Rigaku D/max 2500 X-ray powder diffractometer (Rigaku, Tokyo, Japan) with Nickel filtered Cu Kα 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 to 40° (2θ), with a scanning speed of 8° (2θ)/min and step size of 0.06° (2θ).

Particle size analysis of starch was done using a laser light scattering particle size analyzer (Mastersizer S, version2.15, Malvern instruments Ltd, Malvern, UK). The focal length was 100 mm.

FT-IR spectra of the native starch and starch acetates were recorded with an IR spectrometer (Bruker Vector 22) using potassium bromide (KBr) disks prepared from powdered samples mixed with dry KBr in a ratio of 1:30.

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