Physicochemical Properties of Carboxymethylated Sago (*Metroxylon sagu*) Starch

ZAINAL A. NOOR FADZLINA, ALIAS A. KARIM, TJOON T. TENG

ABSTRACT: Carboxymethyl starch (CMS) with degree of substitution (DS) ranging from 0.1 to 0.32 was prepared from sago (*Metroxylon sagu*) starch in non-aqueous medium using isopropanol as a solvent. The physicochemical, rheological, and thermal properties of the starches were investigated. At room temperature (25 °C), CMS hydrated readily, resulting in higher swelling power compared with native (unmodified) starch. Light microscopy revealed that CMS granules imbibed more water than native starch at room temperature and thus caused a larger increase in granule size. Some of the CMS granules lost their integrity. Scanning electron microscopic observation revealed fine fissures on the surface of CMS (DS 0.32) granules compared with a relatively smooth surface of native starch granules. Carboxymethylated sago starch exhibited excellent dispersibility and cold water solubility as judged by the absence of peak viscosity in the pasting profile (determined by Rapid ViscoAnalyzer). Pasting profile of CMS was qualitatively similar to pregelatinized starch. Despite exhibiting greater swelling power, CMS showed significantly lower pasting viscosity compared with the native starch. Intrinsic viscosity was also greatly reduced by carboxymethylation. Studies using differential scanning calorimetry (DSC) showed that transition temperatures and enthalpies decreased with an increase of degree of substitution. CMS at higher substitution levels (DS 0.27 and 0.32) showed significantly lower retrogradation tendency, as indicated by lower setback, absence of DSC endotherm upon storage at 4 °C and lower syneresis upon repeated freeze-thaw cycles. The results suggested that retrogradation might be effectively retarded by the presence of the bulky carboxymethyl group.

Keywords: carboxymethyl starch, modified starch, sago starch, retrogradation

Introduction

Starch is a versatile and useful polymer not only because it is a cheap, natural material but also because of the ease with which its physicochemical properties can be altered through chemical or enzyme modification and/or physical treatment. Through modification, the properties of native starch can be improved such as decreasing retrogradation, syneresis, and gelling tendencies of pastes, increasing freeze-thaw stability, or adding hydrophobic/hydrophilic groups into the starch chain (BeMiller 1997).

Starch becomes cold-water-soluble by substituting the hydroxyl groups with sodium monochloroacetate (SMCA) to give carboxymethyl starch (CMS). CMS is a water-soluble polysaccharide that finds many applications in the food and nonfood industries (Bhattacharyya and others 1995). The carboxymethyl group is hydrophilic in nature, and when introduced into the starch granule, it weakens or strains the internal bond structure holding the granule together. The reduction in bond strength is reflected in lower starch pasting temperatures. The higher the level of modification, the lower the pasting temperature until the starch granules are rendered soluble or swell in water at room temperature.

CMS can be produced by substitution of the hydroxyl groups with sodium monochloroacetate in the presence of strong alkali. Carboxymethylation can be performed in water as a solvent or in a water-miscible organic solvent containing a small amount of water such as ethanol, isopropanol, methanol, or toluene. The use of organic solvent will preserve the final product in the granular form and the side product can be washed out easily (Tijsen and others 2001). CMS can be produced from many sources of starch such as corn, wheat, potato, high-amylose corn, and tapioca (Bhattacharya and others 1995).

The properties of carboxymethylated starch can be characterized by the degree of substitution (DS), the distribution of functional groups, and molecular weight distribution. The amount of carboxymethyl groups formed is indicated by the degree of substitution. The DS is defined as the average number of substituents per anhydro glucose unit (AGU), the monomer unit of starch. Each AGU contains 3 hydroxyl groups, so the DS lies between 0 and 3 (Tijsen and others 2001).

The major properties of CMS are a lower gelatinization temperature, its ability to swell in cold water, improved freeze-thaw stability, and reduced tendency to retrograde. In this study, sago starch was carboxymethylated and the physicochemical, rheological, and thermal properties of carboxymethylated starch were investigated.

Materials and Methods

Materials

Sago starch was supplied by NITSEI Sago Industries Sdn. Bhd. (Province Wellesley, Penang, Malaysia). The starch was used as provided without any further treatment. Sodium monochloroacetate (SMCA) was used as an etherifying agent, whereas sodium hydroxide (NaOH) was used to provide alkaline condition to facilitate the reaction. All other chemicals were of analytical grade.

Carboxymethylation

For the preparation of CMS, the method of Lazik and others (2002) was followed with minor modification. Five CMS samples with various degrees of substitution were prepared by using different amounts of NaOH (0.24 *M* and 0.55 *M*), SMCA (0.24 *M* and 0.55 *M*), temperature (36 °C and 44 °C), and duration (120 min and 280 min) of the process.

MS 20050417 Submitted 7/12/05, Revised 8/12/05, Accepted 8/29/05. *The authors are with Food Biopolymer Science Research Group, School of Industrial Technology, Univ. Sains Malaysia, 11800 Minden, Penang, Malaysia. Direct inquiries to author Karim (E-mail: nadhaz@streamyx.com).*

Sago starch (100 g, dry basis) was slurried in 400 mL of isopropanol in a 1-L, 3-necked, round-bottom reaction flask equipped with a stirrer, reflux-condenser, and burette. A reflux-condenser was used to prevent the loss of organic liquid. The calculated amount of NaOH was added into the flask over a period of 40 min. The mixture was allowed to stand 1 h for further swelling. Sodium monochloroacetate (in powder form) was then added to the reaction mixture. Subsequently, the flask was heated to the reaction temperature and left for a definite time. After cooling, the reaction was neutralized by adding acetic acid. The precipitate was filtered, washed with 95% ethanol (3×), and dried overnight at 50 °C to obtain a dry powdered product.

Degree of substitution

The degree of substitution of carboxymethyl starches was measured by the titration method as described by Kim and Lim (1999) with minor modification. A sample of CMS (2.5 g, dry basis) was accurately weighed into a 150-mL beaker. Twenty-five milliliters of 0.1 *N* hydrochloric acid was added with stirring for 30 min. The slurry was vacuum-filtered through a small funnel, washed with distilled water (300 mL usually sufficient), and gelatinized in water by boiling for 20 min in a water bath. Carboxymethyl groups were titrated with standardized 0.1 *N* NaOH solution. A blank determination was run on the original sample to correct for native acid substances. Each sample was run in triplicate.

$$\text{Carboxyl content(M)} = \frac{(\text{sample - blank})\,\text{mL} \times 0.0045 \times 100}{\text{sample weight (d.b., g)}}$$

DS was calculated by the following equation (Hebeish and Khalil 1988):

$$\text{degree of substitution, D.S.} = \frac{162 \times M}{4500 - (RM)}$$

where M = carboxyl content (%); R = molecular weight ($-CH_2COOH - 1$)

Swelling power and solubility

Swelling power and solubility were determined in triplicate using the method described by Liu and others (1999). Starch (0.5 g dry basis [db]) was weighed into a centrifuge tube to which 40 mL distilled water/0.1 *M* NaCl solution was added. The tube was heated at temperatures of 30 °C, 50 °C, 70 °C, and 90 °C in a shaking water bath for 30 min. The tubes were cooled to room temperature and centrifuged (1670 × *g*) for 20 min. The supernatant was carefully poured out and dried overnight at 120 °C. Swelling power was determined as a ratio of sediment weight to dry starch (g/g), whereas solubility is a ratio of dried supernatant to dry starch (%). Each sample was run in triplicate to determine the mean value.

Pasting properties

Pasting properties of the samples were determined by a Rapid ViscoAnalyzer (RVA 3D; Newport Scientific, Narrabeen, Australia). Starch sample (2.5 g) was added to 25 g distilled water in a RVA sample cup. The starch slurry was analyzed by the procedure of Kwon and others (1997) with minor modification. The samples were equilibrated at 50 °C for 1 min, heated to 95 °C in 7.5 min, held at 95 °C for 5 min, cooled to 50 °C in 8.5 min, and held at 50 °C for 3 min. Peak viscosity (PV), hot paste viscosity (HPV), breakdown (BD), setback (SB), and cold paste viscosity (CPV) were determined from RVA plots. Each sample was run in triplicate to determine the mean value.

DSC thermal profile

Thermal profile of native and CMS samples was performed by using a differential scanning calorimeter (DSC) (model 2910; DuPont, Wilmington, Del., U.S.A.) equipped with a standard DSC cell and a Thermal Analyst 2000. Starch (db) and deionized water were weighed directly into the hermetic pan to give total weight ±10 mg (starch-water ratio, 1: 2). The mixture of starch-water was then sealed and left at least 1 h for equilibration. An empty pan was used as reference and DSC was carried out from 20 °C to 110 °C with a heating rate of 10 °C/min. From the DSC curve, the transition temperatures (onset temperature, T_o, peak temperature, T_p, and completion temperature, T_c) and gelatinization enthalpy (ΔH) were evaluated as characteristics of the gelatinization process. After completion of the DSC run, the samples were stored at 4 °C for 7 d and then analyzed again by DSC using the same heating program. The ratio of the second gelatinization enthalpy (ΔH_2) to the 1st (ΔH_1) could be regarded as the degree of retrogradation. Each sample was run in triplicate to determine the mean value.

Freeze-thaw stability

The method described by Pal and others (2002) was followed with minor modification. Starch (8%, w/v) was gelatinized at 85 °C for 15 min. The starch paste was then cooled to room temperature and 20 g of it was transferred to the 50-mL centrifuge tube. All samples were frozen at –18 °C for 18 h and then thawed at room temperature for 6 h. The starch suspension was then centrifuged at 1670 × *g* for 20 min. The percentage of water separated after each freeze-thaw cycle was measured and expressed as the percentage of water separated:

$$\text{syneresis (\%)} = \frac{\text{water separated (g)}}{\text{total weight of sample (g)}} \times 100$$

Each sample was run in triplicate to determine the mean value.

Intrinsic viscosity

The determination of intrinsic viscosity was based on the method developed by Ahmad and others (1999) with minor modification. A predetermined weight of starch samples was initially dissolved in 0.5 *M* KOH with addition of sodium chloride (AnalaR grade from BDH) to give final concentrations of 0.10 *M* NaCl. The measurement of all samples was carried out by using an Ubbelohde-type capillary viscometer (Poulten Selfe & Lee Ltd., Essex, U.K.; PSL ASTM-IP IC, constant = 0.03009 [mm^2/s]/s) with a 0.75-mm dia. The capillary viscometer was placed in a constant temperature water bath at 25.0 ± 0.1 °C. Exactly 12 mL of centrifuged solution was transferred to the viscometer using pipette. The sample was further diluted with 0.5 *M* KOH to give 5 different concentrations in the range 0.3% to 0.5%, w/w. The intrinsic viscosity of the starch solution at infinite dilution is obtained by extrapolating the specific viscosity values measured for the successive dilutions. Each sample was replicated 3 times. The average variance was less than 0.01 s.

Microscopy

Light miscroscopy. A light microscope (Olympus, REC, Tokyo, Japan BH-2) fitted with a crossed polar analyzer and a camera was used to observe the starch in distilled water. Starch dispersion was stirred before sampling using a wire loop and transferred onto a microscope slide. Each sample was viewed and photographed.

Scanning electron microscopy. Each starch sample was air-dried, placed on an aluminum stub having double-sided sticky tape on it, coated with gold using a sputter coater, and viewed under a scanning electron microscope (Cambridge S-200; LEO Inc., Thornwood, N.Y., U.S.A.).

Experimental design and statistical analysis

The experiments were designed and conducted at 3 stages: (1) 2^4

full-factorial experiments; (2) a second set of experiments using the method of steepest ascend; (3) optimization of the process using central composite rotatable design. The total of 32 experimental runs included 16 factorial points, 12 axial points, and 4 center points. Experiments were conducted in random order. The 4 independent variables considered to be the most important factors (based on preliminary experiments and data from the literature) having the greatest effect on the carboxymethylation process were as follows: concentration of sodium hydroxide, concentration of sodium monochloroacetate (SCMA), temperature, and duration of the reaction. The range and the level of each independent variable investigated are given in Table 1. Design Expert software version 5.0 (Stat-Ease Inc., Minneapolis, Minn., U.S.A.) was used to produce the design matrix.

The data were statistically analyzed using SPSS (Statistical Package for Social Science) version 10.0 (SPSS Inc., Chicago, Ill., U.S.A.). One-way ANOVA was used to compare means at the 5% significance level.

Table 1—Independent variables and levels for the 2^4 full factorial design

Independent variable	Low value (–)	High value (+)
Concentration of NaOH (*M*)	0.24	0.55
Concentration of SCMA[a] (*M*)	0.17	0.52
Temperature (°C)	36	44
Duration (min)	120	280

[a]SCMA = sodium monochloroacetate

Results and Discussion

The degrees of substitutions (DS) obtained for the carboxymethylated starches were 0.10, 0.17, 0.22, 0.27 and 0.32. Most commercially produced CMS have degrees of substitution generally less than 0.2 to 0.3 (Rutenberg and Solarek 1984). CMS was readily dispersed and produced a clear paste in cold (25 °C) water. From visual observation, increasing DS of CMS resulted in increasing clarity of the paste. It is known that normal starch (containing amylose) will retrograde upon cooling of the starch paste and produce an opaque paste/gel. The reason for higher clarity of CMS could be attributed to the steric hindrance by the bulky carboxymethyl groups, which interfered and hindered the reassociation of intermolecular linkages of starch chains, consequently inhibited retrogradation. Other substituted starches such as cationic starch (Siau and others 2004) and hydroxypropyl starch (Kim and others 1992; Pal and others 2002) also typically exhibited clear paste.

Swelling power and solubility

Swelling power and solubility of native starch and CMS with various degrees of substitution were measured at different temperatures (30 °C, 50 °C, 70 °C, and 90 °C). The purpose was to measure the relative capacity of the starch granules to swell at different temperatures and the amount of soluble materials produced. Figure 1 and 2 show the changes of swelling power and solubility of all starches with respect to temperature. Carboxymethylation significantly ($P < 0.05$) increased swelling power and solubility of native starch, and generally increasing the DS led to an increase in these values. It is evident that CMS granules swelled readily, even at 30 °C, compared with that of native starch. Further increase in granule swelling at elevated temperature was observed for all starches, but, in general, the CMS showed a lower percentage increase in swelling than that of native starch. This may suggest that the CMS can achieve between 60% and 80% of its swelling capacity in water at room temperature (25 °C), depending on the degree of substitution.

The introduction of carboxymethyl groups into the starch granule structure appeared to result in a weakening of the granular structure due to repulsion between neighboring groups, thus inhibiting interchain associations. Data from this study could not identify the actual modification points in the granule but it is suggested that the structural loosening may occur predominantly in the amorphous region (including the branching point of amylopectin), consequently permitting greater water uptake and an increase in the swelling of the granule. Comparatively little is known, however, about the location of the substituents on the starch polymers or inside the starch granules. A conclusion from some studies (Zhu and Bertoft 1997; Kavitha and BeMiller 1998; Manelius and others 2000) is that preferentially amylose and the regions around the branches in amylopectin become modified. This largely corresponds to the amorphous parts in the semicrystalline starch granules. This will also depend on the method of modification. For example, Manelius and others (2000) suggested that dry-cationized starch was preferentially cationized at

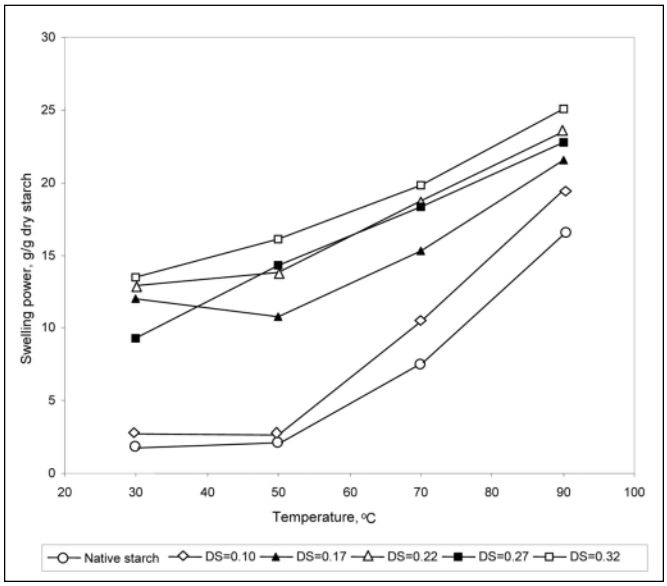


Figure 1—Swelling power for native and carboxymethyl starch (CMS) samples at different temperatures

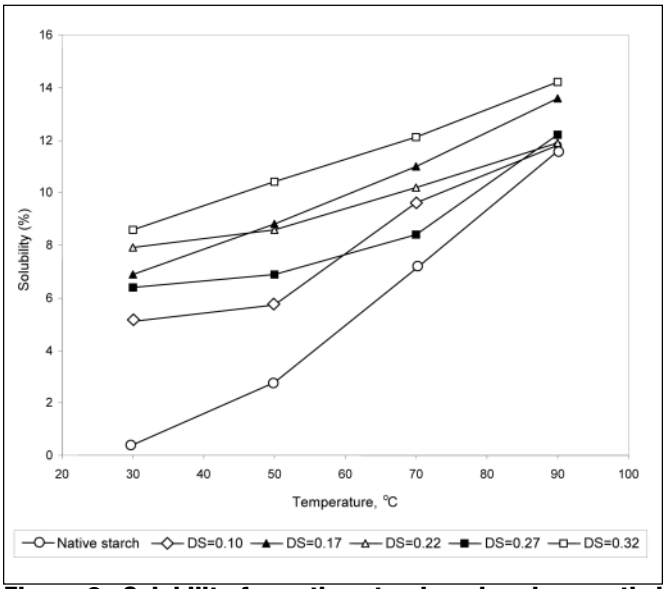


Figure 2—Solubility for native starch and carboxymethyl starch (CMS) samples at different temperatures

the surface of the granules, whereas wet-cationized starch was modified throughout the granules.

Keetels and others (1996) concluded that the phosphate group greatly affects the swelling power of potato starch. Repulsion between these negatively charged groups would be responsible for the more rapid and higher extent of swelling of potato starch granule. The same explanation can be applied to CMS because of the presence of negatively charged carboxymethyl groups in CMS as well. To investigate whether the presence of negatively charged carboxymethyl groups affects the swelling behavior of CMS, another set of experiments was carried out in 0.1 *M* NaCl solution. Evidently, high swelling power of CMS was depressed in 0.1 *M* NaCl compared with the same sample in deionized water (Table 2). In NaCl solution, the electrical double layer around charged groups is compressed, thus CMS swelled less in 0.1 *M* NaCl solution, whereas no significant difference for the swelling power of native starch in both solvents was observed. This experiment confirmed that carboxymethyl groups greatly affect the swelling power of the CMS granules.

Table 2—Swelling power of native and carboxymethyl starch (CMS) in deionized water and 0.1 *M* NaCl solution at different temperatures

		Swelling power(g/g)		
Temp (°C)	Solvent	Native starch	DS = 0.10	DS = 0.32
30	Deionized water	2.1	2.7	13.5
	Deionized water + NaCl	1.8	2.2	10.8
50	Deionized water	1.8	2.5	11.7
	Deionized water + NaCl	1.8	2.5	9.9
70	Deionized water	7.6	2.7	13.5
	Deionized water + NaCl	7.2	3.4	12.3
90	Deionized water	16.3	19.5	25.1
	Deionized water + NaCl	15.4	3.1	16.6

Pasting properties

Figure 3 shows the pasting profile of the native starch and CMS samples as a function of cooking time and temperature. Pasting parameters derived from the curves are shown in Table 3. The pasting profiles of CMS samples were notably different from that of native starch but qualitatively very similar to pre-gelatinized starch (not determined in this work but as reported in the literature). Thomas and Atwell (1999) postulated that pregelatinized starch granules act like a sponge to immediately imbibe water and produce viscosity. As the carboxymethylated starch takes up water more readily than the native starch, the gelatinization can occur more easily and at a lower temperature. Due to the cold-water-soluble properties of CMS, a distinct pasting temperature could not be detected by RVA. It is interesting to note that the CMS with lower DS (0.1 and 0.17) did not exhibit appreciable peak viscosity compared with that of CMS with higher DS (0.22, 0.27, and 0.32). The reasons for this intriguing observation are not clear but it could be related to the distribution pattern of carboxymethyl groups in the granule. Further investigation is warranted to elucidate the mechanism and distribution pattern for such samples.

It is also interesting to note that native starch exhibited lowest swelling power (Figure 1) but highest peak viscosity (Figure 3) when subjected to the heating and cooling cycle. Most of the authors related the greater swelling power with higher peak paste viscosity for the other types of modification (Butler and others 1986; Crosbie 1991; Kim and others 1992; Kweon and others 1997; Bhandari and others 2002; Pal and others 2002). In general, the introduction of ionic groups into the polymer chain induces an increase in viscosity of the solution because the coil expands by the electrostatic repulsion and the osmotic pressure due to the ions in the system. This is true in the system where starch granules are able to swell to the maximum capacity in dilute solutions (as in the measurement of swelling power) and in the absence of mechanical shear. However, in the pasting process in the RVA, the concentration of the system (8% w/w) is much higher and thus represents a close-packed granule assembly. In such system, the elasticity or the rigidity of the granules will have an effect on the viscosity. The fact that the peak viscosity for CMS is lower than the native starch

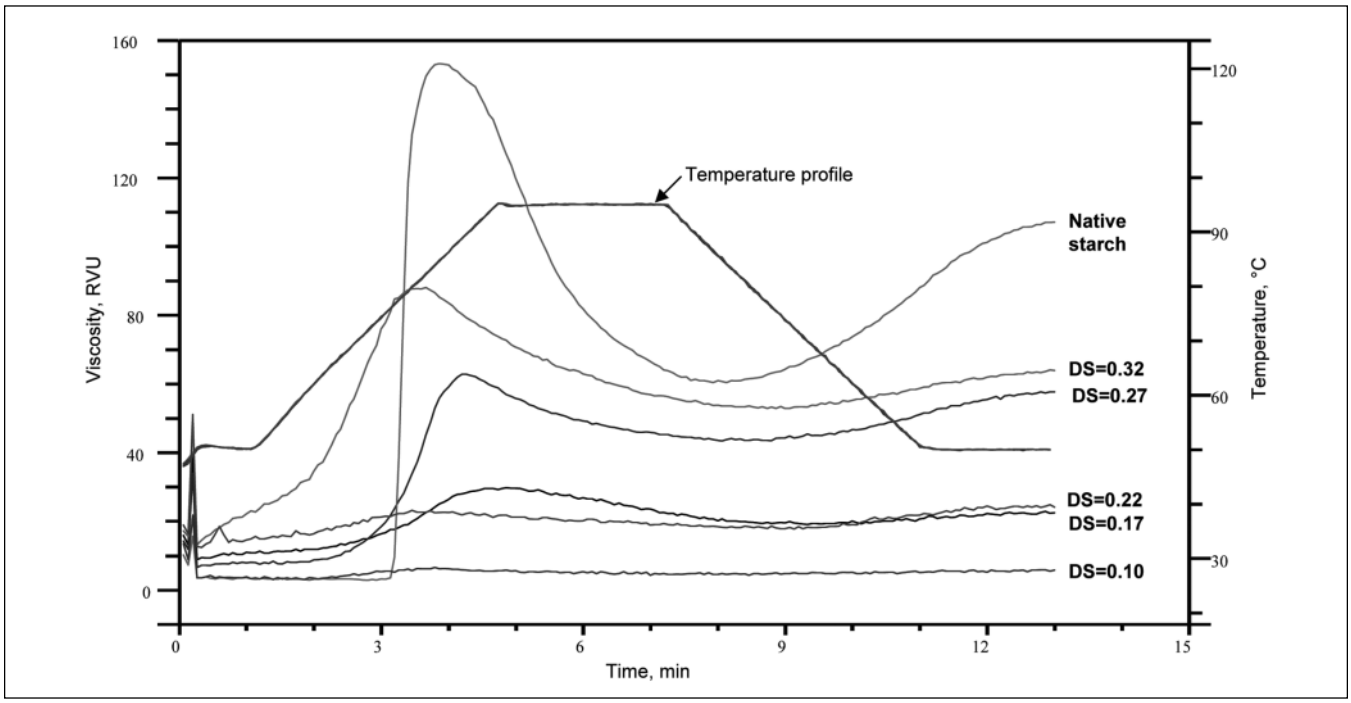


Figure 3—Pasting curves for native and carboxymethyl starch (CMS) with different degrees of substitution

Table 3—Pasting properties of native and carboxymethylated sago starches

		Pasting properties[a]					
DS	Pasting temp (°C)	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Breakdown (RVU)	Cold paste viscosity (RVU)	Setback (RVU)	Peak time (min)
0.0 (native)	76.8 ± 0.1	152.8a ± 0.9	60.6a ± 0.8	92.2a ± 1.7	106.1a ± 1.6	45.5a ± 2.4	3.9bc ± 0.0
0.10	N/A	14.4f ± 0.8	7.8e ± 0.6	6.6f ± 0.7	11.8f ± 0.7	3.9e ± 0.2	3.8bc ± 0.0
0.17	N/A	29.0d ± 1.4	18.5d ± 0.8	10.5d ± 0.6	21.6e ± 1.3	4.8d ± 0.2	4.8a ± 0.2
0.22	N/A	24.8e ± 1.8	19.0d ± 1.4	5.8e ± 0.4	25.3d ± 1.3	6.3d ± 0.1	3.8c ± 0.3
0.27	N/A	61.7c ± 1.4	42.8c ± 1.0	18.9c ± 0.5	56.9c ± 0.9	14.2b ± 0.2	4.2b ± 0.1
0.32	N/A	85.6b ± 2.6	52.3b ± 1.8	35.3b ± 2.8	62.7b ± 1.1	11.2c ± 0.2	3.5c ± 0.1

[a]Values are means ± SD (n = 3). Means within a column with same letter are not significantly different at the 5% level of probability. N/A = no peak observed.

Table 4—Transition temperatures and enthalpy of native starch and carboxymethyl starch associated with gelatinization of 1:2 starch-water systems

	Transition temperatures (°C)[a]			
Degree of substitution	Onset temp, T_o	Melting temp T_p	Comp. temp. T_c	ΔH_1 (J/g dry starch)
DS = 0.00	69.2a ± 0.3	75.5a ± 0.1	88.6a ± 0.2	18.6a ± 2.2
DS = 0.10	67.6b ± 0.2	74.4b ± 0.2	80.5b ± 0.6	1.7b ± 0.6
DS = 0.17	66.3b ± 0.2	71.9c ± 0.2	80.3b ± 0.2	1.7b ± 0.6
DS = 0.22	64.4c ± 0.5	70.3d ± 0.8	78.5c ± 0.2	1.5b ± 0.7
DS = 0.27	61.3d ± 0.3	69.5d ± 0.3	78.4c ± 0.1	2.2b ± 0.1
DS = 0.32	59.3e ± 1.1	66.5e ± 0.1	75.6d ± 0.2	1.1b ± 0.5

[a]Values are means ± SD (n = 3). Means within a column with same letter are not significantly different at the 5% level of probability.

may suggest that the CMS granules were less rigid or more elastic and thus partly contributed to reduce the viscosity of the system. In addition, it can be seen during the early part of the pasting curve, CMS granules were readily swollen (higher viscosity) and as heating proceeded, the granules became progressively fragile and some of them disintegrated due to shearing effect. This presumably caused substantial loss in viscosity of CMS compared with that of native starch. The fragility of the CMS granules is clearly evident when observed under the light microscope (see discussion on light microscopy).

It is noteworthy that the CMS exhibited significantly lower breakdown compared with the native starch. Given the fragile nature of the CMS granules, it was expected that the breakdown value for CMS would be higher than that of native starch, but in this case the opposite results were obtained. The plausible explanation for this effect is as follows: CMS granules readily swell from the beginning of the pasting cycle, at about 30 °C (Figure 3); and as the system (paste) was sheared, some of the more fragile granules were broken and the viscosity of the paste was progressively lowered. The peak viscosity of the CMS samples were consequently lower than that of native starch. On the other hand, native starch granules only attain the peak viscosity after holding at 95 °C with the value of 2 to 10 times higher than the CMS samples. The rapid loss of viscosity only commenced after this point and thus the large difference in the peak viscosity and the trough (hot paste) viscosity gave a large value for breakdown.

DSC thermal profile

Figure 4 shows the DSC thermal profile and Table 4 shows the transition temperatures and enthalpy associated with gelatinization for native starch and CMS at various degrees of substitution. Native starch showed a typical distinctive endotherm having high peak temperature compared with CMS. Transition temperatures associated with gelatinization (onset, peak, and completion temperatures) and enthalpy of gelatinization were significantly lower for CMS than that of native starch. Furthermore, carboxymethylation gives more remarkable effect on the enthalpy (energy) to disrupt the crystallites compared with the effect on the transition temperatures. The decrease in heat of gelatinization of CMS is probably due to a decrease in the number of hydrogen bonds required to be broken for the swelling of starch granules. The introduction of bulky groups into the starch chain appears to have a weakening effect on the granular structure of starch by disrupting the intermolecular and intramolecular hydrogen bonding. Furthermore, the hydrophilic character of the carboxymethyl group helps the easy hydration of starch granules and thus lowers the gelatinization temperature for CMS. These results agree with the observations of Yang and others (1995) for CMS derived from potato.

After storage at 4 °C for 1 wk, samples were evaluated for their retrogradation tendency. Retrogradation ratio was estimated by ΔH_2 divided by ΔH_1 (Yang and others 1995). It is evident that no peak was observed for CMS samples whereas the peak for native starch was shifted to a lower temperature (Figure 4). The onset, peak, and completion temperatures for native starch are 44.9 °C, 54.7 °C, and 68.8 °C, respectively, and the retrogradation ratio is 0.41. The appearance of endothermic peak upon cold storage has been attributed to the retrograded amylopectin rather than amylose (Abd Karim and others 2000). The absence of this peak in CMS starch may suggest that the amylopectin molecules have not been able to reassociate/realign and failed to form sufficiently ordered structure (double helices or crystallites). This could be attributed to the electrostatic repulsion of the carboxyl groups of CMS and perhaps the steric hindrance provided by the bulky carboxymethyl groups. Thus, less perfect or stable structure was formed, which requires less energy to break down the bonding in the starch chain.

Freeze-thaw stability

Freeze-thaw stability for native and CMS was evaluated by subjecting 8% starch paste to repeated cycles of freezing and thawing and measuring the total amount of water separated on centrifuging the thawed paste (Baker and Rayas-Duarte 1998; Yuan and Thompson 1998), and the results are shown in Table 5. With the exception of low DS samples (DS 0.1 and 0.17), CMS showed significantly lower syneresis than the native starch, with the sample of DS = 0.32 showing the lowest (about 2%) syneresis (started from the 7th freeze-thaw cycle). Native starch and 2 CMS samples (DS 0.1 and 0.17) showed significantly higher syneresis, which indicates that an extensive retrogradation had occurred when the starch was subjected to storage at very low temperature. These 2 CMS samples

Table 5—Syneresis (%) for native and carboxymethyl starch (CMS) after storage at –18 °C for 1 wk[a]

DS	Syneresis (%)
0.0 (native)	17.9b ± 1.1
0.10	31.6a ± 5.3
0.17	32.1a ± 1.8
0.22	14.7b ± 1.2
0.27	7.8c ± 0.7
0.32	2.1c ± 0.5

[a]Value is means ± SD (n = 3). Means within a column with same letter are not significantly different at the 5% level of probability.

exhibited syneresis almost twice as high as the native starch. The results were unexpected because these 2 starches showed the lowest setback (Table 3), which means lowest tendency toward retrogradation and, therefore, lowest syneresis.

It should be noted, however, that the setback values can be regarded as an indicator of short-term stability of starch due mainly to retrogradation of amylose. It has been demonstrated that starch retrogradation proceeds biphasically (Miles and others 1985), namely, the rapid early stage and the slow later stage being dominated by recrystallization of amylose and amylopectin, respectively. For the 2 CMS samples with low DS, it is suggested that the carboxymethylation was more confined to the bulk amorphous domain constituted by amylose. As a consequence, the setback values, which measure the rapid early stage of retrogradation due to amylose aggregation, were lower. However, because the amylopectin was probably not carboxymethylated to a large extent (except possibly the branching points), it will be able to retrograde during the long storage and contribute to the high syneresis value observed. On the other hand, CMS with higher DS (especially DS 0.27 and 0.32) were probably more thoroughly carboxymethylated (including the amylopectin), and therefore the retrogradation was largely inhibited.

Retrogradation is responsible for the syneresis of starch pastes and gels when held for a long period of time (Abd Karim and other 2000). The retrogradation properties of the starches are indirectly influenced by the structural arrangement of starch chains within the amorphous and crystalline regions of the ungelatinized granules, which in turn influence the extent of granule breakdown during gelatinization and the interaction that occurs between starch chains during gel storage. Wattanachant and others (2003) also reported that native sago starch had low freeze-thaw stability. However, chemical modification via substitution improved the freeze-thaw stability of starch gel during frozen storage. The syneresis was significantly reduced by the incorporation of the bulky group into the starch chain.

Hoover and others (1988) studied the freeze-thaw stability of hydroxypropyl pea starch and reported that the bulky hydroxypropyl group incorporated into starch chain had improved the stability to frozen storage. The bulky group causes steric hindrance in the starch chain, thus it will prevent the alignment of starch chain, and finally reduced the tendency of native pea starch to retrograde. This improvement was also observed by other authors for hydroxypropyl starch (Wu and Seib 1990; Pal and others 2002; Waliszewski and others 2003; Wattanachant and others 2003), phosphate starch (Waliszewski and others 2003), cross-linked hydroxypropyl starch (Waliszewski and others 2003), and succinate starch (Bhandari and others 2002)

Intrinsic viscosity

The solution properties of conformationally disordered ("random coil") polysaccharides are critically dependent on the volume occupied by the individual coils (Morris and others 1981), which can be conveniently characterized by intrinsic viscosity, [η], the fractional increase in viscosity per unit concentration of polymer in the limit of infinite dilution. For polyelectrolytes such as CMS, there is a progressive reduction in coil volume with increasing ionic strength (Smidsrød and Haug 1971), due to progressive screening of intramolecular electrostatic repulsion. When solutions are prepared in water, the ionic strength from counterions to the polymer changes as the polymer concentration is changed, with consequent variation in coil dimensions. Meaningful values of intrinsic viscosity can therefore be obtained only if the ionic strength is held constant by addition of extraneous salt, the conditions normally chosen being 0.1 M NaCl, as used in the present work.

The intrinsic viscosity [η] of native and CMS having different degrees of substitution at 25.00 ± 0.01 °C are shown in Table 6. The

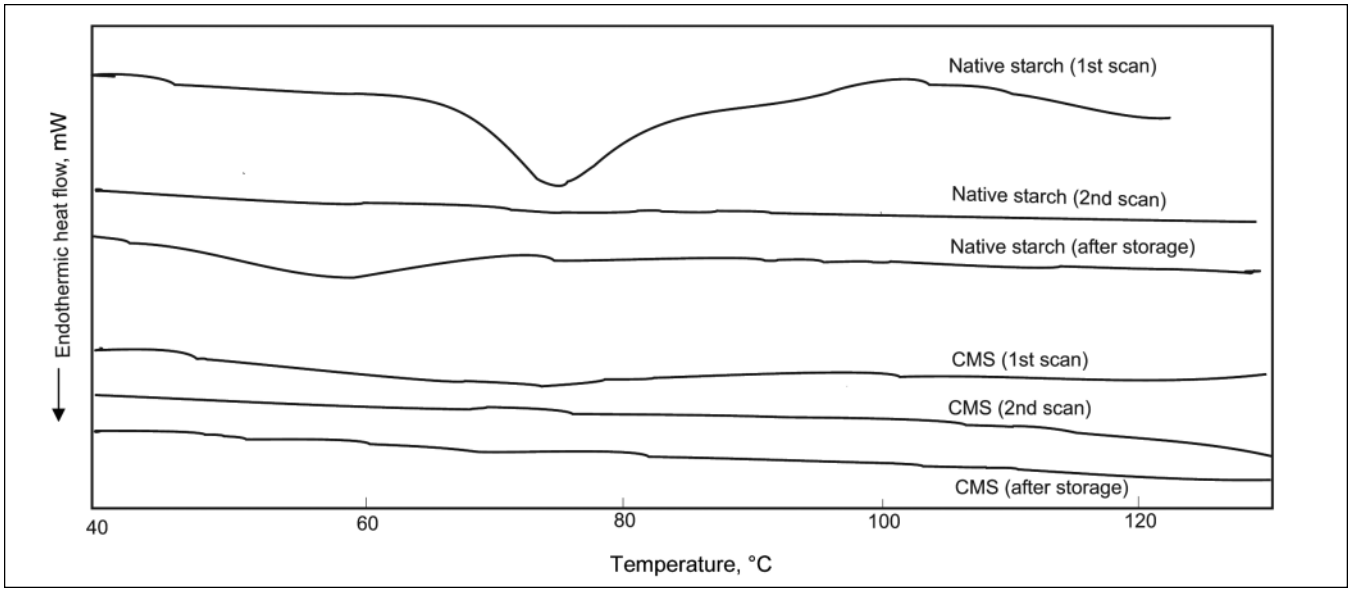


Figure 4—Differential scanning calorimetry (DSC) thermograms of 1:2 starch-water systems for native and carboxymethyl starch (DS 0.10) after the 1st and 2nd scan and after storage for 7 d at 4 °C. (All thermal curves are normalized to 1 g of dry starch.)

[η] for native starch is about 152 mL/g, whereas for CMS the values ranged from 88 to 115 mL/g. Evidently, carboxymethylation reduced the intrinsic viscosity of native starch ($P < 0.05$) but the difference between the CMS samples studied was insignificant ($P > 0.05$). It is expected that the CMS, being a polyelectrolyte, will occupy higher hydrodynamic volume and thus higher [η] values due to the mutual repulsive effect between starch chains in dilute solution. However, the fact that the [η] values were much lower for CMS suggests that some depolymerization of starch polymers have occurred during the carboxymethylation process. The concentration of alkali (NaOH) used in this work was 0.24 *M* and 0.55 *M*. Aqueous NaOH is known to reduce the gelatinization temperature of starches. Jackson and others (1988), Wang and Wang (2002), and Lai and others (2004) have described the subsequent depolymerization of both amylose and amylopectin when treated with aqueous alkali.

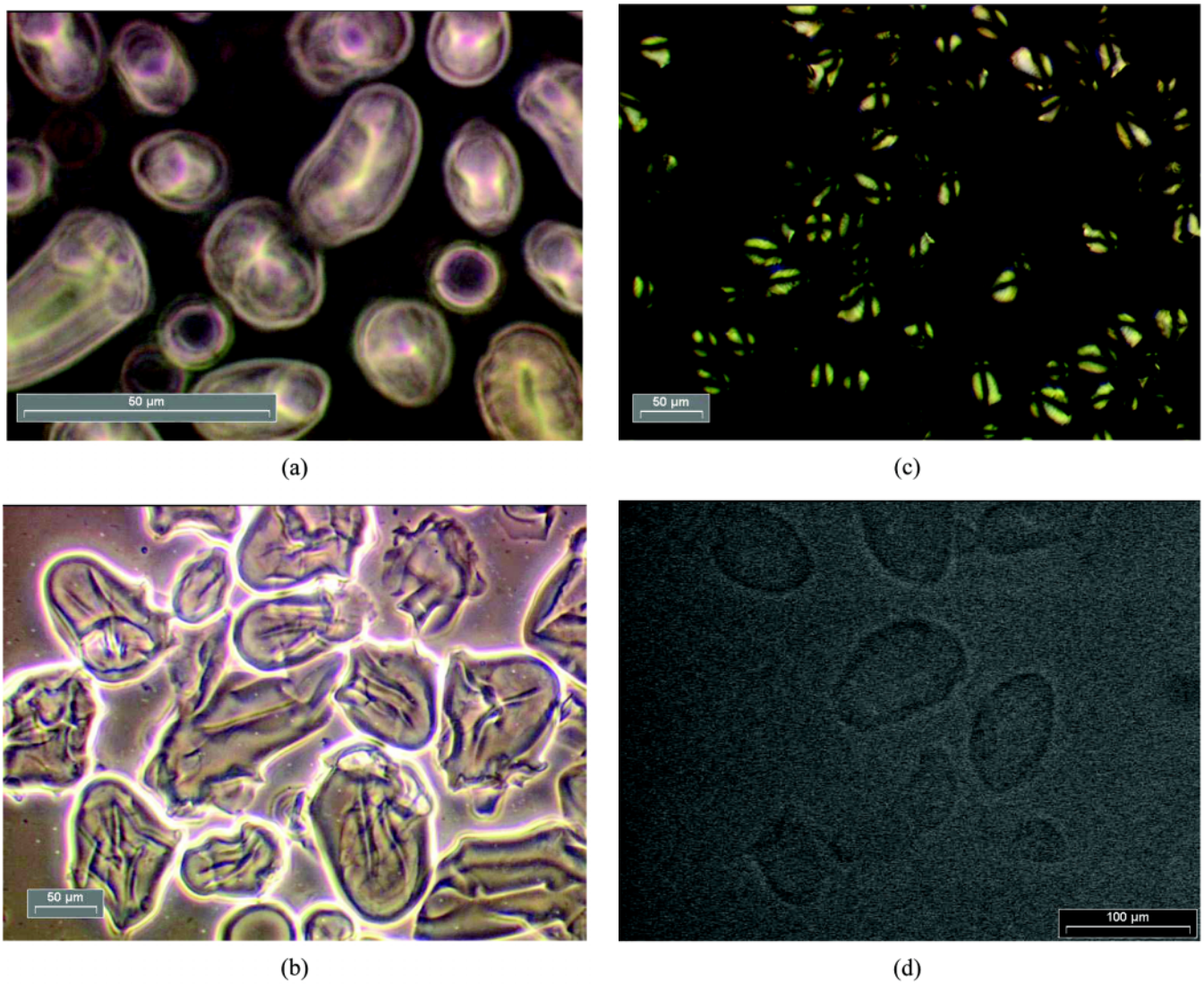
Table 6—Intrinsic viscosity of native starch and carboxymethyl starch

Type		Intrinsic viscosity (mL/g)
Native starch	DS = 0.0	151.6a ± 2.7
	DS = 0.10	108.4c ± 2.8
	DS = 0.17	115.4b ± 2.0
CMS	DS = 0.22	97.6d ± 1.4
	DS = 0.27	108.6c ± 1.7
	DS = 0.32	88.4e ± 3.02

[a]Value is means ± SD ($n = 3$). Means within a column with same letter are not significantly different at the 5% level of probability.

Microscopic observation

At room temperature (or without heating), native starch granules exhibited very limited swelling (Figure 5a). On the other hand, CMS granules exhibited rapid and greater swelling at room temperature, thus caused a larger size as shown in Figure 5b. Most of the granules in CMS appeared to be distorted and wrinkled. Some other granules were slightly folded and broken. When viewed under polarized light (Figure 5c), a dark "Maltese cross" can be observed clearly for native starch granules, but not for CMS samples (Figure 5d). The loss of Maltese cross marking indicated destruction of the ordering of crystallites in the native granules due to the carboxymethylation process. It is also observed that some of the CMS granules lost their integrity due to the conditions used in this process (concentration of NaOH,



(a) (c) (b) (d)

Figure 5—Starch granules viewed under the light microscope: (a) native starch granules viewed under phase contrast; (b) carboxymethyl starch (DS 0.32) under phase contrast; (c) native starch under polarized light; (d) carboxymethyl starch (DS 0.32) under polarized light.

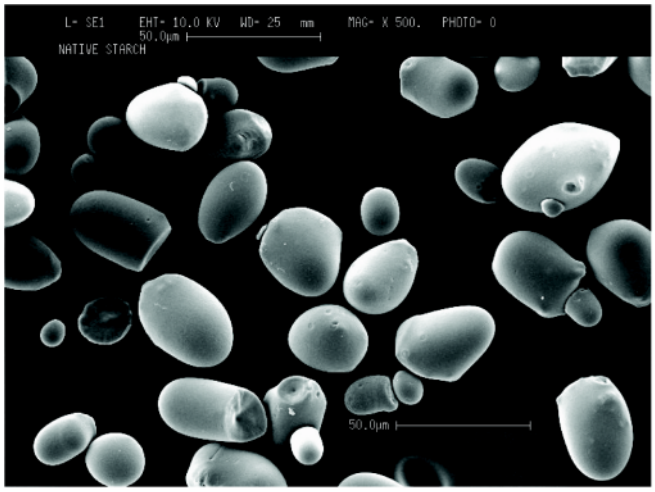
SMCA, stirring speed, and so on). From SEM, some granules of CMS were observed to have fine fissures on it, especially for CMS with the highest degree of substitution (DS = 0.32) (Figure 6b). Compared with the relatively smooth surface of native starch granules (Figure 6a), the surface of CMS granules was rather rough.

Conclusions

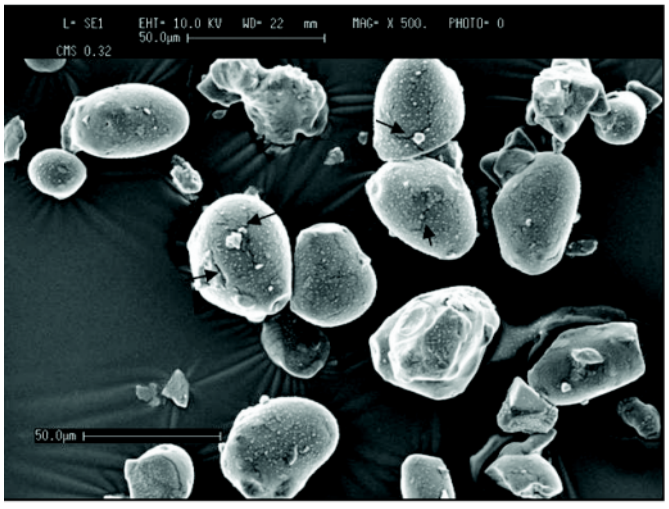
Carboxymethylated sago starch was successfully prepared with the highest degree of substitution of 0.32. The results presented showed that carboxymethylated sago starch exhibited excellent dispersibility, increased swelling power and solubility, and less tendency toward retrogradation. Carboxymethylated sago starch exhibited good freeze-thaw stability, which indicates its stability during storage at low temperature.

Acknowledgments

This work was supported by the 8th Malaysia Plan R&D grant (project nr 03-02-05-2066 EA 004) under the Intensification of Research in Priority Areas (IRPA) Program of the Ministry of Science, Technology and Innovation, Malaysia.



(a)



(b)

Figure 6—Scanning electron micrographs of (a) native starch granules (×500); (b) carboxymethyl starch (DS 0.32) (×500). Arrows indicate fine cracks/fissures on the surface of the granule.

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