Effects of Amylose Content on the Physicochemical Properties of Sodium Carboxymethyl Rice Starches

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ABSTRACT

Nine native rice starches, prepared from different strains of rice with the amylose content between 14.67 and 29.09%, were employed in the preparation of sodium carboxymethyl rice starches (SCMRSs). The reaction was carried out at 50°C for 20 minutes, using monochloroacetic acid as a reagent under alkaline condition and 1-propanol as a solvent. The degree of substitution was determined and the physicochemical properties, including water solubility, pH and viscosity of 1% w/v solution, X-ray diffraction analyses and film-forming property were investigated. The degree of substitution of the prepared SCMRSs ranged from 0.2459 to 0.4034. All SCMRSs were freely soluble in water; the pH of 1% w/v solutions were between 8.6 and 9.9 and the viscosities ranged from 32.7 to 66.0 mPa-s. The degree of substitution showed a significant positive correlation with the amylose content, while the pH and the viscosity did not. The X-ray diffraction of all SCMRSs showed the loss of crystallinity which was possibly due to the pregelatinization of starch molecule by water and heat. At 3% w/v concentration, most SCMRS pastes formed clear films with varying film characteristics, e.g., from flaky and brittle to soft and gluey or elastic, depending on the amylose content of the native starches. The results from this study suggested that amylose content affected the physicochemical properties of not only the native starches but also the corresponding SCMRSs.

Key words: Thai rice, Sodium carboxymethyl rice starch, Amylose content, X-ray diffraction, Viscosity, Physicochemical properties, Compressibility, Film-formation

INTRODUCTION

Rice (*Oryza sativa* L., Gramineae) is the most common crop consumed by people of many Asian countries, including, among others, China, India, Japan and Thailand. Thailand is also one of the major rice growers and exporters of the world. The Royal Thai government has categorized rice as one of the four major produces, along with shrimp, rubber and cassava, that require more research studies to improve the quality, production processes and safety, as well as to create new products and expand their applications. This has led to the establishment of Rice Research Center in many areas throughout the country to conduct field experiments on growing many cultivars of rice. In addition to the grains that are consumed

as food, rice can also be processed into starch which generally contains between 16 to 17% of amylose. Nevertheless, the amylose content in different strains of rice can vary from as low as 5 to 7% to as high as 28 to 30% (Rice Research Institute, 2003) which results in the differences in some characteristics of the cooked grains such as taste and texture. Amylose and amylopectin are known to possess different physicochemical properties such as structural arrangement, size, water solubility and reactivity to iodine, as well as some pharmaceutical characteristics. Schwartz and Zelinskie (1978) studied the properties of corn starch as binder and disintegrant for tablets and suggested that the binding property was due to amylopectin while amylose was responsible for the disintegrating property. Rice and rice starch have long been used mainly in the food industry but, until recently, have not been much studied for their potentials in pharmaceutical industry, partly because starches from other sources such as cassava (tapioca), potato and corn are produced in larger quantities each year. However, the variation of amylose content in different strains of rice and the general differences between rice starch and other starches suggest that rice starch might possess some unique characteristics that makes it more suitable than other starches as some types of pharmaceutical excipients.

The limitation of the use of starch in pharmaceutical industry has been due to some poor physicochemical properties such as solubility in water, flowability and paste strength. In order to overcome these problems, chemical, physical or enzymatic methods have been employed to prepare "modified starches" with improved properties. Sodium carboxymethyl starch (SCMS), a starch ester derivative prepared by a reaction between native starch and chloroacetic acid in an alkaline condition, is among many interesting modified starches. SCMS is soluble in unheated water and yields paste with smoother texture, greater flexibility and strength than those of pregelatinized starches (Mishra et al., 1990). SCMS is officially listed in the United States Pharmacopeia (USP) and the British Pharmacopoeia (BP), is proven to be safe and has been used in many types of foods (Chen and Jane, 1994). Previous studies reported the use of SCMS, prepared mainly from tapioca starch, as binder (Pitaksuteepong, 1995), disintegrant (Theruya, 1995) and directly compressible excipient for tablet (Timaroon and Kulvanich, 1992). SCMSs prepared from mungbean and commercial-grade rice starch were also reported as potential suspending agent (Suwannapakul, 1997). The influence of the amylose content on the properties of the prepared SCMS has not been studied.

The objective of this works is to investigate the physicochemical properties of sodium carboxymethyl rice starches (SCMRSs) prepared from native starches of nine different strains of rice. 1-Propanol is chosen as a solvent. The parameters evaluated include the degree of substitution, solubility in water and the pH of aqueous solutions, X-ray diffraction pattern, viscosity, and film-forming ability and film characteristics. The information obtained from this investigation will facilitate the use of rice starch in pharmaceutical industry and can be used as a guideline for the selection of rice strains with appropriate properties for the preparation of pharmaceutical excipients.

MATERIALS AND METHODS

Materials

Nine strains of rice grains (RD 23, Chai-nat 1, Khao Jow Hawm Phitsanulok 1, RD 21, Suphanburi 60, Suphanburi 2, Pathumthani 1, Khao Dawk Mali 105 and Khao Jow Hawm Khlong Luang 1) were obtained from Chiang Mai Rice Research Center (San Pathong,

Chiang Mai). The starch was extracted from rice grains, using conventional milling method and air-dried. Following a subsequent oven-dry at 50°C for 12 hours, the native rice starch was ground to pass sieve no.80 before use. All chemicals used in the preparation and analysis of modified starches were of AR grade or equivalent. Double-distilled commercial grade methanol was used in the washing of the final products, with the exception of the final wash in which the AR grade methanol was used.

Methods
Analysis of Amylose Content
The amylose content of the native rice starch was determined according to the method described for the analysis of mill-rice amylose by Juliano (1971).

Preparation of Sodium Carboxymethyl Rice Starches (SCMRSs)
SCMRSs were prepared by a substitution reaction as described for sodium carboxymethyl mungbean starch by Kittipongpatana et al. (2005), using 1-propanol as a solvent. In brief, 30 g of monochloroacetic acid was dissolved in 400 mL of 1-propanol. Then, while stirring, 100 g of native starch powder was added into the solution followed by the solution of sodium hydroxide. The mixture was heated up to 50°C and steadily maintained for 20 minutes with continuous stirring. At the end, the reaction was ceased by neutralization with glacial acetic acid. The liquid supernatant was decanted and the powder product was washed several times with 80% methanol and a final wash with 100% methanol. The obtained modified starch was oven-dried at 50°C for 6 hours before passing through sieve no.80.

Determination of Degree of Substitution
The degree of substitution (D.S.) of each MRS was determined by the USP XXIII method described for Croscarmellose Sodium, which included two steps - titration and residue on ignition. The D.S. can be calculated using the following equation:

$$D.S. \ = \ A + S \quad .... (1)$$

when A is the degree of substitution of carboxymethyl acid and S is the degree of substitution of sodium carboxymethyl. A and S can be calculated using the information from the titration and ignition steps:

$$A \ = \ 1150 \ M/(7120\text{-}412M\text{-}80C) \quad .... (2)$$
$$S \ = \ (162+58A)C/(7102\text{-}80C) \quad .... (3)$$

when M is the mEq of base required in the titration to end point. C is the percentage of ash remained after ignition. The reported D.S. values are means of three determinations.

Solubility in Water and pH of the Solution
The solubility in unheated water was tested by adding 0.1 g of SCMRS into 10 mL of water (1% w/v), mixed thoroughly and observed the solubilization and/or swelling of SCMRSs. The pH of water-soluble SCMRS solutions (1%) was determined directly, using a Waterproof pHScan WP 2 (Eutech Instruments, USA).

Paste Clarity

A 1% w/v paste (0.2 g in 20 mL distilled water) was prepared for each SCMRS. The clarity of pastes was determined by placing 2.5 mL in a disposable cuvette and measured the absorption on a spectrophotometer at 650 nm against a water blank.

X-ray Diffraction

X-ray diffraction patterns of native starches and SCMRSs were recorded in the reflection mode on a Siemens D-500 X-ray diffractometer. Diffractograms were registered at Bragg Angle $(2\theta) = 5\text{-}40°$ at a scan rate of 5° per minute.

Viscosity

Apparent viscosity was measured using a Brookfield R/S-CPS rheometer (Bob-and-Cup format) in suspensions of 1.0 g SCMRS in 100 mL deionized water (1% w/v). The samples were prepared by dispersing SCMRS powder onto stirring water, mix thoroughly, and the suspensions were allowed to stand overnight for complete swelling before measurement. The measuring system was CC48 DIN. The mode used was CSR (controlled shear rate). The measured parameters consisted of three steps: (1) an increase of the shear rate from 0 to 1,000 s^{-1} in 1 min, (2) held at 1,000 s^{-1} for 1 min and (3) a decrease of the shear rate from 1,000 to 0 s^{-1} in 1 minute. All measurements were performed in triplicate, at a controlled temperature of 25±1°C. The data were analyzed with a Brookfield Rheo 2000 software. The apparent viscosity for all samples in this study was measured at a shear rate of 1,000 s^{-1}. Viscosity was expressed in mPa.s.

Film-Forming Ability

A 3% w/v paste (0.9 g in 30 mL deionized water) was prepared for each native rice starch and SCMRS. For native starches, the mixture of starch and water was heated to boiling and stirred until paste was formed. The SCMRS was directly dissolved in unheated water and stirred to form paste. The film-forming ability of native starches and SCMRSs was determined by placing the paste in a plastic petri dish, dried at 60°C for 17 hours at 40% humidity. The film formed on the petri dish was peeled off and the characteristics of the film were observed and recorded.

Statistical Analysis

All tests were performed at least in triplicate. The statistical significant tests were performed using Duncan's multiple range test at 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

The amylose contents of the native rice starches used in this study were determined to be in a range from 14.67 to 29.09%. The values are in agreement with those reported in the literature (Rice Research Insitute, 2003). Under the specified preparation conditions, the obtained sodium carboxymethyl rice starches (SCMRSs) have the degree of substitution (D.S.) values between 0.2459 and 0.4034. All SCMRSs were completely soluble in unheated water and formed translucent to clear gels, while native starches were only partially soluble even in hot/boiling water and yielded opaque gels. The pH of the 1% (w/v) solutions ranged between

8.6 and 9.9, as a result of the alkalinized carboxymethyl substitution, while the viscosities ranged between 32.7 to 66.0 mPa.s (Table 1).

Table 1. Rice strains and physicochemical properties of native and sodium carboxymethyl rice starches.

Rice Strains	Reported Amylose (%)[*]	Analyzed Amylose (%)	D.S.	Solubility in water	$A_{650\,nm}$	pH (1% w/v)	Viscosity, 1% w/v (mPa.s)
Chai-nat 1	26-27	29.09	0.4034	✔	0.024	8.6	50.7±0.4
Dawk Mali 105	12-17	16.19	0.3024	✔	0.083	8.8	61.3±0.6
Khlong Luang 1	18-19	17.76	0.3125	✔	0.087	8.9	32.7±0.2
Pathumthani 1	17.8	15.82	0.2623	✔	0.097	9.9	66.0±0.3
Phitsanulok 1	14.9	14.67	0.2459	✔	0.113	9.5	49.0±0.4
RD 21	17-20	18.48	0.3319	✔	0.064	8.6	52.7±0.1
RD 23	25-30	25.80	0.3729	✔	0.033	8.6	47.0±0.2
Suphanburi 2	22-23	25.26	0.3434	✔	0.042	8.6	61.3±0.3
Suphanburi 60	23-27	25.33	0.3538	✔	0.051	8.7	40.7±0.5

* data from Rice Research Institute (2003)

A good positive correlation between the amylose contents in the native rice starch and values of the degree of substitution ($r = 0.9278$, $r^2 = 0.8607$; Figure 1) of the carboxymethylated derivatives suggested that the amylose molecules in starch are likely to be more susceptible to chemical modification than the amylopectin molecules. This could be due to the straight-chain nature of the amylose which allows better access of the reacting chemicals to the –OH groups of the glucose units, while the branched-chain and considerably larger amylopectin structure could limit such access. The ability to dissolve in water of all SMCRSs is also an indication that amylose portions, which are less soluble in water than amylopectin, were involved in the substitution reaction that converted them into a soluble part. On the other hand, the viscosities of 1% w/v solutions do not correlate with the amylose content nor the D.S. values (Figure 2).

Figure 1. Correlation between the amylose content (%) of the native starches and the degree of substitution (D.S.) of the sodium carboxymethyl derivatives. r = 0.9278, r² = 0.8607.
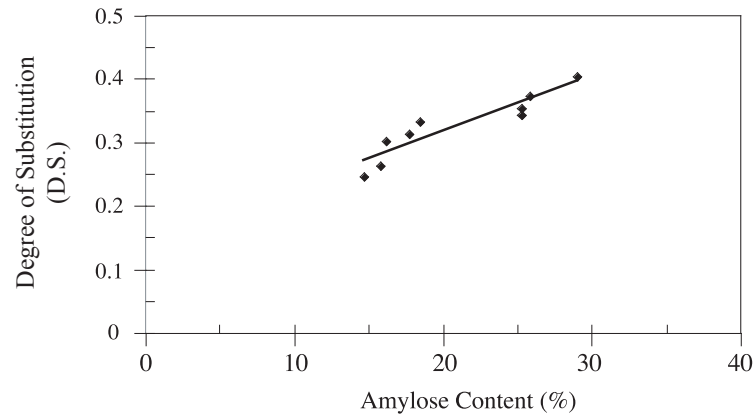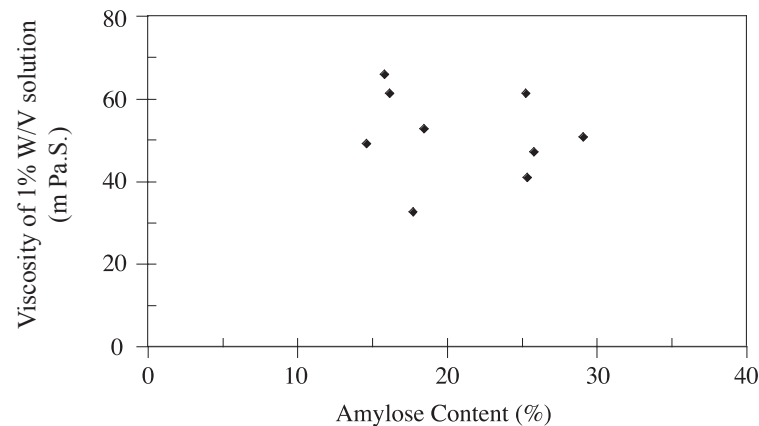

Figure 2. Correlation between the amylose content (%) of the native starches and the viscosity of the sodium carboxymethyl derivatives.

   The X-ray diffractogram of the native rice starch showed an A-type crystal pattern with strong reflections at 14.9,17.8 and 22.8° of diffraction angle 2θ for all strains of rice. These values are in agreement with those reported for cereal starches. Upon chemical modification to SCMRSs, these visible reflections disappeared (Figure 3) which indicated that the crystalline forms in the starch molecules had been removed. Similar finding was previously reported for mungbean starch (Kittipongpatana et al., 2005) and was suggested to be due to the breakage of chemical bonds in starch molecules by heat and water and caused the rupture of starch granules. The X-ray results indicated that the starch granules were not only carboxymethylated but also pregelatinized. This is evident also by the low- to-medium viscosity of the SCMRS

solutions, in contrast to the nature of carboxymethylated starch in which, in the solution, exhibits high viscosity.

Figure 3. Representative X-ray diffractograms of (A) native rice starch, and (B)sodium carboxymethyl rice starch.
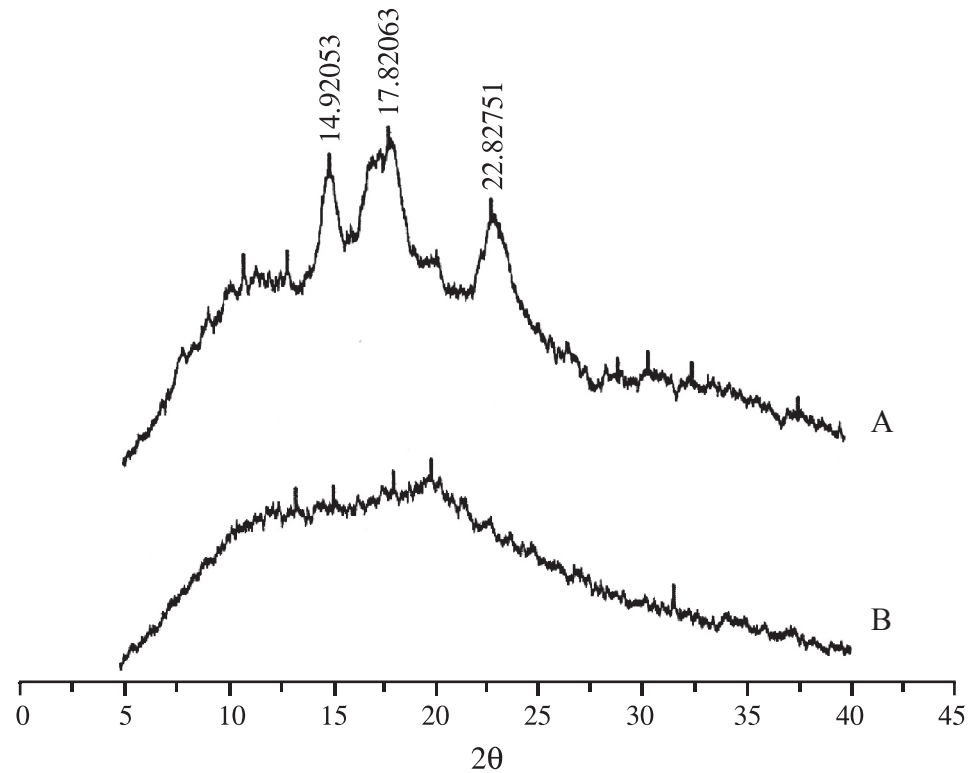
The film-forming ability of various strains of rice starches and SCMRSs is shown in Table 2. All of the native starches formed either flaky or intact films that were opaque and brittle. The films formed from starches with low amylose content were mostly flaky and very brittle, while those formed from starches with higher amylose content showed lesser degree of brittleness and became intact films. The overall film characteristics, however, were not satisfactory. On the other hand, most SCMRS pastes formed clear, intact films with varying characteristics from flaky and brittle to soft and gluey or elastic, depending on the amylose content of the original starches. The results showed that the higher the amylose content of the native starch, the better the characteristics of the film formed from the corresponding SCMRS. Amylose is known for its ability to form gel and, upon drying, becomes intact plate, while amylopectin does not exhibit such property. Since amylose is less soluble in water, its insoluble portion probably contributes to the opaqueness observed in the native starch films, while the brittleness was due to the relatively higher ratio of amylopectin to amylose. When substituted with carboxymethyl groups, amylose molecules become more soluble in water, thus improve the clarity of the pastes. The more-soluble carboxymethylated amylose molecules also conduce the integrity and strength of the SCMRS films (Figure 4).

Table 2. Film-forming ability of SCMRSs prepared from various strains of rice starches.

Strains	Amylose (%)	Film Characteristics	
		Native Starch	**SCMRS**
Phitsanulok 1	14.67	opaque, flaky, very brittle	clear, flaky and brittle
Pathumthani 1	15.82	opaque, flaky, very brittle	clear, flaky and brittle
Dawk Mali 105	16.19	opaque, flaky, very brittle	clear, intact, but fragile
Khlong Luang 1	17.76	opaque, flaky, brittle	clear, intact, but fragile
RD 21	18.48	opaque, flaky, brittle	clear, intact, soft, a little gluey
Suphanburi 2	25.26	opaque, intact, but brittle	clear, intact, soft, gluey
Suphanburi 60	25.33	opaque, intact, but brittle	clear, intact, elastic
RD 23	25.80	opaque, intact, but brittle	clear, intact, elastic
Chai-nat 1	29.09	opaque, intact, but brittle	clear, intact, elastic
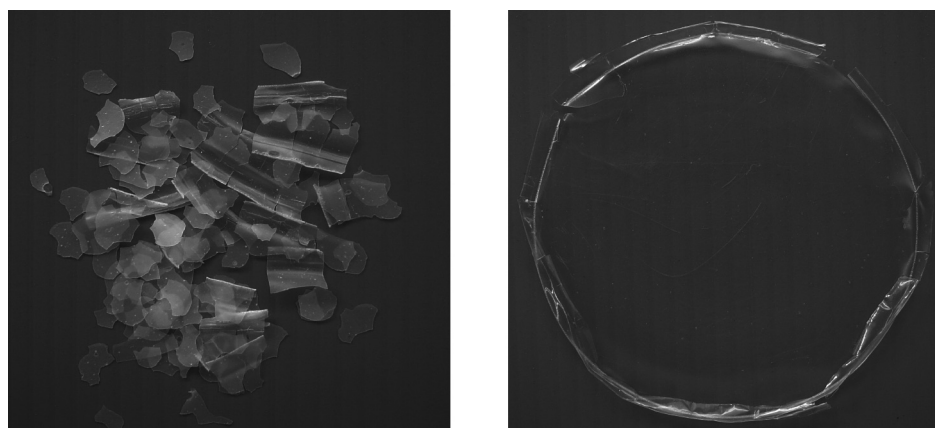
Figure 4. Representative films prepared from 3%w/v solutions of "Chai-nat 1" rice starch (A) native starch, (B) sodium carboxymethyl starch.

CONCLUSION

The modification of native rice starch by carboxymethylation reaction improves several physicochemical properties such as water solubility, clarity and viscosity, as well as film-forming ability. The difference in amylose content in rice starches affects some characteristics of SCMRSs. An increase in amylose content results in an increase in the degree of substitution and an increase in the integrity, softness and elasticity of the films. The pH and the viscosity of SCMRS solutions, in contrast, are independent of the amylose content. The amylose molecules are suggested to be actively involved in the modification by chemical reaction. The structural changes altered the physicochemical properties of the starch molecules which, in turn, resulted in different and broader applications of the modified starch, especially in pharmaceutical industry. Based on these findings, the development of pharmaceutical excipients from rice starch is currently underway in our laboratory.

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