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Influence of reaction parameters on carboxymethylation of rice starches with varying amylose contents

Kunruedee Sangseethong[a], Pathama Chatakanonda[b], Rungtiva Wansuksri[a], Klanarong Sriroth[c,*]

[a] Cassava and Starch Technology Research Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, Pathum Thani, Thailand
[b] Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand
[c] Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

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ABSTRACT

The influence of reaction parameters on the carboxymethylation of rice starches with different amylose contents was investigated. Rice starches with varying amylose contents showed various degrees of susceptibility to the reaction conditions. The maximum degree of substitution (DS) for all three rice starches was obtained under similar reaction conditions which involved a reaction medium consisting of isopropanol–water at the ratio of 90:10, a molar ratio of NaOH:AGU at 1.5 and a reaction temperature and time of 40 °C and 3 h. Under these conditions, the DS for all rice starches was similar; however, when the reaction was performed under conditions using lower NaOH concentration, the effect of starch types on the DS was observed. The results could be explained in terms of the granular/structural features of the different rice starches, their degrees of granular swelling as influenced by the reaction conditions and the accessibility of the etherifying reagents to starch molecules.

1. Introduction

Starch is a major natural polymer that has been used for a long time in both food and non-food applications. However, certain undesirable characteristics of native starch such as insolubility in cold water and sensitivity of cooked starch to high temperature, low pH and shear have limited its wider industrial use. The common approach to overcome such drawbacks is by physical or chemical modification to alter the molecular structure and thus improve the properties to suit specific applications. Carboxymethyl starch (CMS) is an important modified starch widely used in many fields. CMS is used as a disintegrant (called sodium starch glycolate) in the pharmaceutical industry (Shah & Augsburger, 2002), and as a sizing and printing agent in the textile industry (Ragheb, El-Sayiad, & Hebeish, 1997; Roberts, 1967; Tatongjai & Lumdubwong, 2010). Recently, with an increasing interest in novel environmentally friendly products, CMS has found innovative applications as a raw material for the development of biodegradable bio-based hydrogels (Lawal et al., 2009) and of a sustained-release device for drugs and bio-active compounds (Lemieux, Gosselin, & Mateescu, 2009; Mihaela Friciu, Le Tien, Ispas-Szabo, & Mateescu, 2013).

CMS is usually produced by the reaction of starch with monochloroacetic acid or sodium monochloroacetate (SMCA) based on Williamson's ether synthesis. The reaction is carried out in the presence of alkali in order to increase the nucleophilicity of the hydroxyl group and to aid the swelling of the starch granules. However, a side reaction between sodium hydroxide and SMCA can also occur (Bhattacharyya, Singhal, & Kulkarni, 1995). Since CMS becomes soluble in water when the degree of substitution is higher than 0.1 (Roberts, 1967), the reaction is usually carried out in an aqueous organic solvent, in most cases alcohol. The presence of alcohol prevents starch from gelatinizing and preserves the granular form of starch; thus, the side products and reagent residues can easily be washed out (Tijsen, Kolk, Stamhuis, & Beenackers, 2001). However, the alcoholic hydroxyl groups may compete with the starch for etherification with SMCA (Ambjörnsson, Schenzel & Germgård, 2013).

Due to the presence of an anionic functional group, CMS possesses unique properties such as solubility in cold water, improved storage stability of paste and increased paste and film clarity. Beside the botanical source of native starch, the properties of CMS are dependent on the DS (Sangseethong, Ketsilp, & Sriroth, 2005; Spychaj, Wilpiszewska, & Zdanowicz, 2013; Tatongjai &

* Corresponding author. Tel.: +66 2 940 5634; fax: +66 2 940 5634.
E-mail address: aapkrs@ku.ac.th (K. Sriroth).

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Lumdubwong, 2010). Although the synthesis of CMS was first reported in the literature in 1924 (Chowdhury, 1924) and the commercial product has long been available, it has continued to be a subject of many research studies (Bhattacharyya et al., 1995; Heinze, Pfeiffer, & Lazik, 2001; Jie, Wen-ren, Manurung, Ganzeveld, & Heeres, 2004; Kooijman, Ganzeveld, Manurung, & Heeres, 2003; Lawal, Lechner, & Kulicke, 2008; Li et al., 2010; Tijsen et al., 2001; Volkert, Loth, Lazik, & Engelhardt, 2004; Wang, Pan, Hu, Miao, & Xu, 2010). Most of these previous studies have focused on the reaction parameters that affect the DS of CMS and the optimization of CMS production from a specific type of starch; however, studies of the influence of the botanical source and structural parameters of the native starch on the carboxymethylation process have been relatively limited.

Bhattacharyya et al. (1995) studied the conditions for the carboxymethylation of common corn and waxy amaranth starches and found that the optimal reaction conditions for the two starches differed considerably. For comparison, the authors also included rice starch having a granule size similar to amaranth starch but an amylose (AM):amylopectin (AP) ratio similar to corn; and potato starch having a granule size larger than both corn and amaranth starch but an AM:AP ratio close to corn starch. However, it was not possible to draw a conclusion regarding the relationship between the DS of CMS and the size of the starch granule or the AM:AP ratio; probably because all four starches were from different botanical sources. Kittipongpatana, Chaitep, Kittipongpatana, Laenger, & Sriroth (2007) prepared CMS from rice starches and found that there was a positive correlation between the AM content in the native rice starch and the DS of the obtained CMS. On the other hand, Tatongjai and Lumdubwong (2010) reported that AM did not affect the DS of CMS prepared from rice starches with varying AM contents. The discrepancy between the previous reports might be due to the different sets of reaction conditions used in those studies.

The aim of this work was to study the carboxymethylation of rice starches containing different AM contents. The factors expected to affect the carboxymethylation process including the type of solvents, the water content in the reaction medium, the molar ratio of NaOH to AGU (n_{NaOH}:n_{AGU}) and the reaction temperature and time were systematically investigated. The results will provide insights into the structural aspect of starch that affects the carboxymethylation process and allows the determination of the optimal operating conditions to achieve carboxymethyl rice starches (CMRS) with a high degree of substitution.

2. Materials and methods

2.1. Materials

Three types of commercial rice starch were used in this study. Normal rice starch (NRS) was kindly provided by General Food Products Co., Ltd., Nakhon Rachasima, Thailand. Jasmine rice starch (JRS) and glutinous rice starch (GRS) were obtained from Bangkok Starch Industrial Co., Ltd., Nakhon Pathom, Thailand. Sodium monochloroacetate was obtained from Fluka Analytical (Buchs, Switzerland). Ethanol (95% purity) used to wash CMRS was of technical grade. All other chemicals used in the study were of analytical grade.

2.2. General analytical methods

The contents of fat, ash and moisture were examined according to AOAC methods 2003.05, 923.03 and 925.10 (2012). The nitrogen content of the rice starches was determined by the AOAC Kjedahl method 2001.11 (2012) and converted to protein contents using a factor of 5.95. The apparent amylose content was determined according to the colorimetric method of Juliano (1971).

2.3. Preparation of carboxymethyl starch

The method for the carboxymethylation of rice starches was based on our previous report (Sangseethong et al., 2005) with slight modification. The reaction was performed in aqueous-organic liquid media. The organic solvents used in this experiment were methanol, ethanol and isopropanol. The reaction was carried out in a 250 mL two-necked round-bottom flask equipped with a motor-driven stirrer. In the first step, 12.5 g of starch (10% w/w of starch–aqueous–alcohol mixture) was dispersed in the organic solvents (being varied between 70 and 95% according to the specified content of water in the aqueous–alcohol solution). While the starch slurry was vigorously stirred at 250 rpm, an aqueous solution of NaOH (0.5–3.0 mol/mol AGU) was added drop-wise. The amount of water used to prepare the NaOH solution was varied (5–30% w/w of aqueous–alcohol solution) according to the experimental plan in Table 1. The reaction mixture was then heated to the target temperature (30, 40 and 50 °C). After stirring for 10 min, SMCA powder (1 mol/mol AGU) was added to the mixture. The mixture was stirred at the defined experimental conditions for a certain period of time (0.5–5 h). At the end of the reaction period, the starch slurry was filtered, and the recovered product was suspended in 95% ethanol and neutralized (pH 5.5–6.5) with aqueous HCl solution. Following filtration, the starch was dispersed again in 85% ethanol, and the suspension was filtered. This process was continued until the silver nitrate test for chloride in the filtrate was negative. The starch was then washed with absolute ethanol twice and dried in an oven at 40 °C for 16 h. The compilation of experimental conditions for this study is given in Table 1.

2.4. Determination of degree of substitution (DS)

The Na-form of CMRS was first converted to the acid form (H-CMRS) by the method described by Stojanovic, Jeremic, Jovanovic, and Lechner (2005) with slight modification. CMRS (3 g) was dispersed in acetone (90 mL) by stirring with a magnetic stirrer and then converted to the acid form (H-CMRS) by adding 6 M HCl (7.5 mL). After being stirred for 30 min, the dispersion was filtered; the recovered product was redispersed in 80% ethanol, and the product was recovered from the suspension by filtration. This process was repeated until the filtrate became neutral. The H-CMRS was then dispersed in absolute ethanol, recovered by filtration, dried at 50 °C and ground. The H-CMRS was then used for the DS determination by back titration according to ISO-11216 (1998). About 0.5 g (accuracy of weighing ± 0.1 mg) of the H-CMRS was dissolved in 25 mL of 0.1 M NaOH. Deionized water (75 mL) was then added. The excess NaOH was back-titrated with standardized 0.1 M HCl using phenolphthalein as the indicator. A blank titration was also conducted. The DS was calculated from the following equation:

$$\mathrm{DS} = \frac{w_c \times 162}{(100\% - w_c) \times M_c}$$

where 162 g/mol is the molar mass of AGU, M_c is the molar mass of the carboxymethyl group (58 g/mol) and w_c (% by mass) is the carboxymethyl group content calculated from the following equation:

$$w_c = \frac{c_{HC1} M_c \times (V_b - V_s) \times 100\%}{m} \times \frac{100\%}{100\% - w_m}$$

where c_{HCl} (mol/L) is the HCl concentration, V_b (mL) is the volume of HCl used for the blank titration, V_s (mL) is the HCl volume used for

Table 1
Reaction parameters for carboxymethylation of rice starches.

Factor no.	Solvent	Water content (%, w/w of aqueous–alcohol solution)	Temperature (°C)	n_{NaOH}:n_{AGU} (mol/mol)	Time (h)
1	Methanol Ethanol Isopropanol	10	40	1.5	3
2	Isopropanol	5 10 20 30	40	1.5	3
3	Isopropanol	10	30 40 50	1.5	3
4	Isopropanol	10	40	0.5 1.0 1.5 2.0 3.0	3
5	Isopropanol	10	40	1.5	0.5 1 2 3 4 5

the sample titration, m (mg) is the sample mass used for titration and w_m (% by mass) is the moisture content of the test sample.

2.5. Differential scanning calorimetry (DSC)

The gelatinization properties of the rice starches were characterized using a differential scanning calorimeter (DSC7, Perkin-Elmer, Massachusetts, USA) calibrated with indium. A starch suspension (30% w/w) in a stainless steel pan was heated from 0 to 150 °C at a heating rate of 10 °C/min. The onset (T_o), peak (T_p) and conclusion (T_c) gelatinization temperatures and enthalpy (J/g dry starch) of gelatinization were determined from the melting transitions of the endotherms.

3. Results and discussion

3.1. Chemical compositions and thermal properties of native rice starches

The chemical compositions of the native rice starches are shown in Table 2. All rice starch samples had low levels of protein, fat and ash. The apparent AM contents determined by the iodine colorimetric method were in the range 6.52 to 29.60%. Accordingly, the rice starches in the present study were classified as very low AM (6.52% for the glutinous rice starch), low AM (18.10% for the jasmine rice starch) and high AM (29.60% for the normal rice starch) starches based on Juliano (1998). The relatively high apparent AM content (6.52%) in the waxy starch (GRS) could be attributed to the long branched chain of AP which also binds iodine and produces a blue color, resulting in an overestimated amylose content of starch, as suggested by Jane et al. (1999).

The thermal analysis (Table 3) showed that the starch samples were different in their gelatinization characteristics with respect to their AM content. NRS with the highest AM content had the highest T_o and T_p while the lowest gelatinization temperatures were found in GRS (waxy starch). This observation was consistent with previous studies which found that the gelatinization temperatures increased with an increasing apparent AM content in various rice starches (Chung, Liu, Lee, & Wei, 2011; Park, Ibanez, Zhong, & Shoemaker, 2007). The second endothermic peak, which was attributed to the melting of the AM-lipid complex, was observed around 100 °C in the non-waxy rice starches (NRS and JRS), while no corresponding peak was found in the waxy rice starch (GRS). The melting enthalpy of the AM-lipid complex increased with the apparent AM content, being 1.25 and 0.39 J/g for NRS and JRS, respectively.

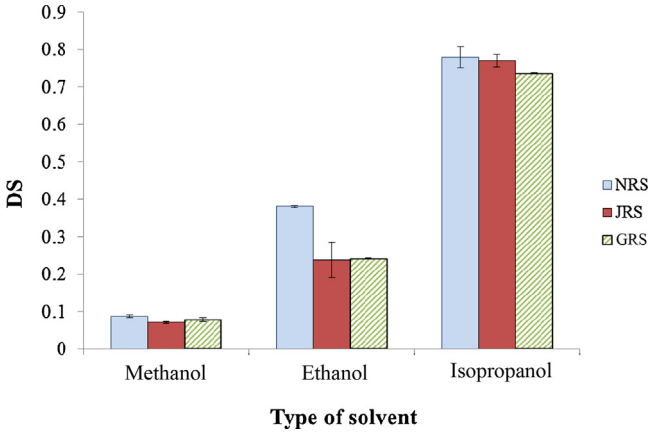

Fig. 1. The effect of various organic solvents on the DS for the carboxymethylation of normal (NRS), jasmine (JRS) and glutinous (GRS) rice starches. The reaction conditions are given in Table 1.

3.2. Effect of solvent types

Various organic solvents were investigated for the carboxymethylation of rice starches. The solvents used were methanol, ethanol and isopropanol. The solvent:water ratio was 90:10 (w/w) while other reaction parameters were kept constant as indicated in Table 1 under Factor 1. The effect of the organic solvent type on the extent of the carboxymethylation of various rice starches expressed by the DS is shown in Fig. 1. A similar trend was observed for all samples of rice starches where the highest DS was obtained in isopropanol and the lowest was obtained in methanol. CMRS prepared from rice starches with different AM contents had similar DS values when the reaction was carried out using the same

Table 2
Chemical composition of native rice starches (mean ± standard deviation).

Sample	Apparent amylose (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
Normal rice starch	29.60 ± 0.39	1.09 ± 0.04	0.28 ± 0.06	0.07 ± 0.01	12.03 ± 0.06
Jasmine rice starch	18.10 ± 0.31	0.25 ± 0.02	0.24 ± 0.02	0.17 ± 0.01	10.78 ± 0.17
Glutinous rice starch	6.52 ± 0.02	0.69 ± 0.02	0.25 ± 0.02	0.11 ± 0.00	10.20 ± 0.17

Table 3
Thermal properties of native rice starches (mean ± standard deviation).

Sample	1st endothermic transition (starch gelatinization)				2nd endothermic transition (amylose–lipid complex)			
	Temperature (°C)			Enthalpy (J/g)	Temperature (°C)			Enthalpy (J/g)
	T_o	T_p	T_c		T_o	T_p	T_c	
Normal rice starch	65.40 ± 1.39	75.09 ± 0.12	82.05 ± 0.18	15.55 ± 0.44	89.29 ± 0.07	100.92 ± 0.35	107.95 ± 0.37	1.25 ± 0.23
Jasmine rice starch	62.31 ± 0.47	69.00 ± 0.24	75.96 ± 0.13	13.65 ± 0.26	88.03 ± 0.23	93.25 ± 0.11	105.27 ± 0.11	0.39 ± 0.05
Glutinous rice starch	58.19 ± 0.30	66.84 ± 0.47	76.19 ± 0.88	15.30 ± 0.45	nd.[a]	nd.[a]	nd.[a]	nd.[a]

[a] Not detected.

solvent system, except for NRS in ethanol which showed a higher DS than the other two types of starch. The results observed were in agreement with the previous studies reported in the literature that aqueous isopropanol was the most efficient reaction medium for the carboxymethylation of various starches (Tijsen et al., 2001; Jie et al., 2004; Lawal, Lechner, Hartmann, & Kulicke, 2007; Lawal et al., 2008) as well as cellulose (Pushpamalar, Langford, Ahmad, & Lim, 2006). The effects of different alcohols on etherifying reactions of polysaccharides have been reported in several literature papers. Covered here are only those related to carboxymethylations. It has been suggested that the effects of the solvent system on carboxymethylation are dependent on the solvent polarity and its ability to dissolved sodium hydroxide (Stigsson, Kloow, & Germgård, 2006). Olaru and Olaru (2001) studied the effect of different solvents on carboxymethylation of cellulose and found that the reaction was more efficient when conducted in aqueous isopropanol than in aqueous ethanol. The results from that study also indicated that the change of crystallinity and polymorphism of cellulose during alkalization caused by different solvent systems was the main reason for different reactivity observed. It was suggested that the change in crystallinity and polymorphism might be due to the partition of sodium hydroxide between the reaction medium and the cellulose chain. This partition occurred when suspending cellulose in a mixture of an organic solvent, water and sodium hydroxide. In such a system, the organic medium played the role of uniformly distributing NaOH with water in cellulose and so to form an 'aqueous' solution of NaOH in the cellulose phase. The nature and composition of the reaction medium played an important part in the structural transformation of the cellulose during alkalization. In the case with more polar solvents like ethanol, NaOH could be easily dissolved in the solvent phase and the concentration of NaOH in an aqueous layer near the polymeric chains was presumably lower; consequently, a minimum modification of the crystalline structure of solid polymer was observed. On the other hand, isopropanol, having lower dielectric constant, was a poorer solvent for NaOH and it favored a higher concentration of NaOH in the vicinity of cellulose, causing a substantial decrystallization of the cellulose (Olaru & Olaru, 2001). Similar effects of solvents could explain the results observed in our study; thus, isopropanol, having lower dielectric constant, might cause more pronounced change in crystalline structure of starch granules resulting in the highest DS of CMRS.

3.3. Effect of water content

The effect of the water content in the reaction medium for the carboxymethylation of rice starches with varying AM contents is presented in Fig. 2. Isopropanol containing various amounts of water between 5% and 30% was used as the reaction medium. For NRS, the results showed that the DS increased as the water content increased from 5 to 10% after which a reduction in the DS was observed. Water plays a significant role in the carboxymethylation of starch by facilitating the dissociation, diffusion and adsorption of etherifying agents and it also facilitates the swelling of starch granules thus making them more accessible to the reagents (Bhattacharyya et al., 1995; Lawal et al., 2007). These factors would be responsible for the initial increase in the DS. A decrease in the DS at the water content higher than 10% could be due to a dilution effect. It has been reported that a mixture of isopropanol, water and NaOH forms a two-phase liquid system due to the low solubility of NaOH in a nonpolar solvent (Yokota, 1985). Consequently, when an aqueous NaOH solution was added to a starch-isopropanol suspension, a heterogeneous system could occur, forming an aqueous layer containing a high concentration of NaOH around the starch particles while the outer layer was an alcohol phase containing very small amount of NaOH. In this case, an increase in the water proportion in the reaction medium would result in the lower concentrations of both the reagent and the catalyst in the aqueous phase; thus the rate of reaction would be decreased. In addition, it has been suggested that at higher water fraction the side reaction between NaOH and SMCA with the formation of sodium glycolate is slightly promoted causing a reduction in the DS (Kooijman et al., 2003).

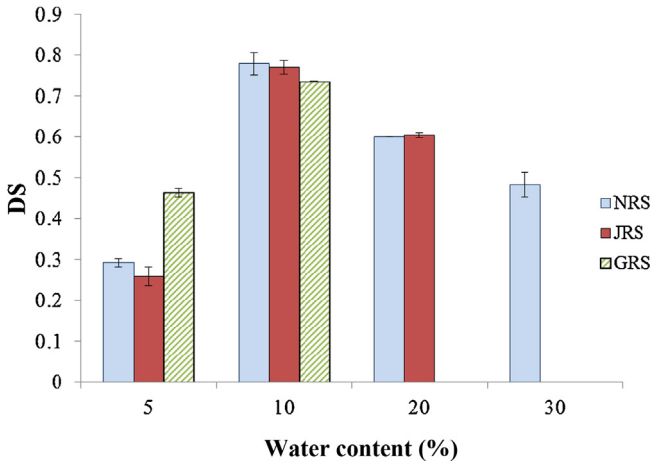

Fig. 2. The effect of the water content in the reaction medium on the DS for the carboxymethylation of normal (NRS), jasmine (JRS) and glutinous (GRS) rice starches. The reaction conditions are given in Table 1.

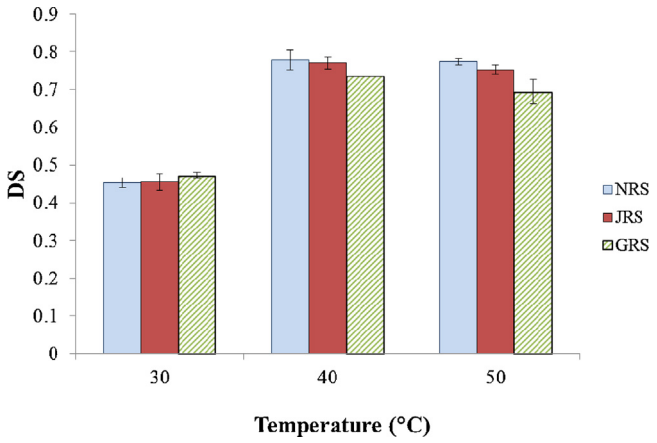

Fig. 3. The effect of the reaction temperature on the DS for the carboxymethylation of normal (NRS), jasmine (JRS) and glutinous (GRS) rice starches. The reaction conditions are given in Table 1.

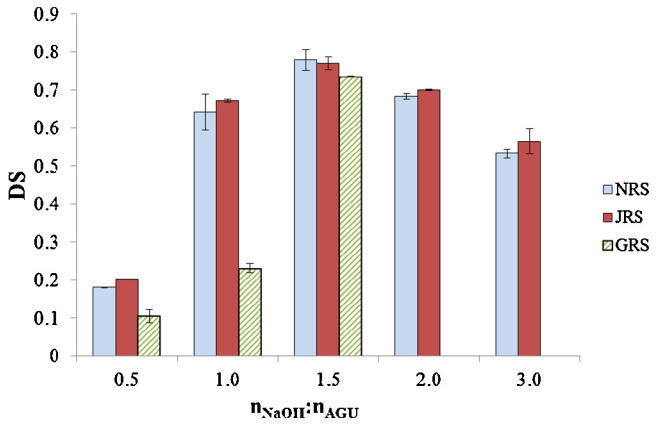

Fig. 4. The effect of the molar ratio of NaOH to AGU on the DS for the carboxymethylation of normal (NRS), jasmine (JRS) and glutinous (GRS) rice starches. The reaction conditions are given in Table 1.

A similar trend was observed for JRS and GRS with the maximum DS attained at 10% water content. However, at a water content of 30% for JRS and 20% for GRS, the gelatinization of starch occurred dramatically leading to starch agglomeration and finally the reaction mixture became a firm, sticky mass which caused the stirrer to stop; thus the reaction was terminated. During the carboxymethylation, an anionic group was introduced into the starch molecules causing the starch granules to become weaker and the starch molecules to become more water soluble. The results indicated that the highest water content at which starch could maintain its granular structure during the carboxymethylation process depended on the starch type. NRS with the highest AM content possessed the strongest granular structure, indicated by the gelatinization temperatures (Table 3), and could tolerate the highest level of water content (at least 30%), while GRS lost its granular structure at the lowest level of water content (20%). It has been suggested that AP mainly contributes to the swelling of starch granules, whereas AM and lipids suppress swelling and maintain the integrity of the swollen starch granules (Tester & Morrison, 1990). This hypothesis could explain the results observed in the present study. The GRS (waxy type) mainly consisted of AP with no measurable AM–lipid complex (as indicated in Table 3), therefore the starch granules swelled rapidly and greatly; as a result, the granular structure was lost sooner than in the other two starches. Regardless of the different degrees of granular susceptibility among the rice starches, the maximum DS for all three starches was obtained at the same water content (10%), which was similar to the values previously reported for potato (Tijsen et al., 2001), cassava (Jie et al., 2004) and amaranth starch (Bhattacharyya et al., 1995).

3.4. Effect of reaction temperature

For all three rice starches, the DS increased when the reaction temperature increased from 30 °C to 40 °C (Fig. 3). No change in the DS was observed when the temperature further increased up to 50 °C. An increase in the reaction temperature enhances the solubility and diffusion of the etherifying reagents and the swelling of the starch granules. In addition, the proportion of molecules with energy higher than the activation energy increases with an increase in temperature which contributes to a higher rate of reaction (Lawal et al., 2007). Under the conditions used in this set of experiments, the AM content in the different rice starches did not have an effect on the carboxymethylation reaction. All three rice starches gave similar DS values when the reaction was carried out at the same temperature. It has been reported that carboxymethylation causes a reduction in the gelatinization temperature of starch (Tijsen et al., 2001). As observed in the present study, at a reaction temperature of 50 °C, partial gelatinization occurred causing a slight degree of agglomeration especially for the GRS. Therefore, in order to avoid the gelatinization and agglomeration of starch granules during the process, the reaction temperature of 40 °C was chosen for the investigation of other reaction parameters.

3.5. Effect of NaOH concentration

The effect of the sodium hydroxide concentration was studied by varying the molar ratio of NaOH to AGU (n_{NaOH}:n_{AGU}) between 0.5 and 3.0 (Table 1). The DS of all starch samples increased as the molar ratio of NaOH:AGU increased from 0.5 to 1.5 (Fig. 4). The initial increase of DS with the NaOH concentration was expected since NaOH was used to activate the starch molecules with the formation of starch alkoxide; it also facilitated the swelling of starch granules enhancing the diffusion and penetration of the etherifying agents into the starch granular structure. The maximum DS of all three starches was attained at the same NaOH concentration which was at n_{NaOH}:n_{AGU} of 1.5. Any further increase in the NaOH concentration caused a decrease in the DS of NRS and JRS, while GRS starch gelatinized and agglomerated into a lump and mixing was no longer possible; thus, the reaction was stopped. A decrease in the DS of NRS and JRS at a higher molar ratio of NaOH to AGU could be explained by the competition between the main reaction of the starch molecules with SMCA and the side reaction of NaOH with SMCA, with the side reaction becoming more significant when the molar ratio of NaOH to AGU was higher than 1.5.

In contrast to NRS and JRS, GRS became gelatinized and agglomerated forming a stiff mass when the molar ratio of NaOH to AGU was higher than 1.5. These results indicated that the granular structure of GRS is weaker than that of NRS and JRS as discussed in Section 3.3; thus, GRS lost its granular structure under the reaction conditions at a lower alkaline concentration when compared with the other two rice starches.

3.6. Effect of reaction time

For all starches, the DS increased with an increasing reaction time between 0.5 and 3.0 h (Fig. 5). An increase in reaction time provided a longer period for the swelling of starch granules as well as for the diffusion of the reagent into starch granules; as a result, contacts between the etherifying agent and the starch molecules could be improved. (Bhattacharyya et al., 1995). Since there was no remarkable increase in the DS observed after 3 h of reaction,

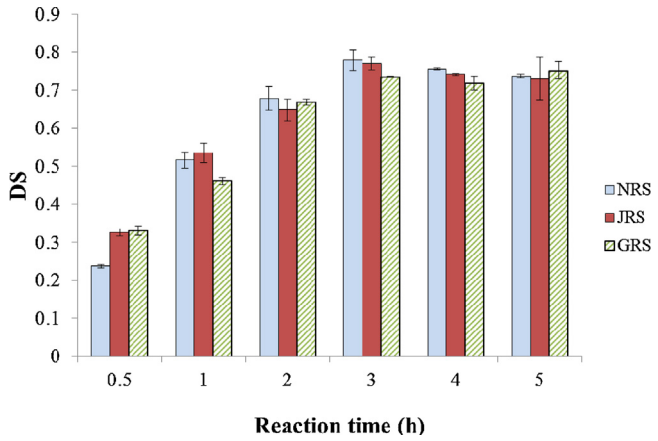

Fig. 5. The effect of the reaction time on the DS for the carboxymethylation of normal (NRS), jasmine (JRS) and glutinous (GRS) rice starches. The reaction conditions are given in Table 1.

it appeared that the reaction time of 3 h was suitable for the carboxymethylation of all three rice starches.

3.7. *Effect of the rice starch type*

Beside the reaction conditions, the structure and compositions of starch (i.e., granule size and shape, AM/AP ratio, crystallinity) have been suggested to affect the carboxymethylation process. The results in this study indicated that, regardless of AM content of the native starch, the reaction conditions to obtain the maximum DS for all three rice starches were similar which were in a reaction medium consisting of isopropanol–water at the ratio of 90:10, a molar ratio of NaOH:AGU at 1.5 and a reaction temperature and time of 40 °C and 3 h, respectively. Interestingly, under these conditions, the CMRS obtained from all rice starches had similar values of the DS (0.78, 0.77 and 0.74 for NRS, JRS and GRS, respectively) suggesting that their reactivity toward the carboxymethylation process was similar. However, under the conditions with the molar ratio of NaOH:AGU lower than 1.5, the effect of the starch type on the carboxymethylation reaction could be observed (Fig. 4).

Stojanovic, Jeremic, and Jovanovic (2000) investigated the carboxymethylation of native, oxidized and high AM starches in pure water and ethanol while keeping the other reaction parameters constant. They reported that the starch type and its history had no influence on the DS values when the carboxymethylation was carried out in water, probably because the starches were completely gelatinized and had lost their granular structure. However, when the reaction was performed in ethanol, the starch maintained its granular structure during the whole reaction, and since the granular structure depends on the starch type, different DS values were obtained. Similar explanation could be used to understand the results observed in the present study. As the carboxymethylation is done under heterogeneous conditions with semi-crystalline starch granules suspended in an aqueous–alcohol mixture, the dense packing of molecules inside the granules might limit access of the etherifying agent to the starch molecules. In this reaction, NaOH serves as a swelling agent modifying the crystalline structure of starch granules and increasing the diffusion and penetration of the etherifying agent into the starch granular structure. Thus, depending on the NaOH concentration, the starch granules swell to various extents. It could be assumed that under the conditions containing higher NaOH concentration (n_{NaOH}:$n_{AGU} \geq 1.5$), the swelling of the starch granules might be higher which could provide sufficient accessibility to the starch molecules by the etherifying agents; thus, the granular structure of the different rice starches had no influence on the DS values. However, under the conditions with the lower NaOH concentration, the compact structure of the starch granules could possibly be preserved; consequently, the supramolecular and morphological structure of native starch could influence the reaction process.

It has been suggested that AP is responsible for the ordered crystalline structure of the starch granules, while AM is associated with amorphous region and tends to disrupt the order in starch crystallites (Cheetham & Tao, 1998a; Cheetham & Tao, 1998b; Hizukuri, 1985). Although AP fine structure plays an important role in the crystalline regions of starch, many studies have reported the invert correlation between the relative crystallinity of starch and the apparent AM content. For maize starch, the degree of granule crystallinity was reported to decrease from 42% to 17% as AM content increased from 0% to 84% (Cheetham & Tao, 1998a). Iturriaga, Lopez, and Añon (2004) reported that the crystallinity degree of waxy rice starches was higher (48%) than that of the non-waxy ones (37% to 40%), with no significant difference was found among the non-waxy varieties. The structural differences between waxy and non-waxy starches reported in the literature could possibly explain the lower DS of the GRS than those of the other two starches when the reaction was carried out under conditions with a molar ratio of NaOH:AGU lower than 1.5 (Fig. 4). Since the GRS (waxy type) consisted mainly of AP, it was supposed to contain a higher proportion of crystalline regions which were less accessible to the etherifying reagents; as a result, it gave lower DS than the other two starches.

These results could explain the conflicting reports in previous studies on the effect of the AM content of rice starches on the carboxymethylation reaction in which Kittipongpatana et al. (2007) found that there was a positive correlation between the AM content and the DS of CMRS while Tatongjai and Lumdubwong (2010) reported that the AM content did not affect the DS values. It is possible that the former study was carried out under conditions that preserved the granular structure of the rice starches (the reaction containing NaOH:AGU at a molar ratio of 1.2:1.0), while the latter study was performed under conditions that provided high granular swelling and accessibility to starch molecules (the reaction containing NaOH:AGU at the molar ratios of 3.0:1.0 and 3.5:1.0).

4. Conclusions

Three rice starches with varying AM contents were modified by the carboxymethylation process. The influence of the key reaction parameters was investigated. The granular structure of rice starch with a lower AM content was more susceptible to higher proportions of water in the reaction medium and NaOH concentrations, as the starch gelatinized and its granular structure was lost. The reaction conditions to obtain the maximum DS were independent of the AM content, with an isopropanol–water ratio of 90:10, a molar ratio of NaOH:AGU at 1.5 and a reaction temperature and time of 40 °C and 3 h, respectively. Under these conditions, the DS values obtained for all three rice starches were nearly the same suggesting that the even accessibility of the starch molecules throughout the granules was achieved. However, under the conditions containing lower concentration of NaOH in which the granular structure of the native starch was partially maintained, different DS values were obtained for different starches.

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References

Ambjörnsson, H. A., Schenzel, K., & Germgård, U. (2013). Carboxymethyl cellulose produced at different mercerization conditions and characterized by NIR FT Raman spectroscopy in combination with multivariate analytical methods. *BioResources*, *8*, 1918–1932.

Association of Official Analytical Chemists (AOAC). (2012). *Official methods of analysis* (19th ed.). Virginia: The Association of Official Analytical Chemists.

Bhattacharyya, D., Singhal, R. S., & Kulkarni, P. R. (1995). A comparative account of conditions for synthesis of sodium carboxymethyl starch from corn and amaranth starch. *Carbohydrate Polymers*, *27*, 247–253.

Cheetham, N. W. H., & Tao, L. (1998a). Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohydrate Polymers*, *36*, 277–284.

Cheetham, N. W. H., & Tao, L. (1998b). Solid state NMR studies on the structural and conformational properties of natural maize starches. *Carbohydrate Polymers*, *36*, 285–292.

Chowdhury, J. K. (1924). Esters of polysaccharides with hydroxyl acid. *Biochemische Zeitschrift*, *148*, 76–97.

Chung, H. J., Liu, Q., Lee, L., & Wei, D. (2011). Relationship between the structure, physicochemical properties and in vitro digestibility of rice starches with different amylose contents. *Food Hydrocolloids*, *25*, 968–975.

Heinze, Th., Pfeiffer, K., & Lazik, W. (2001). Starch derivatives with high degree of functionalization. III. Influence of reaction conditions and starting materials on molecular structure of carboxymethyl starch. *Journal of Applied Polymer Science*, *81*, 2036–2044.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydrate Research*, *141*, 295–306.

ISO-11216. (1998). *Modified starch-determination of content of carboxymethyl groups in carboxymethyl starch*. Switzerland: International Organization for Standardization.

Iturriaga, L., Lopez, B., & Añon, M. (2004). Thermal and physicochemical characterization of seven argentine rice flours and starches. *Food Research International*, *37*, 439–447.

Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, *76*, 629–637.

Jie, Y., Wen-ren, C., Manurung, R. M., Ganzeveld, K. J., & Heeres, H. J. (2004). Exploratory studies on the carboxymethylation of cassava starch in water-miscible organic media. *Starch*, *56*, 100–107.

Juliano, B. O. (1971). A simplified assay for milled rice amylose. *Cereal Science Today*, *16*(10), 334–338.

Juliano, B. O. (1998). Varietal impact on rice quality. *Cereal Food World*, *43*, 207–222.

Kittipongpatana, O. S., Chaitep, W., Kittipongpatana, N., Laenger, R., & Sriroth, K. (2007). Physicochemical and pharmaceutical properties of carboxymethyl rice starches modified from native starches with different amylose content. *Cereal Chemistry*, *84*, 331–336.

Kooijman, L. M., Ganzeveld, K. J., Manurung, R. M., & Heeres, H. J. (2003). Experimental studies on the carboxymethylation of arrowroot starch in isopropanol–water media. *Starch*, *55*, 495–503.

Lawal, O. S., Lechner, M. D., Hartmann, B., & Kulicke, W. M. (2007). Carboxymethyl cocoyam starch: Synthesis, characterisation and influence of reaction parameters. *Starch*, *59*, 224–233.

Lawal, O. S., Lechner, M. D., & Kulicke, W. M. (2008). The synthesis conditions, characterizations and thermal degradation studies of an etherified starch from an unconventional source. *Polymer Degradation and Stability*, *93*, 1520–1528.

Lawal, O. S., Storz, J., Storz, H., Lohmann, D., Lechner, D., & Kulicke, W. M. (2009). Hydrogels based on carboxymethyl cassava starch cross-linked with di- or polyfunctional carboxylic acids: Synthesis, water adsorbent behavior and rheological characterizations. *European Polymer Journal*, *45*, 3399–3408.

Lemieux, M., Gosselin, P., & Mateescu, M. A. (2009). Carboxymethyl high amylose starch as excipient for controlled drug release: Mechanistic study and the influence of degree of substitution. *International Journal of Pharmaceutics*, *382*, 172–182.

Li, X., Gao, W. Y., Huang, L. J., Wang, Y. L., Huang, L. Q., & Liu, C. X. (2010). Preparation and physicochemical properties of carboxymethyl *Fritillaria ussuriensis* Maxim. starches. *Carbohydrate Polymers*, *80*, 768–773.

Mihaela Friciu, M., Le Tien, C., Ispas-Szabo, P., & Mateescu, M. A. (2013). Carboxymethyl starch and lecithin complex as matrix for targeted drug delivery: I. Monolithic Mesalamine forms for colon delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*, 521–530.

Olaru, N., & Olaru, L. (2001). Influence of organic diluents on cellulose carboxymethylation. *Macromolecular Chemistry and Physics*, *202*, 207–211.

Park, I. M., Ibanez, A. M., Zhong, F., & Shoemaker, C. F. (2007). Gelatinization and pasting properties of waxy and non-waxy rice starches. *Starch*, *59*, 388–396.

Pushpamalar, V., Langford, S. J., Ahmad, M., & Lim, Y. Y. (2006). Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste. *Carbohydrate Polymers*, *64*, 312–318.

Ragheb, A. A., El-Sayiad, H. S., & Hebeish, A. (1997). Preparation and characterization of carboxymethyl starch (CMS) products and their utilization in textile printing. *Starch*, *49*, 238–245.

Roberts, H. J. (1967). Starch derivatives. In R. L. Whistler, & E. F. Paschall (Eds.), *Starch: Chemistry and technology* (2) (pp. 293–350). New York, NY: Academic Press.

Sangseethong, K., Ketsilp, S., & Sriroth, K. (2005). The role of reaction parameters on the preparation and properties of carboxymethyl cassava starch. *Starch*, *57*, 84–93.

Shah, U., & Augsburger, L. (2002). Multiple sources of sodium starch glycolate, NF: Evaluation of functional equivalence and development of standard performance tests. *Pharmaceutical Development and Technology*, *7*, 345–359.

Spychaj, T., Wilpiszewska, K., & Zdanowicz, M. (2013). Medium and high substituted carboxymethyl starch: Synthesis, characterization and application. *Starch*, *65*, 22–33.

Stigsson, V., Kloow, G., & Germgård, U. (2006). The influence of the solvent system used during manufacturing of CMC. *Cellulose*, *13*, 705–712.

Stojanovic, Z., Jeremic, K., & Jovanovic, S. (2000). Synthesis of carboxymethyl starch. *Starch*, *52*, 413–419.

Stojanovic, Z., Jeremic, K., Jovanovic, S., & Lechner, M. D. (2005). A comparison of some methods for the determination of the degree of substitution of carboxymethyl starch. *Starch*, *57*, 79–83.

Tatongjai, J., & Lumdubwong, N. (2010). Physicochemical properties and textile utilization of low- and moderate-substituted carboxymethyl rice starches with various amylose content. *Carbohydrate Polymers*, *81*, 377–384.

Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry*, *67*, 551–559.

Tijsen, C. J., Kolk, H. J., Stamhuis, E. J., & Beenackers, A. A. C. M. (2001). An experimental study on the carboxymethylation of granular potato starch in non-aqueous media. *Carbohydrate Polymers*, *45*, 219–226.

Volkert, B., Loth, F., Lazik, W., & Engelhardt, J. (2004). Highly substituted carboxymethyl starch. *Starch*, *56*, 307–314.

Wang, L. F., Pan, S. Y., Hu, H., Miao, W. H., & Xu, X. Y. (2010). Synthesis and properties of carboxymethyl kudzu root starch. *Carbohydrate Polymers*, *80*, 174–179.

Yokota, H. (1985). The mechanism of cellulose alkalization in the isopropyl alcohol–water–sodium hydroxide-cellulose system. *Journal of Applied Polymer Science*, *30*, 263–277.