

Properties of Carboxymethylated-Cassava and Sago Starches Prepared by using Sodium Monochloroacetate

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In the present study, carboxymethyl starch (CMC) were produced from different sources of starch and their physico-chemical properties were evaluated. Carboxymethylation was performed using different concentrations of sodium monochloroacetate (1.1, 1.3 and 1.5 mol/mol of anhydrous glucose units) in a three-necked round-bottom flask (250 mL) for approximately 3 h (250 rpm and 40°C) in a two-stage reaction comprising alkalization and etherification. The introduction of carboxymethyl groups was confirmed in the results by the appearance of a new peak in the FTIR spectrum in 1650.10-1649.76 cm⁻¹ region. In addition, degree of substitution (DS) of produced CMS was ranged from 0.53-0.60. An increase in the concentration of sodium monochloroacetate (1.1, 1.3 and 1.5 mol/mol of anhydrous glucose unit) resulted in greater paste clarity, higher solubility and greater swelling than native starch. Furthermore, cassava starch (tapioca) produced a CMS exhibited greater swelling than sago starch, however by contrast, CMS-sago was more soluble and clearer than CMS-cassava.

Keywords: Carboxymethylation, Cassava, Sago, Starch.

INTRODUCTION

Starch is one of the most crucial and abundant polysaccharides after cellulose and chitin. Starch and its derivatives have numerous industrial applications because they are inexpensive, nontoxic, renewable, and biocompatible with several other materials. Sago palm (*Metroxylon* sp.) and cassava have a high utility value in the manufacturing of some commercial products, such as modified starches, lactic acid, cyclodextrins, and ethanol. In Indonesia, the land under sago forests occupies an area of 1.25 million hectares. Assuming that the productivity of sago is 100 kg per stem; at a population density of 30 stems per hectare, the expected annual production is 6.48 million tonnes of sago starch [1]. Moreover, the annual cassava starch (tapioca) production in Indonesia is approximately 26 million tonnes [2]. However, native starch has some limitations for example, it is insoluble in cold water, its viscosity after gelatinization cannot be controlled easily, aqueous solutions or gels containing starch exhibit turbidity and starch exhibits a tendency to retrogradation [3], thus these properties of starch limit its industrial applications.

The modification of starch improves its suitability for industrial applications. Carboxymethyl starch (CMS) is a modified starch containing anhydrous glucose units (AGUs) that are etherified with carboxymethyl groups. In the present study, three -OH functional groups of AGUs of starch were replaced by an etherification agent at the C6, C2, and C3 positions of each AGU [4].

Several studies on CMS production have reported variations in degree of substitution of CMS. Carboxymethylation using monochloroacetic acid and sodium monochloroacetate (SMCA) in an isopropanol medium resulted in the formation of CMS with degree of substitution (DS) of 0.37-0.85 (from potato starch) and 0.74-0.78 (from rice starch) [5,6]. Some studies have also reported the synthesis of CMS by using a mixture of starch obtained from various sources such as kudzu root, sago and corn [7-9].

Carboxymethylation of starch exhibited significant different properties in comparison with native starch. It was reported that CMS from sago starch (DS < 0.3) exhibited high swelling properties, excellent solubility at ambient temperature, low

intrinsic viscosity and a low retrograde tendency [10]. Furthermore, some CMS characteristics, such as viscosity of aqueous solution, dissolution rate, clarity, film-forming ability, gelatinization temperature, pH and storage stability, depend largely on the DS of glucosidic units [11]. Highly-substituted CMS can be used as thickeners, suspension agents and protective colloids in building materials and in the food, cosmetics and pharmaceutical industries [12]. In addition, introducing carboxymethyl groups probably increases the pH sensitivity of starch, which is a primary requirement of the matrices used for controlled release in oral drug delivery systems [13,14]. In the present study, sago starch and cassava starch (tapioca) were carboxymethylated using different concentrations of sodium monochloroacetate (SMCA) to modify and improve the characteristics of carboxymethyl starch.

EXPERIMENTAL

The raw materials used in this study were cassava starch (tapioca) and sago starch from *Metroxylon* sp., provided by local small medium enterprises in Bogor, Indonesia. Native starch was firstly alkalinized by using NaOH (p.a) followed by etherification using sodium monochloroacetate (p.a). These processes were conducted in an isopropanol (p.a).

Cassava and sago starches were sun-dried (for approximately 7 days) and artificially dried (50 °C for approximately 2 days) to achieve a moisture content of ≤ 10 %. The crude flour was subsequently pulverized and sifted using 100-mesh screens. The results of proximate analysis revealed the following components: moisture, ash, protein, fat, crude fibre, carbohydrate (by differences method), starch (by Luff-Schoorl method) and amylose [15]. The amylopectin content was calculated by subtracting the starch content from the amylose content.

Synthesis of CMS was performed according to the modified method of Sangseethong *et al.* [16]. Cassava and sago starches were carboxymethylated in an aqueous-organic liquid medium containing isopropanol as solvent. The reactions were carried out in a three-necked round-bottom flask (250 mL), equipped with a motor-driven stirrer. Starch (10 %, w/w) was dispersed in isopropanol (with a maximum water content of 17 %). When the slurry was vigorously stirred at 250 rpm, NaOH at a concentration of 1.9 mol/mol of AGU was added and heated the reaction mixture at 40 °C. After stirring for 20-30 min, three concentrations of SMCA, *viz.*, 1.1, 1.3 and 1.5 mol/mol of AGU, were added to the mixture and continued the reaction for 3 h. Next, pH of the reaction mixture was adjusted to 5.5-6.5 by using an aqueous HCl solution. The resulting CMS was purified through filtration and washing with 85 % ethanol until the filtrate exhibited a negative reaction with silver nitrate solution. The CMS obtained was then dried in an oven at 50 °C.

The solubility and swelling property were analyzed using reported method [17]. Other crucial parameters, such as paste clarity and degree of acidity were also observed. The degree of substitution of CMS was determined according to the ISO method 11216 [18]. The carboxymethyl groups in the CMS were first converted to the acid form by acidifying with HCl. The acidified starch (3 g) was then recovered by precipitation with methanol (2 mL) and distilled water (75 mL) and drying in an oven approximately at 90°C. Then, the dried starch was

treated with 25 mL of 0.1 N NaOH and stirred using a magnetic stirrer for 1 h. The starch was then back-titrated with 0.1 N HCl using phenolphthalein as an indicator. The DS values was calculated as described in the ISO method as follows:

$$W_c = \frac{c \times M_c \times (V_b - V_s) \times 100}{m} \times \frac{100}{100 - W_m}$$

$$DS \text{ values} = \frac{W_c \times M_a}{(100 - W_c) \times M_c}$$

where, W_c = percentage of CMS weight, c = HCl concentration, M_c = molar mass of CMS, V_b = titration volume, V_s = titer volume, m = CMS mass, W_m = moisture content and M_a = relative mass of AGU. In addition, reaction efficiency (RE) was defined as a ratio of the experimental degree of substitution (DS) to the theoretical DS (DSt).

$$RE (\%) = \frac{DS \times 100}{DSt}$$

with $DSt = \frac{n_{SMCA}}{n_{AGU}}$ if $n_{NaOH} \geq n_{SMCA}$; $DSt = \frac{n_{NaOH}}{n_{AGU}}$ if $n_{NaOH} < n_{SMCA}$.

Detection method: Starch granule morphology was examined using an EVO MA 10 scanning electron microscope (Carl Zeiss, SMT AG, Oberkochen, Germany). The samples were mounted on studs, sputter-coated with gold. In addition, functional group formation during carboxymethylation was analyzed by using an Fourier transform infrared (FTIR) spectrophotometer (Tensor 37, Boker). For each sample, 32 scans were recorded in the range 4000 to 400 cm^{-1} .

RESULTS AND DISCUSSION

Proximate characteristics of native starches: The proximate data (Table-1) revealed that the starch content of sago and cassava (tapioca) was approximately 96.76 and 97.81 %, respectively, having minor components such as fat, protein, ash and fibre. The small amounts of minor components were not expected to affect carboxymethylation. However, water content was relatively high (11.43-16.42 %), and this could have inhibited the reaction, because the water content is a crucial factor affecting carboxymethylation. Some water is necessary to swell the starch grains to improve their accessibility to the reagent. However, hydrolysis of chloroacetic acid occurs in the presence of water and a base as a side reaction; furthermore, excess water induces

TABLE-1
PROXIMATE CHARACTERISTICS OF NATIVE STARCHES FROM CASSAVA AND SAGO

Components	Composition (%)	
	Cassava starch	Sago starch
Moisture content	16.42	11.43
Ash content (db)	0.11	0.08
Crude fat content (db)	1.56	1.83
Crude fiber content (db)	0.72	0.45
Crude protein content (db)	1.13	0.53
Carbohydrate content by different (db)	80.05	85.69
Starch content (db)	97.81	96.76
Amylose content (db)	23.05	22.08
Amylopectin content (db)	76.95	77.72

agglomeration of starch particles [19]. To overcome this problem, sample was dried for minimizing the moisture content ($\leq 10\%$). The highest tolerable amount of water depends on numerous factors, such as type of starch, pulp density and temperature.

Additionally, amylose content did not differ significantly between sago starch (23.05%) and cassava starch (22.08%). The proportion of amylose content and amylopectin content also plays a critical role because of its crystallinity. Amylose has an amorphous structure while, amylopectin has a crystalline structure. This structural differences affect the characteristics of CMS because alkalization can break intermolecular bonds in the amorphous region of amylose, which tends to enhance swelling properties [20,21].

Degree of substitution of carboxymethyl starch and retrogradation (RE): Degree of substitution is inherently affected by the properties of native starch and reaction conditions [22]. Table-2 shows that the range of DS values of CMS-cassava and CMS-sago was 0.53-0.60. The DS value was comparable to that of CMS prepared using ethanol as a solvent (DS 0.40-0.63) [23] but was higher than that of CMS-sago synthesized using microwave radiation (DS 0.3) [8] and CMS-cassava produced using a mixture of SMCA and water as a solvent (DS 0.32) [4]. Aqueous isopropanol considered to be as medium, which increased the DS values. Isopropanol was reported to produce the highest DS in CMS obtained from rice starch because its lower dielectric constant causes a more pronounced change in the crystalline structure of starch granules [6]. Furthermore, the DS obtained in present study was categorized as medium DS. A DS of 0.39 was classified as medium, whereas a DS of 0.94 was classified as high [12]. However, the values of DS of CMS did not differ substantially between both starches (originated from different sources). Moreover, an increase in SMCA concentration did not significantly affect the DS values of CMS. A study also reported similar DS of CMS from starches obtained from potato, wheat, corn, waxy maize, high amylose and oxidized starch [19].

The efficiencies of carboxymethylation of sago and cassava starch were in the range 38-51% (Table-2), indicating that the conversion process achieved only partially perfect results. Etherification by using organic solvents initiates the process

occurring in the amorphous region of the starch granule, and these processes continue to the crystalline area [24]. This sequence limits the process and reduces the retrogradation (RE). A study also suggested that during etherification, a side reaction occurred to produce sodium glycolate and reduced the yield of carboxymethyl starch [25]. In addition, other significant factors affecting the RE of carboxymethylation were the concentrations of NaOH and water and SMCA [16]. An increase in the SMCA contents reduced the RE, particularly at higher concentrations of NaOH. Reduction in RE is likely to result from some structural changes introduced into the starch molecules as the reaction proceeded. The addition of bulky carboxymethyl group to the starch molecules may cause steric hindrance, thus interrupting the reaction between the etherifying reagent and the remaining -OH groups. This effect was more apparent at high SMCA concentrations, which eventually reduced the RE.

Physico-chemical characteristics of CMS: The CMS was prepared using different SMCA concentrations in the reaction mixture. A control sample was also prepared under the same conditions as those for CMS but without SMCA addition. The properties of the control sample were analyzed along with those of the other CMS samples. This comparison allowed separate evaluation of the effects of carboxymethylation and those of alkalization.

Table-3 illustrates some properties of CMS from sago and cassava (tapioca) starch. In general, most of the CMS physico-chemical properties, such as paste clarity, solubility and swelling properties, were substantially improved compared with native starch. The solubility and swelling property of CMS-cassava significantly increased between 4 and 10 times compared with its native starch, except at an SMCA concentration of 1.5 mol/mol AGU. An increase solubility in cold water occurred because of not only to the introduction of negatively charged carboxymethyl groups but also because of alkalization. The strong alkaline treatment transformed the -OH groups of the starch molecules (St-OH) into alkoxide groups (St-O₂) [26]. The repulsion between negative charges resulted in the dissociation of double-helical regions and the degradation of crystalline structure, which resulted soluble and swellable starch. Moreover,

TABLE-2
DEGREE OF SUBSTITUTION (DS) VALUES AND REACTION EFFICIENCY OF CMS OF CASSAVA AND SAGO

Parameters	Concentration of sodium monochloroacetate (SMCA) (mol/mol AGU)					
	Tapioca			Sago starch		
	1.1	1.3	1.5	1.1	1.3	1.5
DS values	0.57 ^a ±0.00	0.60 ^a ±0.02	0.59 ^a ±0.02	0.56 ^{ab} ±0.01	0.53 ^a ±0.01	0.58 ^b ±0.03
Reaction efficiency (%)	51.69 ^a ±0.05	46.52 ^a ±1.92	38.10 ^a ±0.58	51.46 ^b ±1.02	44.62 ^c ±5.03	38.50 ^d ±1.74

TABLE-3
PHYSICO-CHEMICAL PROPERTIES OF CMS FROM TAPIOCA AND SAGO

Parameters	Native starch	Cassava starch			Native starch	Sago starch		
		CMS (mol/mol AGU)				CMS (mol/mol AGU)		
		1.1	1.3	1.5		1.1	1.3	1.5
pH	6.44 ^a ±0.04	8.23 ^b ±0.06	8.58 ^b ±0.12	7.70 ^c ±0.37	6.68 ^a ±0.22	8.13 ^c ±0.10	7.23 ^d ±0.23	8.68 ^b ±0.12
Paste clarity (%)	77.50 ^a ±0.00	88.55 ^b ±1.34	92.68 ^c ±1.73	93.70 ^c ±0.00	70.27 ^a ±4.20	93.55 ^c ±2.33	98.25 ^c ±0.64	95.78 ^c ±3.78
Solubility (70 °C)	14.00 ^a ±0.00	82.00 ^b ±8.49	81.00 ^b ±1.41	4.00 ^c ±0.00	20.00 ^a ±0.00	76.00 ^b ±8.49	82.00 ^c ±5.66	84.00 ^c ±2.83
Swelling power (70 °C)	9.07 ^a ±0.00	75.22 ^c ±5.35	76.04 ^c ±1.86	98.06 ^d ±2.45	9.3 ^a ±0.00	53.46 ^b ±7.09	29.21 ^c ±4.41	34.73 ^c ±6.25

*[NaOH]: 1.9 mol NaOH/mol AGU

the swelling property increased with the concentrations of NaOH used in alkalisation [21]. In previous study, the swelling property was affected by the stirring process in alkalization because the agitation equipment supplied the energy of mechanical shear and defined the activation of starch carboxyl group [27].

The starches of both origins exhibited specific characteristics of CMS (Table-3). Cassava (tapioca) starch tended to exhibit considerably greater swelling (98 %) than sago starch (34 %). The differences in the crystalline nature of native starches affected the characteristics because the swelling property was strongly affected by amylopectin [8] and the negatively correlated with amylose [28]. Cassava starch exhibits an A type crystalline structure, whereas sago starch exhibits a B type crystalline structure [20]. This differences were also confirmed by FTIR analysis, which revealed that cassava (tapioca) CMS exhibited a sharper peak of ether functional group formation than did CMS-sago. Ether formation was assumed to be the main factor underlying the considerably higher solubility (almost 90 %) of CMS-sago.

Granular morphology of CMS: Starch modification possibly affects its granular morphology. In this study, granular structure was observed using a scanning electron microscope;

in particular, CMS treated by 1.5 mol SMCA/mol AGU owing to its greater swelling property. Originally, native starch has a smooth surface area, specific shape, granules of various sizes, and slight dents in a few starch granules [24]. In this study, native sago starch exhibited a well-defined ellipsoidal shape (Fig. 1a) and a larger granule size (21.66-33.17 μm in diameter) than native cassava (tapioca) starch (12.06-14.18 μm), but native cassava starch exhibited uniformly spherical granule shape and size (Fig. 1b).

In this study, the granule structures of CMS from sago and cassava differed from those of their native starches. Fig. 1c-d illustrates that the granule structure was deformed and disrupted. The granule surface changed rough and exhibited groove and cavities. In addition, granule sizes of both types of CMS were greater than those of the native starches. Similar to the findings of a previous study, granule shape of CMS from sago starch was distorted compared with the native starch, and its surface exhibited greater roughness as well as grooves and cracks [25]. Moreover, a honeycomb-like porous structure was observed on the surface of CMS from *C. angustifolia* [29]. This morphological transformation was suggested to have occurred because of alkalization in which Na^+ ion is released during cleavage of the hydrogen bond. Alkalization disrupts the structure and thus

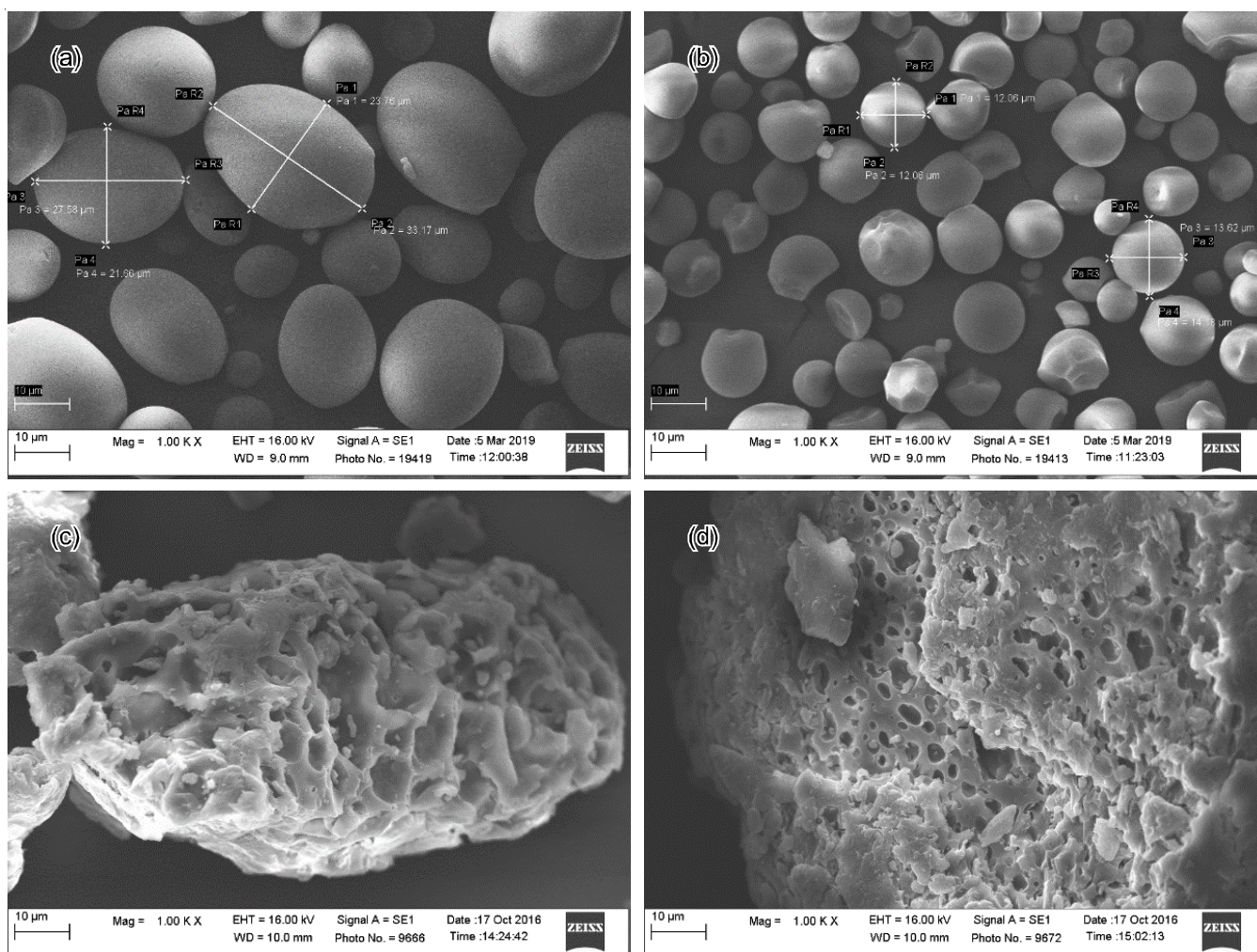


Fig. 1. SEM micrographs of native sago starch (a), native cassava starch (b), CMS from sago starch (c) and CMS from cassava starch (d) with NaOH concentration of 1.9 mol/mol AGU and sodium monochloroacetate acid (SMCA) concentration of 1.5 mol/mol AGU (1000 of multiplication)

the surface of granules appeared eroded [21]. Strong alkaline treatment also disrupts the crystalline structure and the granule surface became shrunken and collapsed [16]. In addition, pore formation also occurred on the entire surface of granule with different shapes and sizes of pores. The pore size of CMS-sago (Fig. 1c) was larger (diameter range 4.133-22.33 μm) than the CMS-tapioca (Fig. 1d) (diameter range 1.899-6.941 μm).

FTIR analysis: The introduction of carboxymethyl groups into recurrent polysaccharide units can be detected through infrared spectroscopy. Fig. 2 illustrates the formation of a functional group at $\sim 1649.76\text{ cm}^{-1}$ (tapioca) and 1650.10 cm^{-1} (sago). These two peaks were close to the absorption band at 1650 cm^{-1} , which correspond to the ether group. The appearance of ether group confirmed successful etherification during carboxymethylation. Previously, sharp peaks at 1603, 1423 and 1324 cm^{-1} indicated carboxymethylation by using monochloroacetic acid [30]. These absorption bands represented asymmetric ($-\text{CO}$) and symmetric (COO^-) carbonyl groups, $-\text{CH}_2$ scissoring and $-\text{OH}$ bending vibrations, respectively. Similarly, another study also reported peaks in the range 1600-1580 and $1410\text{--}1318\text{ cm}^{-1}$ indicated carboxymethylation of potato starch [12], while peaks near 1600 cm^{-1} ($\text{C}=\text{O}$ *str.*) indicated carboxymethylation of Indian palo starch [29].

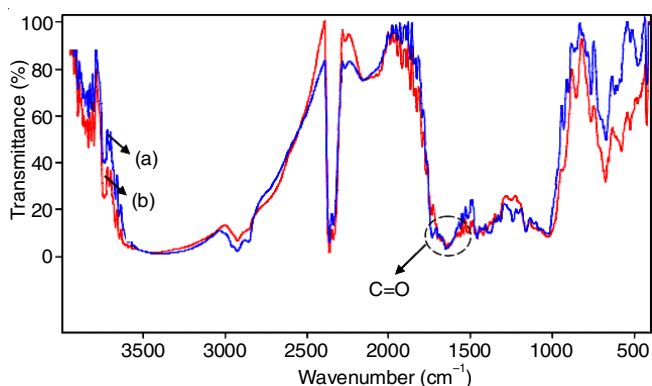


Fig. 2. FTIR spectra of CMS from cassava starch (a) and sago starch (b)

Conclusion

Carboxymethyl starch (CMS) were successfully produced from sago and cassava (tapioca) using sodium monochloroacetate (SMCA). The reaction resulted in CMS exhibited a varied degrees of solubility in cold water and swelling properties which in turn were affected by the concentration of SMCA. In addition, CMS-cassava exhibited greater swelling than CMS-sago.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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