

POLIKARBOKSILAT DARI PATI YANG BIODEGRADABEL DAN LARUT DALAM AIR

(BIODEGRADABLE AND WATER-SOLUBLE POLYCARBOXYLATES DERIVED FROM STARCH)

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ABSTRACT

Utilization of natural resources, like polysaccharide, as a raw material for the preparation of biodegradable and functional polymer was studied. Partially dicarboxylated polysaccharides were prepared using starch from corn, sago and tapioca, and comparison was made of their biodegradability, hydrolytic degradability and calcium ion sequestration capacity. The biodegradability of dicarboxylated starch sodium salt (DC-starch) depended on the dicarboxylation degree. DC-starch containing more than 75-85 mol% of unreacted glucopyranose groups in the polymer chain showed excellent biodegradation. DC-starch with high dicarboxylation degree was resistant to biodegradation but showed good calcium ion sequestration. Among DC-starches from corn, sago and tapioca starches, hydrolytic degradability and calcium ion sequestration performance were essentially the same.

Keywords: biodegradation, dicarboxylated starch, polycarboxylate, water-soluble polymer

ABSTRAK

Penelitian pembuatan polimer fungsional dan biodegradabel dari polisakarida telah dilakukan. Polisakarida yang mengalami dikarboksilasi-sebagian dibuat dari pati yang berasal dari jagung, sagu dan tapioka. Sifat-sifat yang dipelajari adalah biodegradabilitas, degradasi secara hidrolitik dan kapasitas penangkapan ion kalsium. Dari penelitian diperoleh bahwa biodegradabilitas pati-terdikarboksilasi (DC-starch) merupakan fungsi dari derajat dikarboksilasi. DC-starch dengan kandungan gugus glukopiranososa yang tak bereaksi dalam rantai lebih dari 75-85 mol% menunjukkan biodegradabilitas yang sangat baik. DC-starch dengan derajat dikarboksilasi yang tinggi ternyata resisten terhadap biodegradasi, tetapi menunjukkan sifat kemampuan penangkapan ion kalsium yang baik. Diantara DC-starch dengan bahan dasar jagung, sagu dan tapioka tidak menunjukkan perbedaan nyata pada degradasi secara hidrolitik dan kapasitas penangkapan ion kalsium.

Kata kunci : biodegradasi, pati terdikarboksilasi, polikarboksilat, polimer yang larut dalam air.

INTRODUCTION

As well known, biodegradable and water soluble polymer have been widely studied and published in many fields, such as in medical (Luten et.al 2003, Nair et.al, 2007), pharmaceutical (Park et.al, 2005; Gao et.al, 2003), foods (Nussinovitch, 2003), plastics (Anonim, 2002), etc. Water soluble polymer are used in industries such as in water treatment, coating, textile sizing, paints,

detergent applications, etc. Using of sodium tripolyphosphate (STPP), a builder in cleaning industry, was considered promoted environmental imbalance and the death of certain aquatic life-form due to the eutrophication. On the other hand, organic polycarboxylate salts show good chelating properties for ions hard water and may become STTP substitutes.

It is reported that high-molecular weight

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polycarboxylates showed excellent performance as dispersants and detergent builders (Crutchfield, 1978; Freeman and Bender, 1993). However, they are generally highly resistant to biodegradation. It is expected that the developments of biodegradable high-molecular weight polycarboxylates are needed in the industrial field. The introduction of biodegradable segment into the functional polymer chain is one way to design environmentally acceptable biodegradable functional polymers (Matsumura et.al, 1993).

Carbohydrates may serve as an attractive raw material for the preparation of high-molecular weight poly(carboxylic acid)s because they are inexpensive, renewable and biodegradable materials (Swift, 1995). Among the carbohydrates, starch is the most abundant material, and may be one of the most promising raw material in the future. Starch is mainly composed of two types of molecules, amylose and amylopectin. The composition of amylose and amylopectin in the starch has a wide variation depending on the origin of starch, plant variety, geographical conditions, etc (Daniel et.al, 1994). Particularly, corn, sago and tapioca starches, which are the most promising tropical products, attracted attention as a renewable raw material in the industrial field. In 1959, dicarboxylated starches were suggested by Hofreiter et.al. for use with detergents to improve washing performance (Hofreiter et.al, 1959). After this, some investigations of dicarboxylated polysaccharides were reported with respect to the synthetic methods and properties (Nieuwenhuizen et.al, 1985; Floor et.al, 1989). Some properties of polycarboxylates obtained from amylose and amylopectin individually (Dannoue and Matsumura, 1993; Matsumura et.al, 1994) were reported. However, starch composed both of amylose and amylopectin has not yet been extensively studied. In this paper, starches from corn, sago and tapioca were partially dicarboxylated to retain biodegradable glycopyranose groups by the conversion of the vicinal diols of glycopyranose into dicarboxylates via dialdehydes and compared with respect to biodegradability, hydrolytic degradability as well as functionality.

EXPERIMENTAL

Materials and measurements

Starch samples from corn (maize, *Zea mays* L.), sago (from stems of several species of palms, principally *Metroxylon*, *Arenga* and *Mauritia* sp.) and tapioca (*Manihot utilissima* P., *Manihot esculenta*) were used in this research. The other materials were of the highest available purity and were used as purchased.

¹³C NMR spectra were recorded with a JEOL model JNM-FX90A Fourier Transform Spectrometer operating at 22.5 MHz with complete proton decoupling (JEOL Ltd., Tokyo, Japan). Infrared (IR) spectra were measured using a JASCO Fourier Transform Spectrophotometer model FT/IR-5000 (JASCO Ltd., Tokyo, Japan). Number-average molecular weight (*M_n*) and molecular weight distributions were measured by a gel-permeation chromatographic system (GPC) with commercial GPC columns (TSK gel G5000PW+ G2500PW, TOSOH Co. Ltd.) with a 0.1 M phosphate buffer containing 0.3 M NaCl, pH 6.8, as eluent. The system was calibrated with a poly(ethylene oxide) standard (*M_n* : 3000-996000, *M_w/M_n*: 1.02-1.10), purchased from TOSOH Co. Ltd. (Tokyo, Japan).

Preparation of the Partially Dicarboxylated Starches

Partially dicarboxylated starch sodium salt (DC-starch) having various dicarboxylation degree was prepared by the partial conversion of the vicinal diol of the glucopyranose groups of starch into dicarboxylates via dialdehydes as shown in Figure 1. Preparation of dicarboxylated corn starch sodium salt (DC-corn) having a number-average molecular weight (*M_n*) of 19300 and a dicarboxylation degree of 46 mol% [DC-corn-19300 (46)] is described as a representative.

Corn starch (5 gram) was suspended in 0.1 M aqueous sodium periodate (145 mL) and stirred at 4°C for 6 hours in the dark. After the reaction, the suspension was filtered, washed thoroughly with chilled water, dried in vacuum below 40°C to obtain diformylstarch in 92.8% yield (4.64 gram). The dialdehyde content of the product, determined by the

method of Nieuwenhuizen (Nieuwenhuizen, 1985), was 47.5 mol%.

Diformylstarch (4 gram) was suspended in 123 mL water, nitrogen was bubbled into the solution at 0°C for 30 min, and a solution of 26 gram sodium chlorite in 70 mL water was added to the aqueous diformylstarch. The pH of the solution was adjusted to 4 with acetic acid, and the mixture was stirred at 20 °C for 24 hours.

The temperature was then raised to 50°C and the reaction was allowed to continue for another 1 hour. Nitrogen was passed through the solution until a colorless solution was obtained. The pH of the solution was then raised to 10 with 3 M aqueous sodium hydroxide. The solution was poured slowly into a large amount of ethanol (400 mL) with stirring to precipitate the polymer.

The precipitated polymer was dissolved in 50 mL of water, and the solution was then again added slowly to a large amount of ethanol (300 mL) with stirring to precipitate the polymer. The precipitated polymer was dissolved in 50 mL of water and dialyzed against distilled water for 3 days.

The insoluble precipitate was removed by filtration, and the filtrate was evaporated in vacuum before freeze-dried. Further drying was carried out at 70°C in vacuum to obtain DC-corn in 96% yield (3.84 gram) as a white powder. The dicarboxylate content was 41 mol % = 19300 and = 3.6 by GPC.

The following spectral data for DC-Corn-19300(46) confirm the structure.

IR(KBr) : 3333(OH), 2934(CH₂), 1614, 1419 (COONa), 1153, 1024 cm⁻¹ (C-O-C).
¹³C NMR (22.5 MHz : D₂O) : 61.7-63.1(-CH₂-), 72.1-75.0 [>CH-CH(CH₂OH)-O-], 78.2-81.7 [>CH-CH(CH₂OH)-O-, -CH(OH)-], 100.9-101.8 (-O-CH-O-), 174.9-177.1 (COONa)

Dicarboxylated sago starch sodium salt (DC-sago) and dicarboxylated tapioca starch sodium salt (DC-tapioca) having various degree of dicarboxylation were also obtained by a similar procedure.

Typical oxidation condition and analytical data for DC-starch are shown in Table 1.

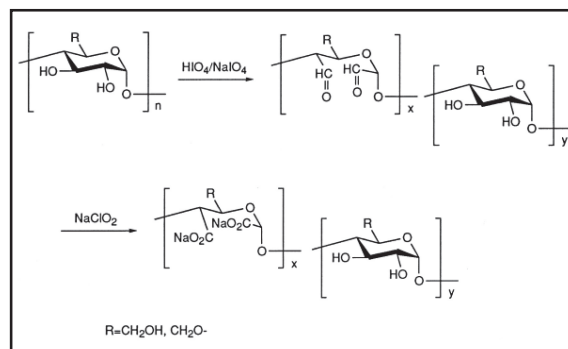


Fig 1. Preparation of partially dicarboxylated starch sodium salts (DC-Starch) by the partial conversion of the vicinal diol of the glucopyranose groups of starch into dicarboxylates *via* dialdehydes.

Biodegradation Test

Biochemical oxygen demand (BOD) was determined with a BOD tester (Model 200 and 100 F; TAITEC Corp., Koshigaya-shi, Japan) by the oxygen consumption method basically according to the OECD Guidelines for Testing of Chemicals (301C, Modified MITI Test) at 25°C (OECD, 1981), using an activated sludge freshly obtained from a municipal sewage treatment plant. The concentration of the polymer in the incubation media was 25 mg/L.

Hydrolytic Degradation

A non-enzymatic degradation test was carried out by dissolving the polymer in buffer solutions with pH values of 4, 5, 6, 8 and 10. The polymers at a concentration of 400 mg/L were incubated at 30°C in an incubator. Solutions of 0.1 M acetate buffer at pH 4 and 5, 0.1 M phosphate buffer at pH 6, 0.1 M tris-HCl buffer at pH 8, and 0.1 M glycine-NaOH buffer at pH 10 were used for the non-enzymatic degradation test. The degradation of the polymer was analyzed by GPC, and the hydrolytic degradability was expressed by molecular weight reduction in percent given by:

$$\text{molecular weight reduction (\%)} = [(M_0 - M_t)/M_0] \times 100$$

where M_0 is the initial molecular weight (maximum molecular weight of GPC peak), and M_t is the molecular weight (maximum

molecular weight of GPC peak) after t days of incubation.

Calcium Sequestration Capacity

A calcium ion electrode (Model 93-20, Orion Research, Inc., Boston, MA) and an ion meter (IM-20E, TOA Electronic, Ltd., Tokyo, Japan) were used to measure the equilibrium calcium ion concentrations. Ten milligrams of the polymer was dissolved in 50 mL of 1×10^{-3} M calcium hardness solution containing a 0.08 M KCl (ion strength, $\mu = 0.08$). The pH of the solution was adjusted to 9 at 30 °C. The electrode was immersed in the solution, which was then stirred. After 10 min, equilibrium free calcium ion concentrations were measured, and the calcium sequestration capacity was expressed as grams of calcium ion sequestered by 100 grams of polymer. As a comparison, the polymer was replaced by sodium tripolyphosphate (STPP) or 3-oxapentanedioate (ODA) with an equal weight of the polymer.

RESULTS AND DISCUSSION

Preparation of Partially Dicarboxylated Starch

DC-starch having a varying amount of Unreacted glucopyranose groups to provide enzymatically cleavable segments was prepared by the partial oxidation of corn, sago and tapioca starch using sodium periodate followed by sodium chlorite oxidation. Because high molecular weight polymer is expected to show better calcium ion sequestration (Dannoue, 1993), oxidative-cleavage reaction of starch by sodium periodate was carried out at low temperature and for a shorter reaction time (6 hours) in order to avoid molecular weight reduction by hydrolytic degradation.

The dicarboxylation degree was controlled by the concentration of sodium periodate.

Table 1 shows the typical oxidation conditions and analytical data for DC-starch.

Table 1. Typical Preparation and Analytical Data of Partially Dicarboxylated Starch (DC-Starch).

Compound ^a	Oxidation of starch to dialdehyde by aqueous sodium periodate ^b		Oxidation of dialdehyde to DC-Starch by aqueous sodium chlorite ^c				
	Concentration of sodium periodate (M)	Dialdehyde		Dicarboxylate			
		Yield (%)	Content (mol%)	Yield (%)	Content (mol%)	\bar{M}_n	\bar{M}_w/\bar{M}_n
DC-Corn-11400 (7)	0.015	93	18	82	7	11400	5.1
DC-Corn-27200 (29)	0.05	93	29	80	29	27200	4.9
DC-Corn-19300 (46)	0.10	93	47	96	46	19300	3.6
DC-Corn- 9400 (77)	0.20	76	96	95	77	9400	1.7
DC-Corn- 7500 (78)	0.30	93	85	61	78	7500	1.8
DC-Sago-14300 (10)	0.015	91	18	57	10	14300	7.6
DC-Sago-18100 (23)	0.05	93	35	73	23	18100	3.8
DC-Sago- 8900 (44)	0.10	97	57	64	44	8900	2.1
DC-Sago-12300 (67)	0.20	92	89	81	67	12300	1.8
DC-Sago-11800 (81)	0.30	93	97	56	81	11800	1.8
DC-Tapioca-21100 (8)	0.015	92	18	87	8	21100	6.3
DC-Tapioca-17800 (24)	0.05	94	32	82	24	17800	2.3
DC-Tapioca-15800 (45)	0.10	95	56	70	45	15800	8.0
DC-Tapioca-15900 (73)	0.20	95	83	83	73	15900	2.3
DC-Tapioca-11400 (78)	0.30	95	96	78	78	11400	1.6

^a Polymer code indicates number average molecular weight () and relative dicarboxylate content in mol % in parenthesis.

^b Dialdehyde of starch was prepared by the reaction of 5 gram starch with 145 mL aqueous NaIO_4 for 6 hours at 4°C.

^c DC-Starch was prepared by the reaction of dialdehyde with aqueous sodium chlorite at pH 4 for 24 hours at 20°C.

The data (in the table) is better presented in the form of graph/picture

Biodegradation of DC-starch

BOD values were measured to evaluate the potential biodegradability of the polymers using a BOD tester with an activated sludge and a test polymer concentration of 25 mg/L. It was found that the biodegradability of DC-starch was dependent on the amounts of the unreacted glucopyranose groups in the polymer. Figure 2 shows the relationship between 28-day biodegradation ($\text{BOD}_{28}/\text{TOD}$), calculated from the BOD values and the theoretical oxygen demand (TOD), and the dicarboxylation degree. Biodegradability is dependent on the dicarboxylation degree, or the content of unreacted glucopyranose groups, showing that the unreacted glucopyranose groups act as a biodegradable segment even in the polycarboxylate chain. However, highly dicarboxylated DC-starch was practically resistant to biodegradation even though the unreacted glucopyranose groups remained in the polymer chain. This fact indicates that a definite block length of successive glucopyranose units is necessary to react with the hydrolyzing enzyme in the polycarboxylate polymer chain. Among the corn, sago and tapioca starches used in this test, a slight difference was observed in the biodegradation results when compared using the same dicarboxylation basis. The biodegradability was better in the order of DC-corn, DC-sago and DC-tapioca. A BOD/TOD value of greater than 60% is regarded to indicate readily biodegradability. The dicarboxylation degrees of DC-corn, DC-sago and DC-tapioca showed that the 60% BOD-biodegradation values were 25%, 20% and 15%, respectively. The difference in

biodegradability of these DC-starches is probably ascribed to the microstructure of amylopectin in the starches.

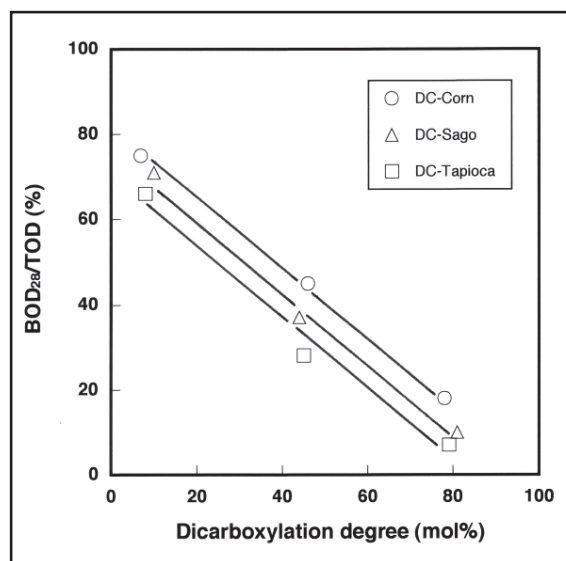


Fig. 2. Biodegradability (BOD/TOD) of DC-Corn, DC-Sago and DC-Tapioca as determined by the biochemical oxygen demand (BOD) as a function of the dicarboxylation degree after 28-day incubation. Biodegradability was calculated from BOD, as measured by the oxygen consumption method in a BOD tester using polymer concentration of 25 mg/L with activated sludge at 25°C, and the theoretical oxygen demand (TOD)

Hydrolytic Degradation

The non-enzymatic hydrolytic degradability of DC-starch was evaluated in aqueous solutions of different pH values in sterile condition. Figure 3 shows the correlation between the dicarboxylation degree of DC-tapioca, as a typical of DC-starch, and the molecular weight reduction which is caused by the hydrolytic cleavage after 30-day incubation in various pH buffer solutions. It was confirmed that DC-tapioca was quite stable against hydrolytic degradation under neutral and alkaline conditions after 10 day incubation at 30 °C. However, after 30-day incubation DC-tapioca tended to be degraded slightly under acidic conditions of pH below 6, and DC-tapioca

with a higher dicarboxylation degree of 78% was more labile to hydrolytic degradation. These results indicate that the hydrolytic cleavage predominantly occurred at the acetal linkages of the dicarboxylated moieties other than the unreacted sugar moieties. Similar tendencies were observed for DC-corn and DC-sago, and among the DC-starches no significant difference in non-enzymatic degradation was observed. We previously reported that dicarboxylated alginic acid with a dicarboxylation degree of more than 42 mol% showed significant molecular weight reduction at pH values below 8 (Winursito, 1996). It was also confirmed that DC-starch was much stable than dicarboxylated alginic acid against the hydrolytic stability. The difference in hydrolytic stability of DC-starch and dicarboxylated alginic acid will be ascribed to the carboxyl groups at the C6 position of alginic acid.

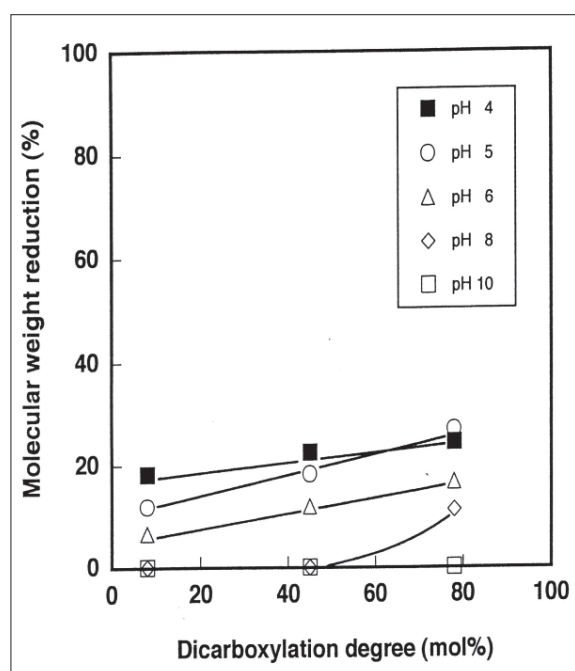


Fig. 3 Molecular weight reduction of 400 mg/L DC-Tapioca with dicarboxylation degree of 8, 45, and 78 mol %, respectively, in 0.1 M acetate buffer (pH 4 and 5), phosphate buffer (pH 6), tris-HCl Buffer (pH 8) and glycine-NaOH buffer (pH 10) solutions after 30-day incubation at 30 °C in the dark.

Calcium ion sequestration capacity

Calcium ion sequestration capacity is shown in Figure 4 as a function of the dicarboxylation degree of DC-starch. It was found that calcium ion sequestration was dependent on the content of the carboxylate groups in the polymer, and a clear relationship between calcium ion sequestration and the carboxylate content was seen when compared on an equal basis.

DC-Starch with high carboxylate contents showed better calcium ion sequestration performance. Among the corn, sago and tapioca starches used in this test, no significant difference was observed in the calcium ion sequestration.

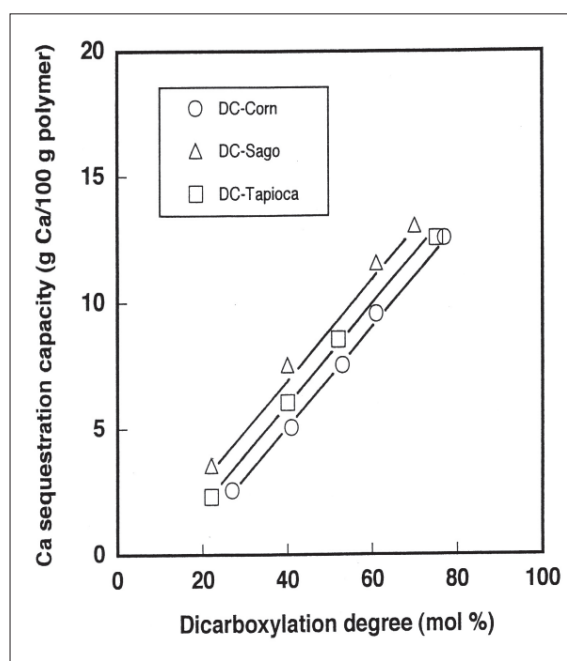


Fig. 4 Calcium ion sequestration capacity of DC-Starch as a function of the dicarboxylation degree. Calcium ion sequestration capacities of STPP and ODA were 18.2 and 13.9, respectively.

CONCLUSION

Partially dicarboxylated starch sodium salt (DC-starch) from corn, sago and tapioca that contained unreacted glucopyranose groups was biodegraded according to the dicarboxylation degree or the content of the unreacted glycopyranose groups. DC-starch

containing more than about 75-85 mol% unreacted glucopyranose groups in the polymer chain showed excellent biodegradation. DC-starch with a high dicarboxylation degree was resistant to biodegradation but showed good calcium ion sequestration. DC-starch was quite stable under neutral and slight alkaline conditions. Among DC-starches from corn, sago and tapioca starch, no significant difference in hydrolytic degradability and calcium ion sequestration was observed.

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