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Noncatalytic hydrolysis of guar gum under hydrothermal conditions

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Abstract—Guar gum, a naturally occurring heteropolysaccharide made of mannose and galactose, was hydrolytically degraded without a catalyst in a batch reactor to produce water-soluble (WS) saccharides including mono- and oligosaccharides. The degradation was carried out under hydrothermal conditions over ranges of temperature from 180 to 240 °C and of reaction time from 3 to 60 min. Guar gum was readily dissolved and hydrolyzed, and the major products identified in the WS components were oligosaccharides with degrees of polymerization up to about 20, monosaccharides containing mannose and galactose, and 5-hydroxymethyl-2-furaldehyde (5-HMF). At 200 °C, the oligosaccharide yield, obtained from the difference between the yields of the total WS saccharides and monosaccharides, showed the highest value of 94.4% at 7 min among all conditions studied, on the basis of the saccharide content in the initial sample. The oligosaccharide yield decreased with reaction time, and the yield of monosaccharides correspondingly increased, and reached the highest value of 34.5% (mannose 22.8%, galactose 11.7%) at 60 min. The monosaccharides produced were further decomposed to secondary products such as 5-HMF. The maximum yield of 5-HMF obtained was 26.3% at 220 °C and 30 min. The production and the decomposition of galactose somewhat preceded those of mannose.

Keywords: Degradation; Galactomannan; Guar gum; Hydrolysis; Hydrothermal; Oligosaccharide; Polysaccharide

1. Introduction

Guar gum, which is a galactomannan derived from the seed of a leguminous plant *Cyamopsis tetragonolobus*, has been widely used as a food additive because of the very high viscosity of guar gum aqueous solutions even at low concentrations.[1] It has been used as a versatile thickener and/or stabilizer for ice cream, baked goods, sauces and beverages.[1] Guar gum is also used in various industries such as mining, paper, textile, ceramic, paint, cosmetic, pharmaceutical, and explosive.[2]

Galactomannan is a heteropolysaccharide consisting of a linear chain of (1→4)-linked β-D-mannopyranosyl residues to which are attached varying proportions of (1→6)-linked α-D-galactopyranosyl groups as single unit side chains.[3,4] A simplified molecular structure of galactomannan is illustrated in Figure 1. The mannose to galactose ratio, distribution of galactose residues along the mannan backbone, molecular weight, and molecular weight distribution (MWD) usually vary according to the origin of the polysaccharide. Structures of commercial galactomannans and their related functions have been studied in detail.[5,6] Most guar galactomannan samples generally contain 38 ± 2% of D-galactose,[4] and the average molecular weight is about 2×10^6 Da.[7]

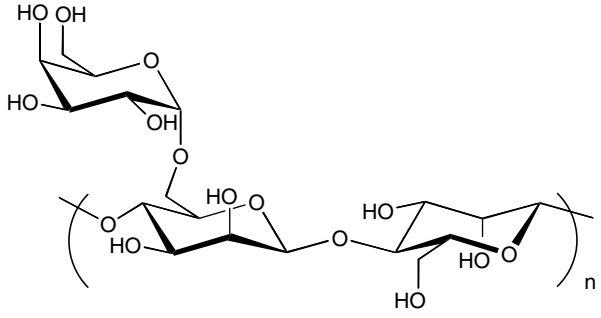

Figure 1. Molecular structure of galactomannan.

Partially hydrolyzed guar gum (PHGG) has attracted attention as a water-soluble (WS) dietary fiber since it

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was first used in Japan as a common food additive.[8] The intake of PHGG shows physiological effects such as increasing defecating frequency and lowering the pH of feces of both healthy men and constipated women,[9,10] and reducing serum cholesterol, free fatty acid, and glucose concentrations in humans.[11] The administration of feed supplemented with PHGG can also prevent the colonization of *Salmonella enteritidis* in young and laying hens, which could be related to improvement in the balance of intestinal microflora.[12]

PHGG is usually produced by hydrolyzing guar gum with enzymes, for example, *endo*-β-D-mannanase,[8–10] or acids. The enzymatic process often requires long reaction times and there are significant costs for obtaining enzymes and reagents for pH control; nevertheless, enzymes are often used in food processing. The acid-catalyzed process leads to high environmental impact and corrosion of instruments because of the use of strong acids.

As compared to both conventional hydrolytic methods, the hydrothermal process appears attractive because of shorter reaction times and the elimination of reagents such as acids.[13–15] The term 'hydrothermal process' generally means a process that occurs under conditions of high-temperature and high-pressure liquid water, where hydrolysis proceeds without a catalyst because of the high reactivity of water.[16–18] Hydrothermal treatment leads to simple processes of low environmental impact. In fact, the hydrolytic degradation of polysaccharides such as cellulose[19–24] and hemicelluloses[25,26] under hydrothermal conditions, including supercritical conditions (above 374 °C) has been extensively studied to produce mono- and oligosaccharides. Recently, we have demonstrated that naturally occurring polysaccharides, that is, pectic acid[27,28] and starch[29,30] can be converted into mono- and oligosaccharides at relatively low temperatures (around 200 °C) without any catalyst under hydrothermal conditions. Moreover, the addition of carbon dioxide instead of common acids has been found to accelerate hydrolyses of various polysaccharides.[31] However, hydrothermal degradation of heteropolysaccharides has been scarcely investigated, except for some hemicelluloses.[25,32] In this work, guar gum was hydrolytically degraded under hydrothermal conditions without any catalyst and the effects of reaction temperature and time on the product distribution were investigated.

2. Materials and methods

2.1. Materials

Guar gum was purchased from Sigma–Aldrich Japan (Tokyo, Japan). Standard samples of mannose and galactose were purchased from Sigma–Aldrich Japan (Tokyo, Japan) and Junsei Chemical (Tokyo, Japan), respectively. The purities of the two standard samples were not mentioned, but no other peaks were observed by high-performance anion exchange chromatographic (HPAEC) analyses. Pullulan standards, with molecular weights from 5900 to 112,000, were obtained from Shodex (Tokyo, Japan). All chemicals were used as received.

2.2. Hydrothermal degradation

The experimental apparatus and the procedure are substantially the same as those in the previous work[22,29,31] and are described below. The hydrothermal degradation of guar gum was carried out in a small bomb-type batch reactor made of stainless steel tubing with an inner volume of ca. 3.6 mL. Guar gum (0.03 g), dried at 100 °C in an air oven, and 3 g of distilled water were loaded in the reactor at room temperature. The solid sample was so small that it was well dispersed in the reactor. The conversion with this sample-to-water ratio was found to be quite reproducible from the preliminary runs. The reactor was sealed, shaken vigorously to homogenize the solution and immersed in a molten salt bath whose temperature had been maintained at the desired value within ±1 °C. After the prescribed time elapsed, the reactor was removed from the bath and rapidly cooled in a water bath at room temperature. In this study, the reaction time was counted from the moment the reactor had been placed in the molten salt bath. The reactor temperature, measured with a thermocouple inserted in the reactor, reached the prescribed values in about 2 min for all reaction temperatures studied. The contents in the reactor were washed with distilled water (ca. 20 mL) and filtered through a 0.3 μm glass filter. The solid residue recovered on the glass filter was weighed after drying in an air oven at 100 °C overnight, and the filtrate was analyzed as described.

2.3. Analytical methods

2.3.1. Initial sample. Guar gum, dried overnight in an air oven at 100 °C, was analyzed by an elemental analyzer (2400 series II, Perkin–Elmer, CT, USA). To determine saccharide composition, the initial sample was completely hydrolyzed in 0.5 M sulfuric acid aqueous solution at 100 °C for 3 h and the completion of hydrolysis was confirmed through preliminary runs at different treatment times. The hydrolyzate was analyzed by high-performance anion exchange chromatography (HPAEC) using an instrument equipped with a pulsed amperometric detector (Dionex, Sunnyvale, CA, USA) and a CarboPac PA1 column (4 mm I.D. × 250 mm long) at 35 °C using both 0.02 M sodium hydroxide solution (eluent A) and 0.02 M sodium hydroxide/1 M sodium acetate solution (eluent B) at a total flow rate of 1 mL/min in a gradient mode. The proportion of

eluent A was changed as follows: 100–88% over 35 min, 0% for 5 min and 100% for 20 min.

2.3.2. Product solution. Total organic carbon (TOC) content in the product solution was measured by a total carbon analyzer (model 5000A, Shimadzu, Kyoto, Japan) and the conversion from guar gum to WS components was determined on a carbon weight basis. The MWD of the product was determined by gel permeation chromatography (GPC) on a HPLC system (Hitachi, Tokyo, Japan) equipped with a UV and a refractive index detector by using a GPC column (SB-803 HQ, Shodex, Tokyo, Japan) at 40 °C and the flow rate of the eluent (0.05 M sodium nitrate solution) of 0.5 mL/min. The absolute MWD of the guar gum hydrolyzate was obtained according to the literature[33] through calibration with the chromatographic retention data of the pullulan standards using the concept of universal calibration through the following equation with the Mark–Houwink–Sakurada constants reported for guar gum[34] and pullulan.[35]

$$M_g = 0.67 M_p^{0.97} \quad (1)$$

where M_g and M_p are the molecular weights of guar gum and pullulan, respectively.

The monosaccharides produced, that is, mannose and galactose and the further decomposition product, 5-hydroxymethyl-2-furaldehyde (5-HMF), were identified and quantified by HPAEC. Furthermore, a fraction of the products was completely hydrolyzed in 0.5 M sulfuric acid aqueous solution at 100 °C for 3 h, and the hydrolyzate was analyzed by HPAEC to quantify the total WS saccharides consisting of mono- and oligosaccharides. The oligosaccharide yield was obtained by subtracting the monosaccharide yield from the total WS saccharide yield.

The production of oligosaccharides was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) using an AXIMA-CFR spectrometer (Shimadzu, Kyoto, Japan) in the linear and the positive ion mode by collecting mass spectra with 100 pulses of a nitrogen laser at 337 nm. The matrix solution, consisting of 2,5-dihydroxybenzoic acid of 80 mM and sodium chloride of 15 mM in aqueous ethanol solution (50% v/v), and the product solution were mixed on a sample plate, co-crystallized by evaporating the solvent under ambient conditions, and then ionized by nitrogen laser pulses.

3. Results and discussion

The saccharide composition of guar gum, shown in Table 1, was determined from the three individual analyses. The total saccharide content in guar gum was determined to be 79.7% on a carbon weight basis and the compositions of the remainder were not identified.

Table 1. Saccharide composition of guar gum

Saccharide composition			
Mannose	Galactose	Total	Mannose/galactose ratio
47.1[a]	32.6[a]	79.7[a]	1.44

[a] (g-carbon in saccharide/g-carbon in guar gum) × 100.

The filtrate of the products recovered from the hydrothermal degradation of guar gum was almost transparent and not viscous whereas crude guar gum hydrates rapidly in cold water to form highly viscous colloidal dispersions[2] and its 1% solution is opaque and about 2000–3000 centi-poises.[8] Hydrothermal degradation significantly reduced the viscosity.

Figure 2 shows the time change of the TOC content recovered in the product solutions at various temperatures. Except at 180 °C, the TOC values reached over 95% within 10 min, indicating guar gum was almost dissolved, and then the TOC values decreased with time. Presumably, the decrease in TOC values at longer reaction times results from the formation of the solid residue recovered on the glass filter. Although gaseous products were not analyzed in this study, their formation was less likely at these temperatures as has been observed for other polysaccharides under hydrothermal conditions[21,29,36] even in the presence of alkali or nickel catalyst.[37] At 180 °C, the lowest temperature studied, a longer time was required to dissolve guar gum.

Figure 3 depicts the time changes of the product distribution at 200 °C. The monosaccharide yield is defined as the sum of mannose and galactose yields and the yield of unknown species represents the difference between the TOC value and the sum of the yields of oligosaccharides, the monosaccharides and 5-HMF. All yields were

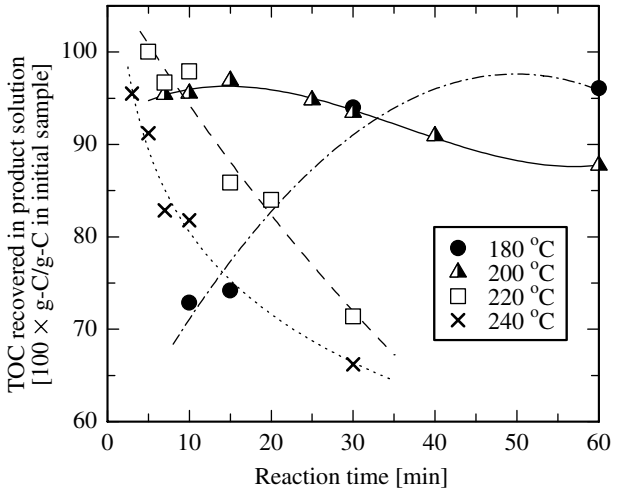

Figure 2. TOC recovered in product solution versus reaction time at various temperatures.

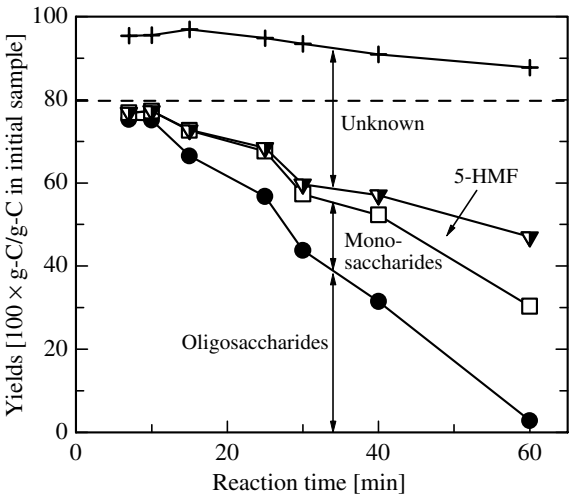

Figure 3. Time changes of product distribution at 200 °C. The horizontal broken line is drawn at the saccharide content of 79.7% in the initial sample.

determined based on the carbon weight of the initial sample loaded in the reactor. At 7 min, the highest oligosaccharide yield obtained was 75.2%, which was equivalent to 94.4% of the saccharide content in the initial sample. The oligosaccharide yield decreased over time and the monosaccharide yield increased correspondingly. At 30 min, the yield of 5-HMF, a major decomposition product of hexoses by the loss of three molecules of water in an acid-catalyzed reaction,[38,39] started to increase. It is clear that the guar gum was hydrolyzed and the hydrolyzates were further decomposed to secondary products at longer reaction times. The consecutive reactions taking place were similarly observed in the hydrothermal degradation of other polysaccharides, that is, cellulose[20,21] and starch.[29,30]

Figure 4 illustrates the MWDs of the products obtained at 200 °C and three reaction times, determined from the GPC chromatograms. The MWD of products shifted to smaller molecular weights with reaction time and correspondingly the peak of monosaccharides (molecular weight = 180) was enhanced. A similar tendency in MWD was observed when the reaction temperature was increased at a fixed reaction time. The molecular weight of the peak top position of the products obtained at 200 °C and 10 min was around 20,000. According to the literature,[33,40] it takes over 10 h to obtain hydrolyzates having similar molecular weights with enzymes even though the treatment time depends on concentration and conditions. The remarkably short reaction time is an advantage of the hydrothermal degradation and is attractive for industrial processes.

The production of oligosaccharides was confirmed by MALDI-TOF-MS. Figure 5 shows the mass spectrum of the products obtained at 200 °C and 15 min. Peaks, increased at a constant increment of 162 Da, were observed for oligosaccharides having degrees of polymerization (DP) up to about 20. While saccharides having higher DPs could also exist in the products according to the MWD (see Fig. 4) they were not detected because the ionization response dropped off with increasing the molecular weight for mixtures of large carbohydrate polymers.[41]

Figure 6 shows the time changes of the total WS saccharide yields at various temperatures. At 200 °C for 7 or 10 min, over 95% of the saccharides contained in the initial sample was recovered in the product solution without decomposition. The yield decreased with time except at 180 °C because of further decomposition. The decomposition rate was faster at higher temperatures and the saccharides were completely decomposed at 240 °C and 30 min. At 180 °C and shorter reaction

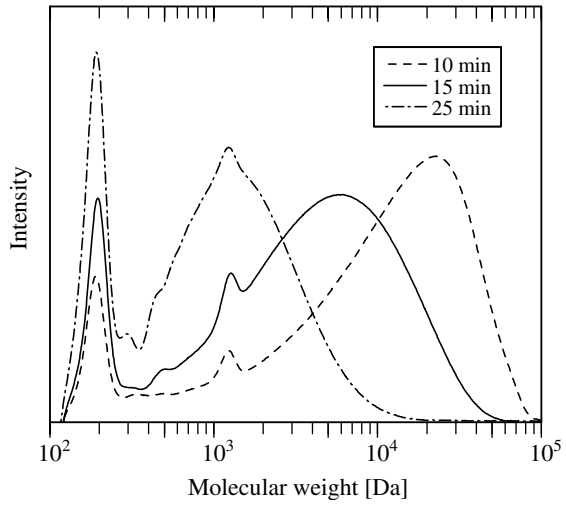

Figure 4. Molecular weight distribution of products obtained at 200 °C and three reaction times.

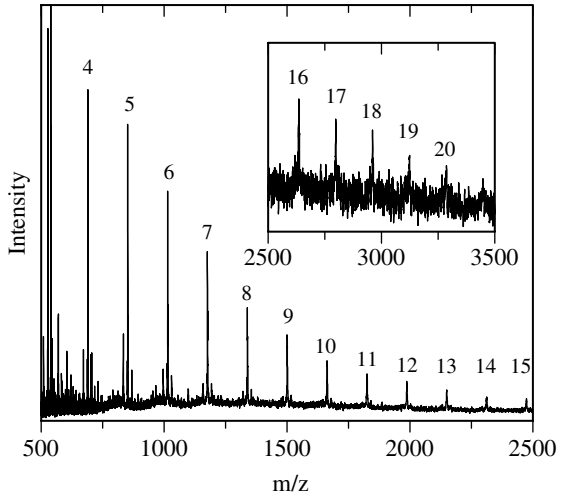

Figure 5. Mass spectrum of hydrolyzates obtained at 200 °C and 15 min. The numerals represent the degree of polymerization for each oligomer peak.

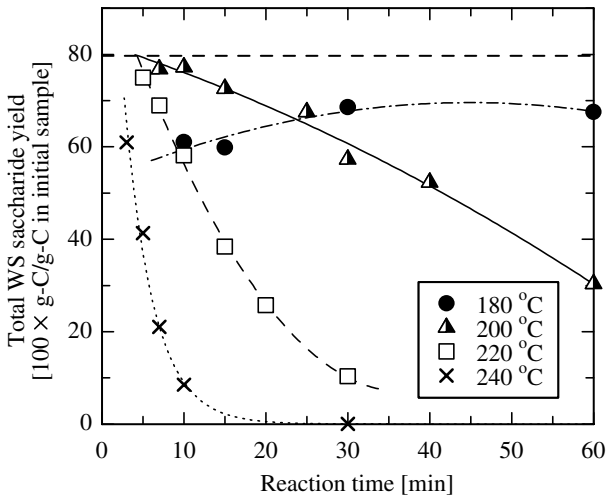

Figure 6. Total WS saccharide yield versus reaction time at various temperatures. The horizontal broken line is drawn at the saccharide content of 79.7% in the initial sample.

times, the yields were relatively low because the dissolution of guar gum was not complete, as seen in Figure 2.

Figure 7 shows the time changes of the yield and the composition of the monosaccharides produced at various temperatures. The total yield of the monosaccharides, that is, mannose and galactose, simply increased with time at 180 and 200 °C, although the yields were low at 180 °C. At higher temperatures, the monosaccharide yield initially increased and then decreased because of the further decomposition. At 200 °C and 60 min, the highest monosaccharide yield was 27.5% (mannose 18.2%, galactose 9.3%) on a carbon weight basis, corresponding to 34.5% (mannose 22.8%, galactose 11.7%) on a saccharide basis. The composition of monosaccharides produced also varied with reaction time. At all temperatures studied, the production of galactose preceded that of mannose to some extent. The difference in the time change of the yields of galactose and mannose at 200 °C is more clearly depicted in Figure 8.

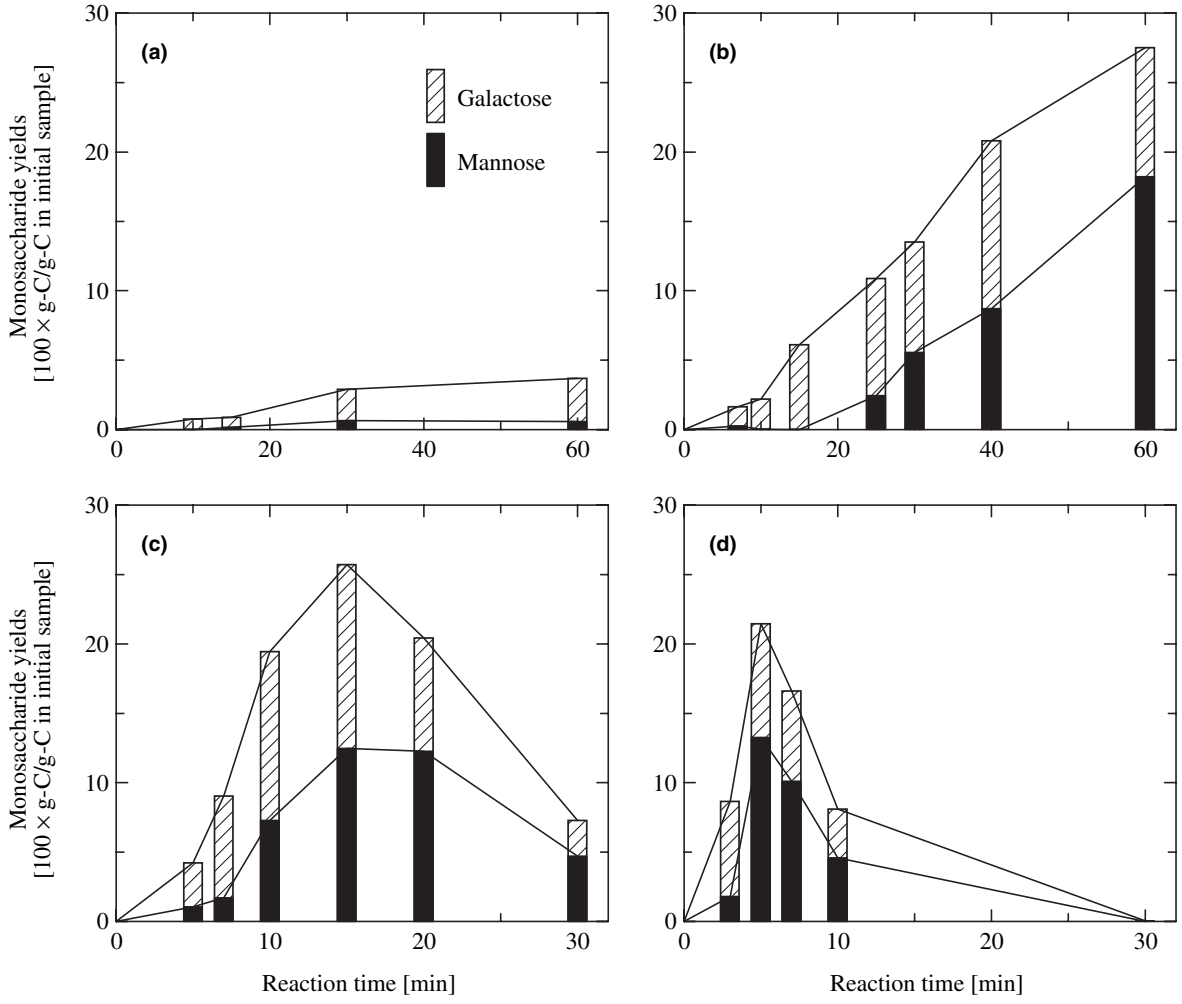

Figure 7. Time changes of yield and composition of monosaccharides produced at (a) 180, (b) 200, (c) 220, and (d) 240 °C.

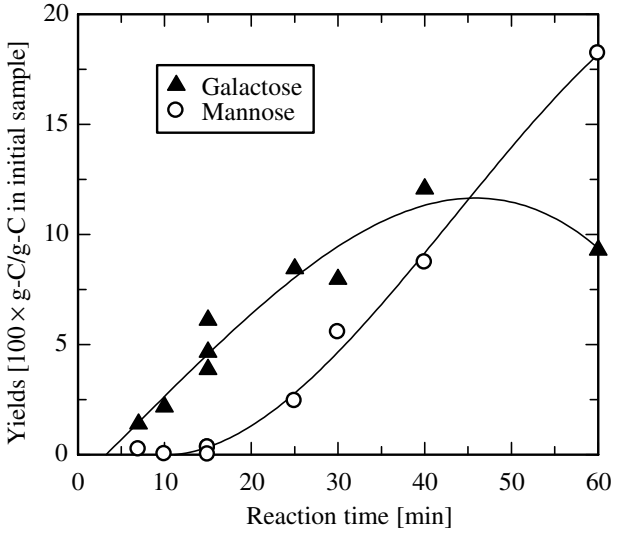

Figure 8. Time changes of galactose and mannose yields at 200 °C.

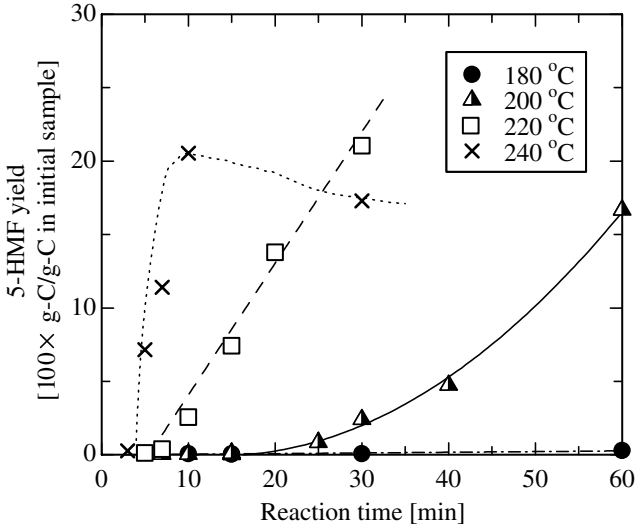

Figure 9. 5-HMF yield versus reaction time at various temperatures.

Obviously the galactose yield increased earlier than the mannose yield as would be expected from the structure of galactomannan (Fig. 1). Because galactose is the single-unit side-chain attached to the mannan backbone, it is liberated by the hydrolysis of every galactose–mannose linkage. However, the hydrolysis of mannose–mannose linkages does not liberate mannose, except at the end of the mannan backbone. As a result, galactose is preferentially produced at the initial stages of the hydrothermal degradation of guar gum. Thus, the observed time change of the monosaccharide composition does not imply that galactose–mannose linkages are more susceptible to hydrolysis than that of mannose–mannose linkages, but rather indicates that random hydrolysis is taking place under hydrothermal conditions. We also observed a similar tendency in the acid hydrolysis of guar gum, that is, galactose was produced followed by mannose (data not shown).

The 5-HMF yield is plotted against the reaction time at various temperatures in Figure 9. Because 5-HMF is a major decomposition product of hexoses, the yield is an index of the degree of the secondary reactions. The 5-HMF yield was very low at 180 °C even for 60 min because monosaccharides were scarcely produced at 180 °C, as seen in Figure 7. The yield simply increased with time at 200 and 220 °C. At 240 °C, the highest temperature in this study, the 5-HMF yield increased and then decreased at 30 min because of the further decomposition. The highest yield of 5-HMF was 21.0% at 220 °C and 30 min on a carbon weight basis of the initial sample.

The total mannose yield was plotted against the total galactose yield in Figure 10. These yields are defined as the mannose or galactose yields determined after the complete acid hydrolysis of the products obtained from the hydrothermal degradation of guar gum. The mannose to galactose ratio was close to the initial value (1.44) at 180 °C, where the decomposition of saccharides barely took place. As the decomposition of saccharides proceeded, that is, as both total yields decreased at higher temperatures or longer times, the ratio shifted to the mannose rich side. This indicates that galactose began to decompose prior to mannose.

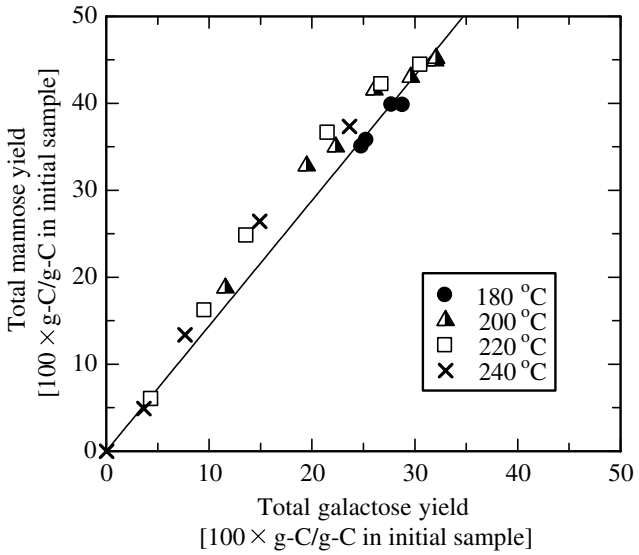

Figure 10. Total mannose yield versus total galactose yield. Both yields were obtained from acid hydrolysis of water soluble saccharides formed by hydrothermal degradation. The slope of the solid line is 1.44, which is the ratio of mannose to galactose contained in the initial sample.

Monosaccharides including galactose and mannose are known to exist as a equilibrium mixture of cyclic and acyclic structures in solution,[42] whereas saccharide units in oligo- and polysaccharides are fixed in a single

ring structure, except at the reducing end. It is reported that saccharides in the open chain form are more readily decomposed when compared to those in the cyclic form under hydrothermal conditions.[43,44] In the present study, since the mannose units remained more stable in the polymerized form than the galactose units because of the slower production of mannose than galactose, mannose decomposition was consequently delayed as compared to galactose.

4. Conclusions

Mono- and oligosaccharides were produced by the non-catalytic hydrolysis of guar gum under hydrothermal conditions. 5-HMF was also obtained from the further decomposition of the monosaccharides produced in the course of the hydrothermal degradation. Among all conditions studied, the highest yields of oligosaccharides, monosaccharides, and 5-HMF on a carbon weight basis of the initial sample were 75.2% (94.4%) at 200 °C and 7 min, 27.5% (34.5%) at 200 °C and 60 min, and 21.0% (26.3%) at 220 °C and 30 min, where the figures in parentheses are on the basis of saccharide content in the initial sample. Hydrolysis of guar gum under hydrothermal conditions was much faster than that with enzymes. The production rate of galactose was somewhat faster than that of mannose and this phenomenon was attributed to the molecular structure of galactomannan. The decomposition of galactose also preceded that of mannose.

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