

亜臨界水中でのD - ガラクツロン酸およびD - ガラクツロン酸ナトリウムの分解動力学

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Degradation Kinetics of D-Galacturonic Acid and Sodium D-Galacturonate in Subcritical Water

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Abstract: The degradation process of D-galacturonic acid in subcritical water was measured from 160 to 220°C. The process at any temperature obeyed first-order kinetics. The temperature dependence of the degradation rate constant could be expressed by the Arrhenius equation, and the activation energy and the frequency factor were estimated to be 131 kJ/mol and $4.81 \times 10^{12} \text{ s}^{-1}$, respectively. The degradation process of sodium D-galacturonate in subcritical water was also measured from 160 to 190°C. Sodium galacturonate was more easily degraded than galacturonic acid. The degradation process of sodium galacturonate did not obey the first-order kinetics, but could be expressed by the Weibull equation. According to the Arrhenius equation, the activation energy and the frequency factor were estimated to be 147 kJ/mol and $1.26 \times 10^{15} \text{ s}^{-1}$, respectively, for the degradation of sodium galacturonate. The pH of the reaction mixture at room temperature was also measured for the degradation of both the galacturonic acid and sodium galacturonate.

Key words: galacturonic acid, sodium galacturonate, subcritical water, degradation

Water, which maintains its liquid state in the temperature range from its normal atmospheric boiling point (100°C) to its critical temperature (374°C) under pressurized conditions, is called subcritical water or compressed hot water. Subcritical water possesses two distinct features from ambient water; one is a lower dielectric constant and the other is a higher ion product at higher temperature.^{1–3)} The latter property indicates the possibility that the water can act as an acid or base catalyst because of the high concentrations of both hydrogen and hydroxide ions.^{4–6)} The decomposition or hydrolysis of polysaccharides, which are renewable bioresources, by subcritical water has been investigated.^{7–10)} We also reported the decomposition kinetics of monosaccharides,¹¹⁾ disaccharides,^{12–14)} trisaccharides¹⁵⁾ and oligosaccharides¹⁶⁾ in subcritical water. Although all the saccharides used in the previous studies were non-electrolytes, bioresources include cationic or anionic saccharides. Polyanionic saccharides, such as pectin, alginic acid and hyaluronic acid, contain hexuronic acids, such as D-glucuronic, D-galacturonic, D-mannuronic and L-guluronic acids. Knowledge of the degradation of the uronic acids, which are produced by the hydrolysis of the polyanionic saccharides, would be required for recovering the constituent uronic acid by the hydrolysis of the polyanionic saccharides in subcritical water.

In this context, the degradation kinetics of galacturonic acid, which is a constituent of pectin, in subcritical water was examined in the temperature range of 160 to 220°C. The degradation kinetics of sodium galacturonate was also measured in order to examine the effect of the counter-ion form on the degradation.

MATERIALS AND METHODS

Materials. D-Galacturonic acid monohydrate (purity > 98%) and sodium D-galacturonate (> 98%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Sigma-Aldrich Co. (Tokyo, Japan), respectively.

Decomposition of galacturonic acid or sodium galacturonate in subcritical water. Galacturonic acid or sodium galacturonate was degraded using a continuous flow system, which has been previously reported.^{11,14)}

Galacturonic acid or sodium galacturonate was dissolved in distilled water at 0.5% (w/v), 1.0% or 2.5% and the solution was sonically degassed under reduced pressure. The solution in a glass bottle was connected to a helium gasbag to prevent the re-dissolution of oxygen in the solution and delivered at a constant flow rate with an LC-10ATvp HPLC pump (Shimadzu Corporation, Kyoto, Japan) to give a specific residence time of 10 to 300 s in the reaction coil (SUS 316), which was immersed in a silicon oil bath (Toray-Dow-Corning Silicone, Dow Corning Toray Co., Ltd., Tokyo, Japan) and the temperature was regulated between 160 to 220°C. To rapidly terminate the reaction, the reaction mixture leaving the reactor was passed through a cooling coil immersed in an iced water bath. The high pressure adjustable back-pressure valve (Upchurch Scientific Inc., Oak Harbor, USA) was connected to the line after the cooling coil to control the pressure in the system and keep it at the constant pressure of 10 MPa. The effluent was collected in a sampling vessel for analysis after about 5 to 10 residence times had elapsed.

The concentration of the remaining galacturonic acid or sodium galacturonate in the effluent was analyzed using a

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Shimadzu LC-10AS HPLC with an RID-10A refractometer. The columns used for the analysis were two Supelco-gel Ca columns (300 mm × 7.8 mm I.D.; Sigma-Aldrich Co., Japan) in series with a Supelguard Ca guard column (50 mm × 4.6 mm I.D.; Sigma-Aldrich Co.). The eluent was distilled water and the flow rate was 0.5 mL/min. The determination was carried out at room temperature at least in triplicate and averaged.

The pH of the effluent was also measured at room temperature using a Horiba F-13 pH meter (Horiba Ltd., Kyoto, Japan).

FT-IR spectrum was observed by the ATR method using a Shimadzu FTIR-8300 (Shimadzu Corporation) for the original galacturonic acid and that reacted at 190°C and at the residence time of 150 s and for the original sodium galacturonate and those reacted at 180°C and at the residence times of 100 and 200 s. A sample was placed in the ATR cell and was diluted 5-fold with acetone. The mixture was dried by blowing N₂ gas. The FT-IR spectrum of the thin-layered sample was observed in the wave-number range of 650 to 4000 cm⁻¹.

RESULTS AND DISCUSSION

Degradation of galacturonic acid in subcritical water.

Figure 1 shows the degradation processes at 190°C of galacturonic acid at three different concentrations. The changes in pH of the reactor effluent are also shown in the figure. The plots for the degradation at 0.5, 1.0 and 2.5% (w/v) lie on a straight line on the semi-logarithmic scale, indicating that the degradation processes of galacturonic acid can be expressed by simple first-order kinetics:

$$C/C_0 = \exp(-k\tau) \quad (1)$$

where C is the concentration of the remaining substrate in the reactor effluent, C_0 is the substrate concentration in the feed, τ is the residence time in the reactor and k is the rate constant for the degradation. Some degradation products were recognized on the HPLC charts, but their identification was unsuccessful.

The pH gradually increased as the degradation of galac-

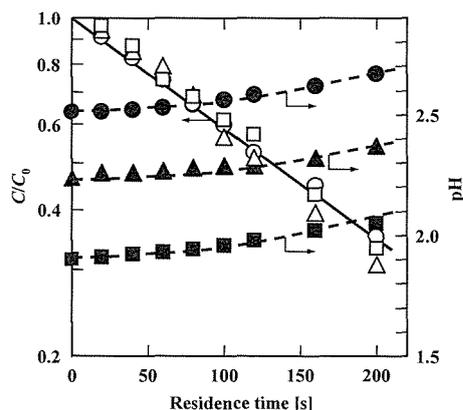


Fig. 1. Degradation processes of galacturonic acid in subcritical water at 190°C.

The concentration of galacturonic acid in the feed, C_0 , was (○, ●) 0.5% (w/v), (△, ▲) 1.0% and (□, ■) 2.5%. Open and closed symbols indicate the concentration of galacturonic acid in the reactor effluent C , which was normalized by C_0 , and pH of the effluent, respectively.

turonic acid proceeded. The decarboxylation of D-galacturonic acid in acidic solution was reported.¹⁷⁾ Therefore, the increase in pH might be ascribed to the decarboxylation of D-galacturonic acid or to the ketone formation under the given reaction conditions. In the FT-IR spectra of unreacted and reacted galacturonic acids, the absorption for C=O stretching of carboxyl group scarcely changed before or after the reaction, but the absorption for the O-H stretching of the group decreased. These facts do not conflict with the above-mentioned inference.

Because the degradation of D-galacturonic acid could be expressed by first-order kinetics, the degradation of galacturonic acid was measured at a constant concentration of 0.5% (w/v) and at different temperatures (Fig. 2). The degradation proceeded faster at the higher temperature. At any temperature, the degradation process obeyed first-order kinetics, and the rate constant k was evaluated from the slope. The pH values of the reaction mixtures are plotted versus the decrease in the concentration of D-galacturonic acid in Fig. 3. Irrespective of the temperature, all the plots lie on a curve. This fact would indicate the degradation mechanism is the same in the range of the tested temperatures.

As shown in Fig. 4, the temperature dependence of the rate constant k could be expressed by the Arrhenius equation:

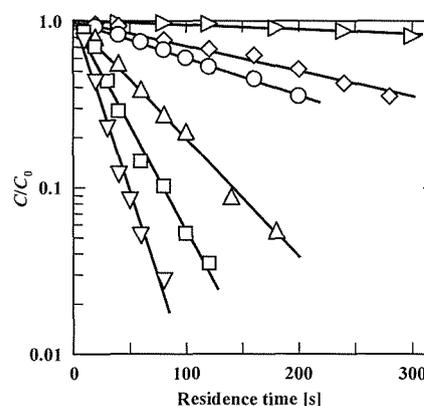


Fig. 2. Degradation processes of galacturonic acid at (▷) 160°C, (◊) 180°C, (○) 190°C, (△) 200°C, (□) 210°C and (▽) 220°C.

The concentration of galacturonic acid in the feed, C_0 , was 0.5% (w/v).

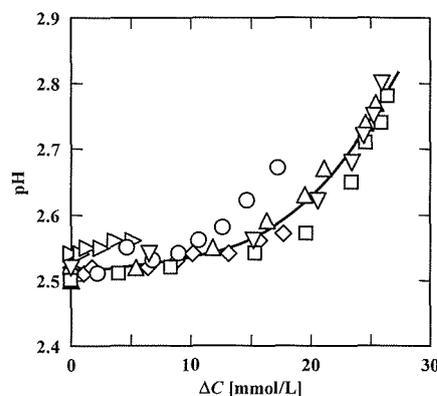


Fig. 3. Relationships between the decrease in the concentration of galacturonic acid, ΔC , and the pH of the reaction mixture at room temperature.

The symbols are the same as in Fig. 2.

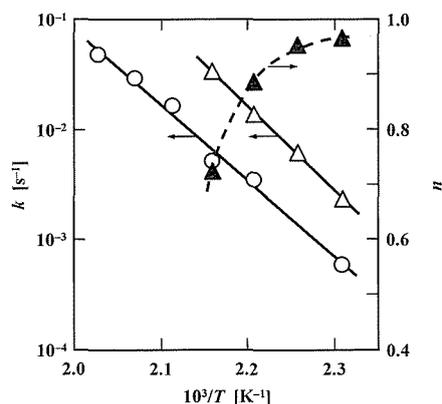


Fig. 4. Temperature dependences of (○) the rate constant k for the degradation of galacturonic acid and of (△) the rate constant k and (▲) the shape constant n for the degradation of sodium galacturonate.

$$k = k_0 \exp(-E/RT) \quad (2)$$

where E and k_0 are the activation energy and the frequency factor for the degradation of galacturonic acid, and were estimated to be 131 kJ/mol and $4.81 \times 10^{12} \text{ s}^{-1}$, respectively. The activation energy was almost the same as those for the degradation of hexoses in subcritical water, *i.e.*, 90 to 170 kJ/mol.¹¹⁾

Degradation of sodium galacturonate in subcritical water.

Figure 5 shows the degradation processes of sodium galacturonate at 180°C, the concentration of which was 0.5, 1.0 and 2.5% (w/v). Compared to the degradation of galacturonic acid at this temperature, sodium galacturonate was more easily degraded. The concentration dependence was not significant. The plots of the normalized concentration of the remaining sodium galacturonate against the residence time did not lie on a straight line on the semi-logarithmic scale. This fact indicates that the degradation processes do not obey first-order kinetics. Therefore, the Weibull equation, which reasonably expresses many kinds of decomposition and deterioration processes,^{18,19)} was adopted to describe the disappearance of sodium galacturonate in the subcritical water:

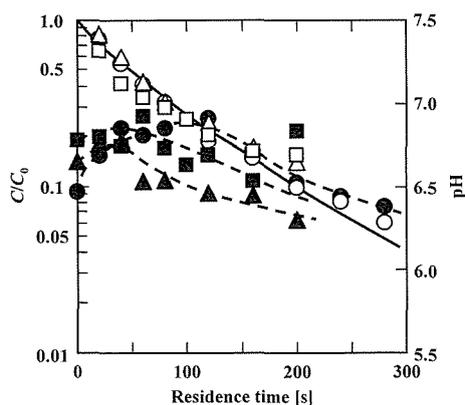


Fig. 5. Degradation processes of sodium galacturonate in subcritical water at 180°C.

The concentration of galacturonic acid in the feed, C_0 , was (○, ●) 0.5% (w/v), (△, ▲) 1.0% and (□, ■) 2.5%. Open and closed symbols indicate the concentration of galacturonic acid in the reactor effluent C , which was normalized by C_0 , and pH of the effluent, respectively.

$$C/C_0 = \exp[-(k\tau)^n] \quad (3)$$

where k and n are the rate and shape constants, respectively. When $n < 1$, the degradation rapidly proceeds during the early stage of the reaction or at short residence times, and its rate gradually becomes slower. The k and n values were estimated to minimize the sum of the residual squares between the experimental C/C_0 value and the calculated one using the Solver of Microsoft Excel[®]. The solid curve in the figure was drawn using the estimated parameters.

Figure 5 also shows the changes in the pH at room temperature of the reaction mixture. The pH increased at the short residence times, and then decreased at the longer residence times. The reason for the pH change remains unclear. The conversion of glucuronic acid to its corresponding lactone even occurs at room temperature.²⁰⁾ The subcritical water treatment of glucose brought about the decrease in pH of the reaction mixture due to the formation of acidic compounds.¹³⁾ Therefore, possible reasons for the pH change during the degradation of sodium galacturonate might be ascribed to the formation of lactone at the short residence times and the formation of acidic compounds due to further degradation at the long residence times. The absorption for the C-H stretching significantly increased with the increase of the reaction time in the FT-IR spectra of the sodium galacturonate treated at 180°C, although the absorption for the C=O stretching scarcely changed. These observations were not inconsistent with the above interpretation.

The degradation processes of 0.5% (w/v) sodium galacturonate were measured at various temperatures (Fig. 6). The pH of the reaction mixtures was also measured. The degradation processes at any temperature were analyzed using the Weibull equation by the method described above to estimate the k and n values. The k and n values are plotted *versus* the reciprocal of the absolute temperature in Fig. 4. The temperature dependence of the k value could be expressed by the Arrhenius equation, and the activation energy and the frequency factor were evaluated to be 147 kJ/mol and $1.26 \times 10^{15} \text{ s}^{-1}$, respectively, from the straight line drawn in the figure. The n value was higher at the lower temperature, and it asymp-

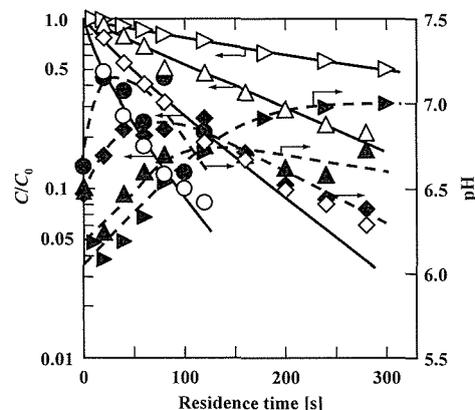


Fig. 6. Degradation of sodium galacturonate at (▷, ▶) 160°C, (△, ▲) 170°C, (◇, ◆) 180°C and (○, ●) 190°C.

Open and closed symbols indicate the concentration of galacturonic acid in the reactor effluent C , which was normalized by C_0 , and pH of the effluent, respectively.

totically increased to unity as the reaction temperature decreased. We have no definite explanation for the temperature dependence of the n value. A possible reason is that sodium galacturonate is degraded through at least two mechanisms, the activation energies of which are different from each other.

At any temperature, the pH of the reaction mixture increased at the short residence time and then declined. The change in the pH might be explained by the above-mentioned reasons.

The degradation kinetics for galacturonic acid was different from that for sodium galacturonate. The reason for the difference remains unclear. Naturally occurring acidic polysaccharides also contain other hexuronic acids as well as galacturonic acid. As described above, sodium galacturonate degraded at a lower temperature than galacturonic acid. The galacturonic acids in acid and sodium-salt forms were more susceptible to degradation than hexoses such as galactose, which are scarcely degraded at 180°C or lower temperatures.¹¹⁾ At those temperatures, the hydrolysis of the glycosidic bond very slightly proceeds.¹²⁻¹⁶⁾ Thus, uronic acids are more fragile in subcritical water than neutral mono- and oligosaccharides. However, the reason for the difference in the susceptibility to degradation among the saccharides also remains unclear. Further investigations are required for understanding the phenomena occurring during the subcritical water treatment of the polysaccharides.

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亜臨界水中での D-ガラクトツロン酸

および D-ガラクトツロン酸ナトリウムの分解動力学

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D-ガラクトツロン酸および D-ガラクトツロン酸ナトリウムの 160-220°C または 160-190°C の亜臨界水中での分解動力学について検討した。D-ガラクトツロン酸の分解過程は 1 次反応速度式で表現できた。速度定数の温度依存性はアレニウス式に従い、活性化エネルギーおよび頻度因子はそれぞれ 131 kJ/mol と $4.81 \times 10^{12} \text{ s}^{-1}$ であった。一方、D-ガラクトツロン酸ナトリウムは D-ガラクトツロン酸よりも分解されやすく、その過程は 1 次反応速度式に従わなかった。そこで、分解過程に Weibull 式を適用し、速度定数と形状係数を算出した。速度定数の温度依存性はアレニウス式で表現でき、活性化エネルギーおよび頻度因子はそれぞれ 147 kJ/mol と $1.26 \times 10^{15} \text{ s}^{-1}$ であった。また、D-ガラクトツロン酸および D-ガラクトツロン酸ナトリウムの分解に伴う反応液の室温における pH についても測定した。