Kinetic analysis for the isomerization of glucose, fructose, and mannose in subcritical aqueous ethanol

Da-Ming Gao, Takashi Kobayashi and Shuji Adachi*

Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

Received November 6, 2014; Accepted December 17, 2014

---

*Corresponding author: Tel.: +81-75-753-6286; Fax: +81-75-753-6285;

*E-mail: adachi@kais.kyoto-u.ac.jp

Abstract:

Fructose, glucose, and mannose were treated with subcritical aqueous ethanol for ethanol concentrations ranging from 0 to 80 % (v/v) at 180–200 °C. The aldose–ketose isomerization was more favorable than ketose–aldose isomerization and glucose–mannose epimerization. The isomerization of the monosaccharides was promoted by the addition of ethanol. In particular, mannose was isomerized most easily to fructose in subcritical aqueous ethanol. The apparent equilibrium constants for the isomerizations of mannose to fructose, $K_{\mathrm{eq,M}\rightarrow\mathrm{F}}$, and glucose to fructose, $K_{\mathrm{eq,G}\rightarrow\mathrm{F}}$, were independent of ethanol concentration and increased with increasing temperature. Moreover, the $K_{\mathrm{eq,M}\rightarrow\mathrm{F}}$ value was much larger than the $K_{\mathrm{eq,G}\rightarrow\mathrm{F}}$ value. The enthalpies for the isomerization of mannose to fructose, $\Delta H_{\mathrm{M}\rightarrow\mathrm{F}}$, and glucose to fructose, $\Delta H_{\mathrm{G}\rightarrow\mathrm{F}}$, were estimated to be 18 and 24 kJ/mol, respectively, according to van't Hoff equation. Subcritical aqueous ethanol can be used to produce fructose from glucose and mannose efficiently.

Keywords: fructose; glucose; isomerization; mannose; subcritical aqueous ethanol

Introduction

Fructose is a common reducing ketose that is used commercially as a sweetener in beverages to enhance our enjoyment of foods. Fructose has positive health effects in humans as it enhances glucocorticoid action(1) and insulin resistance.(2) Recently, fructose has been used as a renewable resource to synthesize biofuels and biochemical(3,4) such as 5-hydroxymethylfurfural and methyl lactate. Nowadays, fructose plays an important role in food and related industries.

The transformation of other monosaccharides to fructose is mainly performed using alkalis, metals, and enzymes. The alkali-catalyzed isomerization has been well known as the Lobry de Bruyn–Alberda van Ekenstein transformation (LBAE transformation). In the LBAE transformation, fructose is produced from glucose in high yield (*ca*. 38 % at 25 °C) and from mannose in relatively low yield (*ca*. 25 % at 25 °C).(5) However, alkali-catalyzed isomerization usually results in many by-products, thus restricting its application. The product distribution in metal-catalyzed isomerization depends on the type of metal ion, saccharides, cosolvents, and metal ion carrier.(6–8) Metal-catalyzed isomerization cannot meet commercial demands because of the low feed concentration of the substrates or the difficulty in synthesizing the catalysts. The current industrial isomerization process to produce fructose involves the utilization of an immobilized glucose isomerase. This enzymatic isomerization is reversible (the equilibrium constant, K_{eq}, is *ca*. 1 at 25 °C) and slightly endothermic, indicating that the maximum attainable yield of fructose is governed by the reaction temperature.$^{8)}$

Many researchers have investigated the isomerization of saccharides such as glucose, mannose, and fructose in 100 % (v/v) subcritical water.$^{9–11)}$ It was reported that aldoses isomerized easily than ketoses in 100 % (v/v) subcritical water.$^{10)}$ Although kinetic analysis revealed that mannose was isomerized most easily to fructose among glucose, mannose, and fructose, the yields of fructose and glucose from mannose were still low. It was also reported that increasing the temperature shifted the chemical equilibrium constant for the isomerization of aldoses to ketoses to a higher value.$^{10)}$

In general, solvent variation significantly affects the reaction rate and apparent chemical equilibrium. Ethanol has been widely investigated in the isomerization of aldoses at ambient temperature using bases, enzymes, or metals.$^{12–14)}$ Adding ethanol to the solvent significantly promoted these isomerizations. The promoting effect of ethanol at low temperatures is usually attributed to changes in both the conformation and configuration of saccharides in aqueous ethanol$^{15)}$ and the change in the apparent chemical equilibrium.$^{7)}$

We have previously reported that the degree of hydrolysis of sucrose changed by the addition of ethanol under subcritical conditions.[16)] The yield of fructose was lower than that of glucose, and this difference increased with increasing ethanol concentration, possibly because of the isomerization between the two saccharides.[16)] However, the mechanism of isomerization has not been clarified. Therefore, we analyzed the kinetics of the isomerization of glucose, mannose, and fructose in subcritical aqueous ethanol.

Materials and Methods

Materials. Ethanol (purity, 99.5 %), D-fructose (> 99 %), and D-glucose (> 99 %) were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Mannose (> 99 %) was purchased from Nacalai Tesque (Kyoto, Japan).

Isomerization among fructose, glucose, and mannose in subcritical aqueous ethanol. Each monosaccharide was dissolved in distilled water, and ethanol was added to produce the substrate solution with a final saccharide concentration of 0.5 % (w/v), at which it was possible to detect saccharides and derivatives using an RI detector. In addition, this concentration would not be greatly deviated from the ideal solution. The solution was sonically degassed under reduced pressure before the subcritical treatment. The solution reservoir was connected to a helium gasbag to prevent the redissolution of oxygen, because saccharides may be oxidized into side products, which is not expected for the isomerization process.17) The solution was delivered into a coiled stainless steel tubular reactor (0.8 mm I.D. × 1.0 m length) immersed in an SRX 310 silicone oil bath (Toray-Dow-Corning Silicone, Tokyo, Japan) with the residence time of 30–500 s using an LC-10AD VP HPLC pump (Shimadzu, Kyoto, Japan). The reaction temperature was in the range 180–200 °C. To rapidly terminate the reaction, the reactor effluent was directly introduced to a stainless steel tube immersed in an ice bath. The pressure inside the tube was regulated at *ca*. 10 MPa using a back-pressure valve (Upchurch Scientific Inc., Oak Harbor, WA, USA). Finally, the effluent was collected into a test tube for the HPLC analysis.

The residence time was calculated according to the method reported previously.16)

HPLC analysis. The concentrations of unreacted substrate and produced monosaccharides were quantified using an HPLC system equipped with an LC-10ADVP HPLC pump (Shimadzu), an RI-101 refractometer (Showa Denko, Tokyo, Japan), and a SUPELCOGEL Ca^{2+} column (7.8 mm I.D. × 300 mm length, Sigma-Aldrich, Tokyo, Japan) equipped with a guard column (4.6 mm I.D. × 50 mm length, Sigma-Aldrich, Japan). Distilled water was used as the eluent at a flow rate of

0.25 mL/min. The columns were maintained at 30 °C in a CTO-10AVP column oven (Shimadzu).

Results and discussion

Effect of ethanol concentration on glucose isomerization

Figure 1 shows the effect of ethanol concentration on glucose isomerization at 180 °C. When the ethanol concentration exceeded 40 % (v/v), glucose isomerization significantly increased with increasing ethanol concentration. Fructose and mannose were competitively produced from glucose both in subcritical water and in subcritical aqueous ethanol. The yield of fructose was almost seven-fold higher than that of mannose at 500 s in 60 % (v/v) aqueous ethanol. Mannose was not detected when the ethanol concentration was below 40 % (v/v). These results were in contrast to those of the hydrolysis of sucrose in subcritical aqueous ethanol. The hydrolysis of sucrose decreased with increasing ethanol concentration, and the isomer of sucrose was not obtained.16) This indicates that only the isomerization of reducing sugars is promoted in subcritical aqueous ethanol.

Figure 1 also shows that ethanol affected the isomerization and decomposition behaviors of glucose. The yield of fructose increased with increasing ethanol concentration at 500 s (Fig. 2). The selectivity of fructose also increased by the addition of ethanol and reached the highest value in 60 % (v/v) aqueous ethanol, where the selectivity was defined as the molar ratio of the produced monosaccharide to the consumed substrate. Although the selectivity of mannose was lower than that of fructose, it reached *ca*. 0.1 when the ethanol concentration was >60 % (v/v). The total saccharide concentration was maintained at a high level, which was slightly lower than the feed glucose concentration and scarcely depended on the ethanol concentration at 180 °C, regardless of the increase in the conversion of glucose. This indicates that the side reaction of glucose was not significantly accelerated, and that most of the consumed glucose was converted to fructose and mannose in subcritical aqueous ethanol at 180 °C.

The contribution of ethanol in promoting glucose isomerization under subcritical aqueous conditions is still unclear. It is known that the pK_a values of glucose in aqueous ethanol and methanol-d_4 decrease with increasing alcohol concentration at ambient temperature, and that the initial reaction rate of alkali-catalyzed glucose isomerization is positively related to the ionization constant.12) Therefore, an increase in ethanol concentration would accelerate glucose isomerization. Another important role of ethanol is that it changes the anomeric equilibrium of glucose, thus changing the apparent chemical equilibrium and facilitating the isomerization of glucose.15) Ethanol not only changes the physical properties of the sugar solution but also participates in hydrogen atom migration during the isomerization.(6) On the other hand, a recent study reported that the initial presence of water in aqueous alcohol suppressed the aluminum-containing zeolite-catalyzed isomerization of glucose to fructose at 120 °C, and that the one-pot synthesis of fructose from glucose could not be performed using aqueous alcohol.(18) However, these reported results are different from our results. One of the reasons may be that the reaction temperature was different in our study, and the role of ethanol in promoting glucose isomerization may change under subcritical conditions.

Temperature dependence of glucose isomerization

Figure 3 shows the effect of temperature on glucose isomerization in 80 % (v/v) subcritical aqueous ethanol. Increasing reaction temperature increased the conversion of glucose and the yields of fructose and mannose at a given residence time. The maximum yield of fructose was achieved faster at higher temperatures. However, the total saccharide concentration at a given residence time decreased with increasing reaction temperature (Fig. 2), indicating that the fraction of disappeared hexoses increased at higher temperatures. Another disadvantage of increasing the reaction temperature is that the selectivity of fructose decreased. A higher maximum yield of fructose was realized at higher temperatures even though the selectivity was lower. In contrast, the selectivity increased at lower temperatures even though a longer residence time was required. Therefore, an appropriate temperature should be selected for the efficient production of fructose from glucose. Similar temperature effects were also observed at other ethanol concentrations. To achieve the maximum yield of fructose within a short time, the isomerization was performed in 80 % (v/v) aqueous ethanol at 180 °C in the subsequent studies.

Mutual isomerization of hexoses

To investigate the mutual isomerization among glucose, mannose, and fructose, the latter two saccharides were also treated under the same conditions as those for glucose. Fig. 4 shows the isomerizations of fructose to glucose and mannose, glucose to fructose and mannose, and mannose to fructose and glucose in 80 % (v/v) aqueous ethanol at 180 °C. The consumption of fructose was the slowest among the three saccharides. Although mannose and glucose were produced from fructose almost in the same yields, the yields were low. These results indicated that fructose significantly decomposed under these conditions.

The isomerization of mannose proceeded faster than that of fructose and glucose.

Fructose was most produced from mannose. However, when mannose was treated with 80% (v/v) aqueous ethanol at 200 °C, the yield of fructose first reached the highest value at a residence time of *ca*. 150 s and then decreased at longer residence times, indicating that the fructose obtained was consequently decomposed (Fig. 4(b)). The yield of fructose produced from mannose was higher than that of fructose produced from glucose within the investigated residence time. Glucose was produced from mannose in a relatively low yield; however, the yield was more than that of mannose produced from glucose.

The substrate-dependent chemoselective isomerization of the investigated monosaccharides in subcritical aqueous ethanol was unique compared to the alkali- and metal-catalyzed isomerizations.5–7) In alkaline aqueous solutions, fructose isomerized to glucose with the highest rate and excellent selectivity. However, the isomerization of mannose was the slowest, and both glucose and fructose were produced almost in the same yield.5) Moreover, mannose isomerized slower than glucose in many ethanolic and methanolic solutions of metal ions.6,7) Many studies reported that glucose was isomerized to fructose more easily than mannose under alkaline conditions.15,19,20) These studies were carried out at low reaction temperatures. The unique product distribution among the investigated saccharides in subcritical aqueous ethanol can be attributed to the difference in the temperature dependence of the rate constants of the reactions.

Kinetic analysis of the isomerization and decomposition of monosaccharides

As shown above, glucose, mannose, and fructose were isomerized and decomposed in parallel in subcritical aqueous ethanol. The probable reaction pathways are shown in Scheme 1, where k_i (i = F→G, F→M, Fd, G→F, G→M, Gd, M→F, M→G, and Md) are the rate constants, and F, G, M, and d represent fructose, glucose, mannose, and the decomposition of these three monosaccharides, respectively. When the decomposition and isomerization of the monosaccharides were assumed to follow first-order kinetics,10) the reaction rate of each monosaccharide can be expressed as follows:

$$\frac{dC_{\mathrm{F}}}{dt} = -(k_{\mathrm{F\to G}} + k_{\mathrm{F\to M}} + k_{\mathrm{Fd}})C_{\mathrm{F}} + k_{\mathrm{G\to F}}C_{\mathrm{G}} + k_{\mathrm{M\to F}}C_{\mathrm{M}} \quad (1)$$

$$\frac{dC_{\mathrm{G}}}{dt} = -(k_{\mathrm{G\to F}} + k_{\mathrm{G\to M}} + k_{\mathrm{Gd}})C_{\mathrm{G}} + k_{\mathrm{F\to G}}C_{\mathrm{F}} + k_{\mathrm{M\to G}}C_{\mathrm{M}} \quad (2)$$

$$\frac{dC_{\mathrm{M}}}{dt} = -(k_{\mathrm{M\to G}} + k_{\mathrm{M\to F}} + k_{\mathrm{Md}})C_{\mathrm{M}} + k_{\mathrm{F\to M}}C_{\mathrm{F}} + k_{\mathrm{G\to M}}C_{\mathrm{G}} \quad (3)$$

where C_{F}, C_{G}, and C_{M} are the concentrations of fructose, glucose, and mannose, respectively.

The rate constants for the reaction of each substrate were evaluated by minimizing the sum of the residual square between the experimental and calculated C_i values using the Solver of Microsoft® Excel 2010. The curves in Figs. 1, 3, and 4 were drawn using the estimated rate constants. The rate constants obtained for the isomerization and decomposition of each substrate at 180, 190, and 200 °C were plotted against the ethanol concentrations shown in Fig. 5. The values of $k_{M \to F}$, $k_{G \to F}$, and k_{Fd} were larger than the others under any condition, and the $k_{M \to F}$ value was the largest, indicating that mannose was most easily isomerized to fructose. In contrast, the rate constants for the reverse reactions, $k_{F \to M}$ and $k_{F \to G}$, were much smaller. The rate constants for the isomerizations between mannose and glucose, $k_{M \to G}$ and $k_{G \to M}$, were also smaller than $k_{M \to F}$ and $k_{G \to F}$. These results indicated that the isomerization of mannose and glucose to fructose was faster than the reverse reactions and the isomerization between mannose and glucose, and that these isomerizations were accelerated by the addition of ethanol and increase in temperature. Although decompositions were also promoted by increase in temperature and ethanol concentration, they were less sensitive to temperature and ethanol concentration than isomerizations. The $k_{M \to F}$ was the most sensitive to the change in ethanol concentration.

To investigate the temperature dependence of the rate constants, the k_i values were plotted against the reciprocal of absolute temperature (Arrhenius plot). Fig. 6 shows the typical Arrhenius plots of the rate constants obtained in 80 % (v/v) subcritical aqueous ethanol. The rate constants of the respective reaction steps separately lie on the straight lines. The frequency factors and activation energies were calculated to be in the ranges of 10^8–10^{12} s^{-1} and 90–130 kJ/mol, respectively. The activation energies of the isomerizations were similar to those of the monosaccharide decompositions in 100 % (v/v) subcritical water,[21)] thus indicating that the isomerization and decomposition of monosaccharides would have a similar energy barrier.

Reaction equilibrium for the isomerization of monosaccharides

Figure 5 also shows that the isomerizations of mannose to fructose and glucose to fructose have high reaction equilibrium constants, K_{eq}. The K_{eq} values were calculated from the rate constants ($K_{eq,M\to F} = k_{M\to F}/k_{F\to M}$ and $K_{eq,G\to F} = k_{G\to F}/k_{F\to G}$); they were not affected by ethanol concentration. The equilibrium constants for each reaction at different ethanol concentrations were averaged and plotted against the reciprocals of absolute temperature according to van’t Hoff equation:

$$\ln K_{eq} = -\frac{\Delta H}{RT} \tag{4}$$

where ΔH and R are the change in enthalpy and gas constant, respectively (Fig. 7). The equilibrium constants for the isomerization of mannose to fructose, $K_{eq,M\to F}$, were higher than those for the isomerization of glucose to fructose, $K_{eq,G\to F}$. The plot of the equilibrium constant for each isomerization lies on a straight line with high correlation coefficients (0.997 for $K_{eq,M\to F}$ and 0.996 for $K_{eq,G\to F}$). The enthalpies for the isomerizations of mannose to fructose, $\Delta H_{\mathrm{M}\rightarrow\mathrm{F}}$, and glucose to fructose, $\Delta H_{\mathrm{G}\rightarrow\mathrm{F}}$, were calculated to be 18 and 24 kJ/mol, respectively. The positive enthalpies indicate that both the isomerizations were endothermic reactions. Therefore, the yield of fructose would increase with increasing temperature.

In conclusion, the isomerizations among glucose, mannose, and fructose were significantly promoted in subcritical aqueous ethanol. Mannose and glucose were easily isomerized to fructose. However, the isomerizations of fructose to glucose and mannose and that between glucose and mannose were not favorable in subcritical aqueous ethanol. Fructose mainly underwent decomposition when it was used as a substrate. The kinetic study showed that $k_{\mathrm{M}\rightarrow\mathrm{F}}$, $k_{\mathrm{G}\rightarrow\mathrm{F}}$, and k_{Fd} were larger than the other rate constants. Both the isomerizations of mannose to fructose and glucose to fructose had high equilibrium constants, indicating that subcritical aqueous ethanol may be a useful reaction medium to produce high fructose syrup.

Acknowledgement

This work was partly supported by the Tojuro Iijima Foundation for Food Science and Technology (D.G.) and by Japan Society for the Promotion of Science (Grant Number 26870296; T. K.).

References

[1] Bursać BN, Djordjevic AD, Vasiljević AD, Vojnović Milutinović DD, Veličković NA, Nestorović NM, Matić GM. Fructose consumption enhances glucocorticoid action in rat visceral adipose tissue. J. Nutr. Biochem. 2013;24:1166–1172.

[2] Akram M, Hamid A. Mini review on fructose metabolism. Obes. Res. Clin. Pract. 2013;7:e89–e94.

[3] Román-Leshkov Y, Chheda JN, Dumesic JA. Phase modifiers promote efficient production of hydroxymethylfurfural from fructose. Science 2006;30:1933–1937.

[4] Holm MS, Saravanamurugan S, Taarning E. Conversion of sugars to lactic acid derivatives using heterogeneous zeotype catalysts. Science 2010;328:602–605.

[5] EI Khaden HS, Ennifar S, Isbell HS. Contribution of the reaction pathways involved in the isomerization of monosaccharides by alkali. Carbohydr. Res. 1987;169:13–21.

[6] Tanase T, Takei T, Hidai M, Yano S. Substrate-dependent chemoselective aldose–aldose and aldose–ketose isomerizations of carbohydrates promoted by a combination of calcium ion and monoamines. Carbohydr. Res. 2001;333:303–312.

[7] Yanagihara R, Soeda K, Shiina S, Osanai S, Yoshikawa S. C-2 epimerization of aldoses by calcium ion in basic solutions. A simple system to transform D-glucose and D-xylose into D-mannose and D-lyxose. Bull. Chem. Soc. Jpn.

1993;66:2268–2272.

[8] Moliner M, Román-Leshkov Y, Davis ME. Tin-containing zeolites are highly active catalysts for the isomerization of glucose in water. Proc. Natl. Acad. Sci. U.S.A. 2010;107:6164–6168.

[9] Kabyemela BM, Adschiri T, Malaluan RM, Arai K. Kinetics of glucose epimerization and decomposition in subcritical and supercritical water. Ind. Eng. Chem. Res. 1997;36:1552–1558.

[10] Usuki C, Kimura Y, Adachi S. Isomerization of hexoses in subcritical water. Food Sci. Technol. Res. 2007;13:205–209.

[11] Ellis AV, Wilson MA. Carbon exchange in hot alkaline degradation of glucose. J. Org. Chem. 2002;67:8469–8474.

[12] Vuorinen T, Sjöström E. Kinetics of alkali-catalyzed isomerization of D-glucose and D-fructose in ethanol-water solutions. Carbohydr. Res. 1982;108:23–29.

[13] Visuri K, Klibanov AM. Enzymatic production of high fructose corn syrup (HFCS) containing 55% fructose in aqueous ethanol. Biotechnol. Bioeng. 1987;30:917–920.

[14] Nilsson KGI, Mosbach K, Sakaguchi K. Increased yield of fructose from glucose employing thermophilic xylose isomerase in water-ethanol mixtures. Biotechnol.

Lett. 1991;13:787–792.

[15] Angyal SJ. The composition and conformation of sugars in solution. Angew. Chem. Int. Ed. 1969;8:157–166.

[16] Gao D, Kobayashi T, Adachi S. Kinetics of sucrose hydrolysis in a subcritical water-ethanol mixture. J. Appl. Glycosci. 2014;61:9–13.

[17] Akiya N, Savage PE. Roles of water for chemical reaction in high-temperature water. Chem. Rev. 2002;102:2725–2750.

[18] Saravanamurugan S, Paniagua M, Melero JA, Riisager A. Efficient isomerization of glucose to fructose over zeolites in consecutive reactions in alcohol and aqueous media. J. Am. Chem. Soc. 2013;135:5246–5249.

[19] Topper YJ, Stetten DW. The alkali-catalyzed conversion of glucose into fructose and mannose. J. Biol. Chem. 1951;189:191–202.

[20] Isbell HS, Frush HL, Wade CWR, Hunter CE. Transformations of sugars in alkaline solutions. Carbohydr. Res. 1969;9:163–175.

[21] Haghighat Khajavi S, Kimura Y, Oomori T, Matsuno R, Adachi S. Degradation kinetics of monosaccharides in subcritical water. J. Food Eng. 2005;68:309–313.

Scheme 1. Simplified reaction pathways for the isomerization of three hexoses in subcritical aqueous ethanol.

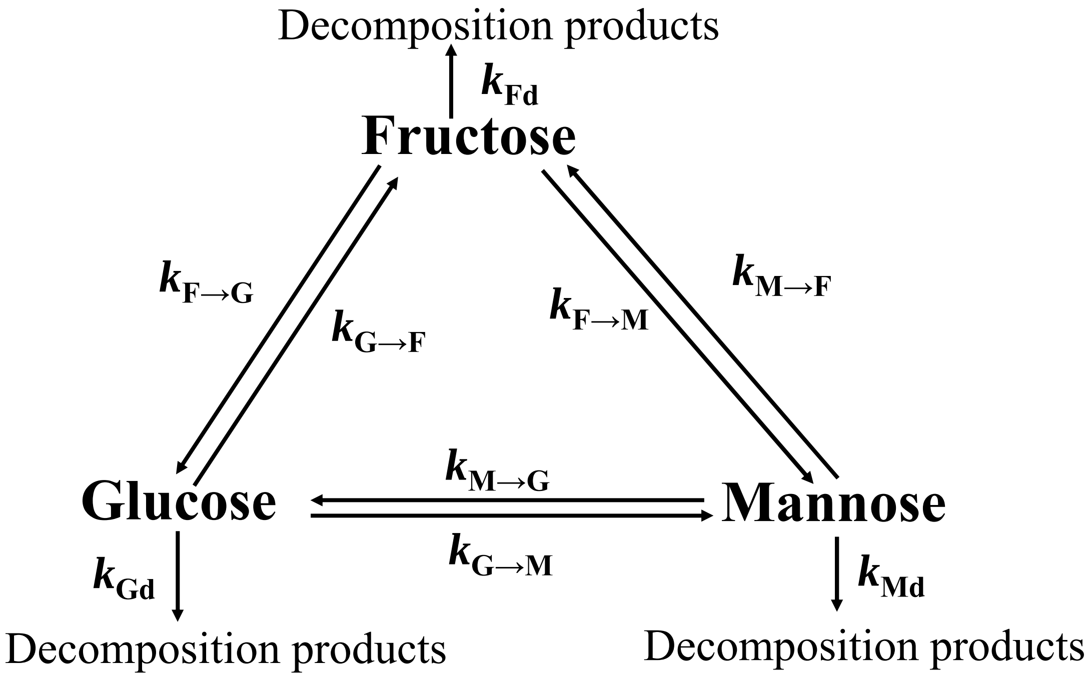

Figure Captions

Fig. 1. Changes in (a) the fraction of remaining glucose treated at 180 °C in (△) 0 % (v/v) (subcritical water alone), (○) 20 %, (▷) 40 %, (□) 60 %, and (◇) 80 % subcritical aqueous ethanol and (b) the yields of the fructose (open symbols) and mannose (closed symbols) obtained with residence times. The symbols in (b) are the same as those in (a). The curves show the calculated results.

Fig. 2. Effect of ethanol concentration on the (◆, ◇) selectivity and (●, ○) yield of fructose, (■, □) selectivity of mannose, and (▲, △) the total saccharide content when glucose was treated at 180 °C (closed symbols) and 190 °C (open symbols) for 500 s.

Fig. 3. Changes in (a) the fraction of remaining glucose at (○)180 °C, (□) 190 °C, and (◇) 200 °C in 80 vol % subcritical aqueous ethanol and (b) the yields of fructose (open symbols) and mannose (closed symbols) obtained with residence times. The symbols in (b) are the same as those in (a). Curves show the calculated results.

Fig. 4. Changes in (a) the fractions of remaining (◇) fructose, (□) glucose, and (△)

mannose at 200 °C in 80 % (v/v) subcritical aqueous ethanol and (b) the yields of the hexoses obtained with residence times. Symbols △ and ▲ represent fructose and glucose produced from mannose; □ and ■ represent fructose and mannose produced from glucose; and ◇ and ◆ represent mannose and glucose produced from fructose, respectively.

Fig. 5. Dependencies of the rate constants of the respective reaction steps on the ethanol concentration at (a) 180 °C, (b) 190 °C, and (c) 200 °C. The rate constants are expressed as follows: (◆) $k_{M\rightarrow F}$, (◇) k_{Fd}, (△) $k_{G\rightarrow F}$, (▲) k_{Md}, (○) k_{Gd}, (●) $k_{M\rightarrow G}$, (□) $k_{F\rightarrow M}$, (■) $k_{F\rightarrow G}$, and (▼) $k_{G\rightarrow M}$.

Fig. 6. Arrhenius plot for the rate constants of the respective reaction steps in 80 % (v/v) subcritical aqueous ethanol. Symbols are the same as those shown in Fig. 5.

Fig. 7. Temperature dependence of the equilibrium constants for the isomerization of mannose to fructose (□, $K_{eq,M\rightarrow F}$) and glucose to fructose (◇, $K_{eq,G\rightarrow F}$).

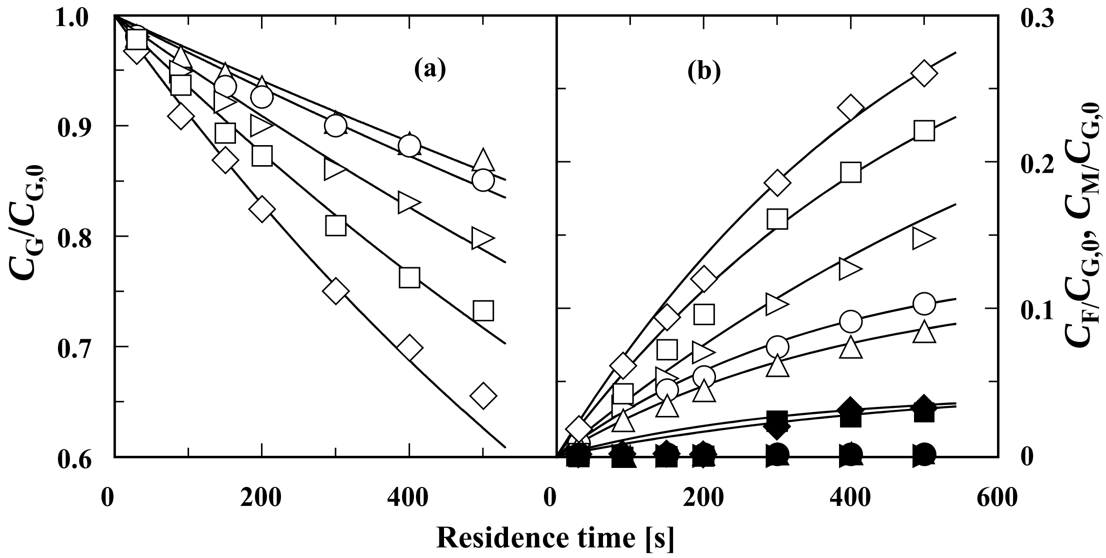

Fig. 1 Gao *et al*.

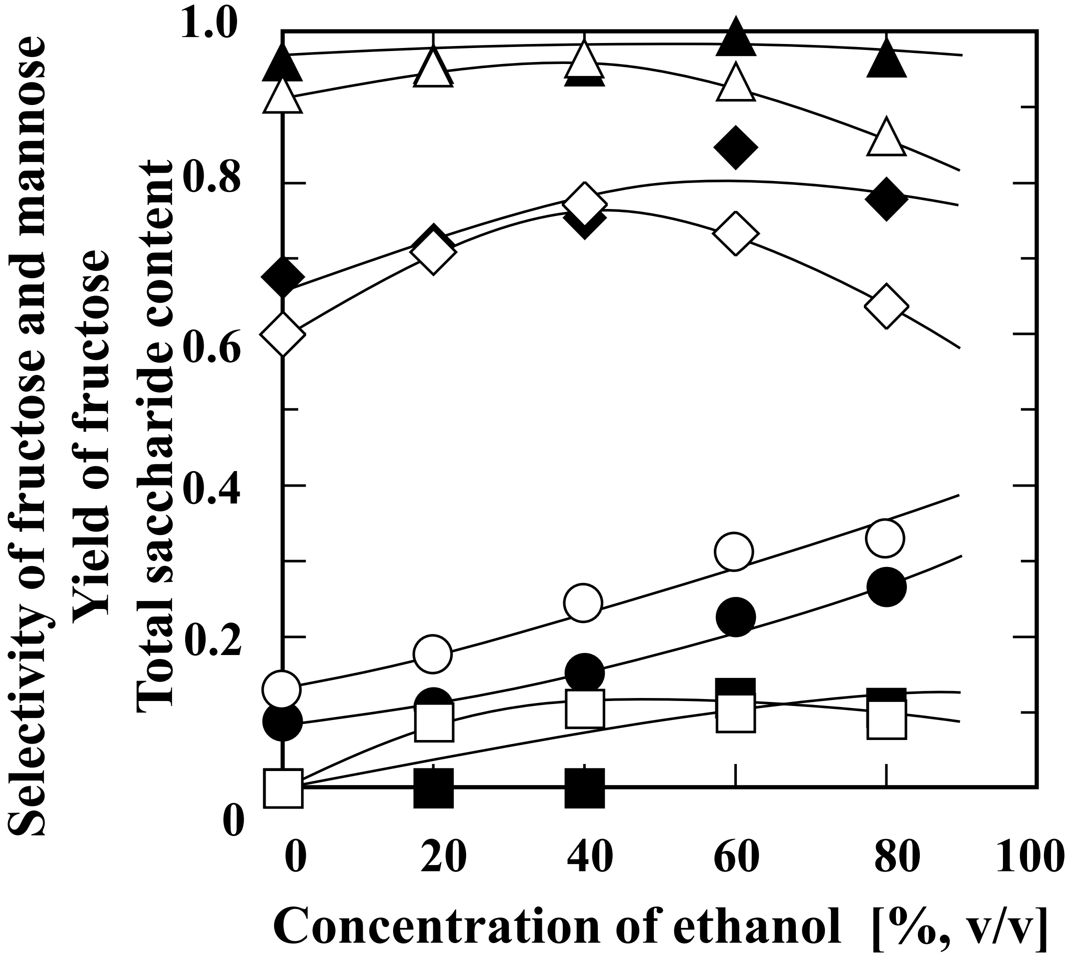

Fig. 2 Gao *et al*.

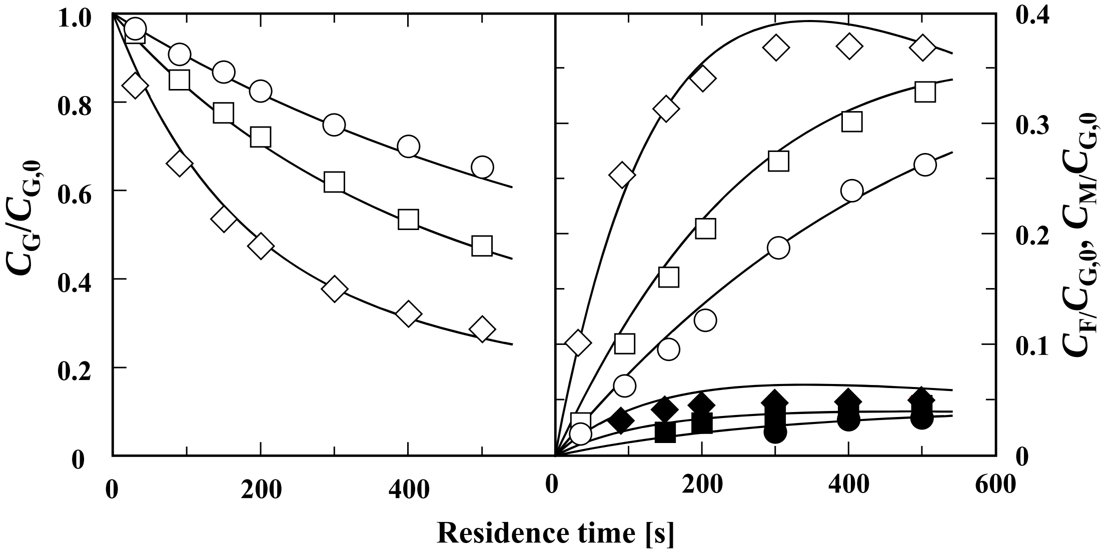

Fig. 3 Gao *et al*.

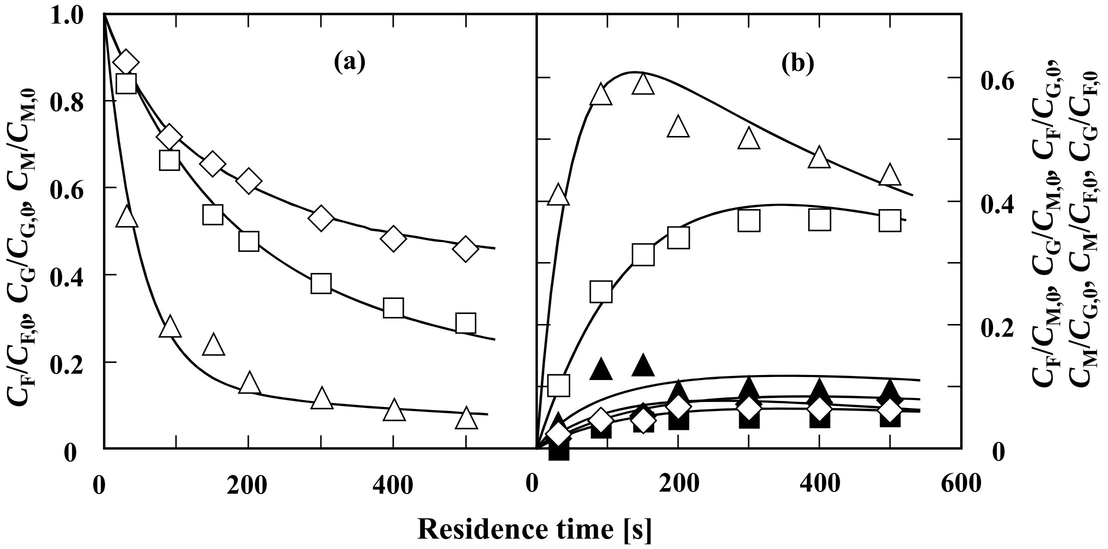

Fig. 4 Gao *et al*.

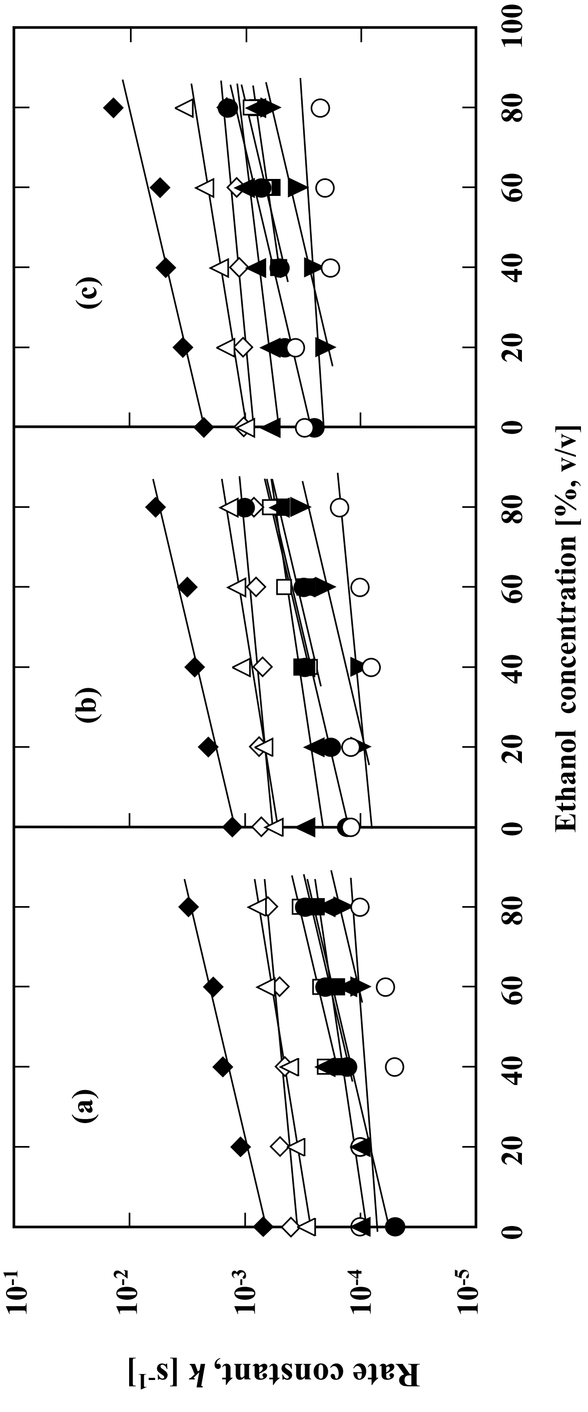

Fig. 5 Gao *et al*.

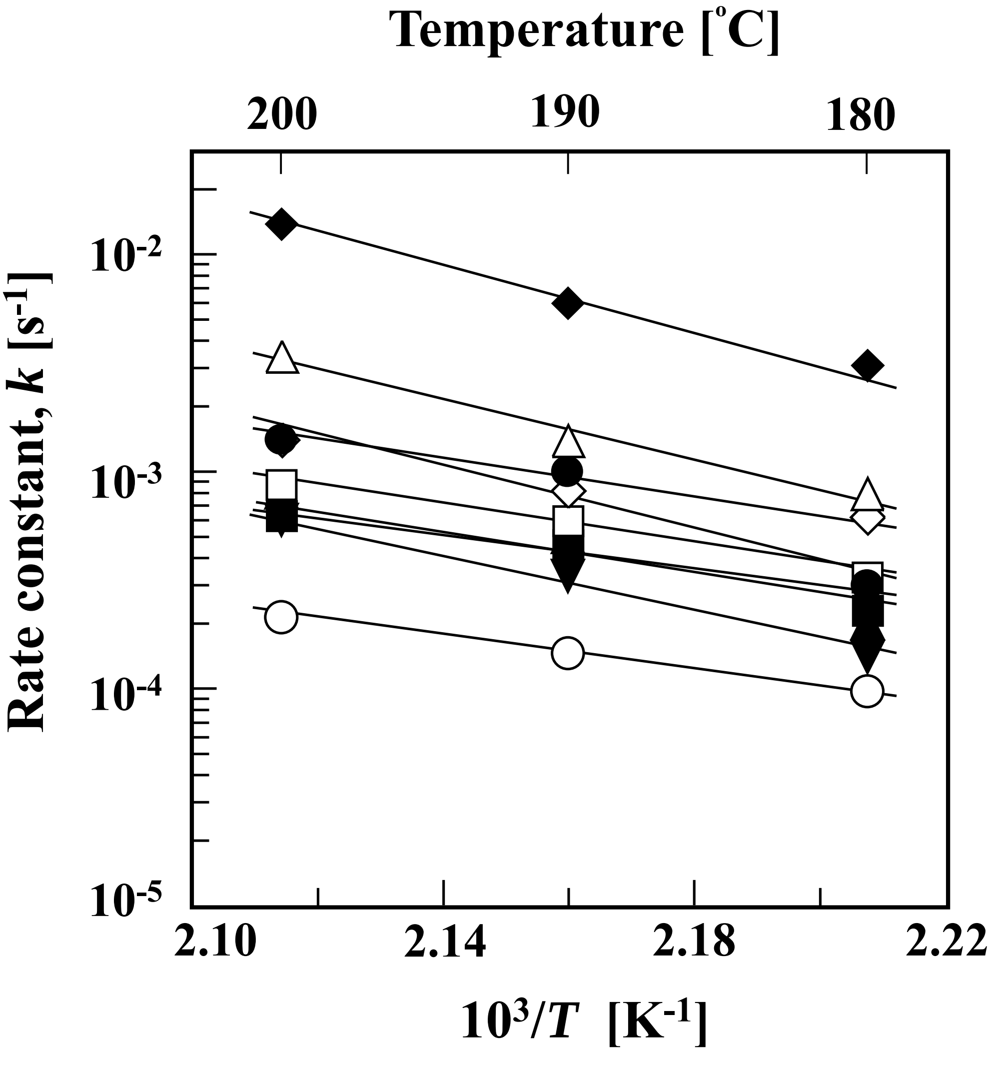

Fig. 6 Gao *et al*.

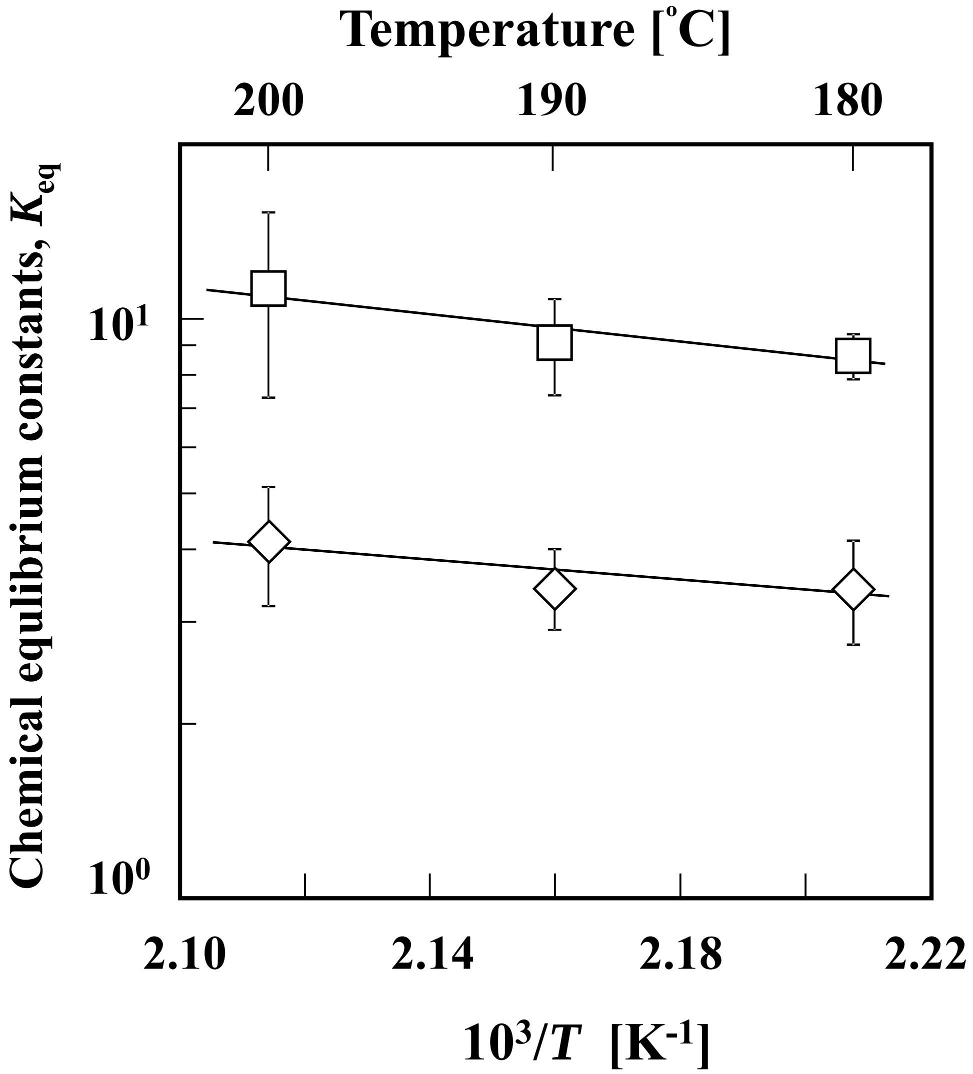

Fig. 7 Gao *et al*.