Effect of phosphate buffer concentration on the isomerization of galactose to rare sugars under subcritical water conditions

Yuichiro Onishi [a], Shuji Adachi [b], Fumito Tani [a], Takashi Kobayashi[*,a]

[a] *Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

[b] *Department of Agriculture and Food Technology, Faculty of Bioenvironmental Sciences, Kyoto University of Advanced Science, Kameoka, Kyoto 621-8555, Japan*

*Corresponding author:

E-mail: kobayashi.takashi.6w@kyoto-u.ac.jp (T. Kobayashi)

Fax: +81-75-753-6285

Abstract

Galactose was treated in sodium phosphate buffer at various concentrations (0.1 to 500 mmol/L) under subcritical water conditions (140°C), and the effects of the buffer concentration and reaction time (0 to 300 s) on the reaction behavior were evaluated. The reaction proceeded rapidly at higher buffer concentrations. Rare sugars (tagatose, talose, and sorbose) were formed from galactose by isomerization. The highest yield of the main product, tagatose, was approximately 14% in 50 mmol/L buffer. However, the tagatose yield did not increase further with increasing buffer concentration. On the other hand, the formation of talose and sorbose was accelerated at higher buffer concentrations, with the highest yields of approximately 5% and 12%, respectively, in 500 mmol/L buffer. At the same time, the formation of byproducts (organic acids and colored substances) was also accelerated in high-concentration buffers. These results suggest that phosphate buffer promoted all reactions occurring under subcritical water conditions.

Keywords: isomerization, organic acids, phosphate buffer, rare sugar, subcritical water

1. Introduction

D-Tagatose (hereafter referred to as tagatose) is a GRAS (Generally Recognized as Safe) substance and has attracted attention as a sugar substitute sweetener (Nagata, Mizuta, Kanasaki, & Tanaka, 2018; Salonen, Salonen, Leisola, & Nyyssölä, 2013). Compared with sucrose, it has 92% sweetness but less than half the calories (Choi & Chung, 2015; Torrico, Tam, Fuentes, Gonzalez Viejo, & Dunshea, 2019). Tagatose also has a low glycemic index (Guerrero-Wyss, Durán Agüero, & Angarita Dávila, 2018), meaning that its ingestion causes a slow increase in blood glucose level. Hence, it is valuable as an alternative sweetener that could treat type 2 diabetes (Lu, Levin, & Donner, 2008). It has also been reported to help maintain dental health (Kim, 2004), and to help reduce body weight while increasing high-density lipoprotein cholesterol levels (Donner, Magder, & Zarbalian, 2010). D-Talose and D-sorbose are also rare sugars. D-Talose is reported to show antitumor and antimicrobial activities (Xiao, Wang, Wang, & Li, 2010), and D-sorbose possesses inhibitory activity of disaccharidase (Oku, Murata-Takenoshita, Yamazaki, Shimura, & Nakamura, 2014). Therefore, it is anticipated that these rare sugars will be used in various products, such as health foods and functional beverages.

As a naturally occurring rare ketohexose, tagatose is present in very small amounts in *Sterculia setigera* gum and in dairy products (Hirst, Hough, & Jones, 1949; Kim, 2004; Mendoza, Olano, & Villamiel, 2005). Due to its scarcity in nature, its artificial production is required, but no method has yet been established for the mass production of tagatose to enable its commercial use. Until now, methods for the mass production of tagatose have been studied using chemical (de Bruijn, Kieboom, & van Bekkum, 1986; Drabo & Delidovich, 2018; Nagasawa, Sato, & Kasumi, 2017), enzymatic (Cheng, Metzger, & Martínez-Monteagudo, 2020; Zhang, Zhang, Jiang, & Mu, 2017), or biotechnological

67 approaches (Oh, 2007; Roy et al., 2018). However, each of these approaches has its
68 challenges. Chemical methods have drawbacks such as the need for multi-step
69 purification and their inapplicability to food production processes, depending on the
70 catalyst used. Enzymatic methods require the purification of enzymes, long reaction
71 times, and, in some cases, genetically modified organisms.

72 Subcritical water is water maintained in a liquid state under pressurized conditions.
73 It is characterized by a high ion product and is capable of isomerizing reducing sugars
74 (Usuki, Kimura, & Adachi, 2007). For example, galactose isomerizes to tagatose and
75 talose during subcritical water treatment (Gao, Kobayashi, & Adachi, 2015). However,
76 there have been reports that the degradation reaction of sugars is pronounced in
77 subcritical water, resulting in low isomerization yields of sugars (Hirayama & Kobayashi,
78 2019; Usuki et al., 2007). This is thought to be due to the formation of organic acids as
79 the sugars degrade, lowering the pH of the reaction solution (Jin, Yun, Li, Kishita, Tohji,
80 & Enomoto, 2008; Kishida, Jin, Yan, Moriya, & Enomoto, 2006; Kruse & Gawlik, 2003;
81 Sınağ, Gülbay, Uskan, & Canel, 2010). As a result, the Lobry de Bruyn–Alberda van
82 Ekenstein (LBAE) transformation reaction, which occurs under alkaline conditions and
83 is the main pathway for the formation of rare sugars, is inhibited.

84 To overcome this problem, we previously investigated the production of rare sugars
85 (tagatose and talose) by the isomerization of galactose using phosphate buffer solution
86 under subcritical water conditions and succeeded in greatly improving the yield at pH
87 7.0 (Onishi, Furushiro, Adachi, & Kobayashi, 2021; Onishi, Furushiro, Hirayama,
88 Adachi, & Kobayashi, 2020). However, the effect of the buffer concentration on the
89 reaction behavior is currently unknown. Its clarification would help to make the
90 production of rare sugars more efficient. In this study, galactose was treated under
91 subcritical water conditions in sodium phosphate buffer at various buffer concentrations,

and the reaction was evaluated in terms of rare sugar yields and side reactions.

2. Materials and methods

2.1. Materials

D-Galactose, D-tagatose, D-talose, and D-sorbose were purchased from Fujifilm Wako Pure Chemicals (Osaka, Japan). Other chemicals were purchased from Wako, Dojin (Kumamoto, Japan) or Nacalai Tesque (Kyoto, Japan).

2.2. Subcritical water treatment of galactose in sodium phosphate buffer

Subcritical water treatment of galactose was performed using the same reaction apparatus as previously reported (Onishi, Adachi, Tani, & Kobayashi, 2022; Onishi et al., 2021; Onishi et al., 2020). Galactose was dissolved at 5 wt% in 0.1–500 mmol/L (0, 0.1, 0.5, 1, 2, 5, 10, 50, 100, 200, 500 mmol/L) sodium phosphate buffer adjusted to pH 7.0 or in pure water (control), to prepare a substrate solution. Nitrogen gas was then blown into the substrate solution to remove oxygen from the solution, and a bag containing nitrogen gas was connected to the solution reservoir to prevent re-dissolution of oxygen. A PEEK high-performance liquid chromatography (HPLC) tube (0.8 mm internal diameter (I.D.), 1.9 mL volume) was used as the reactor. The reaction temperature was fixed at 140°C based on the previous results (Onishi et al., 2020) . The reactor was coiled and immersed in an oil bath maintained at 140°C, and the inlet side was connected to an HPLC pump (LC-10ADVP, Shimadzu, Kyoto, Japan). The outlet side was connected to a cooling water bath, to terminate the reaction. A back pressure valve (P-880, Upchurch, IDEX Health & Science, Oak Harbor, WA, USA) was installed at the outlet of the reactor to maintain the internal pressure at 3 to 4 MPa. Subcritical water treatment was performed by varying the flow rate of the galactose solution to the reactor

to achieve a prescribed reaction time in the range of 30 to 300 s. After reaching a steady state, approximately 1.5 mL of the reactor outlet solution was collected for analysis. The untreated solution was used as a sample with a reaction time of 0 s.

The treatment was performed in triplicate for the galactose concentrations of 5, 10, and 50 mmol/L, and standard deviations for the remaining galactose, yields of tagatose and talose were almost within 2%. Because these deviations were not so large, the treatment with other galactose concentrations were performed once.

2.3. Analysis

Sugars in the treated solution were quantified by HPLC, using an HPLC pump (LC-20AD, Shimadzu) connected to a COSMOIL Sugar-D column (3.0 mm I.D. × 250 mm, Nacalai Tesque) and a refractive index detector (RID-20A, Shimadzu). The column temperature was controlled at 40°C, and 80% (v/v) acetonitrile was used as the mobile phase with a flow rate of 0.4 mL/min. The pH of the treated solution was measured at room temperature using a pH meter (D-71, HORIBA, Kyoto, Japan).

To evaluate coloration, absorption spectra (wavelength 200–700 nm) of the treated solutions were measured using a spectrophotometer (U-5100, Hitachi High-Tech Science, Tokyo, Japan). During preliminary experiments, a peak was observed near 440 nm in the spectra, and the absorbance at 440 nm increased with increasing reaction time. Therefore, absorbance at 440 nm was used as an index for the degree of coloration.

3. Results and discussion

3.1. Effect of buffer concentration on the degradation and isomerization behavior of galactose

Figure 1 shows the effect of buffer concentration (0.1–500 mmol/L) on the formation

142 of tagatose, talose, and sorbose from galactose in sodium phosphate buffer maintained
143 at 140°C (subcritical conditions). Galactose began to decrease from the beginning of the
144 reaction (reaction time < 1 min). However, the degree of galactose decrease was rather
145 small at 30 s, with a steep decrease observed after 60 s. The lower decrease at 30 s would
146 be due to the effect of heat transfer between the oil bath and the reaction medium.
147 In the early stage of the reaction (<180 s), the remaining galactose rapidly decreased
148 with increasing buffer concentration (Fig. 1a). After 180 s, the decrease leveled off. The
149 decrease in galactose at the initial stage was particularly pronounced with buffer
150 concentrations of 10 mmol/L or higher. Finally, at 300 s, the remaining galactose was
151 96% at a buffer concentration of 0.1 mmol/L and 75% at 10 mmol/L; however, it had
152 decreased to 17% at 500 mmol/L. Therefore, sodium phosphate promoted the conversion
153 of galactose to other substances at 140°C, even at low buffer concentrations, and the
154 conversion was considerably higher at higher buffer concentrations. Although the
155 conversion of galactose was indeed low at low buffer concentrations, it would be
156 considerably higher than in pure water (~1%), clearly indicating the effect of phosphate.
157 Next, we discuss the formation of rare sugars (tagatose, talose, and sorbose) by
158 isomerization or epimerization (Fig. 1b–d). The yield of tagatose at 300 s was 2.6% in 0.1
159 mmol/L and 14% in 50 mmol/L phosphate buffer. The yield of tagatose increased
160 significantly with increasing buffer concentration (Fig. 2); this increase was most
161 pronounced in the buffer concentration range of 0.1 to 10 mmol/L.
162 In parallel with the formation of tagatose, the formation of talose was also observed
163 (Fig. 1c). As previously reported, no talose was detected in the absence of buffer (Onishi
164 et al., 2020). However, with phosphate buffer, the yield of talose increased with
165 increasing buffer concentration (Fig. 2), with the highest yield being approximately 5%;
166 previously reported yields of talose were at most 1.5% (Gao et al., 2015; Onishi et al.,

2021; Onishi et al., 2020). In contrast to the formation of tagatose, the talose yield significantly increased at buffer concentrations of more than 50 mmol/L. Presently, although the yield of talose is only 4% at most, we expect that increasing the buffer concentration will become a new method for producing talose in the future if the process will be further improved.

Sorbose was also detected in the galactose-treated solution (Fig. 1d). The effect of buffer concentration was considerable above 50 mmol/L, and the concentration contributed to increase in the yield (Fig. 2); 500 mmol/L produced the highest yield, of approximately 13%. However, the effect of buffer concentration was negligible between 0 and 2 mmol/L. In terms of the time courses, there was a slight time lag for the formation of sorbose (Fig. 1d). The yield of sorbose at 30 s was at most approximately 1%; however, after 60 s, the yield increased rapidly with prolonged reaction time. In other words, the formation of sorbose was less likely to occur at the beginning of the reaction and occurred somewhat later. This contrasts with the formation of tagatose and talose, which proceeded immediately after the start of the reaction. These results suggest that sorbose is formed through consecutive reactions (Fig. S1). These consecutive reactions would be composed of a multi-step LBAE transformation, as discussed in more detail later.

3.2. Kinetic analysis of isomerization

To further explore the various degrees of reaction progress described above, the initial reaction rate of each sugar (initial rate of decrease for galactose and initial rates of formation for the rare sugars) was analyzed. For the kinetic analysis, the initial reaction rate was calculated from the slope at the beginning of the reaction for each sugar shown in Fig. 1; in calculating the slope, we excluded the data at 30 s, when the effect of heat transfer was obvious.

Figure 3 shows a log–log plot of the dependence on the buffer concentration of the reaction rates for the formation or decrease in each sugar. In all reactions, the rate increased with increasing buffer concentration. In other words, phosphate promoted both isomerization and other side reactions. However, the degree of this promotion was heavily dependent on the type of sugar. The rate for tagatose formation was maintained to a certain degree even at low buffer concentrations, i.e., the use of low-concentration buffer solution selectively produced tagatose. On the other hand, the rates for the formation of sorbose and talose increased with increasing buffer concentration. These results suggest that high-concentration buffer would promote isomerization to various rare sugars. The rate of decrease in galactose was greater than the sum of the rates of the rare sugar formation, suggesting the occurrence of side reactions. We discuss this in more detail in the following section.

3.3. Selectivity of rare sugars during isomerization

During the isomerization of galactose, rare sugars can be efficiently synthesized if no side reactions occur. However, as already noted, the degradation of tagatose and talose also occur, decreasing the selectivity of rare sugars. In this section, we discuss the selectivity of rare sugars, which represents the ratio of the formation of rare sugars to the decrease in galactose (selectivity= $Y_{Tag}/X_{Gal} \times 100\%$ or $Y_{Tal}/X_{Gal} \times 100\%$ or $Y_{Sor}/X_{Gal} \times 100\%$), where X_{Gal} is the conversion of galactose; and Y_{Tag}, Y_{Tal}, and Y_{Sor} are the yields of tagatose, talose, and sorbose, respectively. Figure 4 shows relationships between the conversion of galactose and yields of rare sugars, including the total yield of rare sugars ($Y_{Tag}+Y_{Tal}+Y_{Sor}$). The dashed line represents the relationship between the conversion and the yield when no side reactions occur (selectivity=100%); the closer the plot is to the dashed line, the higher the selectivity of rare sugars. Conversely, the further away

217 from the dashed line, the higher the priority of the side reactions.

218 Overall, as the reaction progressed (X_{Gal}>15%), the plot of total rare sugars deviated
219 more from the dashed line, suggesting increased progression of side reactions. However,
220 if the reaction was stopped in the range of X_{Gal}<10%, the product would be almost
221 exclusively rare sugars. The plot for tagatose was also very close to the dashed line when
222 X_{Gal}<10%, but it deviated sharply at higher galactose conversion. In addition, the
223 tagatose yield was almost constant when X_{Gal}>30%, probably indicating the presence of
224 an equilibrium between the galactose–tagatose conversion reaction. As a result, a
225 mixture comprising almost exclusively galactose and tagatose was obtained when
226 X_{Gal}<10%.

227 On the other hand, talose and sorbose showed different behaviors. For talose, the
228 yield increased gradually and almost linearly when X_{Gal}<50%, then leveled off when
229 there was greater galactose conversion (X_{Gal}≥50%). Unlike these rare sugars, the yield
230 of sorbose always increased linearly and was almost the same as that of tagatose at
231 X_{Gal}~80%. As described above, the yield of tagatose reached a plateau at X_{Gal}>20%. One
232 of the reasons for this plateau would be the consecutive isomerization of tagatose to
233 sorbose.

234 It was reported that Y_{Tag}=16% when X_{Gal}=51% in the presence of base (Drabo et al.,
235 2018). Sn-BEA zeolite (solid Lewis acid) gave Y_{Tag}=24% at X_{Gal}=29%. The selectivity of
236 tagatose in this study somewhat resembled the case using base but was different from
237 that using Lewis acid. This reflects that the reaction mechanism in this study is indeed
238 essentially base-catalyzed reaction (LBAE transformation).

239 As described above, the behavior for the formation of each rare sugar was strongly
240 influenced by the buffer concentration, and the degree of influence was heavily
241 dependent on the type of sugar. In addition, there was considerably greater production

242 of sorbose at higher buffer concentrations. One possible explanation for these results is
243 as follows. Each of these isomerization reactions is caused by LBAE transformation (Fig.
244 S1). During this transformation, the formation of 2,3-endiols may be kinetically
245 predominant at higher buffer concentrations. There may also be a slight predominance
246 of talose formation (epimerization). However, the yield of tagatose did not change much
247 in the higher buffer concentration range, partly due to the chemical reaction equilibrium
248 mentioned above. Therefore, the formation of 2,3-enediol from tagatose would also be
249 predominant. The detailed reasons for these phenomena will be clarified in future
250 investigations.

251 Figure 5 shows the effect of buffer concentration on the composition of sugars and
252 byproducts at the reaction time of 300 s. In pure water, the byproducts were less than
253 0.5 wt%, but as the buffer concentration increased, the byproduct content increased. In
254 addition, more than half of the galactose was converted to byproducts at 500 mmol/L.
255 These findings suggest that sodium phosphate buffer does not separately promote
256 isomerization, epimerization, or decomposition; rather, it uniformly promotes all possible
257 reactions when galactose is subjected to subcritical water treatment. Therefore, we
258 compared the results with those of other factors such as pH and UV absorbance and
259 discuss their effects on the byproducts in the following section.

260

261 *3.4. Changes in pH and absorbance following subcritical water treatment*

262 To examine in detail how the buffer concentration influences pH and the degree of
263 coloration of the treated solution, the relationships between buffer concentration, yield
264 of 5-hydroxymethylfurfural (HMF), pH, and absorbance at 440 nm, with a reaction time
265 of 300 s, are depicted in Fig. 6. When the buffer concentration was low (0–2 mmol/L), the
266 pH decreased with increasing buffer concentration, indicating that a low concentration

267 of phosphate buffer possessed little buffering capacity, and the phosphate promoted some
268 reactions, resulting in lower pH (pH 4.96 at 2 mmol/L). These results indicate that
269 phosphate has some catalytic ability.

270 At buffer concentrations of more than 5 mmol/L, the decrease in pH was suppressed,
271 depending on the buffer concentration. This would reflect emerging the buffering
272 capacity of phosphate and also indicate that the effective concentration of organic acids
273 was lower than 5 mmol/L. In addition, the treated solution became colored as the buffer
274 concentration increased. The reasons for this coloration are as follows. It has been
275 reported that there are various pathways for the degradation of sugars and byproduct
276 formation (Kabyemela, Adschiri, Malaluan, & Arai, 1999; Peterson, Vogel, Lachance,
277 Fröling, Antal Jr, & Tester, 2008; Richards & Sephton, 1957; Sasaki et al., 1998) (Fig.
278 S2). During the degradation observed in the present study, organic acid formation and
279 other side reactions will have occurred in addition to isomerization. As shown in Fig. 5,
280 a greater proportion of byproducts formed at higher buffer concentrations; naturally,
281 organic acids would also be formed at higher concentrations. These organic acids would
282 autocatalytically promote the formation of HMF, a starting material for the formation of
283 caramel and humin, from ketoses. It is thought that this increased formation of organic
284 acids facilitates the formation of humin (van Zandvoort, van Eck, de Peinder, Heeres,
285 Bruijnincx, & Weckhuysen, 2015; van Zandvoort et al., 2013) and caramel (Baltes, 1985;
286 Yaylayan & Kaminsky, 1998) from HMF. The humin and caramel formed would result in
287 a high degree of coloration, especially at buffer concentrations of 100 mmol/L or higher.
288 The yield of HMF was kept low (<0.01%) within the buffer-concentration range of 0.5–
289 500 mmol/L. This result indicates that HMF would be immediately converted to humin
290 or caramel at higher buffer concentrations.

291 Considering that decolorization of the treated solution is necessary during the

purification of rare sugars, it is desirable to avoid coloration as much as possible. In addition, from the perspective of production costs, the buffer concentration should be low. Based on the above results and the additional background details, a buffer concentration of 10–50 mmol/L would be considered desirable.

In conclusion, sodium phosphate buffer under subcritical water conditions greatly affects the isomerization of galactose and production of rare sugars, depending on the buffer concentration. The formation of tagatose was promoted at low buffer concentrations, but higher concentrations were required to produce talose and sorbose. In addition, byproduct formation was promoted, especially at higher buffer concentrations.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

T. Kobayashi: Conceptualization, Methodology, Writing - Original draft preparation, Supervision; Y. Onishi: Investigation, Data curation; S. Adachi: Validation, Writing - Reviewing & Editing; F. Tani: Validation - Reviewing & Editing.

Acknowledgments

This study was financially supported by JSPS KAKENHI [grant number 21K05469; T. K.].

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

References

Baltes, W. (1985). Application of pyrolytic methods in food chemistry. *Journal of Analytical and Applied Pyrolysis, 8*, 533-545. https://doi.org/10.1016/0165-2370(85)80050-4.

Cheng, S., Metzger, L. E., & Martínez-Monteagudo, S. I. (2020). One-pot synthesis of sweetening syrup from lactose. *Scientific Reports, 10*(1), 2730. https://doi.org/10.1038/s41598-020-59704-x.

Choi, J.-h., & Chung, S.-j. (2015). Sweetness potency and sweetness synergism of sweeteners in milk and coffee systems. *Food Research International, 74*, 168-176. https://doi.org/https://doi.org/10.1016/j.foodres.2015.04.044.

de Bruijn, J. M., Kieboom, A. P. G., & van Bekkum, H. (1986). Alkaline degradation of monosaccharides III. Influence of reaction parameters upon the final product composition. *Recueil des Travaux Chimiques des Pays-Bas, 105*(6), 176-183. https://doi.org/https://doi.org/10.1002/recl.19861050603.

Donner, T. W., Magder, L. S., & Zarbalian, K. (2010). Dietary supplementation with D-tagatose in subjects with type 2 diabetes leads to weight loss and raises high-density lipoprotein cholesterol. *Nutrition Research, 30*(12), 801-806. https://doi.org/https://doi.org/10.1016/j.nutres.2010.09.007.

Drabo, P., & Delidovich, I. (2018). Catalytic isomerization of galactose into tagatose in the presence of bases and Lewis acids. *Catalysis Communications, 107*, 24-28. https://doi.org/10.1016/j.catcom.2018.01.011.

Gao, D.-M., Kobayashi, T., & Adachi, S. (2015). Production of rare sugars from common sugars in subcritical aqueous ethanol. *Food Chemistry, 175*, 465-470.

https://doi.org/10.1016/j.foodchem.2014.11.144.

Guerrero-Wyss, M., Durán Agüero, S., & Angarita Dávila, L. (2018). D-Tagatose is a promising sweetener to control glycaemia: a new functional food. *BioMed Research International, 2018*, 8718053. https://doi.org/10.1155/2018/8718053.

Hirayama, Y., & Kobayashi, T. (2019). Kinetics for the subcritical treatment of glucose solution at various concentrations. *Food Science and Technology Research, 25*(3), 383-389. https://doi.org/10.3136/fstr.25.383.

Hirst, E. L., Hough, L., & Jones, J. K. N. (1949). Composition of the gum of Sterculia setigera : occurrence of D-tagatose in nature. *Nature, 163*(4135), 177-177. https://doi.org/10.1038/163177b0.

Jin, F., Yun, J., Li, G., Kishita, A., Tohji, K., & Enomoto, H. (2008). Hydrothermal conversion of carbohydrate biomass into formic acid at mild temperatures. *Green Chemistry, 10*(6), 612-615. https://doi.org/10.1039/B802076K.

Kabyemela, B. M., Adschiri, T., Malaluan, R. M., & Arai, K. (1999). Glucose and fructose decomposition in subcritical and supercritical water: detailed reaction pathway, mechanisms, and kinetics. *Industrial & Engineering Chemistry Research, 38*(8), 2888-2895. https://doi.org/10.1021/ie9806390.

Kim, P. (2004). Current studies on biological tagatose production using l-arabinose isomerase: a review and future perspective. *Applied Microbiology and Biotechnology, 65*(3), 243-249. https://doi.org/10.1007/s00253-004-1665-8.

Kishida, H., Jin, F., Yan, X., Moriya, T., & Enomoto, H. (2006). Formation of lactic acid from glycolaldehyde by alkaline hydrothermal reaction. *Carbohydrate Research, 341*(15), 2619-2623. https://doi.org/10.1016/j.carres.2006.06.013.

Kruse, A., & Gawlik, A. (2003). Biomass conversion in water at 330– 410 C and 30– 50 MPa. Identification of key compounds for indicating different chemical reaction pathways.

Industrial & Engineering Chemistry Research, 42(2), 267-279. https://doi.org/10.1021/ie0202773.

Lu, Y., Levin, G. V., & Donner, T. W. (2008). Tagatose, a new antidiabetic and obesity control drug. *Diabetes, Obesity and Metabolism, 10*(2), 109-134. https://doi.org/https://doi.org/10.1111/j.1463-1326.2007.00799.x.

Mendoza, M. R., Olano, A., & Villamiel, M. (2005). Chemical indicators of heat treatment in fortified and special milks. *Journal of Agricultural and Food Chemistry, 53*(8), 2995-2999. https://doi.org/10.1021/jf040406l.

Nagasawa, T., Sato, K., & Kasumi, T. (2017). Efficient alkaline isomerization of lactose to lactulose in the presence of an organogermanium compound. *Journal of Applied Glycoscience, 64*(2), 27-32. https://doi.org/10.5458/jag.jag.JAG-2016_018.

Nagata, Y., Mizuta, N., Kanasaki, A., & Tanaka, K. (2018). Rare sugars, d-allulose, d-tagatose and d-sorbose, differently modulate lipid metabolism in rats. *Journal of the Science of Food and Agriculture, 98*(5), 2020-2026. https://doi.org/https://doi.org/10.1002/jsfa.8687.

Oh, D.-K. (2007). Tagatose: properties, applications, and biotechnological processes. *Applied Microbiology and Biotechnology, 76*(1), 1-8. https://doi.org/10.1007/s00253-007-0981-1.

Oku, T., Murata-Takenoshita, Y., Yamazaki, Y., Shimura, F., & Nakamura, S. (2014). D-Sorbose inhibits disaccharidase activity and demonstrates suppressive action on postprandial blood levels of glucose and insulin in the rat. *34*(11), 961-967. https://doi.org/https://doi.org/10.1016/j.nutres.2014.09.009.

Onishi, Y., Adachi, S., Tani, F., & Kobayashi, T. (2022). Insight into formation of various rare sugars in compressed hot phosphate buffer. *The Journal of Supercritical Fluids, 186*, 105621. https://doi.org/10.1016/j.supflu.2022.105621.

392 Onishi, Y., Furushiro, Y., Adachi, S., & Kobayashi, T. (2021). Isomerization and epimerization
393 of galactose to tagatose and talose in a phosphate buffer containing organic solvents
394 under subcritical water conditions. *Industrial & Engineering Chemistry Research, 60*(14), 5084-5089. https://doi.org/10.1021/acs.iecr.1c00682.
396 Onishi, Y., Furushiro, Y., Hirayama, Y., Adachi, S., & Kobayashi, T. (2020). Production of
397 tagatose and talose through isomerization of galactose in a buffer solution under
398 subcritical water conditions. *Carbohydrate Research, 493*, 108031.
399 https://doi.org/10.1016/j.carres.2020.108031.
400 Peterson, A. A., Vogel, F., Lachance, R. P., Fröling, M., Antal Jr, M. J., & Tester, J. W. (2008).
401 Thermochemical biofuel production in hydrothermal media: a review of sub-and
402 supercritical water technologies. *Energy & Environmental Science, 1*(1), 32-65.
403 https://doi.org/10.1039/B810100K.
404 Richards, G., & Sephton, H. (1957). The alkaline degradation of polysaccharides. Part I.
405 Soluble products of the action of sodium hydroxide on cellulose. *Journal of the*
406 *Chemical Society (Resumed)*, 4492-4499.
407 Roy, S., Chikkerur, J., Roy, S. C., Dhali, A., Kolte, A. P., Sridhar, M., & Samanta, A. K. (2018).
408 Tagatose as a potential nutraceutical: Production, properties, biological roles, and
409 applications. *Journal of Food Science, 83*(11), 2699-2709. https://doi.org/10.1111/1750-
410 3841.14358.
411 Salonen, N., Salonen, K., Leisola, M., & Nyyssölä, A. (2013). D-Tagatose production in the
412 presence of borate by resting Lactococcus lactis cells harboring Bifidobacterium
413 longuml-arabinose isomerase. *Bioprocess and Biosystems Engineering, 36*(4), 489-
414 497. https://doi.org/10.1007/s00449-012-0805-2.
415 Sasaki, M., Kabyemela, B., Malaluan, R., Hirose, S., Takeda, N., Adschiri, T., & Arai, K.
416 (1998). Cellulose hydrolysis in subcritical and supercritical water. *The Journal of*

Supercritical Fluids, 13(1), 261-268.

Sınağ, A., Gülbay, S., Uskan, B., & Canel, M. (2010). Biomass decomposition in near critical water. *Energy Conversion and Management, 51*(3), 612-620. https://doi.org/10.1016/j.enconman.2009.11.009.

Torrico, D. D., Tam, J., Fuentes, S., Gonzalez Viejo, C., & Dunshea, F. R. (2019). D-Tagatose as a sucrose substitute and its effect on the physico-chemical properties and acceptability of strawberry-flavored yogurt. *Foods, 8*(7), 256. https://doi.org/10.3390/foods8070256.

Usuki, C., Kimura, Y., & Adachi, S. (2007). Isomerization of hexoses in subcritical water. *Food Science and Technology Research, 13*(3), 205-209. https://doi.org/10.3136/fstr.13.205.

van Zandvoort, I., van Eck, E. R. H., de Peinder, P., Heeres, H. J., Bruijnincx, P. C. A., & Weckhuysen, B. M. (2015). Full, reactive solubilization of humin byproducts by alkaline treatment and characterization of the alkali-treated humins formed. *ACS Sustainable Chemistry & Engineering, 3*(3), 533-543. https://doi.org/10.1021/sc500772w.

van Zandvoort, I., Wang, Y., Rasrendra, C. B., van Eck, E. R. H., Bruijnincx, P. C. A., Heeres, H. J., & Weckhuysen, B. M. (2013). Formation, molecular structure, and morphology of humins in biomass conversion: Influence of feedstock and processing conditions. *ChemSusChem, 6*(9), 1745-1758. https://doi.org/10.1002/cssc.201300332.

Xiao, H., Wang, G., Wang, P., & Li, Y. (2010). Convenient Synthesis of D-Talose from D-Galactose. *Chinese Journal of Chemistry, 28*(7), 1229-1232. https://doi.org/https://doi.org/10.1002/cjoc.201090213.

Yaylayan, V. A., & Kaminsky, E. (1998). Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system. *Food Chemistry, 63*(1), 25-31. https://doi.org/10.1016/S0308-8146(97)00237-9.

442 Zhang, W., Zhang, T., Jiang, B., & Mu, W. (2017). Enzymatic approaches to rare sugar
443 production. *Biotechnology Advances,* *35*(2), 267-274.
444 https://doi.org/10.1016/j.biotechadv.2017.01.004.
445

Figure captions

Fig. 1. Effects of phosphate buffer concentration on the time courses of (a) decrease in galactose, (b) formation of tagatose, (c) formation of talose, and (d) formation of sorbose, under subcritical water conditions at 140°C.

Fig. 2. Effects of buffer concentration on the fraction of remaining galactose, yield of tagatose, yield of talose, and yield of sorbose at 300 s. The "//" in the figure represents the omission of a concentration range.

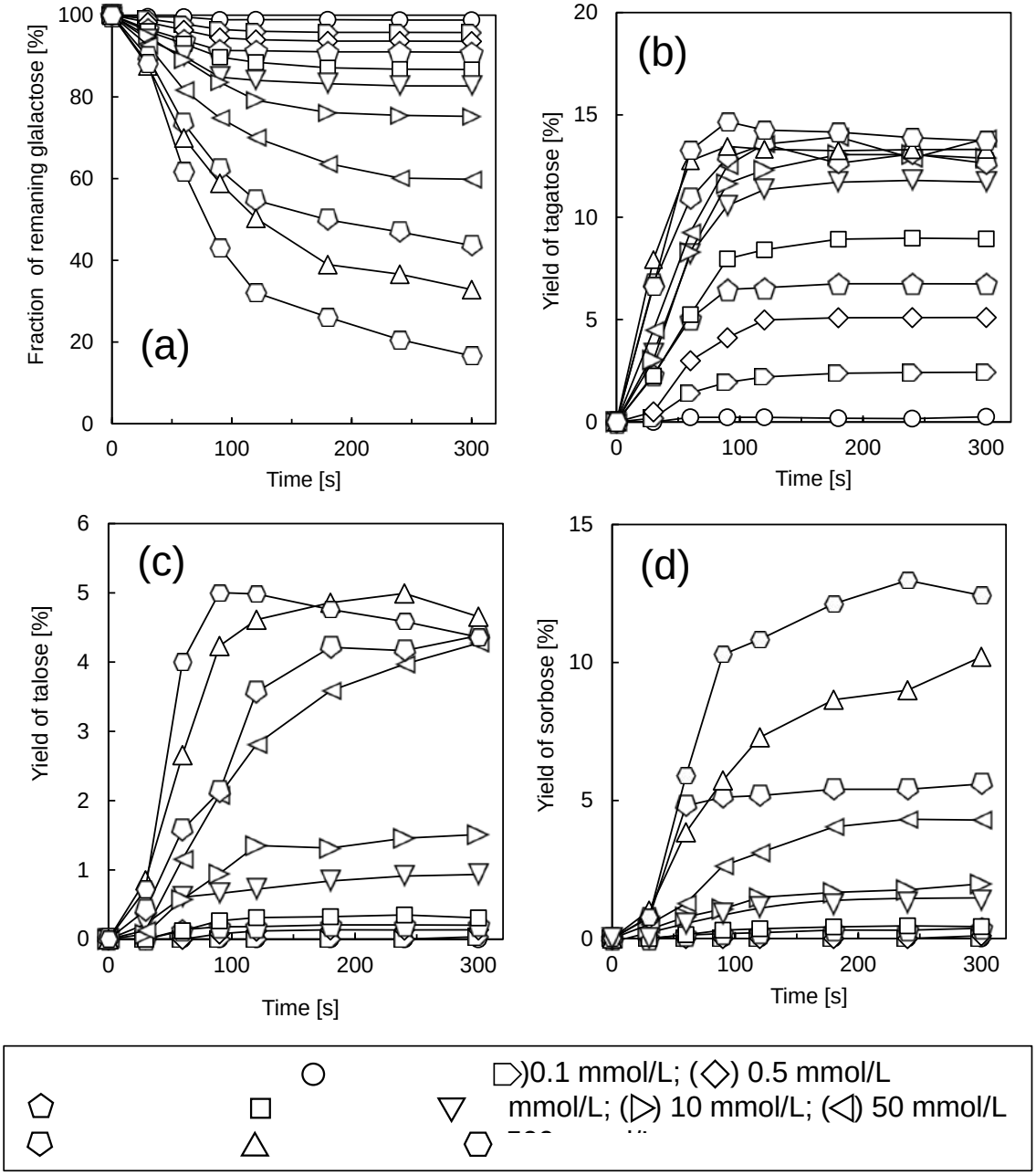
Fig. 3. Effect of buffer concentration on the reaction rate of galactose degradation, tagatose formation, talose formation, and sorbose formation. The "//" in the figure represents the abbreviated concentration range.

Fig. 4. Relationship between the conversion of galactose and yield of rare sugars. Open, gray, and closed symbols represent tagatose, talose, and sorbose, respectively. The dashed line is the set of points corresponding to 100% selectivity.

Fig. 5. Effect of buffer concentration on the composition of treated solutions at the reaction time of 300 s.

Fig. 6. Effects of buffer concentration on the pH (○) and absorbance at 440 nm (Δ), yield of HMF (□) of the treated solutions at the reaction time of 300 s. The "//" in the figure represents the abbreviated concentration range.

1



2

3 Fig. 1. Effects of phosphate buffer concentration on the time courses of (a) decrease in
4 galactose, (b) formation of tagatose, (c) formation of talose, and (d) formation of sorbose,
5 under subcritical water conditions at 140°C.

6

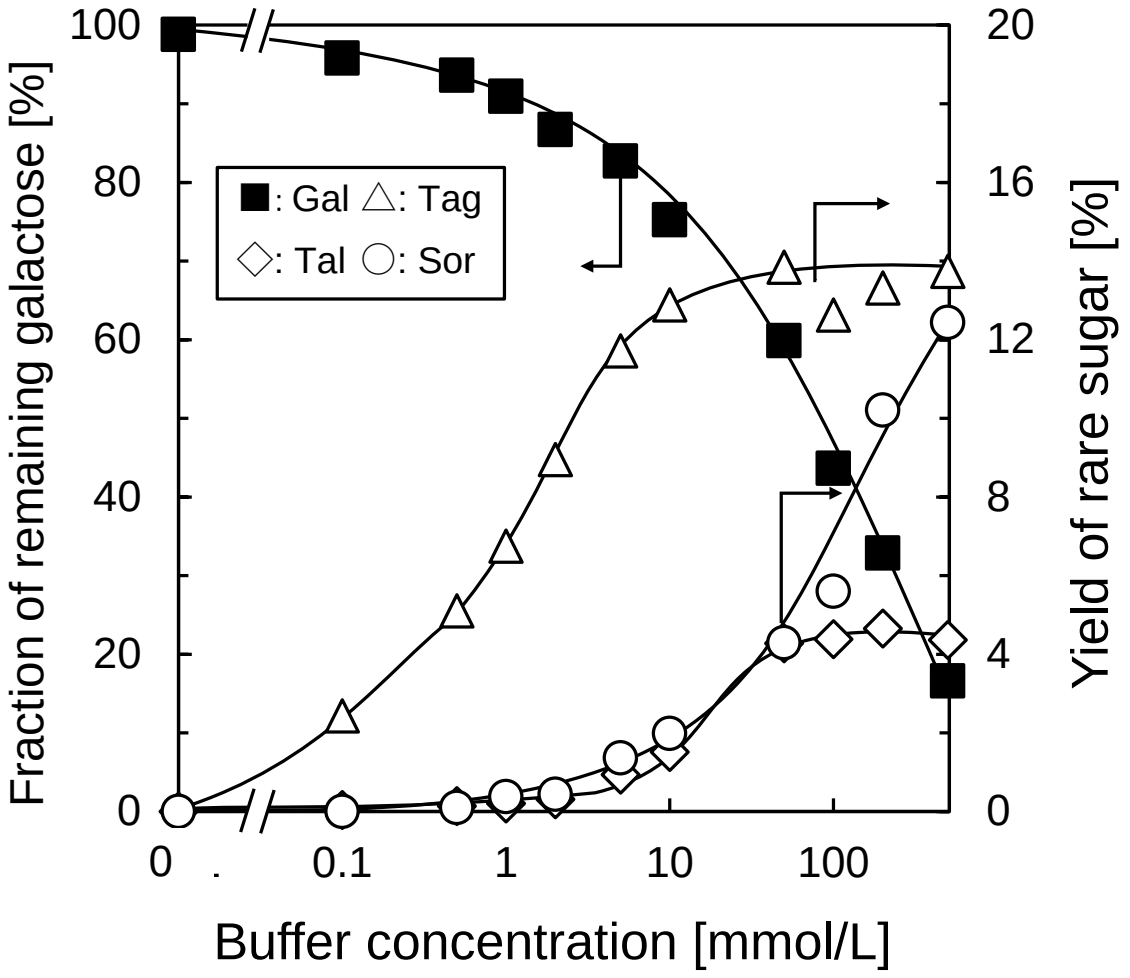


Fig. 2. Effects of buffer concentration on the fraction of remaining galactose, yield of tagatose, yield of talose, and yield of sorbose at 300 s. The "//" in the figure represents the omission of a concentration range.

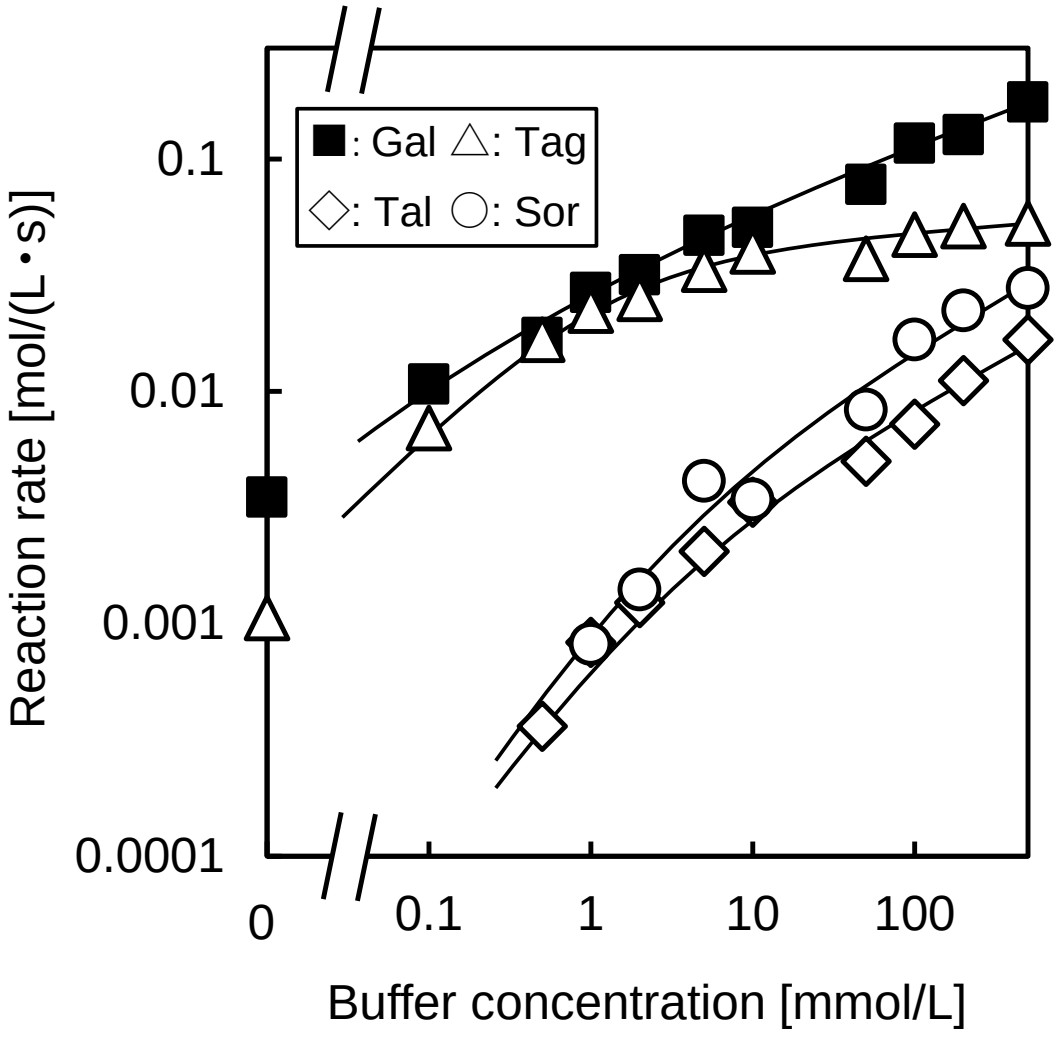


Fig. 3. Effect of buffer concentration on the reaction rate of galactose degradation, tagatose formation, talose formation, and sorbose formation. The "//" in the figure represents the abbreviated concentration range.

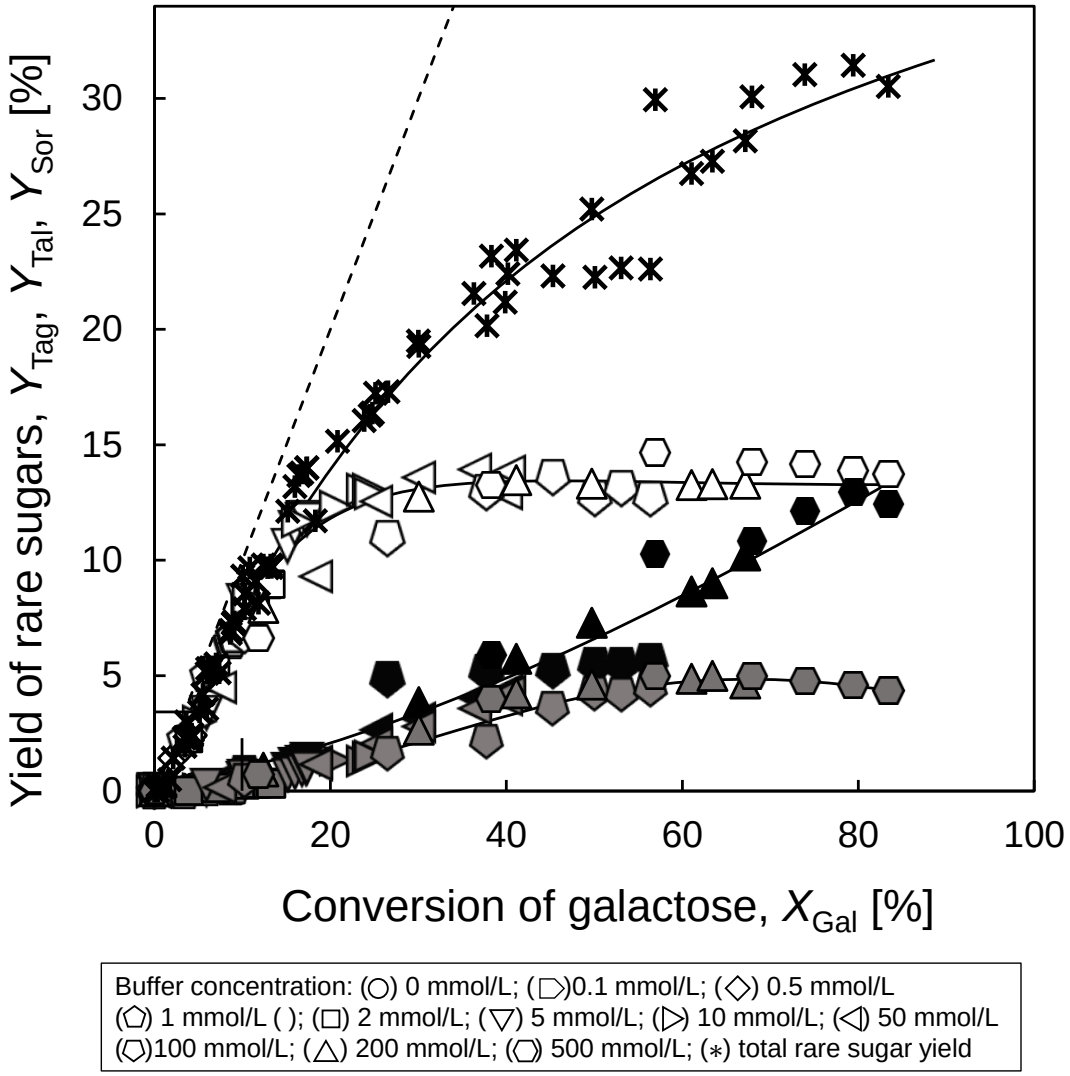


Fig. 4. Relationship between the conversion of galactose and yield of rare sugars. Open, gray, and closed symbols represent tagatose, talose, and sorbose, respectively. The dashed line is the set of points corresponding to 100% selectivity.

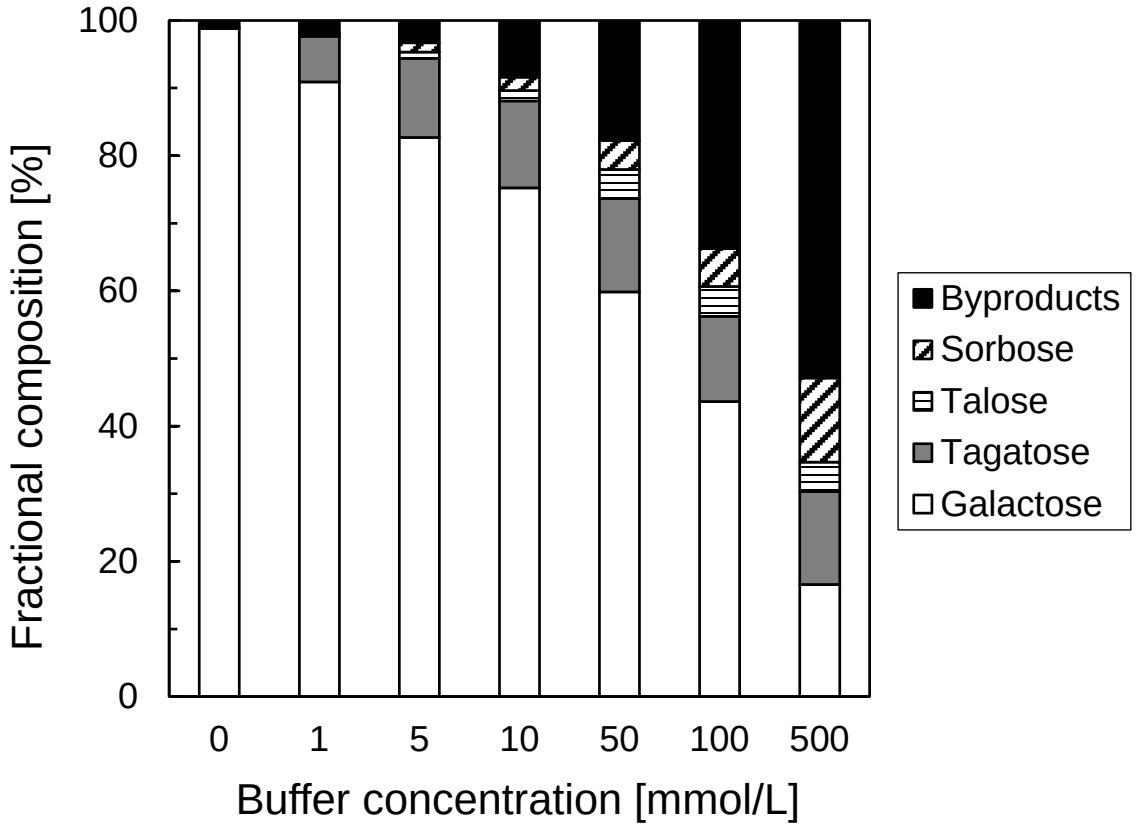


Fig. 5. Effect of buffer concentration on the composition of treated solutions at the reaction time of 300 s.

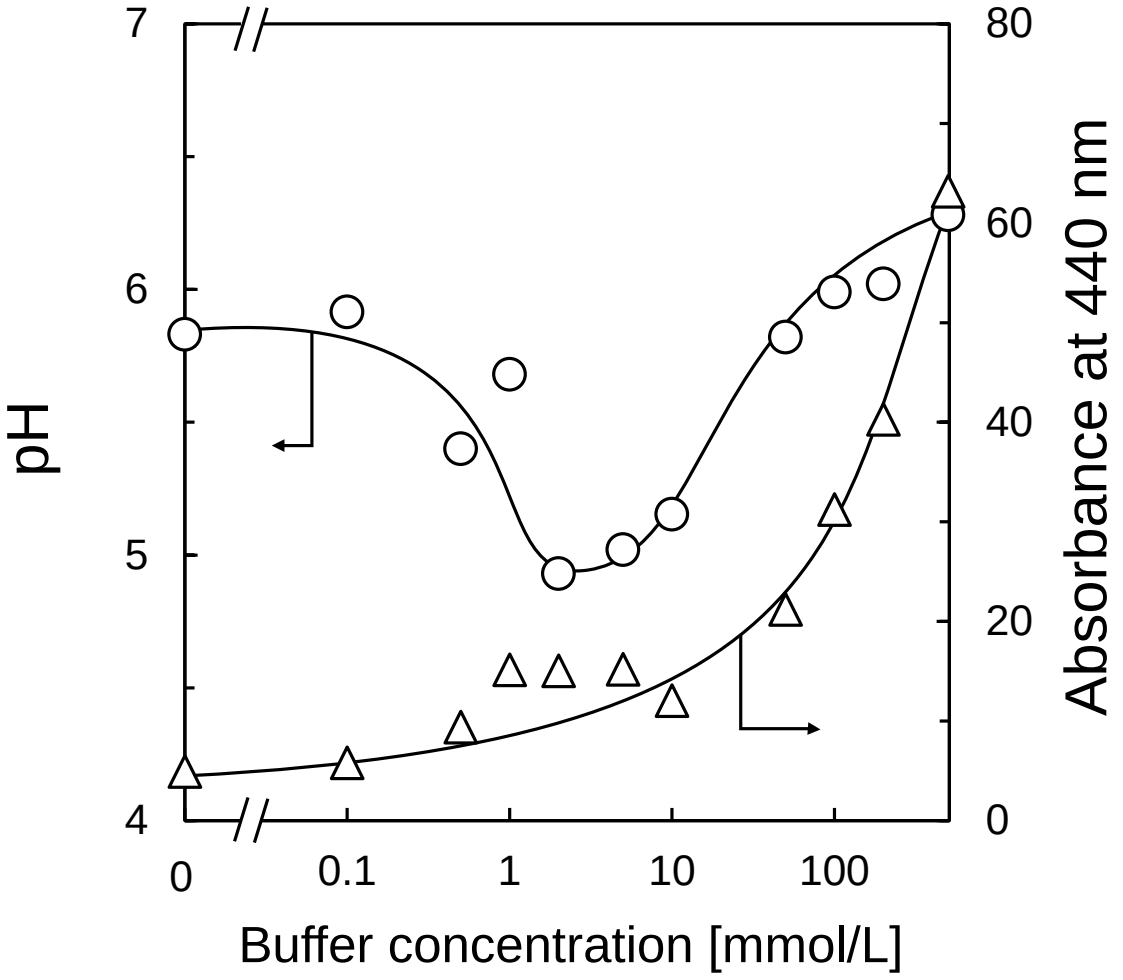


Fig. 6. Effects of buffer concentration on the pH (○) and absorbance at 440 nm (Δ) of the treated solutions at the reaction time of 300 s. The "//" in the figure represents the abbreviated concentration range.

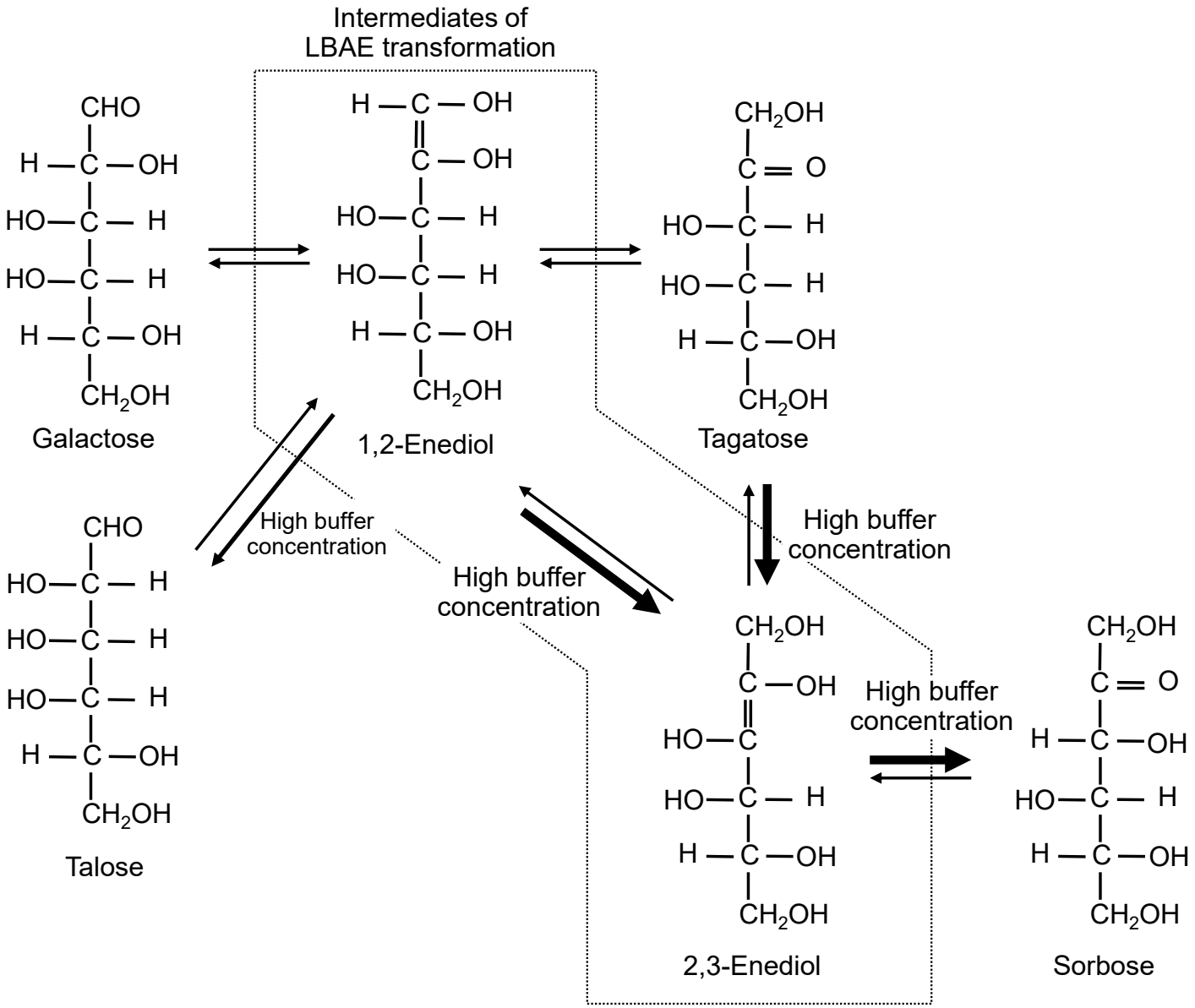


Fig. S1. Pathways for the isomerization of galactose to rare sugars

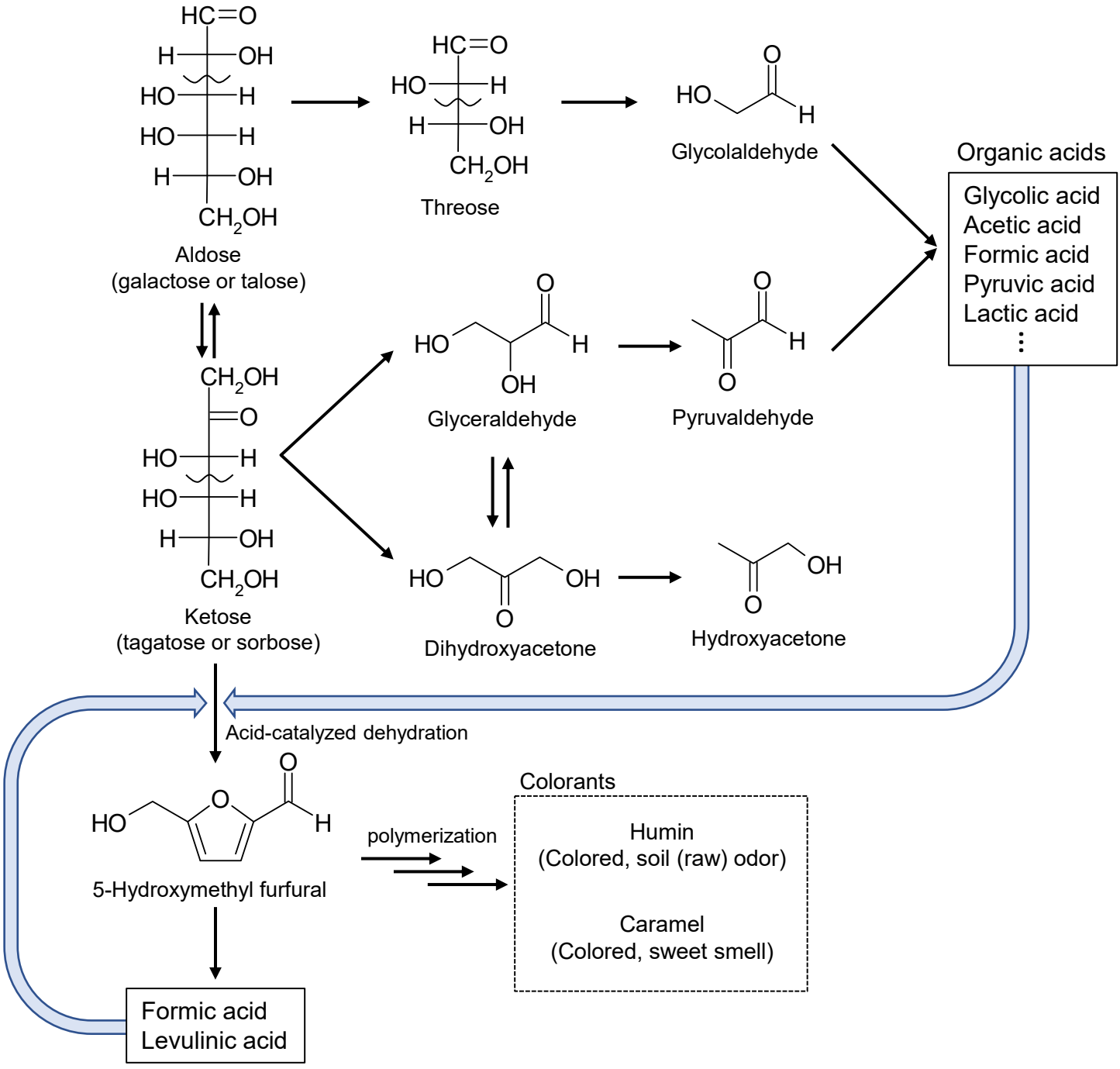


Fig. S2. Promotion of the formation of byproducts by autocatalytic reaction