Production of tagatose and talose through isomerization of galactose in a buffer solution under subcritical water conditions

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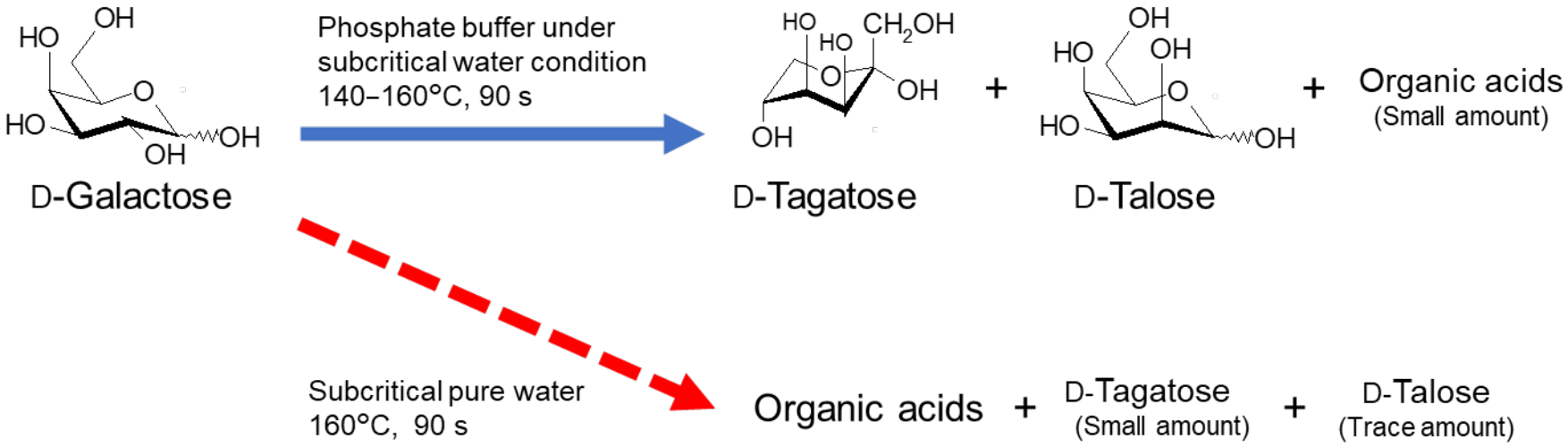
Graphical abstract



Highlights

- Galactose isomerized efficiently in a subcritical buffer solution.
- Tagatose and talose were obtained in high yield.
- Type of the buffer affected the isomerization.
- pH of the solution affected the yield of rare sugars.

Abstract

Galactose was isomerized in pure water or in 10 mmol/L sodium phosphate buffer at 160°C under pressurized conditions. The isomerization of galactose to tagatose and talose in phosphate buffer resulted in 14% and 1.4% yields, respectively, which were significantly higher than those obtained in subcritical pure water (0.6% and <0.1%, respectively). The effect of the temperature on isomerization was examined between 100 and 160°C. The most remarkable isomerization was observed at 120°C or higher. The effect of the buffer solution type was also examined. The pH drop of the treated solution was lesser in MOPS and PIPES buffers than in the phosphate buffer; however, the isomerization was less likely to occur in MOPS and PIPES buffers. The relationship between the pH drop during the reaction in phosphate buffer and the yields of tagatose and talose revealed that the isomerization proceeded only when the pH was >6.3. Our results indicate that the galactose isomerization by Lobry de Bruyn-Alberda-van Ekenstein transformation rarely occurred at low pH due to the formation of organic acids.

Keywords: buffer; galactose; isomerization; rare sugar; subcritical water

1. Introduction

Rare sugars are saccharides that are present in very small quantities in nature. Some of them are low in calories and have a sweet taste [1-3]. In addition, various beneficial physiological functions have been reported for rare sugars, such as anti-tumor [4, 5], anti-inflammatory [4, 6], anti-hypertensive effects [4]. Moreover, rare sugars are reported to prevent obesity [5] and diabetes [1, 7]. Therefore, they have attracted much attention as functional food ingredients. However, practical methods for the mass production of most rare sugars have not yet been developed. Several studies have been reported to produce rare sugars, using enzymatic methods [1, 3-5], alkaline isomerization [8-10], and isomerization using subcritical fluids [11-15].

Subcritical water is the water that maintains liquid state at 100°C or higher under pressurized conditions. Reducing monosaccharides are known to isomerize to other sugars in subcritical water [11]. When heated under high pressure, aqueous alcohols also become subcritical state. It has been reported that high yields of rare sugars can be obtained from common reducing sugars in subcritical aqueous alcohol than in subcritical water [12-15]. For example, galactose isomerizes to rare sugars of tagatose and talose [12]. Isomerization by subcritical aqueous alcohol treatment is simple and can shorten the reaction time. However, concentrations of the rare sugars cannot be increased by the subcritical aqueous alcohol treatment due to the limited solubility of galactose in an aqueous alcohol [16]. For example, tagatose and talose were produced at the concentration of 11 g/L and 2.6 g/L at most, respectively, in aqueous ethanol [12]. In addition, alcohols are flammable and expensive for industrial production. Therefore, to produce rare sugars practically, there is a need to develop efficient methods using subcritical water that can dissolve sugars at high concentrations.

However, the isomerization yield of sugars was reportedly low even in subcritical

water, since most of the sugars is mainly decomposed [11]. When sugars decompose, release of some organic acids during the reaction leads to the pH drop [17-21]. It is expected that a buffer solution may counter the pH drop and improve yields of rare sugars. Therefore, in this study, rare sugars were synthesized using galactose as an inexpensive raw material, and by treating it in a buffer solution under subcritical water conditions.

2. Results and Discussion

2.1. Isomerization in the subcritical buffer solution

The isomerization of galactose to tagatose and talose in a sodium phosphate buffer was investigated under pressurized conditions. The concentration of the buffer was adjusted at lower concentration (10 mmol/L, pH 7.0) because the downstream process (industrial separation and purification of rare sugars) will be simplified, contributing the low-cost production of rare sugars. When galactose (5 wt%) was treated with phosphate buffer at 160°C, the conversion of galactose was significantly increased compared to the treatment with subcritical pure water (Fig. 1). Some time courses for the formation of tagatose showed downward convex at 30 s, showing the effect of heat transfer between the heating medium (silicone oil) and reaction mixture. The effect became, however, negligible at treatment times longer than 60 s. The concentration of galactose in the buffer solution decreased by ca. 25% within 60 s. In contrast, when the treatment time was extended, no further decrease was observed in the galactose content. After 60 s, 14% tagatose and 1.4% talose were formed by the isomerization of galactose, and no further increase in tagatose yield was observed even after extending the treatment time to 120 s. In fact, further prolonging the treatment caused a slight decrease in the tagatose yield although the yield of talose (ca. 1.6%) remained unchanged.

In contrast, the tagatose yield was only 0.6% in subcritical pure water at 60 s, and

prolonged treatment did not increase the efficiency of its formation. After the subcritical pure water treatment, talose was present only in trace amounts (yield <0.1%). These results suggest that the isomerization of galactose to the rare sugars is enhanced in the phosphate buffer even at low concentrations.

2.2. Effects of temperature on the yield and selectivity of rare sugars

The yield of tagatose gradually decreased at treatment times longer than 120 s at 160°C in phosphate buffer (Fig. 1). This decrease could be ascribed to the gradual thermal decomposition of tagatose at high temperatures. Therefore, the reaction temperature in the phosphate buffer was lowered. Hence, the isomerization of galactose was evaluated in the range of 100–160°C. Our results showed that the progress of the reaction was significantly low at 120°C or lower, and more than 90% of galactose remained in solution even after 300 s or longer treatment. The remaining fraction of galactose gradually decreased at 140°C; it reached about 75% after 180 s but did not decrease afterwards. The yields of tagatose and talose at 140°C after 180 s were 13% and 1.3%, respectively, which were comparable to those obtained after the 60-second treatment at 160°C.

The yield of the rare sugars and isomerization selectivity were evaluated at 90 s (Fig. 2). The selectivity was defined as the ratio of the amount of the formed rare sugar to the amount of the consumed galactose. At temperatures below 120°C, the yield of tagatose was low (<1.0%), but at temperatures between 120 to 150°C, the yield sharply increased to about 13%. At temperatures higher than 150°C, the change in yield was insignificant.

The selectivity of galactose isomerization to tagatose was rather low below 110°C. When the treatment temperature was increased to 140°C, the selectivity increased to about 70%, but did not increase further with the increase in the temperature. This could be due to the decomposition of tagatose at higher temperatures.

In contrast, both, the yield and selectivity of galactose isomerization to talose, gradually increased between 130 and 145°C and remained almost constant above 145°C. We observed a difference in the temperature dependence between isomerization to tagatose and talose. This could reflect the difference in the activation energy for the isomerization between these two saccharides. Based on these results, subsequent studies were performed at 140°C, unless otherwise specified.

It was reported that yields of tagatose and talose obtained by subcritical aqueous-ethanol treatment were 14% and 3%, respectively, with tagatose selectivity of 58%, and were almost comparable to results in this study [12]. However, the previous method required the treatment temperature of 180°C and time of 500 s. On the other hand, the 140°C treatment gave almost the same yield within 120 s in phosphate buffer. In addition, the solubility of galactose in 60% (v/v) ethanol in water is ca. 10 wt%. On the other hand, the solubility in water is ca. 32 wt% [22]. Therefore, to improve the productivity (concentration) of rare sugars, it is essential to use water as the reaction medium.

Delidovicha et al. reported the efficient combination of conversion of glucose to fructose in a phosphate buffer (0.3–0.7 mol/L, initial pH 7.3–8.5) and its extractive recovery using 1-octanol and *o*-hydroxymethyl phenylboronic acids (final yield = 51%), indicating the effectiveness of phosphate [23]. Meanwhile, although the yield was lower, isomerization proceeded even at neutral pH of 7.0 in a phosphate buffer with much lower concentration (10 mmol/L) without any other additives in this study.

2.3. Effect of buffer type on the isomerization

The use of other neutral pH buffers was investigated to further improve the yield of the rare sugars. We investigated PIPES (piperazine-*N*,*N*'-bis(2-ethanesulfonic acid), pK_a=6.80) and MOPS (3-(*N*-morpholino)propanesulfonic acid, pK_a=7.20) buffers (10

144 mmol/L) at 140°C because they have the pK_a values of ca. 7 and would further suppress
145 the side reactions and improve the selectivity of rare sugars (Fig. 3). However, compared
146 with the phosphate buffer, PIPES and MOPS buffers suppressed the isomerization of
147 galactose; moreover the suppression was more remarkable for the MOPS buffer. The
148 yields of tagatose and talose at 300 s in PIPES buffer were 9.6% and 0.6%, respectively,
149 which were lower than observed in the phosphate buffer. The yields of the rare sugars
150 were even lower in MOPS (yield of tagatose = 3.6% at 300 s; yield of talose = 0.3% at
151 300 s), although they were higher than by the subcritical pure water treatment. Therefore,
152 it was suggested that the type of buffer influenced the efficiency of galactose conversion
153 to rare sugars. Although the role of the buffer solution on the reaction mechanism is not
154 clear, our results show that the presence of the buffer solution even at low concentrations
155 affects the efficiency of galactose isomerization.
156
157 **2.4. Changes in pH during the treatment**
158 Figure 4 shows the pH change during the treatment under subcritical water conditions.
159 When galactose was treated with subcritical pure water at 160°C, the pH dropped sharply
160 at the initial period of the reaction (0–60 s, pH 4.7 at 60 s), while after 60 s, the drop in
161 pH was less rapid. This could be explained by the immediate formation of organic acids
162 by galactose decomposition. The buffering capacity of phosphate buffer was limited at
163 160°C, i.e. the drop in pH was comparable to that in the pure water treatment, indicating
164 that the buffer solution was too dilute to buffer optimally. We speculate that organic acids
165 formed even in the presence of the buffer, thereby resulting in the loss of the buffering
166 capacity.
167 We tried to lower the reaction temperature to suppress the formation of organic acids.
168 Our results showed that the buffering capacity was maintained at 140°C during the first

60 s (pH was maintained in the range from 6.7 to 6.8 during the first 60 s); however, the formation of organic acids resulted in a pH drop during prolonged treatments. At 300 s or longer, the pH dropped to 5.2, and the buffering capacity was completely lost. The magnitude of pH drop was, however, smaller than observed at the 160°C treatment. Further, the decrease of pH was much lesser in the PIPES and MOPS buffers at 140°C, and at lower temperatures in the phosphate buffer (pH > 6.5 at 300 s). Moreover, under these conditions, the decrease in galactose concentration was also comparatively small (Fig. 1 and Fig. 3). Collectively, these results show that suppression of organic acids formation facilitated the maintenance of the buffering capacity.

As discussed above, the pH change was negligible in PIPES or MOPS buffer. In contrast, the phosphate buffer showed almost no buffering capacity. Nevertheless, the yields of the rare sugars were lower in the PIPES and MOPS buffers than in the phosphate buffer. These results demonstrate that the phosphate buffer not only acts as a weak buffering solution, but also as a catalyst. Therefore, unless otherwise noted, subsequent studies were performed in the phosphate buffer.

2.5. Relationship between pH of the treated solution and the yields of the rare sugars

Formation of the rare sugars in the phosphate buffer proceeded rapidly at the early stage of the reaction, and continued at a decreased rate in the latter half of the treatment (Fig. 1). This was presumably due to the pH drop caused by the loss of buffering capacity. Therefore, we next focused on elucidating the relationship between the degree of reaction progress and pH.

In the phosphate buffer, the relationship between the remaining fraction of galactose and pH could be expressed by a single curve independent of the temperature (Fig. 5, Fig. S1). The reaction started at pH ~7. The fraction of galactose and pH decreased with the

reaction progress, while the yield of the rare sugars increased. The tagatose yield increased sharply and reached about 10% until the pH dropped from 7.0 to approximately 6.5. However, with the further drop in pH, the yield of tagatose was slowed down, and it stabilized when pH dropped below 6.2. The formation of talose showed a similar trend, although the talose yield did not exceed 1.5%. In contrast, the remaining fraction of galactose decreased slightly even at the pH below 6.2. This indicates that galactose could be directly decomposed into organic acids, or that its isomerization to the rare sugars and the formation of organic acids by decomposition of the produced rare sugars were balanced.

As described above, the relationship between the remaining fraction of galactose or the yields of the rare sugars and the pH of the reaction mixture, did not depend on the temperature. These results suggest that pH drop, due to the formation of organic acids, rather than the temperature of the treatment was the crucial factor in determining the overall reaction efficiency. This also indicates that the isomerization of galactose rarely occurred if organic acids were present at a certain level. It has been proposed that the isomerization in subcritical water occurs through Lobry de Bruyn-Alberda-van Ekenstein (LBAE) transformation due to the formation of hydroxide ions [8, 9, 20, 24, 25]. In this study, the fact that the isomerization was suppressed by the acidity of the phosphate buffer clearly indicates that the isomerization occurred through LBAE transformation, and that LBAE transformation occurred even in neutral or weakly acidic pH range (6.3–7.0). This could be due to the high ion product and increase in hydroxide ion concentration under the subcritical state of the buffer solution, which showed a slightly acidic pH of 6.3 at room temperature. Taken together, these results indicate that maintaining a pH above 6.3 in phosphate buffer is effective for the production of rare sugars.

In contrast, in subcritical pure water, a slight decrease in the galactose concentration

(conversion <3%) caused a sharp drop in pH, and the yields of tagatose and talose remained very low. These results differed from those obtained in the phosphate buffer treatment, suggesting that the treatment of galactose with subcritical pure water results mainly in the decomposition of sugars into organic acids, rather than isomerization.

3. Conclusion

Our results showed that when galactose was treated with a buffer solution under subcritical water conditions, tagatose and talose were produced through isomerization at higher yields than under a treatment with subcritical pure water. The isomerization of galactose in sodium phosphate buffer at 140°C resulted in high yields of tagatose and talose (13% and 1.3%, respectively), although the buffering capacity of the solution was low. On the other hand, when MOPS and PIPES buffers were used, the yields of the rare sugars were not so high in spite of the lower pH drop in comparison to the pH drop in the phosphate buffer. Therefore, the subcritical phosphate buffer treatment was effective for the efficient production of the rare sugars. Moreover, maintaining the pH of the reaction mixture at 6.3 or higher was essential for the isomerization in our study.

4. Experimental

4.1. Materials

D-Galactose, D-tagatose, and D-talose were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). Other chemicals (PIPES, MOPS, sodium hydroxide, and acetonitrile) were purchased from FUJIFILM Wako, Dojindo (Kumamoto, Japan) or Nacalai Tesque (Kyoto, Japan).

4.2. Treatment of galactose with a buffer solution under subcritical water conditions

Briefly, galactose was dissolved at a concentration of 5 wt% in 10 mmol/L sodium phosphate buffer, PIPES buffer, or MOPS buffer (pH 7.0) to prepare the starting mixture. PIPES buffer was prepared as follows. PIPES (3.02 g, 10 mmol) was dissolved in 1 L of 10 mmol/L of sodium hydroxide aqueous solution. To the solution, was added 10 mmol/L sodium hydroxide solution to adjust pH at 7.0. MOPS buffer (pH 7.0) was prepared by mixing 10 mmol/L MOPS aqueous solution and 10 mmol/L sodium hydroxide solution. Nitrogen was then sufficiently blown into the starting mixture to remove dissolved oxygen, and its reservoir was connected to a nitrogen gas bag to prevent redissolution of oxygen.

The treatment of galactose was performed using a tubular reactor (reactor volume = 1.9 mL), which was similar to those reported in previous studies [26, 27]. The reactor consisted of a stainless-reinforced PEEK tube (0.8 mm I.D.) immersed in a silicone oil bath and back-pressure regulator (P-880, Upchurch Scientific, Oak Harbor, WA, USA). The temperature of the reactor varied between 100 and 160°C. The starting mixture was delivered into the reactor using HPLC pump (LC-10ADVP, Shimadzu, Kyoto, Japan). The flow rate was adjusted to provide a residence time (reaction time) of 30–300 s. The pressure inside the reactor was maintained at approximately 5 MPa. The outlet side of the reactor was immersed in a water bath to terminate the reaction. The effluent coming out of the reactor was collected and analyzed by HPLC. All experiments were done in triplicate.

4.3. Analysis

The contents of the effluent were analyzed by HPLC. Galactose, tagatose, and talose were quantified by an HPLC system equipped with the RID-20A refractive index detector (Shimadzu) and the LC-20AD HPLC pump (Shimadzu) connected to the COSMOSIL

Sugar-D column (3 mm I.D. × 250 mm, Nacalai Tesque). The eluent was 80% acetonitrile (v/v) at a flow rate of 0.4 mL/min. The products (rare sugars) were confirmed by comparing the retention times of the present study and previous report [12].

pH of the effluent was measured using a D-71 pH meter (HORIBA, Kyoto, Japan) by dipping a pH electrode (9680-10D, HORIBA) into ca. 1 mL of the effluent.

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Figure legends

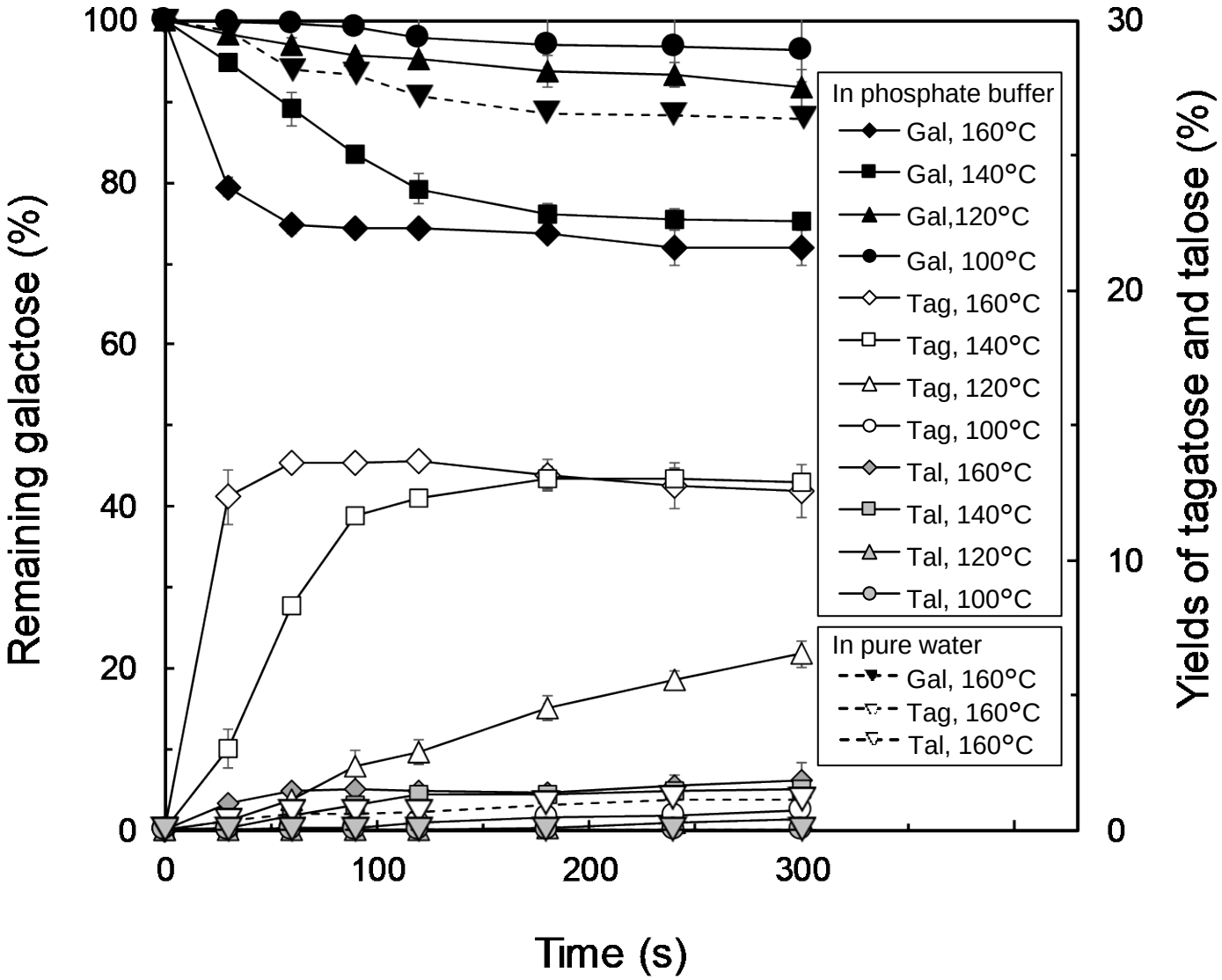
Fig. 1. Time courses for the conversion of galactose into tagatose and talose in the phosphate buffer or in pure water under subcritical water conditions.

Fig. 2. Dependence of the yield of the rare sugars and isomerization selectivity on the treatment temperature at 90 s.

Fig. 3. Time courses for the isomerization of galactose in various buffers at 140°C.

Fig. 4. Change in pH of the treated solution obtained at 100–160°C.

Fig. 5. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 100–160°C.



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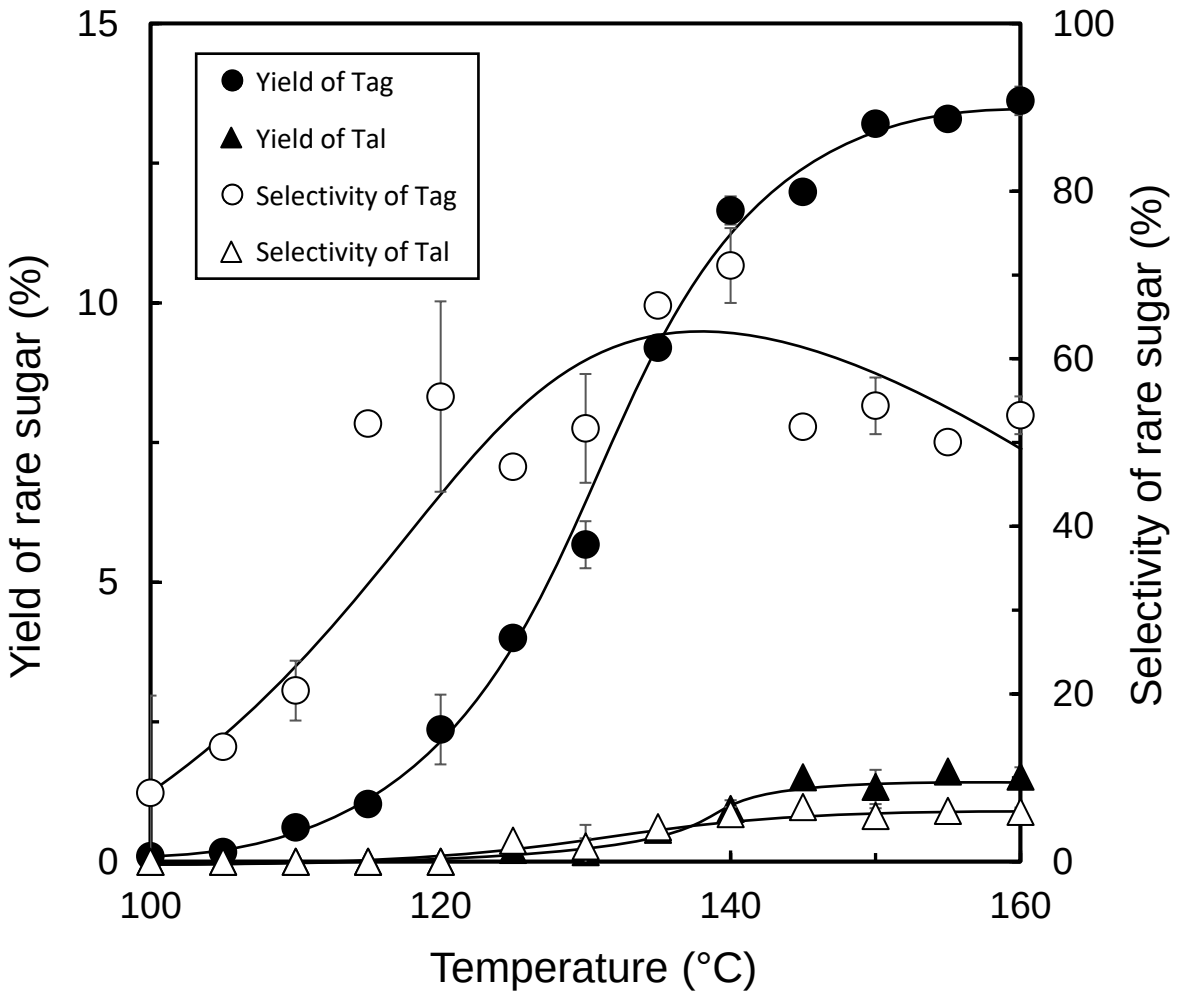

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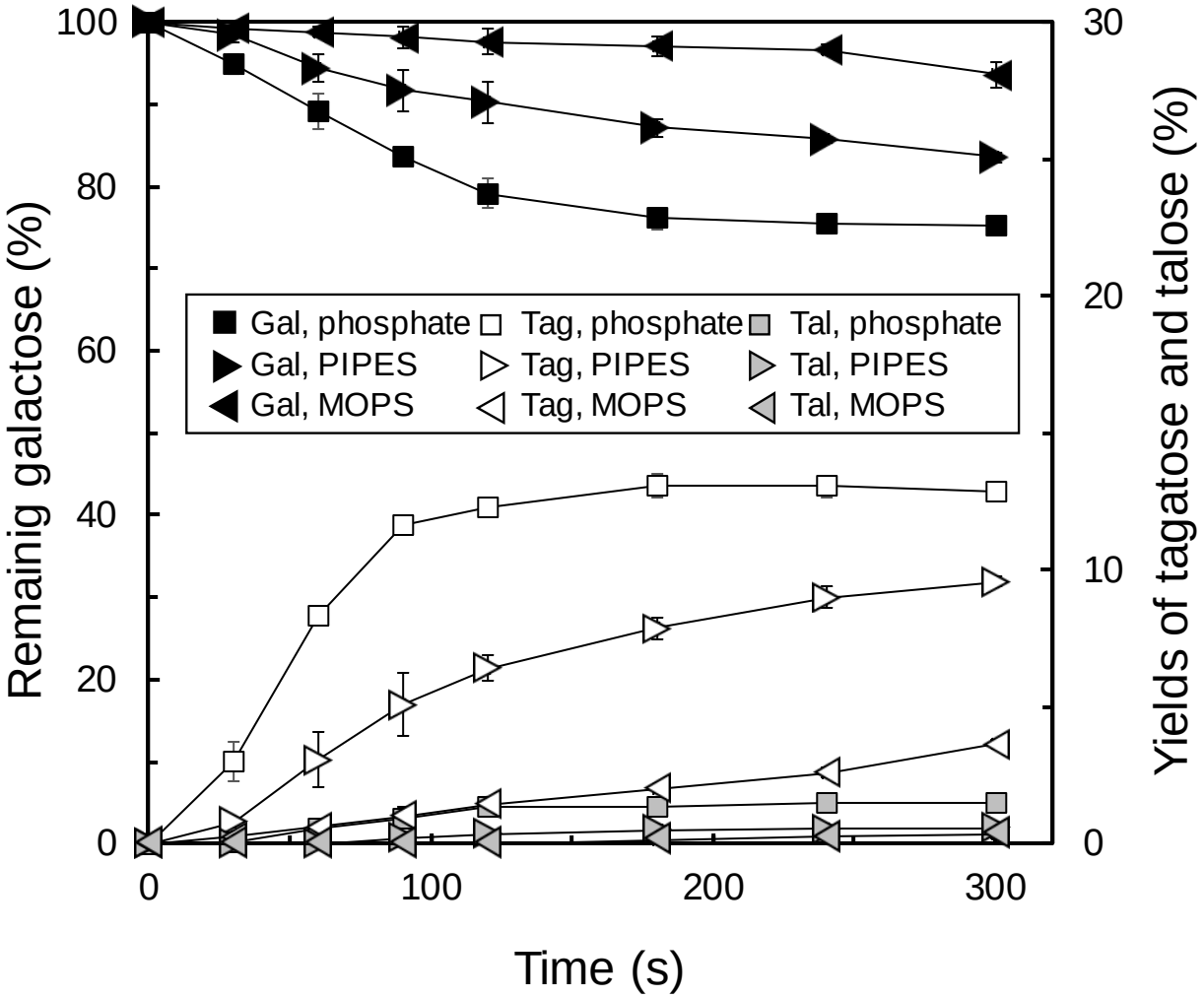

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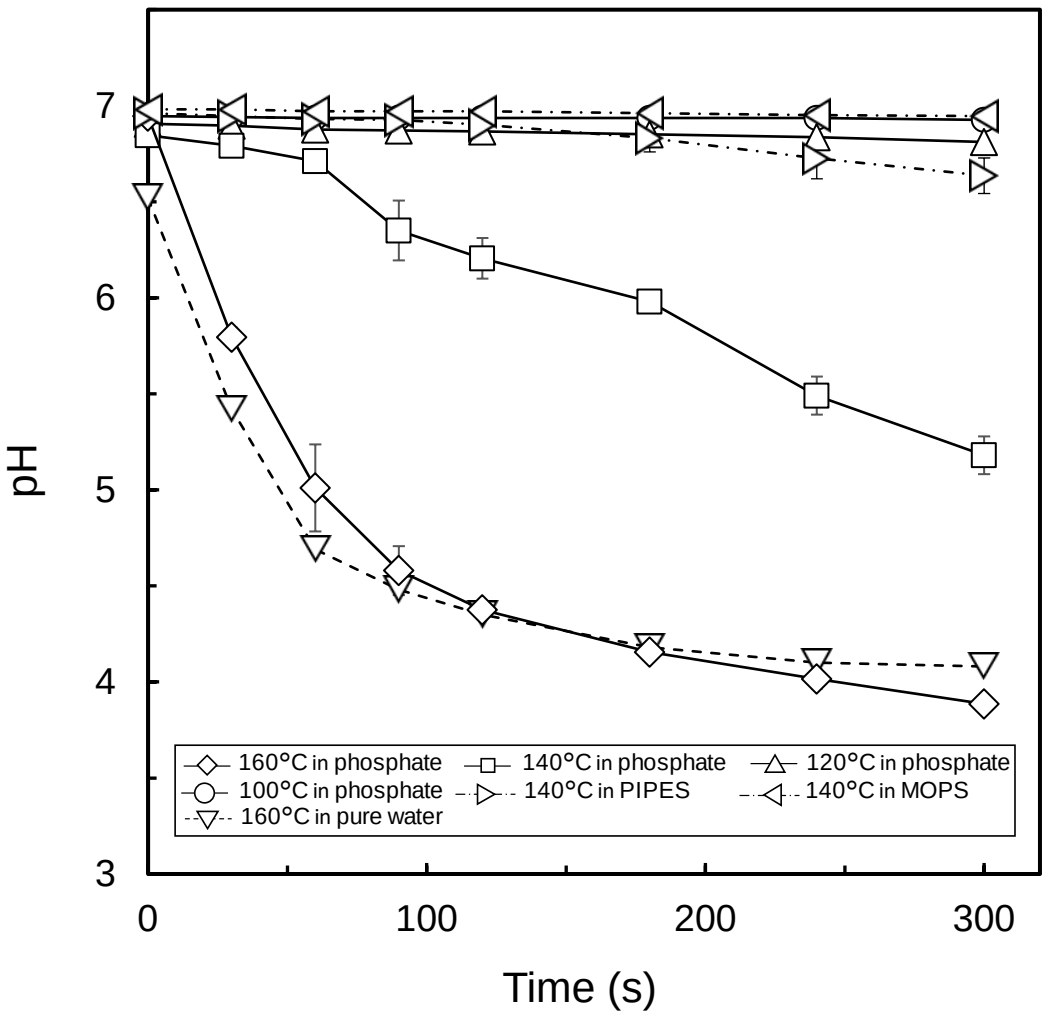

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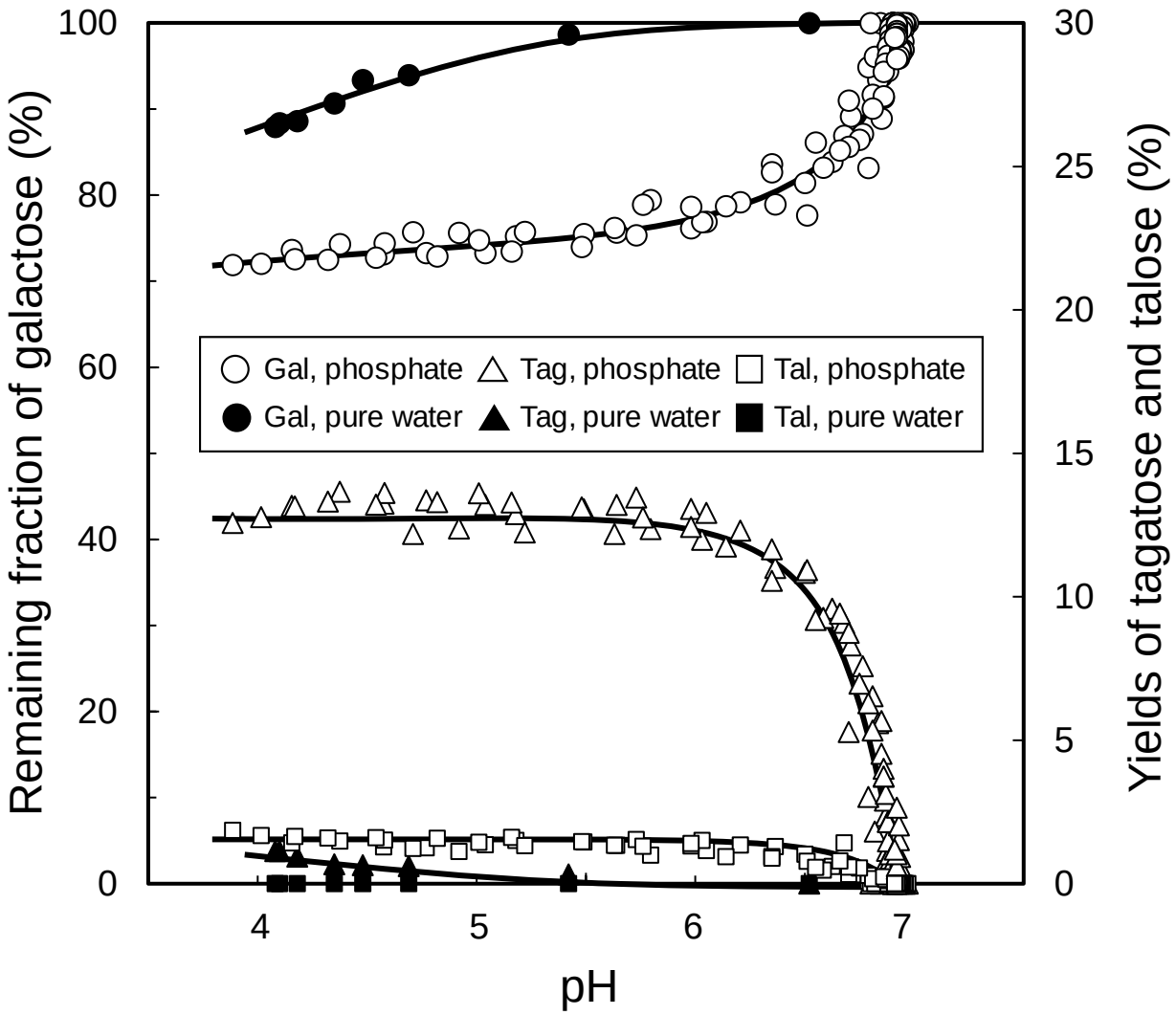

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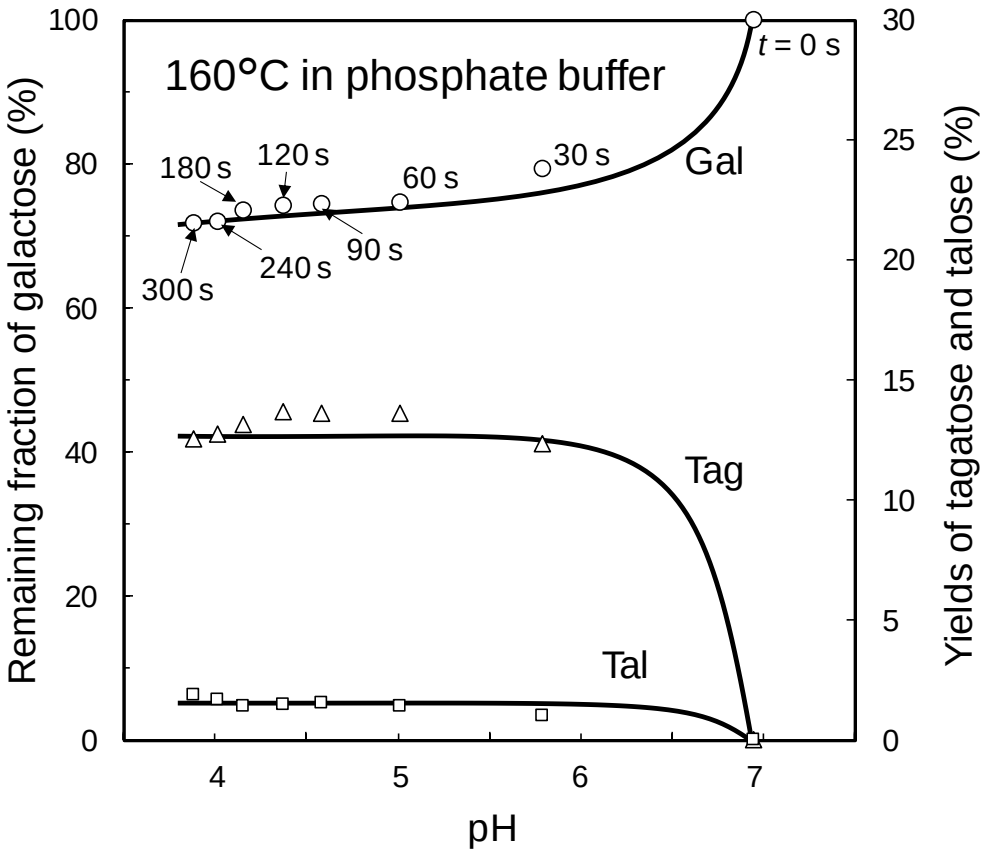
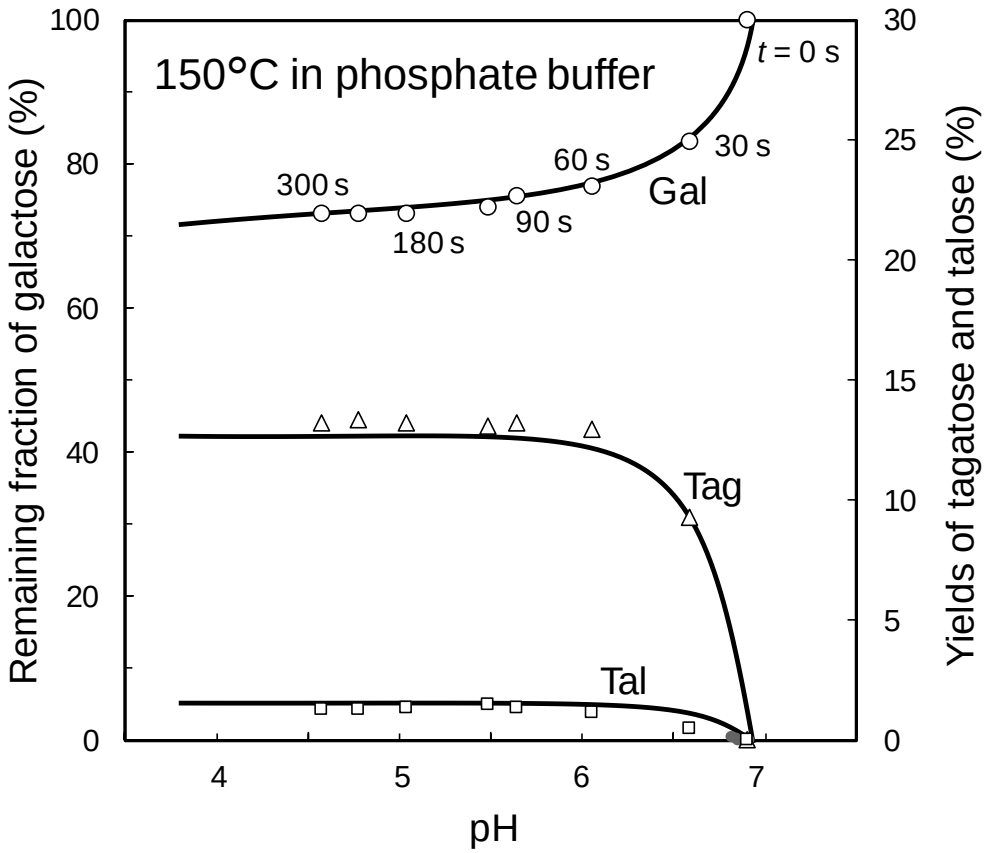


Fig. S1a. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 155 or 160°C in phosphate buffer.



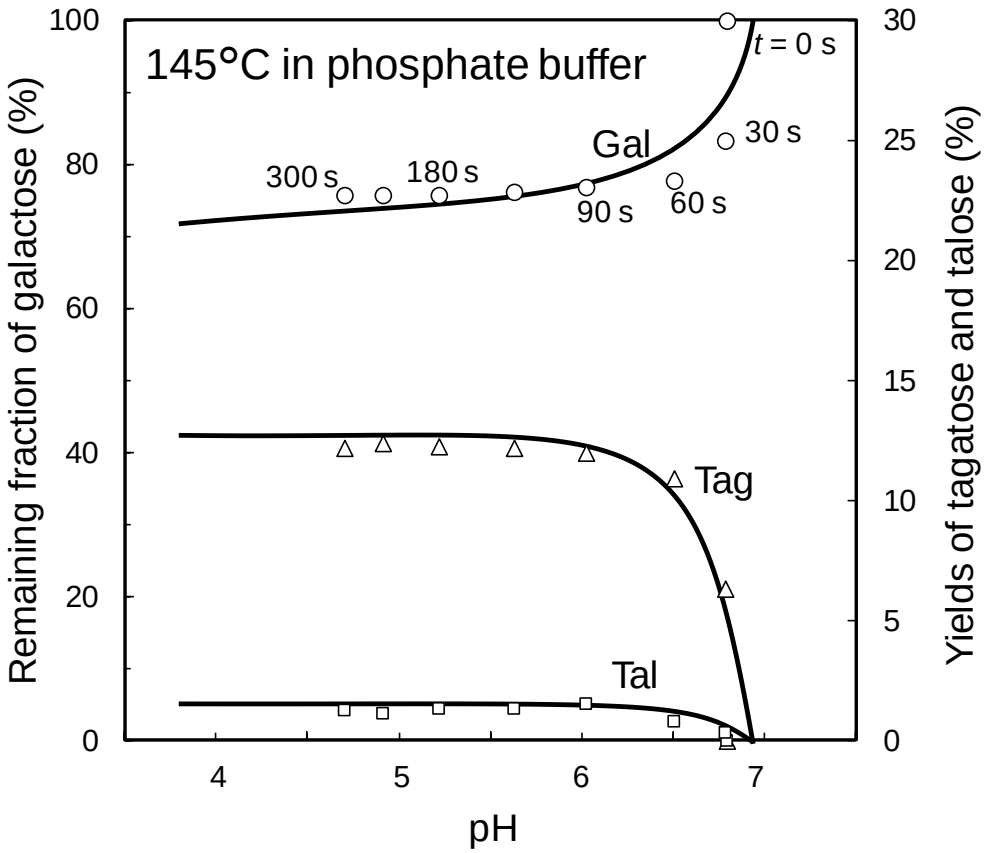
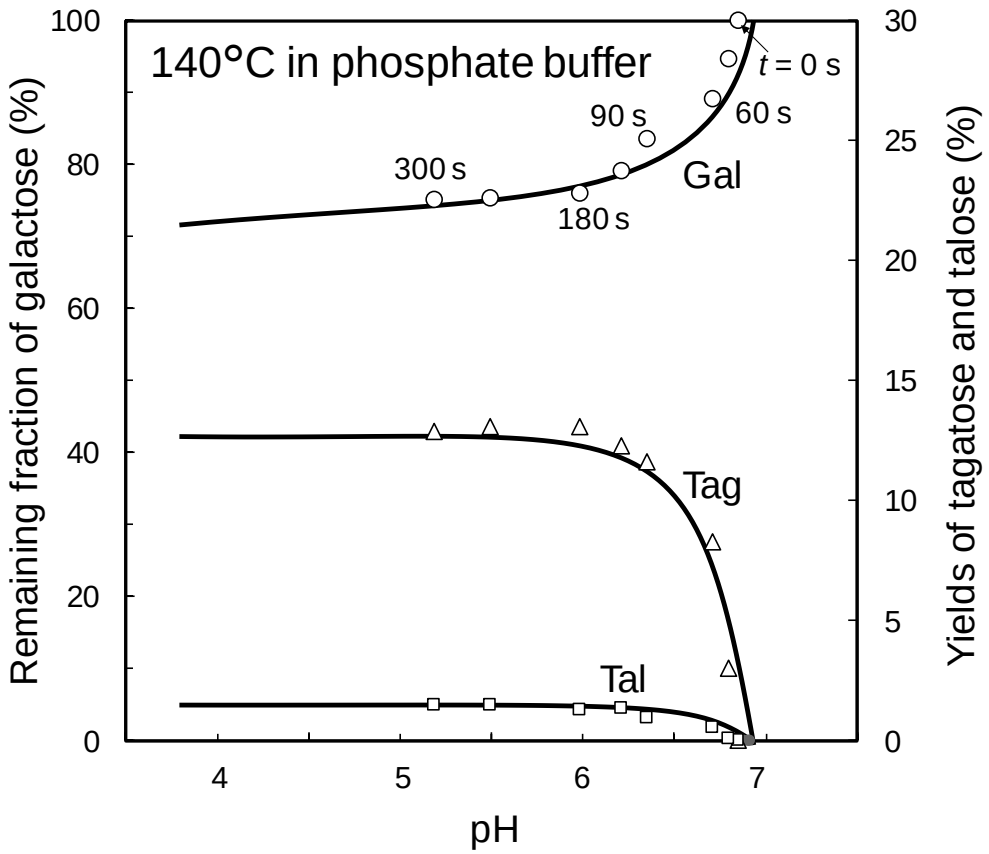
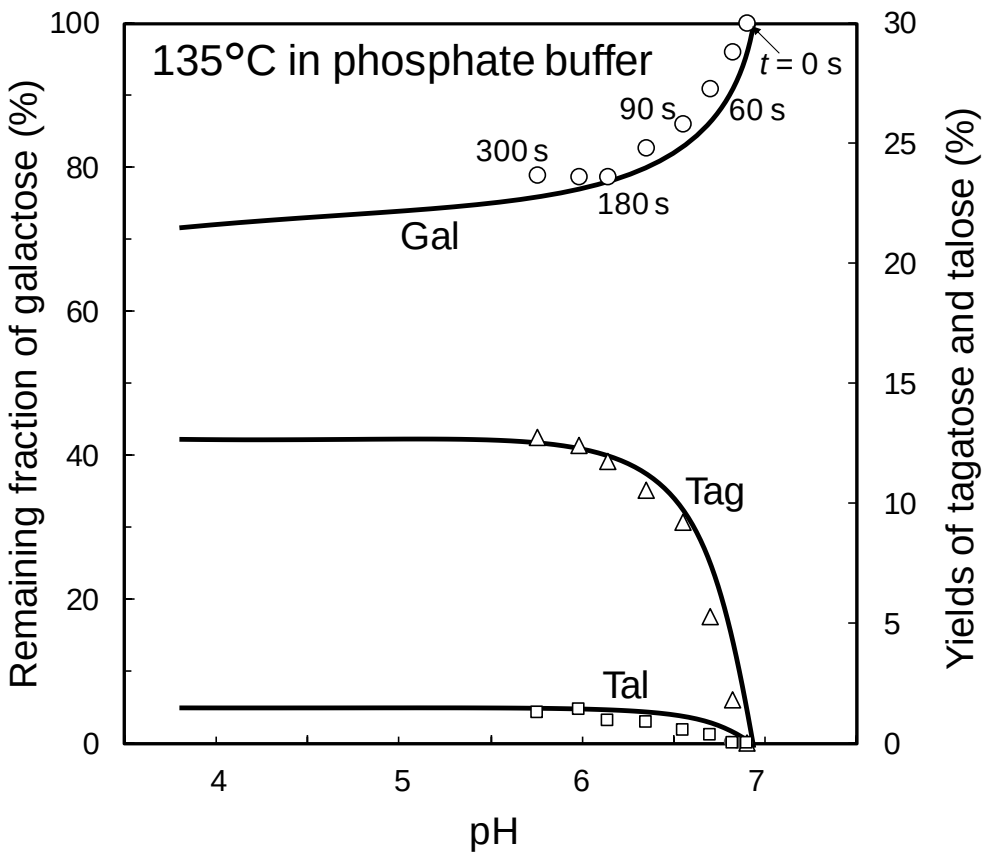


Fig. S1b. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 145 or 150°C in phosphate buffer.





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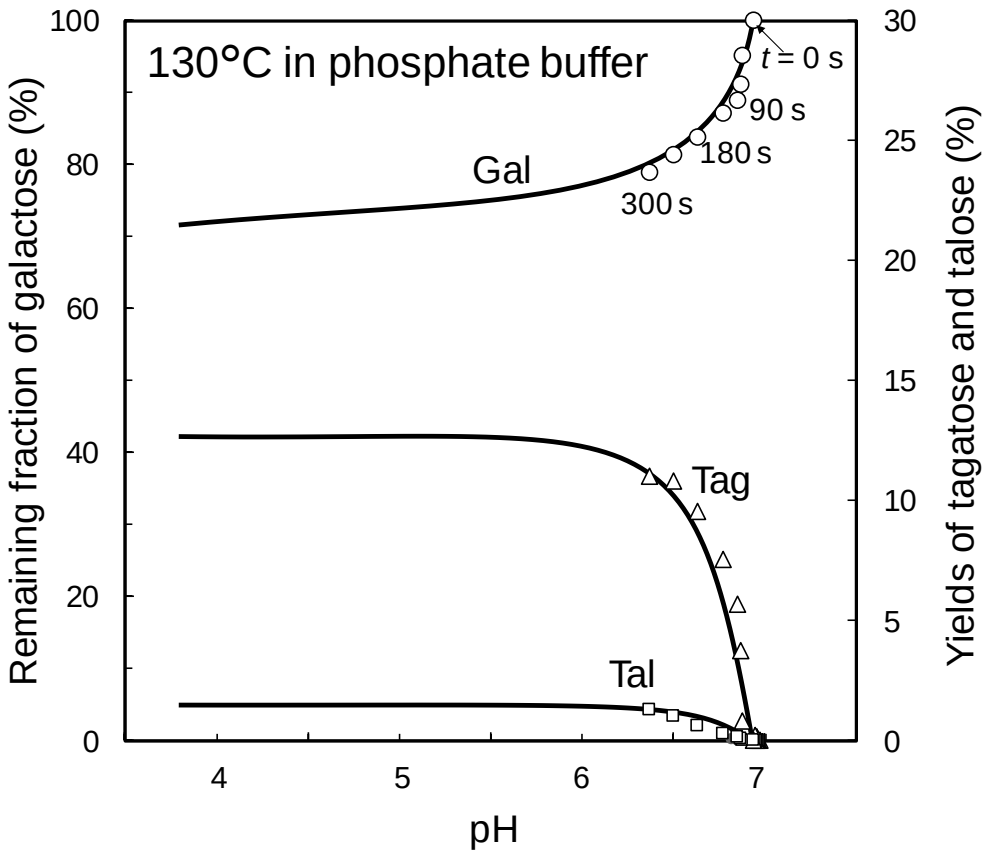


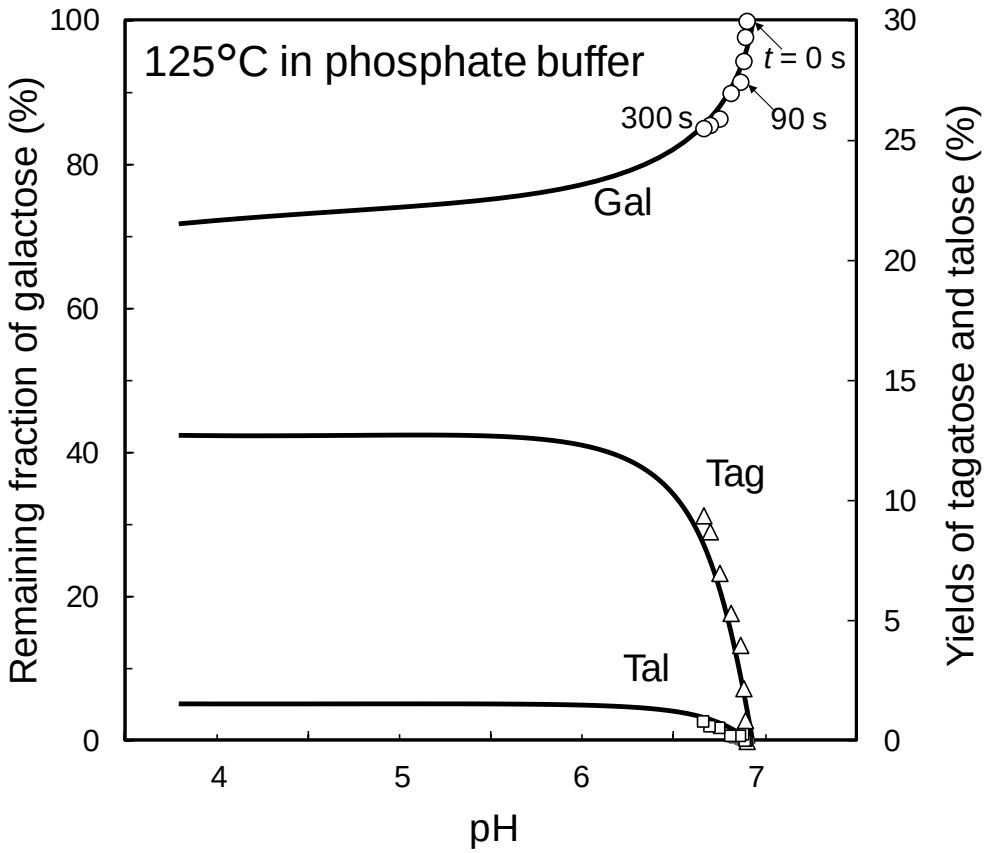
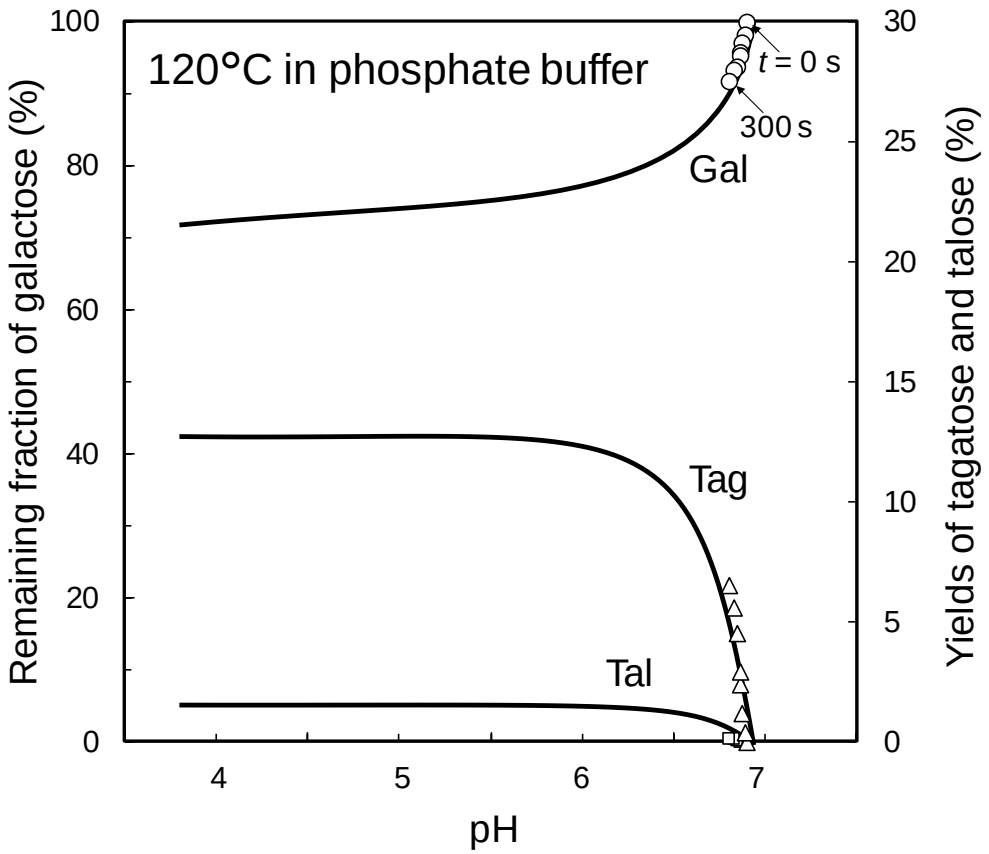


Fig. S1d. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 125 or 130°C in phosphate buffer.



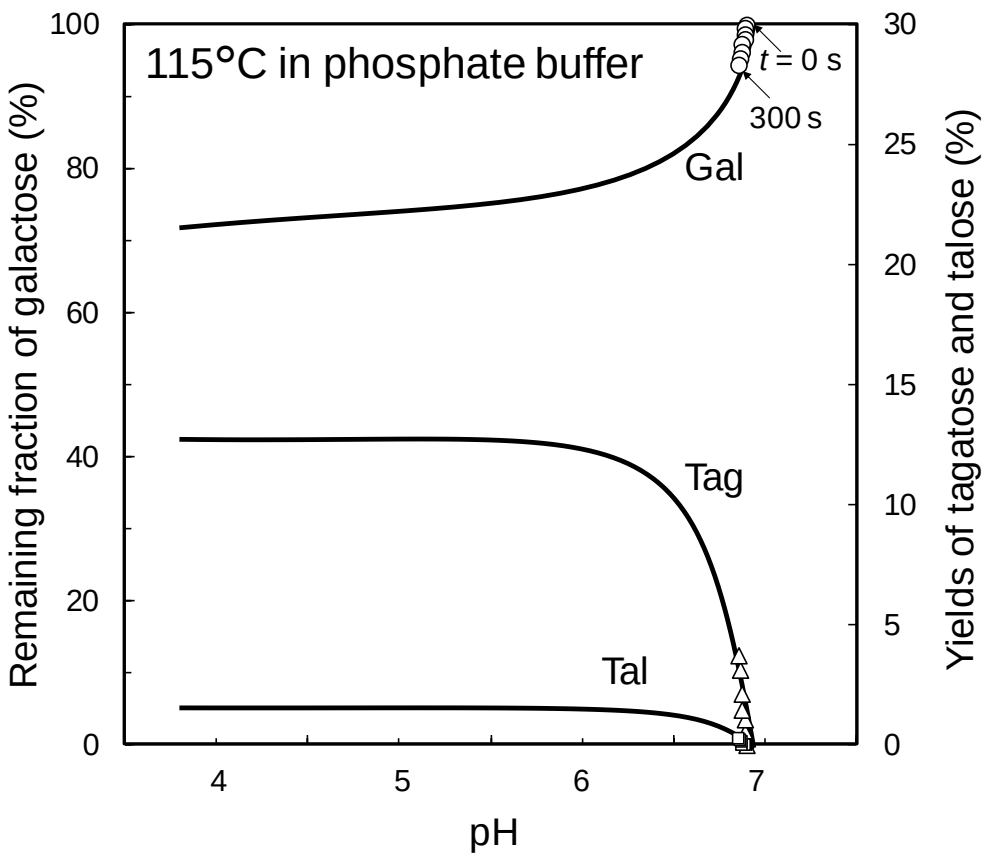
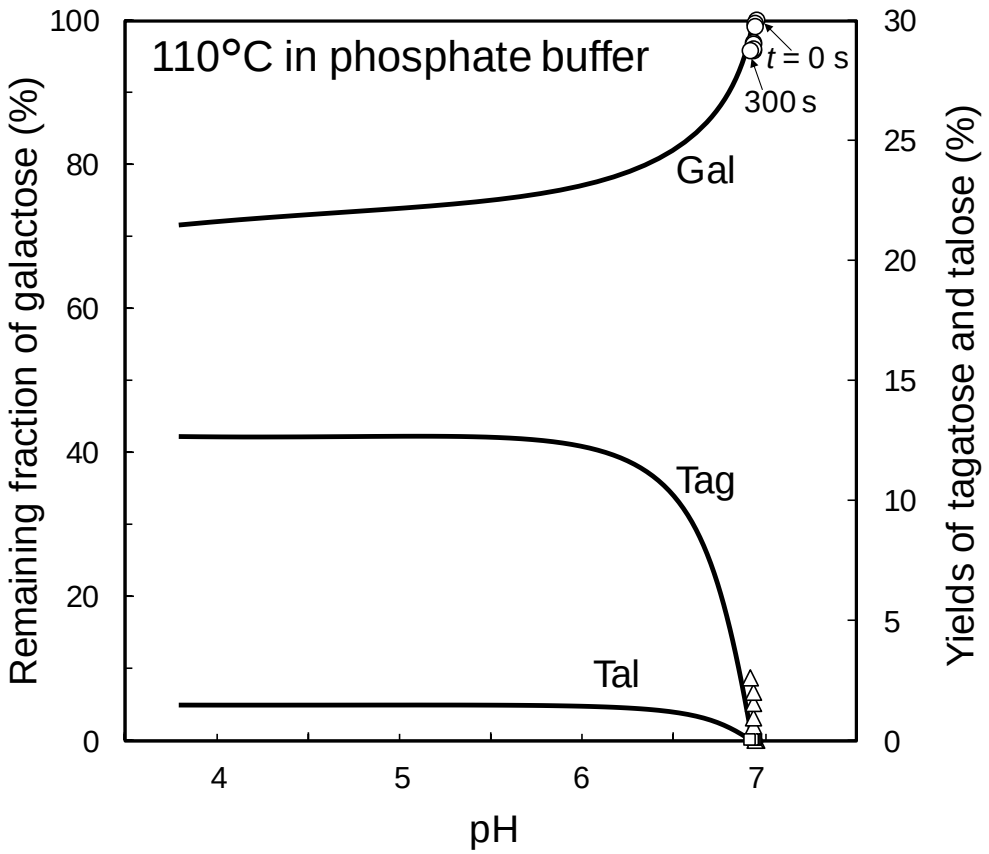


Fig. S1e. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 115 or 120°C in phosphate buffer.



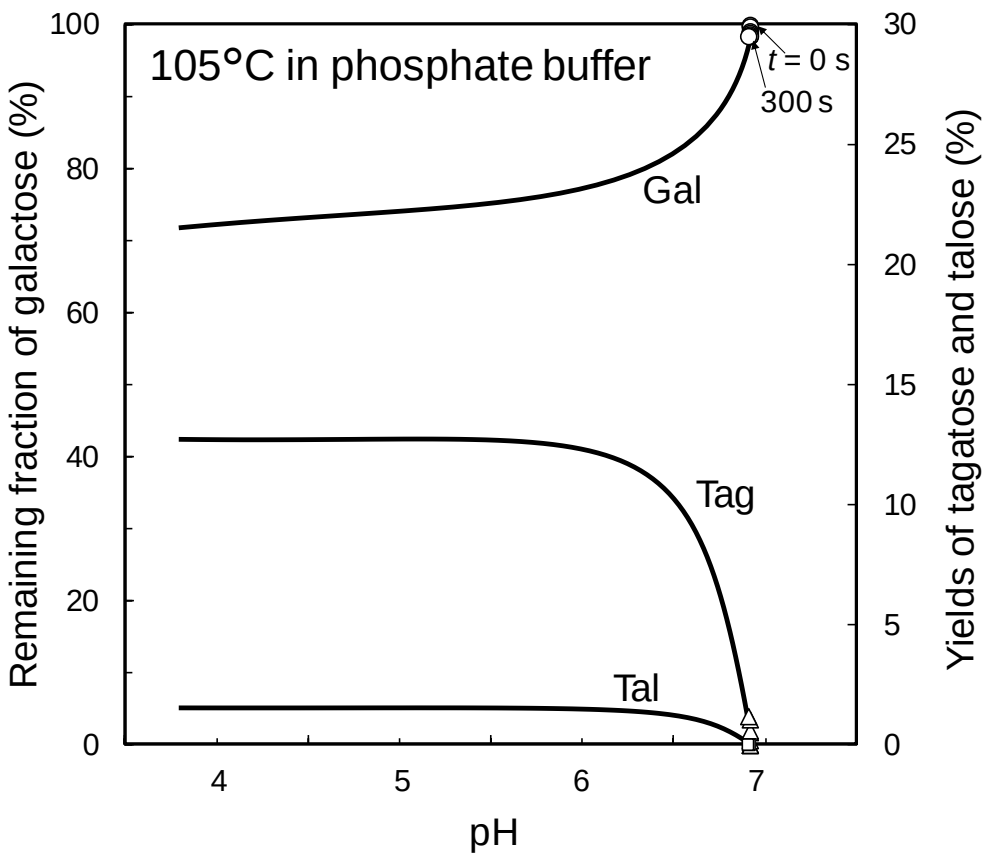


Fig. S1f. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 105 or 110°C in phosphate buffer.

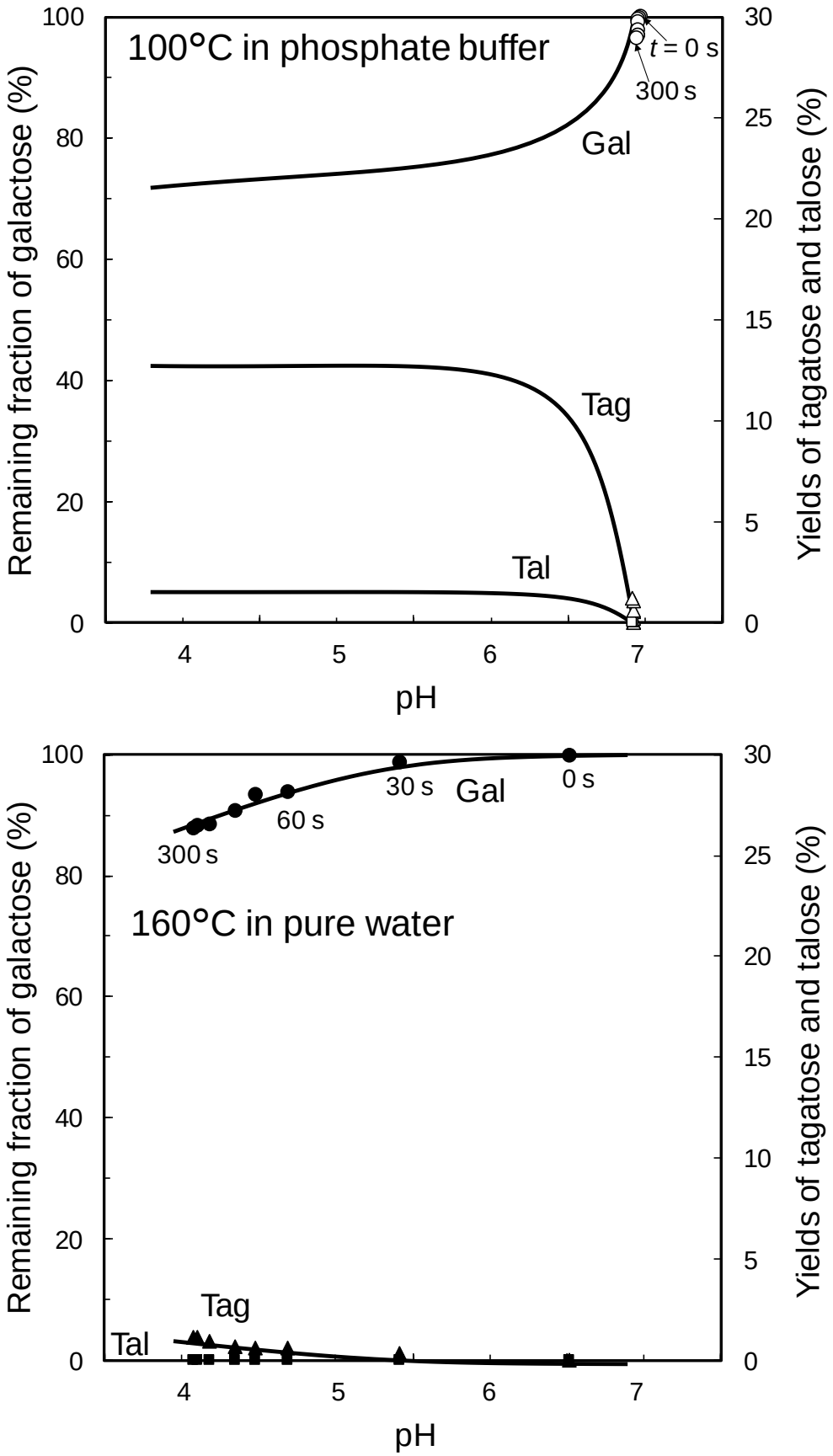


Fig. S1g. Dependence of the remaining fraction of galactose and the yields of tagatose and talose on the pH of the solutions at 100°C in phosphate buffer or at 160°C in pure water.