Isomerization and Epimerization of Galactose to Tagatose and Talose in a Phosphate Buffer Containing Organic Solvents under Subcritical Water Conditions

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ABSTRACT

In this study, galactose was treated under subcritical water conditions at 140 °C and 5 MPa in a mixture of 10 mmol/L sodium phosphate buffer (pH 7.0) and ethanol using a tubular reactor. The effects of ethanol concentration (0–60 wt%) on the isomerization and epimerization of galactose, respectively, to tagatose and talose were investigated. Both ethanol and buffer synergistically improved the isomerization behavior. Increasing the ethanol concentration up to 60 wt% improved the tagatose yield from 13% to 16% at 300 s. In contrast, the yield of talose slightly decreased from 1.5% to 1.2% at 300 s when ethanol was added to the phosphate buffer. The pH of treated solution at 25°C decreased with an increase in the duration of treatment under each treatment condition. However, the decrease in pH was moderately suppressed by the addition of ethanol, which could be one of the reasons for the improvement in tagatose yield. The addition of other organic solvents, such as alkanols, polyols, acetonitrile, and pyridine, also improved the tagatose yield.

1. INTRODUCTION

Rare sugars, rarely present in nature, have a sweet taste but are low in calories.[1-5] They are also known to have various beneficial physiological functions, such as lowering the risk of diabetes,[5, 6] preventing obesity,[7] anti-tumor activity,[8-10] and antioxidant activity.[11] Although they have attracted attention as ingredients for functional foods and pharmaceuticals,[5] practical methods for the large-scale production of rare sugars have not yet been developed to facilitate their commercial use. Several studies have developed methods for producing rare sugars by chemical synthesis including alkali isomerization,[8, 12, 13] by biotechnological methods,[2-5, 10, 14] and by chemo-enzymatic processes.[15, 16] There are many rare sugars, such as D-tagatose,[2, 5, 12, 14-16] D-talose,[8] D-allose,[3, 4, 14] D-psicose[3, 14] lactulose,[13, 15] that were produced through these processes. However, there would be disadvantage in terms of labor or cost: The chemical methods often require multi-step purification and are not applicable to the food production processes in some cases; the enzymatic methods would contain the purification of the enzyme, would have longer reaction time, or in some cases would require genetically modified organisms.

Subcritical water is a type of water that maintains its liquid state under pressurized conditions. It has a high ion product and a low dielectric constant. Sugar is reported to be isomerized or epimerized in subcritical water.[17, 18] Subcritical water treatment of common sugars can be isomerized or epimerized to rare sugars, such as tagatose and talose, respectively, from galactose. However, in pure subcritical water, most sugars are usually decomposed into organic acids, and the yield of isomerized sugars is generally very low.[18-20] Therefore, to overcome the low yield, we attempted subcritical water treatment of common sugars in aqueous ethanol[19, 21-23] or in sodium phosphate buffer[24, 25] to demonstrate the efficiency of these solutions. However, the synergistic effects in the presence of both ethanol and phosphate buffer have not been clarified.

Therefore, in this study, we examined the effects of the addition of ethanol on the subcritical water treatment of galactose in a phosphate buffer for aiming at the practical production of rare sugars (tagatose and talose). The effects of some organic solvents on the treatment were also examined, and we demonstrated the effectiveness of mixing organic solvents and phosphate buffer on the isomerization and epimerization of galactose during the subcritical water treatment.

2. MATERIALS AND METHODS

2.1. Materials

Galactose, tagatose, and talose were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). Phosphate buffer solution and ethanol were purchased from FUJIFILM Wako, and acetonitrile was from Tokyo Chemical Industry (Tokyo, Japan).

2.2. Subcritical water treatment of galactose in a phosphate buffer containing an organic solvent

Sodium phosphate buffer (10 mmol/L, pH 7.0) was mixed with ethanol at the ethanol concentration of 0–60 wt% or organic solvent (50 wt%). Galactose was dissolved in the solution at a concentration of 5 wt% to prepare the starting mixture. Then, nitrogen was sufficiently blown into the mixture to prevent oxidative degradation. Galactose was treated at 140 °C using a tubular reactor as follows. The tubular reactor was made of a PEEK tube (I.D.: 0.8 mm; inner volume: approximately 1.9 mL) immersed in a silicone oil bath, which was similar to those reported in previous studies.[24, 26, 27] The starting mixture was pumped into the tubular reactor using an HPLC pump (LC-10ADVP, Shimadzu, Kyoto, Japan) at a flow rate of 0.35–3.5 mL/min. These flow rates

corresponded to the reaction times (residence times) of 30–300 s. The pressure inside the tubular reactor was maintained at approximately 5 MPa using a back-pressure regulator (P-880, Upchurch Scientific, Oak Harbor, WA, USA). The effluent from the reactor was collected, and its composition was determined by HPLC. The experiments were performed in triplicate for the cases involving ethanol and in n = 1 for the cases involving other organic solvents.

The yields of tagatose and talose were calculated by dividing the concentration of formed sugar in the reactor effluent by the initial concentration of galactose. The selectivity of tagatose was calculated by dividing the amount of formed tagatose by that of consumed galactose.

2.3. Analysis of the treated solution

The composition of the treated solution was determined by injecting the effluent (10 μL) into an HPLC system equipped with an LC-20AD HPLC pump (Shimadzu) connected to a COSMOSIL Sugar-D column (3.0 mm I.D. × 250 mm, Nacalai Tesque, Kyoto, Japan). Galactose, tagatose, and talose were quantified using an RID-20A refractive index detector (Shimadzu). The eluent was a mixture of acetonitrile and water (80:20, v/v) at a flow rate of 0.4 mL/min.

The pH of the treated solution obtained as a reactor effluent was measured at 25°C using an F-74 pH meter (HORIBA, Kyoto, Japan) with a 9681S-10D pH electrode (HORIBA).

3. RESULTS AND DISCUSSION

3.1. Effect of ethanol addition on the yield of rare sugars

Galactose was treated under subcritical water conditions at 140 °C in pure water, in 50 wt% ethanol in water, or in 10 mmol/L sodium phosphate buffer containing 0–60 wt% ethanol to evaluate the effects of ethanol concentration and phosphate buffer on the yield of rare sugars. Figure 1A shows the time course of the remaining fraction of galactose. Galactose barely reacted at 140 °C in pure subcritical water (the remaining fraction of galactose at 300 s: approximately 99%). This result is consistent with those reported by previous studies, which require a temperature of 180 °C or higher for the isomerization or epimerization in pure subcritical water.[18] However, in the presence of ethanol in pure water, the galactose content decreased significantly even at 140 °C; approximately 94% of galactose remained at 300 s. From previous studies, it is reported that the decrease in galactose content was further promoted in subcritical water containing 10 mmol/L sodium phosphate buffer (hereafter, the solution was referred to as buffer-subcritical water),[24, 25] and 79% of galactose remained at 120 s. After 120 s, the decrease in galactose content leveled off, and the remaining fraction of galactose was approximately 75% at 300 s in buffer-subcritical water. These results suggest that both ethanol and phosphate buffer promote the reaction of galactose.

Therefore, to investigate the synergistic effects of ethanol and phosphate on isomerization, the reaction behavior of galactose was observed in the reaction system where ethanol was added to the 10 mmol/L sodium phosphate buffer. When 1 wt% ethanol was added to the buffer, a similar behavior was observed as in buffer-subcritical water. In contrast, when galactose was added to the phosphate buffer containing 5–60 wt% ethanol, the decrease in the galactose content proceeded slightly faster in the initial stage of the reaction (<120 s) than in buffer-subcritical water. However, in the latter half of the reaction (120–300 s), the remaining fraction of galactose barely changed with time and was independent of the ethanol concentration. These results suggest that the addition of ethanol to the phosphate buffer synergistically promotes the reaction of galactose.

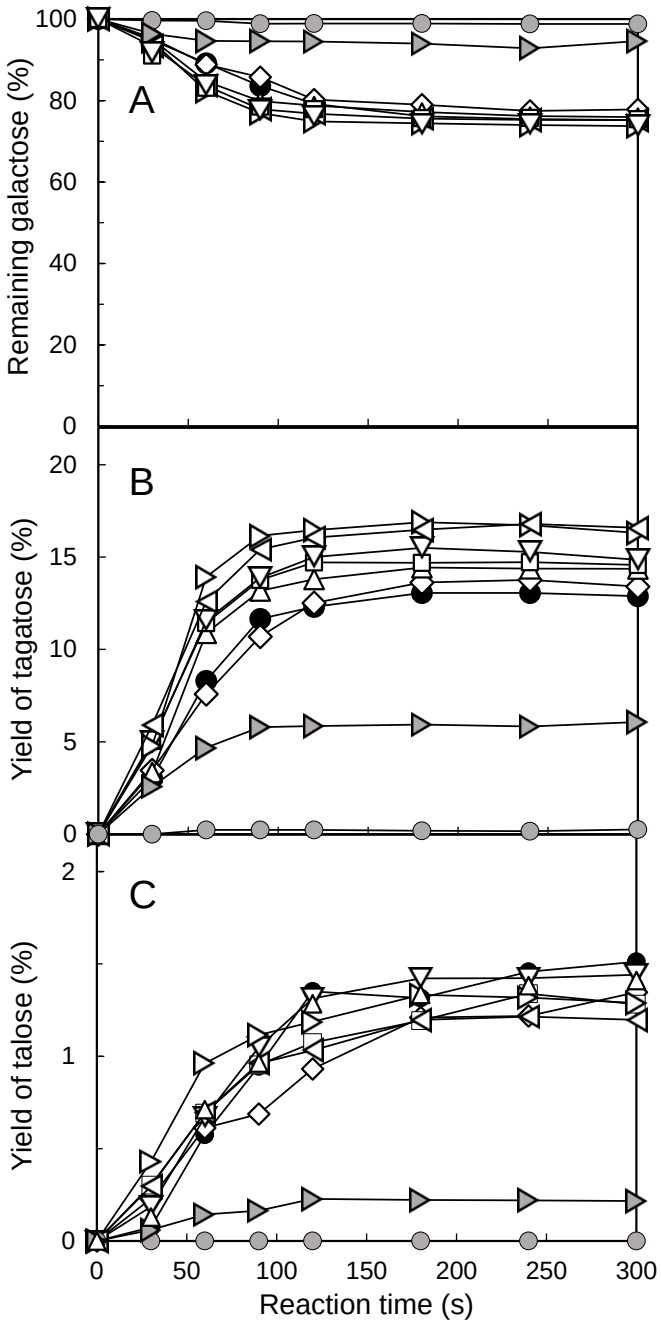

Figure 1. Time courses for (A) remaining galactose, (B) formation of tagatose, and (C) formation of talose in pure water, in 50 wt% ethanol in water, or in the mixture of ethanol and 10 mmol/L sodium phosphate buffer (pH 7.0) at 140 °C under subcritical water condition. Each symbol represents the conditions listed as follows: ●: cited results in 10 mmol/L sodium phosphate buffer;[24] ◇: in 1 wt% ethanol in buffer; △: in 5 wt% ethanol in buffer; □: in 10 wt% ethanol in buffer; ▽: in 20 wt% ethanol in buffer; ▷: 50 wt% ethanol in buffer; ◁: in 60 wt% ethanol in buffer; ○: in pure water; and ▶: in 50 wt% ethanol in pure water.

Figure 1B shows the effects of ethanol addition on tagatose yield. Due to the negligible reaction of galactose in pure subcritical water, almost no tagatose formed within 300 s (tagatose yield <0.3%). In contrast, approximately 6% of tagatose was formed at 300 s in 50 wt% ethanol; this yield was lower than that reported previously (22% in 80 wt% ethanol at 180 °C),[22] which is mainly attributed to the low treatment temperature and higher galactose concentration. In buffer-subcritical water, the yield of tagatose soared within the first 90 s.[24] Then, the yield leveled off at approximately 12% between 90 and 120 s. After 120 s, the yield of tagatose barely changed at approximately 13%, and no remarkable tagatose decomposition was observed.

The addition of 1 wt% ethanol to buffer affected the tagatose yield less than that without ethanol. However, when 5–50 wt% of ethanol was added to buffer, the tagatose yield increased remarkably at the initial stage of the reaction (<90 s). The higher the ethanol concentration in the phosphate buffer, the higher the yield of tagatose at the initial stage of the reaction. However, the tagatose yield was almost the same in 60 wt% ethanol in buffer as in 50 wt% ethanol. As shown above, the yield of tagatose at 300 s improved by 1.25 times from 13.0% (in buffer-subcritical water) to 16.5% (in 60 wt% ethanol in buffer-subcritical water) by adding ethanol to buffer. Therefore, it is suggested that the addition of both phosphate and ethanol synergistically promote tagatose formation.

Figure 1C shows the time courses of talose formation. Yields of talose in 50 wt% ethanol or in phosphate buffer were higher than those in pure subcritical water (0.2% in 50 wt% ethanol and 1.5% in buffer-subcritical water at 300 s). However, unlike tagatose, the addition of ethanol to buffer did not improve the talose yield. Rather, talose yields were slightly lower at particularly higher ethanol concentrations (50–60%) than in buffer-subcritical water.

3.2. Effect of ethanol addition on the tagatose selectivity

The yield of tagatose increased with increasing ethanol concentration. These results suggest that the addition of ethanol to buffer could suppress the decomposition of tagatose. Here, the yield of tagatose was plotted against the conversion of galactose to discuss the suppression of the decomposition (Fig. 2). If the isomerization of galactose to tagatose proceeds efficiently and the decomposition of tagatose is quite suppressed (high selectivity of tagatose = low degree of side reaction), the plot approaches the dashed line in Fig. 2. Conversely, if the decomposition surpassed the isomerization and selectivity was low, the plot deviated below the dashed line. For the treatment in buffer-subcritical water, the higher the conversion of galactose, the farther the black circle symbol was from the dashed line, resulting in lower selectivity of tagatose. In contrast, the plot approached the dashed line by the addition of ethanol to the buffer. Particularly at higher ethanol concentrations, the plot further approached the dashed line. These results suggest that the addition of ethanol tends to suppress the side reactions and increase the selectivity. One of the reasons for these results would be the formation of ethoxide anion, which is a stronger base than hydroxide anion, enhancing the Lobry de Bruyn-van Ekenstein (LBAE) transformation; i.e., addition of ethanol enhances ionization of sugars to form enolate anion.[28]

The addition of ethanol positively affected the yield of tagatose, but negatively affected the yield of talose. There may be another reason for these changes. Tagatose is a ketose, and talose is an aldose. It is considered that the addition of ethanol changed the properties of the reaction medium and facilitated the formation of tagatose in terms of reaction equilibrium. Similarly, it can

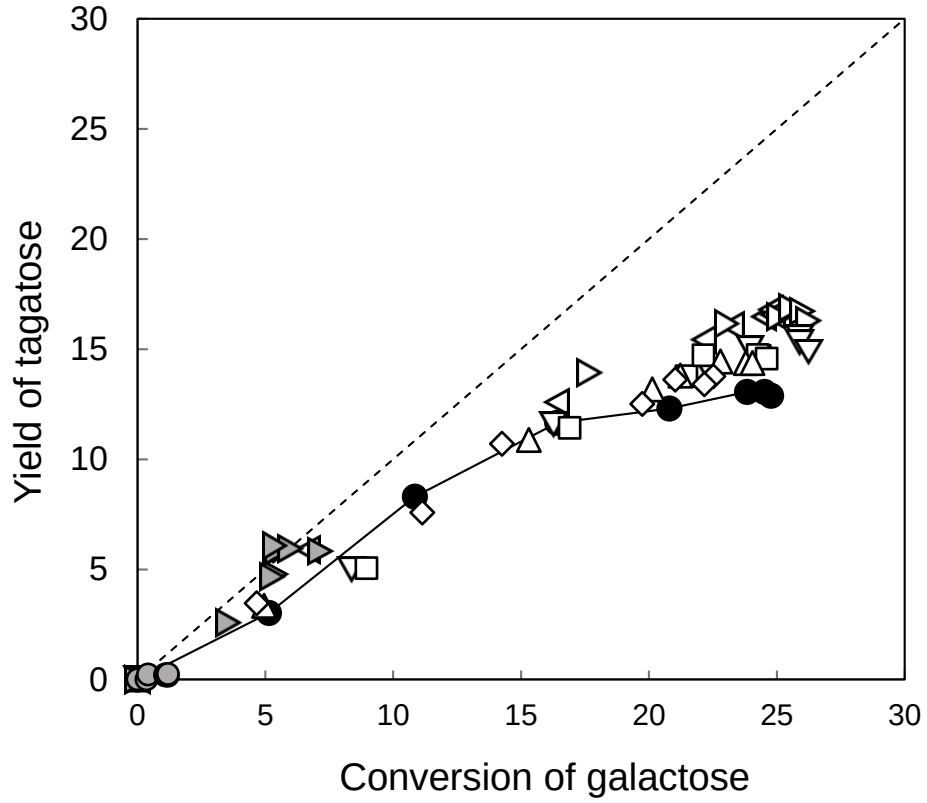

Figure 2. Relationship between the tagatose yield and conversion of galactose. The dashed line indicates the set of points at which galactose was completely isomerized to tagatose without any side reactions. The symbols are the same as those in Fig. 1.

be said that the addition of ethanol shifted the reaction equilibrium toward ketose formation rather than aldose one.

It was reported that reaction equilibrium shifted to the formation of fructose for the glucose-to-fructose isomerization with increasing the temperature.[29] It was also reported that addition of ethanol enhanced the reaction rates for the glucose-to-fructose isomerization and reverse reaction by alkali-catalyzed isomerization.[28] From these facts, the addition of ethanol improves the

isomerization yield of fructose. If this is also true for galactose, the reaction equilibrium would be shifted to the formation of tagatose.

Another reason would be also suggested. It was reported that pK_a value of glucose in the equi-volume mixture of CD_3OD-D_2O was lower than that in D_2O.[30] Ethanol would also affect the pK_a value of sugar, and degree of this effects would depend on the type of sugars. This could change the reaction equilibrium.

3.3. Changes in pH during the treatment

Figure 3 shows the time courses of the pH of the treated solutions at various ethanol concentrations at 25°C. Although the pH was measured at 25°C and was not the results under subcritical water conditions, giving the limited information, the results would, at least, reflect the some kind of reaction behaviors of galactose under subcritical water conditions. At any ethanol concentration, the pH decreased with the passage of time. This could be explained by the immediate formation of organic acids during galactose decomposition. Although the buffering capacity of the phosphate buffer was relatively limited even in the presence of ethanol, the decrease in pH tended to be suppressed as the ethanol concentration increased. In particular, at higher ethanol concentrations (>50 wt%), the decrease in pH was remarkably suppressed.

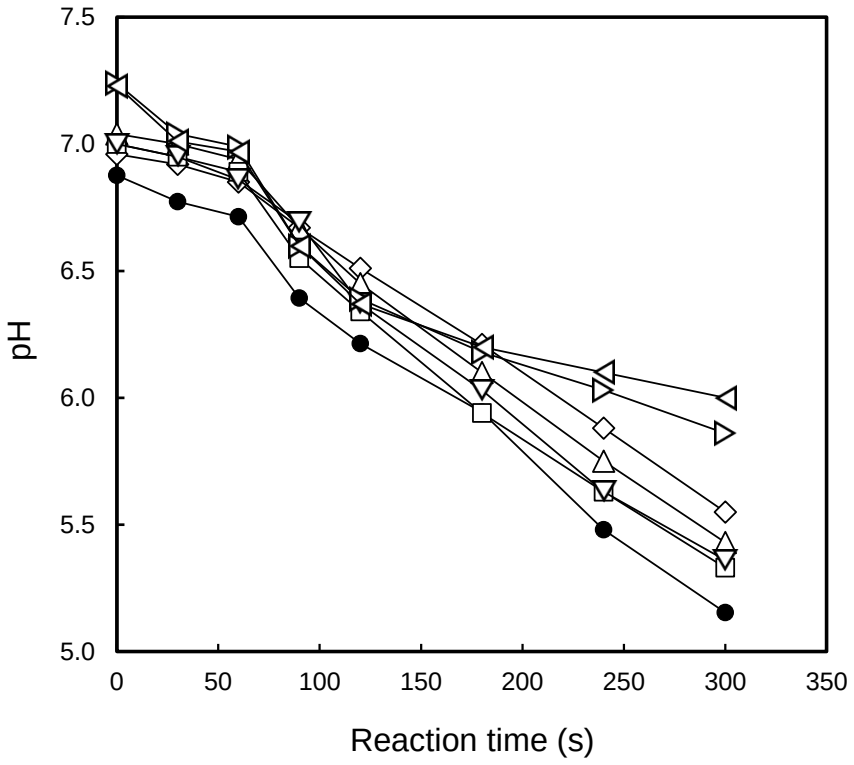

Figure 3. Time courses for the pH of the treated solutions obtained by the subcritical water treatment of galactose in 10 mmol/L sodium phosphate buffer containing ethanol at 140 °C. The measurement of pH was performed at 25°C. The symbols are the same as those in Fig. 1.

To discuss the effect of pH on tagatose formation, tagatose yield was plotted against the pH of the treated solution (Fig. 4). The reaction started at pH ~7, and the pH decreased with increasing tagatose yield. At any ethanol concentration, the yield increased sharply when the pH was approximately 7, indicating that most of the isomerization progressed while the pH change was small (difference between the pH values of the treated and untreated solutions < 0.5). When the pH change was larger than 0.5, tagatose formation reduced. When 20 wt% or more ethanol was added, more than 12% of tagatose yield was achieved within a pH change smaller than 0.3.

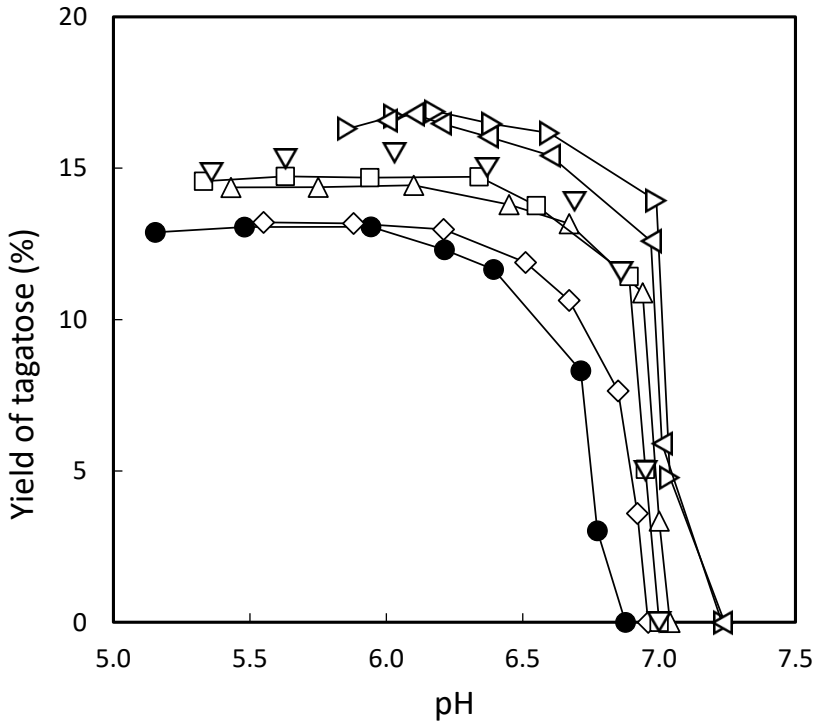

Figure 4. Relationship between the yield of tagatose and pH of the treated solutions obtained during the subcritical water treatment of galactose in 10 mmol/L sodium phosphate buffer containing ethanol at 140 °C. The measurement of pH was performed at 25°C. The symbols are the same as those in Fig. 1.

As described above, it was observed that the addition of ethanol suppressed pH change and improved the efficiency of tagatose production. In addition, when the pH was lower than 6.3–6.5, the tagatose yield barely increased. In our previous study in buffer-subcritical water, isomerization rarely occurred when the pH dropped below 6.3.[24] Similarly, it is necessary to maintain the pH higher than 6.5, even in the presence of ethanol, for the efficient production of tagatose.

3.4. Isomerization and epimerization in phosphate buffer containing various organic solvents

The addition of 50 wt% ethanol promoted the formation of tagatose. To confirm whether this effect is unique to ethanol, we investigated the production behavior of rare sugars when 50 wt% of organic solvents were added. Table 1 shows the remaining fraction of galactose, yields of tagatose, and talose at 120 s in 10 mmol/L phosphate buffer containing 50 wt% of various organic solvents. Thus, the tagatose yields were higher than that in buffer-subcritical water, except when 2-propanol was added. These results confirm that many organic solvents will also promote isomerization.

Table 1. Effects of Organic Solvents on the Isomerization and Epimerization of Galactose at 120 s in 10 mmol/L Sodium Phosphate Buffer at 140 °C.

Solvent	Remaining galactose (%)	Yield of tagatose (%)	Yield of talose (%)
(10 mmol/L Phosphate buffer)	79.2	12.3	1.4
Methanol:Buffer = 1:1 (v/v)	74.9	16.5	1.2
Ethanol:Buffer = 1:1 (v/v)	75.9	16.1	1.1
1-Propanol:Buffer = 1:1 (v/v)	77.0	14.1	1.2
2-Propanol:Buffer = 1:1 (v/v)	89.9	8.9	0.7
t-Butyl alcohol:Buffer = 1:1 (v/v)	80.6	13.3	0.9
Ethylene glycol:Buffer = 1:1 (v/v)	76.4	15.2	1.1
Propylene glycol:Buffer = 1:1 (v/v)	75.6	15.6	1.1
Acetonitrile:Buffer = 1:1 (v/v)	75.9	16.6	1.1
Pyridine:Buffer = 1:1 (v/v)	70.2	14.6	2.2

In 2-propanol, the conversion of galactose at 120 s was low, and the yields of both tagatose and talose were also low. Therefore, 2-propanol would suppress the reaction of galactose under subcritical water conditions. However, the reason for this is currently unknown.

Next, we describe the results when using solvents other than 2-propanol. When alkanols, diols, acetonitrile, or pyridine were added, the yield of tagatose was higher than that in buffer-subcritical water (yield = 13.3–16.5%). In contrast, except for pyridine, the yield of talose was lower than that in buffer-subcritical water, which can be attributed to the reaction equilibrium shifting to the ketose formation side, as described above. When pyridine was added, the behavior was slightly different from those containing other solvents; the conversion of galactose was remarkably higher than that in buffer-subcritical water. In addition, the formation of both rare sugars (tagatose and talose) was promoted. This would be due to the basicity of pyridine, which promoted alkali isomerization and epimerization.

4. CONCLUSION

The addition of various organic solvents to sodium phosphate buffer changed the behaviors for isomerization and epimerization of galactose. The formation of tagatose was promoted, and a large amount of ethanol could further improve the yield of tagatose. In contrast, talose formation was suppressed by the addition of organic solvent due to the equilibrium shift toward tagatose formation. As shown above, the production of rare sugars could be controlled by adding organic solvents to the phosphate buffer.

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Author Contributions

T. Kobayashi conceived and planned the research. Y. Onishi and Y. Furushiro performed the experiments and made equal contributions. S. Adachi contributed to the discussion.

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Notes

The authors declare no competing financial interest.

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Graphical abstract