# PRODUCTION OF TAGATOSE FROM GALACTOSE USING ENVIRONMENTALLY FRIENDLY CATALYST

By
MISS Neeranuch MILASING

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy FOOD TECHNOLOGY
Department of FOOD TECHNOLOGY
Silpakorn University
Academic Year 2024

# การผลิตน้ำตาลทากาโทสจากน้ำตาลกาแล็คโทสโดยใช้ตัวเร่งที่เป็นมิตรต่อสิ่งแวดล้อม

โดย
นางสาวณีรนุช มิละสิงห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปรัชญาดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีอาหาร แบบ 2.2
ภาควิชาเทคโนโลยีอาหาร
มหาวิทยาลัยศิลปากร
ปีการศึกษา 2567

# PRODUCTION OF TAGATOSE FROM GALACTOSE USING ENVIRONMENTALLY FRIENDLY CATALYST

By

MISS Neeranuch MILASING


A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy FOOD TECHNOLOGY
Department of FOOD TECHNOLOGY
Academic Year 2024

| | |
|---|---|
| Title | Production of tagatose from galactose using environmentally friendly catalyst |
| By | MISS Neeranuch MILASING |
| Field of Study | FOOD TECHNOLOGY |
| Advisor | Associate Professor Pramote Khuwijitjaru, Ph.D. |

Faculty of Engineering and Industrial Technology, Silpakorn University in Partial Fulfillment of the Requirements for the Doctor of Philosophy

.....
(Assistant Professor Arunsri Leejeerajumnean, Ph.D.) Dean of Faculty of Engineering and Industrial Technology

Approved by

.....
(Associate Professor Busarakorn Mahayothee, Ph.D.) Chair person

.....
(Associate Professor Pramote Khuwijitjaru, Ph.D.) Advisor

.....
(Associate Professor Jintana Wiboonsirikul, Ph.D.) External Examiner

60403801 : Major FOOD TECHNOLOGY
Keyword : Tagatose, Isomerization, Galactose, Maillard reaction, Arginine, Ethanol, Lactose, Lactulose, Buffers

MISS Neeranuch MILASING : Production of tagatose from galactose using environmentally friendly catalyst Thesis advisor : Associate Professor Pramote Khuwijitjaru, Ph.D.

Tagatose is a rare sugar with health benefits, including low calories, a low glycemic index, and prebiotic properties. This study explored environmentally friendly catalysts, including arginine and buffers, for producing tagatose. The production of tagatose by heating galactose with arginine in aqueous solution under various conditions was investigated. The effects of temperature (90-120 °C), reaction time (0-20 min), arginine concentration (0.01-0.15 mol/mol of galactose), and galactose concentration (5-20% w/v) were evaluated. The highest yields of tagatose (16.8%), talose (2.7%), and sorbose (3.3%) were achieved at 120 °C after 20 min. Talose and sorbose are by-products of this process. The reaction at 120 °C for 4 min provided the highest productivity of 92.4 g/(L·h). Increasing the arginine concentration enhanced the isomerization and the Maillard reaction, turning the solution from clear to yellow-brown and lowering the pH. Higher galactose concentrations reduced tagatose yield but increased productivity to 278 g/(L·h) at an initial galactose concentration of 20% w/v, which is favorable for large-scale production. Additionally, the potential use of lactose, which is much cheaper than galactose, as a raw material for the direct production of tagatose was also investigated. Experiments were conducted at 100-120 °C revealed that lactose primarily converted to lactulose, achieving a maximum yield of approximately 26%. The formation of galactose, however, was almost undetectable. The kinetics of tagatose formation were then studied when galactose underwent isomerization with arginine at temperatures ranging from 90 to 120 °C over a period of 15 to 120 min. The initial rate of tagatose formation increased significantly with temperature, with an activation energy of 66.24 kJ/mol. Arginine decomposed during the reaction, with more degradation occurring at higher temperatures. Furthermore, ethanol (0-40% w/w) was tested as a modifier to enhance the isomerization. Adding 10% ethanol to the reaction solution increased the yield from 17% to 19%, whereas a 40% concentration of ethanol resulted in significant reduction of the yield (15%). Lastly, the isomerization of galactose in buffer systems was explored. Various buffers were tested for their catalytic activity, including CAPS, carbonate, triethylamine, quinuclidine, and L-arginine. The maximum yields of tagatose were 15.0% with CAPS, 15.2% with carbonate, 19.3% with triethylamine, 19.6% with quinuclidine, and 18.1% with L-arginine. However, L-arginine resulted in highest browning reaction. Notably, the initial rate of tagatose formation with carbonate buffer was 3 to 8 times higher than with CAPS, despite both having the same pH. For catalysis involving carbonate buffer, the reaction orders for hydroxide anions and carbonate species were approximately first and zeroth, respectively. *Operando* NMR studies of deuterated galactose isomerization in both carbonate and CAPS buffers indicated similar tautomeric distributions. The deuterium kinetic isotope effect study suggested that carbonate facilitates isomerization through a proton transfer mechanism, with hydroxide anions acting as the catalytically

active species, while carbonate anions stabilize the enediolate anion and/or the transition state.

# ACKNOWLEDGEMENTS

First of all, I gratefully acknowledge the Royal Golden Jubilee Ph.D. Program from the Thailand Research Fund (PHD/0140/2560) for scholarship and research grant support.

I would like to express my sincere gratitude to my thesis advisor, Associate Professor Dr. Pramote Khuwijitjaru, for his supervision, guidance, and suggestions all the time as a Ph.D. student.

I am grateful to Professor Dr. Shuji Adachi, Department of Agriculture and Food Technology, Faculty of Bioenvironmental Sciences, Kyoto University of Advanced Science, Japan for providing valuable guidance and feedback on my manuscripts

I am grateful to Professor Dr. Irina Delidovich, and Valérie Toussaint, Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität, Wien, Austria for allowing me to be a visiting postgraduate student and working there for 12 months (May 2023 - April 2024) on the isomerization of galactose in buffers solution.

Finally, I wish to thank my family and friends who always support and encourage me.

Neeranuch MILASING

# TABLE OF CONTENTS

**Page**

มหาวิทยาลัยศิลปากร

# LIST OF TABLES

**Page**

# LIST OF FIGURES

**Page**

# CHAPTER 1
# General introduction

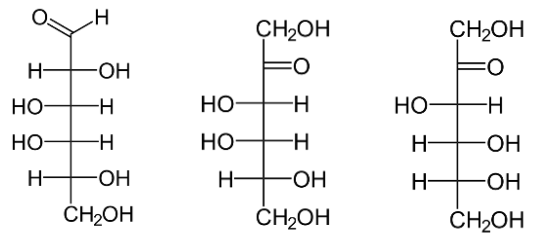
## 1.1 Research problem and its significance

D-Tagatose is a rare hexose and an isomer of galactose with a chemical structure that is an epimer of fructose at the C4 position. Tagatose is found only in trace amounts in some fruits and exudates from plants such as *Stercula setigera* and is produced in very small quantities during the heat processing of dairy products. Tagatose is 92% as sweet as sucrose and has a low caloric value of only 1.5-2.4 kcal/g. It can be used as a drug to control type-2 diabetes and obesity, and as a low-calorie sweetener substitute for sucrose. Tagatose can be produced by an isomerization reaction through either an enzymatic or chemical process. L-Arabinose isomerase is normally used to directly isomerize galactose to tagatose. This method is effective and produces no by-products, but it is usually expensive due to the production cost associated with enzyme instability in various conditions, metal ion requirement, and long production times. The chemical method has been applied in commercial processes using an alkali-catalyzed reaction known as the Lobry de Bruyn–Alberda van Ekenstein transformation. The chemical catalyst produces a high yield (72%); however, it generates many by-products and involves complex purification processes. Recently, the “green method” has gained interest for production of rare sugars. Among several basic amino acids, arginine showed the greatest ability as an alkaline catalyst for the isomerization reaction. Moreover, the limitation of basic catalysts is that acidic by-products are formed during the reaction, causing a drop in pH and suppressing tagatose yield. Thus, using a buffer to maintain the pH of the solution is one of the alternative ways to improve the production of tagatose.

## 1.2 Tagatose

D-Tagatose is a ketohexose, and its empirical formula is $C_6H_{12}O_6$. Tagatose is an isomer of galactose and epimer of the fructose at the fourth carbon atom, as shown in Figure 1.1. It naturally occurs in *Sterculia setigera* gum and is also found in small amounts in heated cow's milk, as well as in various other dairy products (O'Brien-Nabors, 2012). Due to its limited present in the nature, tagatose has been classified as a rare sugar by the International Society of Rare Sugars. Tagatose is approximately 92% as sweet as sucrose in a 10% aqueous solution, without cooling effect, and it has a low-calorie value of 1.5 to 2.4 kcal/g (Kim, 2004; Lamothe et al., 2019). The important properties of tagatose are shown in Table 1.1. Tagatose partly absorbed (about 20%) in the intestine, while the unabsorbed portion can be fermented, giving it prebiotic potential (Lipinski, 2006). Furthermore, tagatose also has other health benefits, such as a low glycemic, and it helps prevent obesity and type-2 diabetes. Tagatose is primarily used as a low-caloric sweetener and flavor enhancer in various applications, such as beverage systems and confectionery products. Moreover, tagatose can be used as a food additive for several functions, such as a texturizer, stabilizer, humectant, formulation aid (Vera & Illanes, 2016).

Tagatose has been categorized as a Generally Recognized as Safe (GRAS) substance under U.S. Food and Drug Administration (FDA) regulations since 2001. The use of tagatose in food products is accepted in several countries, including the European Union, Australia, New Zealand, South Africa, South Korea, Brazil and recently, in Canada since 2020 (Armstrong et al., 2009; Health-Canada, 2024; Lipinski, 2006; Skytte, 2006).




D-galactose D-tagatose D-fructose

Figure 1.1 Chemical structures of D-galactose, D-tagatose, and D-fructose

Nowadays, tagatose is available on the market in table sugar form (Figure 1.2a) and is applied in several food products (Figure 1.2b). The price of tagatose on the market is much higher compared to ordinary table sugar (sucrose), which is sold at $0.42 per pound (32 THB/kg). Damhert Nutrition is selling tagatose in table dispenser form for $4.64 per 39 g (4,086 THB/kg), while Nuprevento sells it for $43.48 per 500 g (2,986 THB/kg). Recently, Alusweet by Biofoods has been selling tagatose at $7.19 per 500 g (494 THB/kg) and $4.99 for 360 mL (476 THB/L) of tagatose drops (syrup). These trends show that the price of tagatose is becoming cheaper, but it is still relatively expensive.


Figure 1.2 Commercial tagatose (a) and products containing with tagatose (b)

Source: www.english.cj.net, www.damhert.be, www. alusweet.com, www.gesundheitsmanufaktur.de

Table 1.1 Some chemical and biological properties of tagatose

| Property | Value |
|---|---|
| Energy (kcal/g) | 1.5 – 2.4 |
| Glycemic index (Glucose = 100) | 3±1 |
| Sweetness | 92 of sucrose in 10% w/w solution |
| Solubility in water (g/100 g water at 20 °C) | 58 |
| Stable pH | 5-7 |
| Cooling effect | No |
| Maillard reaction and caramelization | Yes |
| Potential field of application | Low-calorie dietary food, diabetic food, adjuvant |

Source: Kim (2004)

### 1.3 Production of tagatose

Tagatose can be produced through an isomerization reaction of common monosaccharide, especially galactose. Galactose is an aldohexose that can be obtained by hydrolyzing the disaccharide lactose, which is available from whey as a by-product of the dairy industry. Moreover, galactose can also be obtained from the hydrolysis of hemicellulosic polysaccharides, which are found in agricultural and paper wastes (Drabo & Delidovich, 2018).The processes for tagatose production can be divided into two categories.

#### 1.3.1 Enzymatic process

The enzymatic method is a common pathway for tagatose production. The enzyme L-arabinose isomerase has become the enzyme of interest for producing tagatose from galactose because it demonstrates environmentally friendly and high

selectivity, however, due to the equilibrium shift of the isomerization reaction often being higher at elevated temperatures, using L-arabinose isomerase, which is unstable at high temperatures, can result in a lower yield of tagatose (Kim et al., 2003; Oh et al., 2001). Various sources of L-arabinose isomerase from microbial have been reported for use in tagatose production. As high as 68% yield can be obtained from *Thermotoga neapolitana* under the conditions of pH 7.0, 80 °C (Kim et al., 2002). Recently, Zhang et al. (2020) used L-arabinose isomerase from *Bifidobacterium adolescentis* to convert galactose to tagatose, achieving a yield of 56.7%. However, the use of enzymes requires metal co-factors such as $Co^{2+}$ and $Mn^{2+}$, which are restricted in the food industry (Zhao et al., 2023).

### 1.3.2 Chemical process

Besides using enzymatic processes, tagatose can also be produced by chemical methods. The isomerization under chemical catalysts can be divided into two types of mechanisms.

1. Isomerization in the presence of basic catalyst

This well-known theory of carbohydrates conversion under alkaline conditions is referred to as the Lobry de Bruyn–Alberda van Ekenstein transformation. The reaction involves galactose transforming into enediol under basic conditions, which serves as a key intermediate for isomerization. The isomeric form of galactose, tagatose, is formed after protonation at the C-1 atom of the enediol. In addition, the epimeric aldose of galactose, talose, is also formed by protonation at the C-2 atom of the enediol (see Figure 1.3).

2. Isomerization in the presence of Lewis acid catalyst

The isomerization of sugar can also occur through Lewis acid (an electron acceptor compound) catalysis, such as with tin-containing β zeolite (Sn-BEA). It was reported that galactose in its open form is isomerized to tagatose through a hydride shift from the C-2 to C-1 atoms (Figure 1.4). Talose also forms in this reaction as a minor byproduct via a hydrogen atom transfer back from the C-1 to the C-2 atom of tagatose.

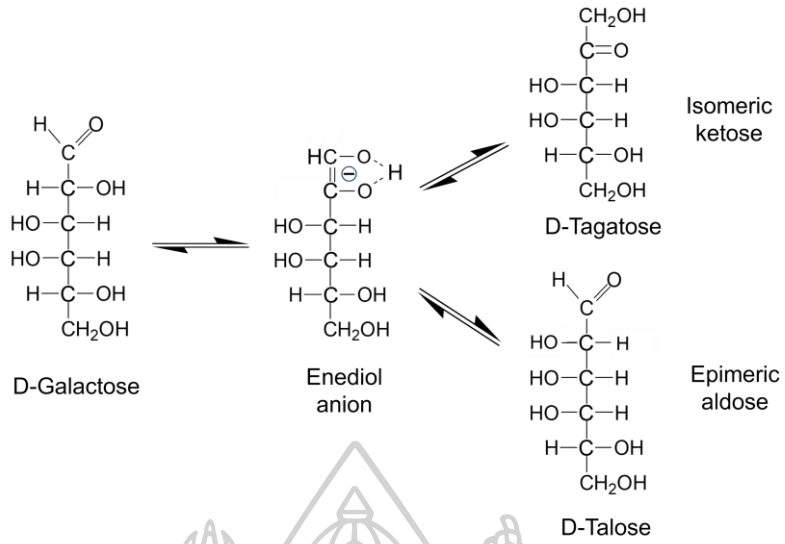



Figure 1.3 Mechanism of galactose isomerization catalyzed by bases through the enediol anion

Source: Delidovich and Palkovits (2016a)

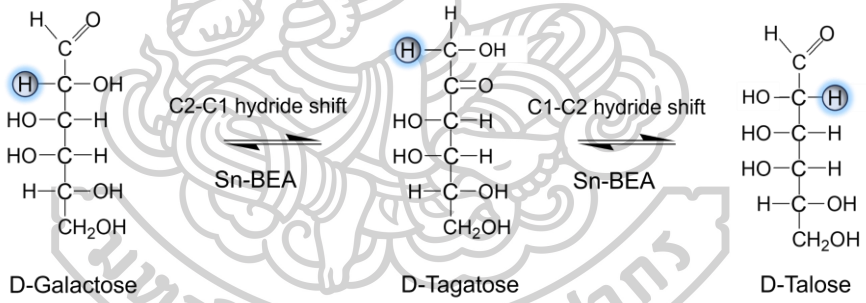



Figure 1.4 Mechanism of galactose isomerization into tagatose by hydride shift

Source: Drabo and Delidovich (2018)

A chemo-catalyst has been applied to commercial processes for the production tagatose using an alkali catalyst since 1992. First, lactose is hydrolyzed by the enzyme lactase into galactose and glucose. The galactose is separated by chromatography and then isomerized using $Ca(OH)_2$ and $Ca_2Cl$ as a catalyst under alkaline condition to obtain calcium hydroxide - tagatose complex, which is neutralized by $CO_2$ to separate tagatose. This process provides 72% yield of tagatose (Beadle et al., 1992). Although this process provided a very high yield, however it had several disadvantages, including

high production costs and low selectivity towards tagatose, because of the formation of numerous by-products such as epimeric counterparts, furfural compounds, and organic acids (Drabo & Delidovich, 2018). Using Lewis acid, it has been reported that 50 mg of Sn-BEA with 10 wt% galactose at a temperature of 80-110 °C for 1-3 h yielded tagatose with a yield of 9-26%. Mg-Al hydrotalcite, a solid base catalyst, yields a tagatose yield of 9-11% at a temperature of 90-100 °C for 1-3 h (Drabo & Delidovich, 2018).

Recently, the 'green' method and catalysts have become a focus of interest for researchers in tagatose production. Subcritical water is water that remains in a liquid state at 100 °C or higher under pressurized conditions. Without any catalyst, tagatose was obtained at only approximately 1% yield from the reaction at 250 °C under subcritical water conditions (Lü & Saka, 2012). Subcritical aqueous ethanol uses ethanol which can be easily removed from the solution and is commonly used in food manufacturing. A 24% yield of tagatose was achieved under conditions of 80% v/v aqueous ethanol at 180 °C (Gao et al., 2015c). Moreover, this technique has been applied to several carbohydrates, such as the isomerization of disaccharides, cellobiose, and lactose (Gao et al., 2016; Soisangwan et al., 2016; Soisangwan, Khuwijitjaru, et al., 2017).

Basic amino acids (lysine, histidine, arginine) are recognized as green catalysts because they are abundant in nature, relatively safe, and environmentally friendly. Recently, isomerization of glucose to fructose with arginine, showing remarkable catalytic activity and achieving a 31% yield of fructose (Yang, Sherbahn, et al., 2016). Similar results have been observed in the isomerization of the pentose ribose to ribulose (Khuwijitjaru & Adachi, 2023a, 2023b).

One of the weaknesses of using basic catalysts is that the pH of the reaction drops during treatment because of the formation of organic acids as by-products of isomerization and degradation of saccharides. This unfavorable reaction might be alleviated by using a buffer. A buffer is a solution that can maintain the pH of a solution. Drabo and Delidovich (2018) uncovered the catalytic activity of a phosphate buffer ($NaH_2PO_4 + Na_2HPO_4$) for the isomerization of galactose, achieving a 10-16% yield of tagatose under reaction conditions of 90-110 °C for 0.5-3 h. Recently, Adachi et al.

(2022; 2021) observed that a pressurized hot phosphate buffer (pH 7.0) could promote the isomerization of maltose to maltulose in batch and continuous reactors, respectively.

For the isomerization of galactose to yield tagatose in a batch reactor at 110 °C, the yields were 16% in arginine and 10% in phosphate buffer solution, respectively (Kobayashi et al., 2023). In a subcritical water continuous reactor containing 10 mM phosphate buffer (pH 7.0), the tagatose yield was reported to be 13% at a reaction temperature of 140 °C (Onishi et al., 2022). Moreover, the tagatose yield did not increase further with buffer concentrations exceeding 50 mM (Onishi et al., 2024), however, the yield could be improved by using a higher temperature of 160 °C to achieve a tagatose yield of 14% (Onishi et al., 2020). In addition, increasing the ethanol concentration up to 60 wt% improved the yield of tagatose from 13% to 16% at 140 °C under subcritical water containing 10 mM phosphate buffer (pH 7.0) (Onishi et al., 2021).

Nowadays, people are placing greater emphasis on health consciousness. To meet the increasing demand for this rare sugar, new practices for tagatose production are needed to make it easier and more practical for commercial scale. Studying on using arginine and buffer as a green catalyst for tagatose production is still limited, therefore this research was aimed to investigate their catalytic activity as eco-friendly alternatives to traditional catalysts, focusing on their efficiency, yield, selectivity and potential to advance the field of tagatose production.

## 1.4 Objectives

1.4.1 To investigate the isomerization of galactose and lactose to tagatose using arginine as a catalyst

1.4.2 To study tagatose formation kinetics and investigate the effect of ethanol concentration on the isomerization of galactose into tagatose

1.4.3 To study the catalytic activity of buffers on the isomerization of galactose to tagatose

# CHAPTER 2
# Isomerization of galactose to tagatose using arginine as a green catalyst

This work investigated the isomerization of galactose to tagatose, a low caloric rare sugar, using arginine as a catalyst. Galactose (5% w/v) and arginine (0.10 mol/mol-galactose) in water were treated at 90–120 °C. The results showed that as the temperature and time increased, galactose was continuously consumed. Rare sugars namely tagatose, talose, and sorbose were formed with the highest yield of 16.8, 2.7, and 3.3%, respectively at 120 °C, 20 min. High temperature and short time conditions resulted in lower Maillard reaction extent. The arginine concentrations at 0.05, 0.10, and 0.15 mol/mol-galactose resulted in a slight increase in tagatose yield while an increase of the initial galactose concentration from 5 to 20% resulted in a decrease in tagatose yield, although the tagatose concentration increased. The highest tagatose productivity of 278 g/(L·h) was obtained using galactose of 20% w/v and arginine of 0.10 mol/mol-galactose at 120 °C and 4 min.

## 2.1 Introduction

Several catalysts which promote the isomerization reaction of common monosaccharides to their isomers via Lobry de Bruyn-van Ekenstein (LBE) transformation have been investigated in the last decade to valorize abundant common monosaccharides and to obtain new functional ingredients (Delidovich, 2021; Delidovich & Palkovits, 2016a).

Among several catalysts, however, relatively safe and environmentally friendly ones or the so-called ‘green catalysts’ are receiving much interest from researchers. Previously, a process using benign solvents, particularly aqueous ethanol, at above their boiling temperatures (so-called subcritical fluid treatment) to produce tagatose has been reported (Gao et al., 2015c; Khuwijitjaru et al., 2018). In addition, treating galactose in phosphate buffer with 60% ethanol at 140 °C provided the highest tagatose yield of 16% (Onishi et al., 2020).

Recently, basic amino acids including arginine, lysine, and histidine have been investigated as catalysts for the isomerization of glucose to fructose, and arginine was found to be the most effective to catalyze the reaction with the highest yield of fructose (Yang, Sherbahn, et al., 2016). It should be noted that, even though there are several studies on the Maillard reaction between various saccharides and amino acids/proteins, only few works have quantified the occurrence of sugar isomerization during the reaction. Isomerization between fructose and glucose during the reaction at various pH was studied by (Kim & Lee, 2008), while the isomerization of glucose to fructose and xylose to lyxose was also reported in the reaction between glucose and xylose with arginine, glutamic acid, glutamine, leucine, lysine, phenylalanine, and γ-aminobutyric acid at pH 6.0 (Lamberts et al., 2008), but a detailed study was not undertaken.

To date, applying arginine to isomerize other common sugars into rare isomers, particularly high-value ones, has never been studied. Therefore, in this study, the application of arginine as a green catalyst for producing high-value tagatose from galactose using a simple batch reaction system was investigated and reported for the first time. In addition, because the Maillard reaction between arginine and reducing sugar is an unavoidable parallel reaction, the extent of the Maillard reaction has to be quantified to find the feasible conditions to obtain high tagatose with low by-products contents.

## 2.2 Materials and methods

### 2.2.1 Materials

D-Galactose (purity > 99%), D-tagatose (purity > 98.5%), and D-talose (purity > 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-Sorbose (purity > 98%) was purchased from TCI (Tokyo, Japan), while l-arginine (purity > 98%) was purchased from Merck (Darmstadt, Germany). Deionized water was used throughout the study.

### 2.2.2 Batch-type galactose isomerization

The galactose isomerization reaction was conducted according to the method used for glucose-fructose isomerization described by Yang, Sherbahn, et al. (2016) with some modifications. Firstly, the effects of temperature and time on isomerization and Maillard reactions were investigated. A solution containing galactose (5% w/v) and arginine (0.10 mol/mol-galactose) was prepared with deionized water. The solution (3 mL) was transferred into several 4 mL-screw cap glass vials and heated in an aluminum block heater (Major Science, Taoyuan City, Taiwan) at different temperatures (90–120 °C). After specific reaction times (0–20 min), three bottles of the treated solution were randomly taken out and cooled down immediately with water to stop the reaction. During the experiment, one bottle with the same solution was inserted with a type-K thermocouple to monitor the temperature during the reaction.

### 2.2.3 Effects of arginine and galactose concentrations

The effects of arginine concentration on galactose isomerization and Maillard reaction were studied using 0.01, 0.05, 0.10, and 0.15 mol/mol-galactose at 5% w/v galactose and 120 °C, while the effect of initial galactose concentration was studied using 5, 10, and 20% w/v at 120 °C with arginine concentration of 0.10 mol/mol-galactose.

## 2.3 Analysis

### 2.3.1 Determination of galactose and its isomers

The treated solution was diluted with 80:20 v/v acetonitrile:water and analyzed for the remaining galactose, the main product, tagatose, and the side products, talose and sorbose, using high-performance liquid chromatography (HPLC). The HPLC system was comprised of an LC-20A pump and a RID-20A refractive index detector (Shimadzu, Kyoto). The sugars were separated on the COSMOSIL Sugar-D column (4.6 mm i.d. × 250 mm) with a guard column (4.6 mm i.d. × 10 mm) (Nacalai Tesque, Kyoto) using 80:20 v/v acetonitrile:water as a mobile phase at a flow rate of 1 mL/min.

The column temperature was maintained at 30 °C using a column heater and the injection volume was 20 µL. The concentration of sugar was calculated from the calibration curve of each standard sugar (Figure A1). Yield (% w/w), conversion of galactose (% w/w), and productivity of tagatose (g/(L·h)) were calculated using the following equations:

$$\text{Yield (\%)} = \frac{\text{Concentration of isomerization product}}{\text{Initial concentration galactose}} \times 100 \quad (2.1)$$

$$\text{Conversion of galactose (\%)} = \frac{\text{Concentration of galactose consumed}}{\text{Initial concentration of galactose}} \times 100 \quad (2.2)$$

$$\text{Productivity of tagatose (g/(L·h))} = \frac{\text{Concentration of tagatose (g/L)}}{\text{Reaction time (h)}} \quad (2.3)$$

### 2.3.2 Determination of Maillard reaction products

Early, advanced, and final products of the Maillard reaction were qualitatively monitored by measuring the absorbance values at 280, 325, and 420 nm, respectively (Echavarría et al., 2014). The treated solution was measured for pH using a pH meter (SevenCompact S220, Mettler Toledo, Columbus, OH, USA).

## 2.4 Statistical analysis

Analysis of variance (ANOVA) and significant differences between means from the triplicate experiment at a significant level of 0.05 by Duncan's multiple range test were performed using PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

## 2.5 Results and discussion

### 2.5.1 Effects of temperature and time on isomerization of galactose to tagatose

The reaction temperature started at room temperature (~32 °C), rapidly increased in the first 5 min, and eventually reached the desired temperatures within 10 min. The degradation of galactose occurred immediately after the initial heating and

also during the temperature-rising period. The result showed that galactose continuously decreased with the reaction time and temperature. It was clearly seen from the reaction at 120 °C that galactose decreased slowly at the first 2 min, which might be because the temperature was relatively low, rapidly decreased during 2–6 min, and then started to level off thereafter. At 20 min, the remaining galactose were 80.2, 73.6, 66.8, and 61.5% for the reaction temperature of 90, 100, 110, and 120 °C, respectively.

Three isomers of galactose, namely tagatose, talose, and sorbose were detected and quantified. Tagatose was the main product of the isomerization reaction, and its amount increased with the temperature and reaction time corresponding to the decrease of galactose. At 90 and 100 °C, the yields of tagatose continuously increased with time, while at higher temperatures, particularly at 120 °C tagatose rapidly increased in the first 6 min and then reached the plateau after 10 min. The reaction at 110 and 120 °C gave the highest yield of 16.5 and 16.8%, respectively, which were not significantly different ($p < 0.05$).

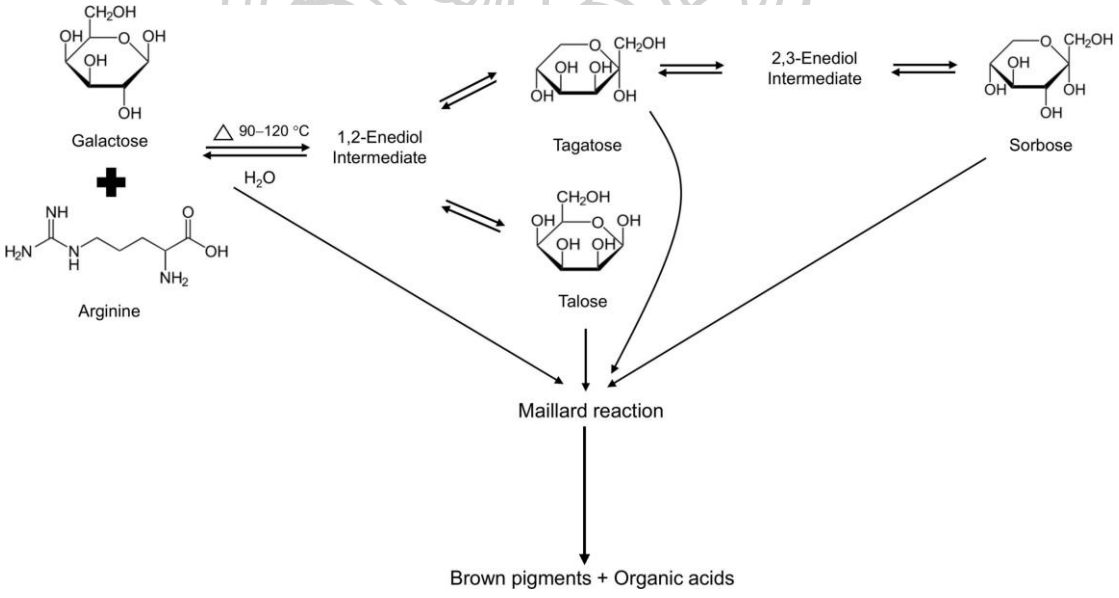



Figure 2.1 Reaction scheme for isomerization of galactose to tagatose, talose, and sorbose and Maillard reaction in aqueous solution with arginine

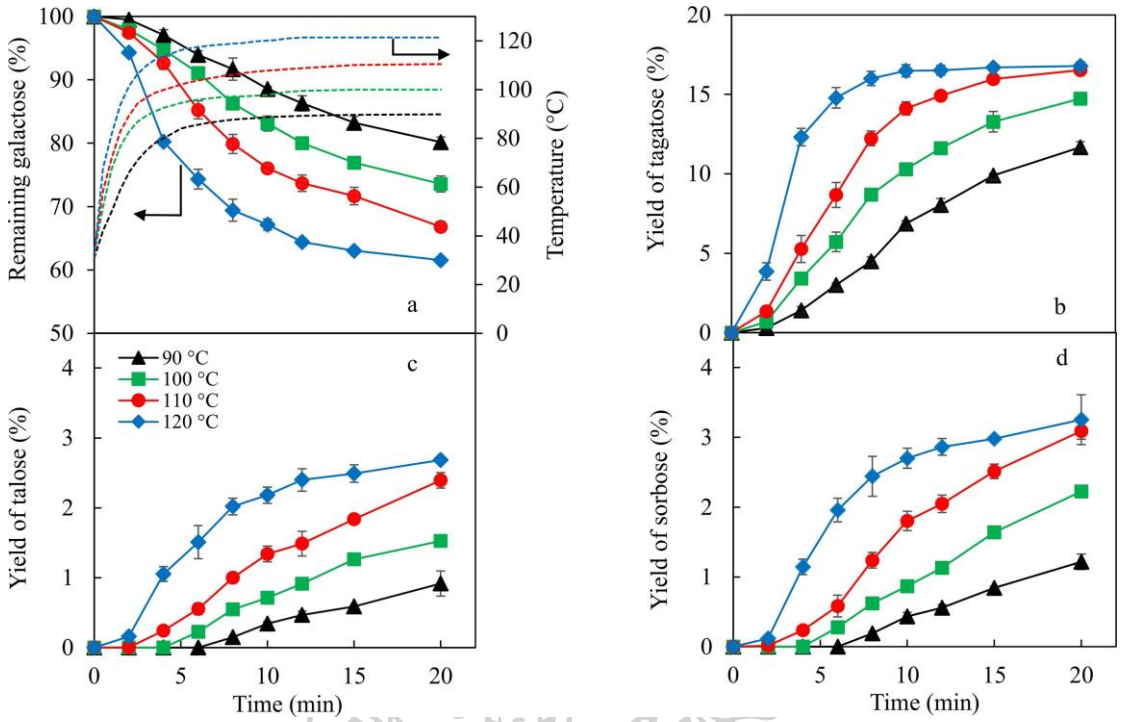



Figure 2.2 Isomerization of galactose (5% w/v) using arginine (0.10 mol/mol-galactose) as a catalyst at different temperatures: (a) remaining galactose, (b) yield of tagatose, (c) yield of talose, (d) yield of sorbose

This limitation of tagatose yield has been reported in other base-catalyzed isomerization of galactose to tagatose as well. For example, the highest tagatose yield of 16.5% was reported for the subcritical phosphate buffer (10 mmol/L, pH 7.0) with 60% ethanol at 140 °C (Onishi et al., 2021). In addition, using phosphate and Mg-Al hydrotalcite as base catalysts also provided the highest tagatose yield of 16 and 11%, respectively (Drabo & Delidovich, 2018). Using KOH as a classical catalyst for LBE transformation at pH 11.5 and 25 °C for 14 days resulted in a slightly higher tagatose yield of 18% (El Khadem et al., 1989). The results demonstrated that the production of tagatose from galactose can be accomplished using arginine as a catalyst within a shorter time by raising the temperature to above 100 °C.

Other isomerization products, talose and sorbose were also determined. The yields of talose and sorbose increased with the temperature and reaction time as well. The yield of sorbose was slightly higher than that of talose. The highest yields of

sorbose (3.3%) and talose (2.7%) were obtained at 120 °C after 20 min of reaction. These values were slightly higher than those reported by other authors (Gao et al., 2015c; Onishi et al., 2021; Onishi et al., 2020). These results suggested that under the alkaline conditions of arginine solution, galactose isomerized to tagatose and other isomers via LBE transformation (Figure 2.1). The reaction started with the transformation of cyclic to acyclic galactose, then deprotonation of galactose to 1,2-enediol intermediate, and formation of tagatose (ketose isomer) after protonation at C-1. Sorbose and talose are side-reaction products of the isomerization of galactose. Sorbose (C-3 epimer of tagatose) was formed after the rearrangement of tagatose into 2,3-enediol intermediate (Drabo & Delidovich, 2018), while talose (C-2 epimer of galactose) occurred by the protonation at C-2 of the 1,2-enediol intermediate (Delidovich & Palkovits, 2016a).

From the relationships between the yields of the rare isomers and the conversion of galactose in Figure 2.3a, it can be seen that when the conversion of galactose was 25% or less, the yield of tagatose was proportional to the conversion of galactose regardless of the temperature, and its slope, that is, the selectivity of tagatose was approximately 0.61. The dashed line indicates the relationship between the conversion of galactose and the yield when the selectivity is one. However, after the reaction progressed further, the plot eventually reached a plateau at approximately 16%, which was the maximum yield found in this study. Similarly, when the conversion of galactose was 25% or less, the yields of both talose and sorbose were proportional to the conversion of galactose. At the galactose conversion of 25% or more, the yields gradually approached respective constant values. Nevertheless, all data from different temperatures aligned on the same line for each monosaccharide, which suggested that the mechanisms of tagatose, talose, and sorbose formation were similar at these temperatures.

To demonstrate the efficiency of the reaction system for tagatose production in this study, the productivity of tagatose is shown in Figure 2.3b. Initially, the productivity increased rapidly with time at every reaction temperature and much faster at higher temperatures. After that, the productivity reached a peak and began to decline with time. The low productivity at 2 min is ascribed to the low concentration of

produced tagatose because the reaction temperature did not reach the preset one. The result indicated that the reaction at 120 °C and 4 min provided the highest productivity of 92.4 g/(L·h). This value was relatively high compared to other studies. Gao et al. (2015c) reported the productivity of 50 g/(L·h) in the subcritical aqueous ethanol treatment (60% v/v) at the residence time of 8.33 min and 180 °C using a continuous tubular reactor. The enzymatic process is usually low in productivity because of the long reaction time. In the enzymatic isomerization using an L-arabinose isomerase, the highest productivity of tagatose was 15.3 g/(L·h) for the reaction time of 20 h (Lim et al., 2007) and 6.66 g/(L·h) for the reaction time of 48 h (Bober & Nair, 2019). In addition, the patented process (Beadle et al., 1989), which used 15 kg galactose in 150 L of reaction mixture with calcium hydroxide to form calcium hydroxide-tagatose complex and calcium chloride as a catalyst, provided a very high yield of tagatose (72%) within 1.5 h of the reaction, however, from our calculation, the productivity was approximately 48 g/(L·h) and the method also needed an additional neutralization step. The results clearly showed that the method in this study was simpler and more efficient for tagatose production.

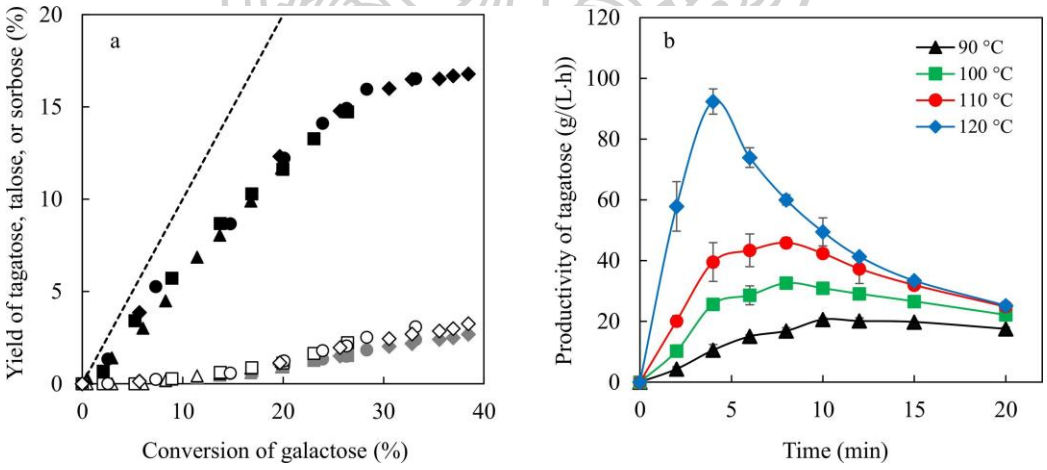



Figure 2.3 (a) Relationship between yields of tagatose (black), talose (gray), and sorbose (white) (%) and conversion of galactose (%), (b) Productivity of tagatose for the isomerization of galactose (5% w/v) using arginine (0.10 mol/mol-galactose) as a catalyst at 90–120 °C, 0–20 min. Triangle, square, circle, and diamond symbols represent the results obtained at 90, 100, 110, and 120 °C, respectively. The dashed line

indicates where galactose is completely converted to another isomer without any side reactions

### 2.5.2 Changes in pH

Arginine contains a guanidinium group in its side chain which gives strong basicity to the molecule (Xu et al., 2017). The pH of the untreated solution containing 5% of galactose and arginine of 0.10 mol/mol-galactose was 10.01 ± 0.04. After heating, the pH value of the treated solution almost linearly decreased with the reaction time and the rates tended to be higher at higher temperatures as shown in Figure 2.4a. The final pH values after 20 min of reaction were 9.46, 9.12, 8.47, and 7.20 at 90, 100, 110, and 120 °C, respectively. These facts suggest that acidic substances, which are by-products, are continuously produced even after the isomerization reached equilibrium. The arginine molecules themselves were degraded during the Maillard reaction through thermal degradation and condensation reactions with carbonyl compounds (Körner, 2021). The decrease in pH could also be attributed to the formation of carboxylic acids from monosaccharide degradation under alkaline conditions (De Bruijn et al., 1987). In addition, organic acids such as levulinic acid, formic acid, and acetic acid could be formed during the Maillard reaction (Martins et al., 2000). The reduction of pH was found in the isomerization of sugars in other base-catalyzed reactions as well (Carraher et al., 2015; Onishi et al., 2021; Renn & Sathe, 1997).

### 2.5.3 Maillard reaction

Maillard reaction is a non-enzymatic browning reaction between reducing sugar and amino acids during heating treatment, and a high pH condition normally enhances the reaction (Damodaran & Parkin, 2017). Figure 2.4b−d show the absorbance values of the treated solution after multiplication with the dilution factors, the absorbance values at 280, 325, and 420 nm, which have been reported to be related to the Maillard reaction products at different stages (Delgado-Andrade et al., 2010; Echavarría et al., 2014). In particular, absorbance at 420 nm has been generally measured as an indicator of browning development (Ashoor & Zent, 1984; Kim & Lee, 2008). The absorbance

values at 280 nm and 325 nm were much higher than those at 420 nm, which were similar to other studies (Echavarría et al., 2014; Kim & Lee, 2008). Figure A2 illustrates the appearances of the treated solutions and they clearly reflected the changes in browning during the isomerization reaction of galactose under different conditions. The formation of brown pigments during the isomerization of sugar at high temperatures is generally of concern. Color removal with activated carbon is one of the major downstream processing steps to obtain target substances from the hydrothermal process, including tagatose production (Vera & Illanes, 2016). A treated solution with lower color pigments is therefore preferred. Similarly to the discussion in the previous paragraph, it was found that at 120 °C and 20 min, which provided the highest yield of tagatose, the absorbance at 420 nm was also the highest (5.76), while at 120 °C and 4 min, which provided the highest productivity, the absorbance at 420 was only 0.43.

Figure A3 shows the changes in galactose and the isomerized sugar with the pH of the solution. Although previous studies (Onishi et al., 2021; Onishi et al., 2020) suggested that the LBE transformation of galactose in a phosphate buffer system did not proceed at pH 6.5 or less, in this study, it was found that the yields of isomerization products were almost constant at a much higher pH of 8.5 or less. This suggested that the isomerization reactions already reached equilibrium at pH 8.5. On the other hand, the remaining galactose gradually decreased even at pH 8.5 or less. Furthermore, even at pH 8.5 or less, the Maillard reaction products increased linearly as the pH decreased. Therefore, the reaction that produces the by-products can proceed independently of pH change.

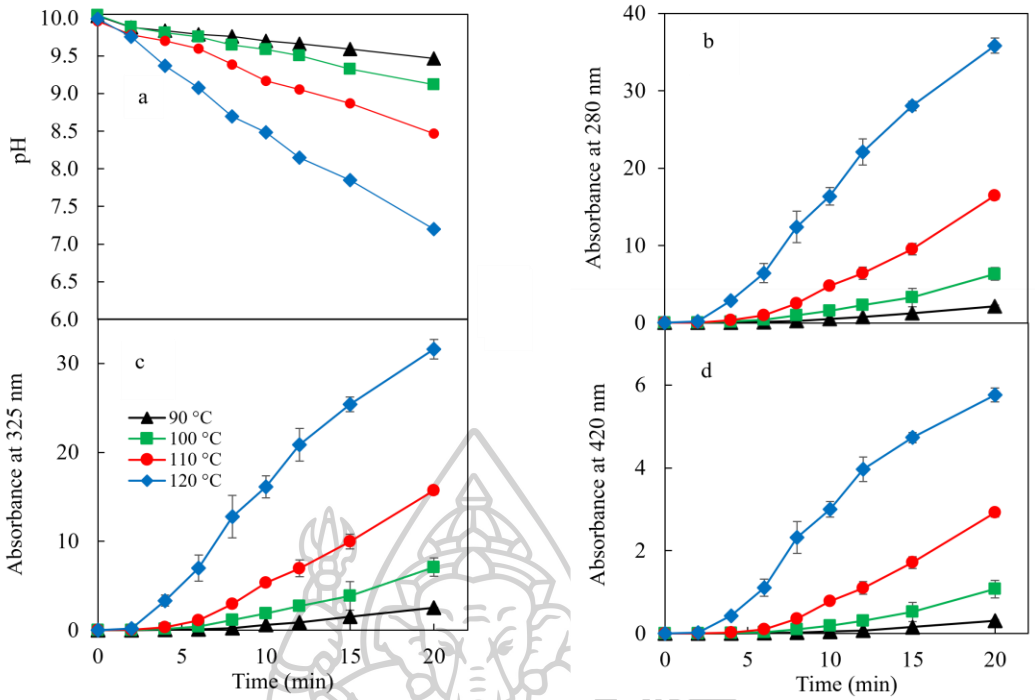



Figure 2.4 (a) pH; the absorbance values at (b) 280, (c) 325, and (d) 420 nm of the treated solution with galactose (5% w/v), arginine (0.10 mol/mol-galactose)

### 2.5.4 Effect of arginine concentration on isomerization of galactose

The effect of arginine concentration on the isomerization of 5% w/v galactose at 120 °C is shown in Figure A4. The values at 20 min of the reaction are also shown in Table 2.1. Similar patterns of time-course of galactose consumption and the formation of tagatose, talose, and sorbose were found for every arginine concentration. The initial pH of the solution was significantly higher at higher arginine concentrations but was all in a high alkalinity range (9.52 to 10.06). The results showed that increasing arginine concentration promoted the consumption of galactose and the formation of tagatose, talose, and sorbose. Increasing the arginine concentration from 0.01 to 0.15 mol/mol-galactose resulted in decreasing in galactose from 85.3 to 57.9% and increased the yield of tagatose from 11.5 to 17.3% at 20 min of the reaction. The highest productivity was obtained at 4 min of the reaction for every arginine concentration (Figure A5). Yang, Sherbahn, et al. (2016) also reported an increase in fructose yield

from glucose when the arginine concentration was increased from 0.05 to 0.10 mol/mol-glucose.

Even though the initial pH values of the solutions were different, the pH of all solutions linearly decreased during the reaction (Figure A6). The arginine concentration also significantly affected the degree of Maillard reaction. The results showed that increasing arginine concentration resulted in the absorbance values at 280, 325, and 420 nm of the treated solution. This indicated that the formation of the Maillard reaction products was accelerated at higher arginine concentrations. Because of the non-significant differences in the yield and productivity of tagatose that were obtained at the arginine concentrations of 0.10 and 0.15 mol/mol-galactose ($p > 0.05$), 0.10 mol/mol-galactose was chosen to investigate the effect of initial galactose concentration.

### 2.5.5 Effect of initial galactose concentration

Table 2.1 also shows the influence of the initial galactose concentration with arginine of 0.10 mol/mol-galactose at 120 °C, 20 min. The time courses of reaction at 0–20 min are shown in Figure A7. The results showed that increasing the initial galactose concentration resulted in significantly lower consumption of galactose ($p < 0.05$). Regarding the yield of tagatose, it was found that the yield was also significantly lower at higher galactose concentrations, with the highest yield of 16.8% at 5% w/v galactose. This could be attributed to the higher rates of pH lowering during the reaction at higher initial galactose concentrations, as can be seen in Table 2.1 and Figure A8a. The final pH at 20 min of reaction for 10 and 20% w/v initial galactose was as low as 6.63 and 6.23, respectively, compared to the value of 7.20 for initial galactose concentration of 5% w/v. This could be attributed to the higher concentration of acidic products during the reactions, as discussed earlier. However, the tagatose productivity significantly increased with the initial galactose concentration.

At the reaction time of 4 min, which provided the highest productivity at every galactose concentration, the productivity linearly increased with the initial galactose

concentration i.e. 92.4, 160.1, and 278 g/(L·h) for the initial galactose concentrations of 5, 10, and 20% w/v, respectively (Figure 2.5).

The time courses of the Maillard reaction (Figure A8b–d) revealed that the absorbance values at 280, 325, and 420 nm of the treated solutions also increased with increasing galactose concentrations, and the color of the solution changed to dark brown at a higher initial galactose concentration. However, at 4 min of reaction, which provided the highest productivity of tagatose, the absorbance at 420 nm were 0.43, 1.08, and 1.13 at the initial galactose of 5, 10, and 20% w/v, respectively, which were approximately 1/14–1/19 of those at 20 min.

In conclusion, the best conditions to provide the highest yield of tagatose were 5% w/v galactose with arginine of 0.15 mol/mol-galactose at 120 °C and 20 min. However, using 20% w/v galactose with arginine of 0.10 mol/mol-galactose at 120 °C and 4 min provided the highest productivity of tagatose with low Maillard reaction product contents.

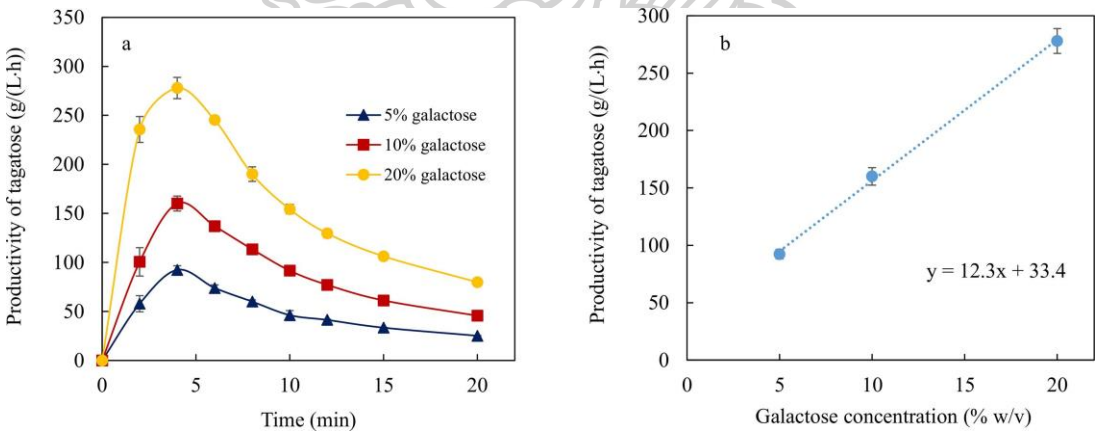



Figure 2.5 (a) Productivity of tagatose in the solution containing different initial concentrations of galactose (5–20% w/v) with arginine of 0.10 mol/mol-galactose at 120 °C. (b) Relationship between productivity of tagatose at the reaction time of 4 min and initial concentration of galactose

Table 2.1 Isomerization of galactose with arginine at 120 °C, 20 min

| Galactose/arginine concentration | Remaining galactose (%) | Yield of tagatose (%) | Yield of talose (%) | Yield of sorbose (%) | Productivity of tagatose (g/(L·h) | Initial pH | pH |
|---|---|---|---|---|---|---|---|
| 0.01 | 85.3±1.15$^{a}$ | 11.5±0.63$^{c}$ | 0.8±0.01$^{d}$ | 0.7±0.10$^{d}$ | 17.3±0.94$^{c}$ | 9.52±0.01$^{d}$ | 6.80±0.14$^{c}$ |
| 0.05 | 70.9±1.46$^{b}$ | 16.1±0.36$^{b}$ | 2.1±0.12$^{c}$ | 2.3±0.22$^{c}$ | 21.4±4.17$^{b}$ | 9.86±0.01$^{c}$ | 7.06±0.05$^{b}$ |
| 0.10 | 61.5±0.21$^{c}$ | 16.8±0.28$^{ab}$ | 2.7±0.01$^{b}$ | 3.3±0.36$^{b}$ | 25.2±0.41$^{ab}$ | 9.99±0.02$^{b}$ | 7.20±0.07$^{ab}$ |
| 0.15 | 56.9±0.09$^{d}$ | 17.3±0.21$^{a}$ | 3.3±0.09$^{a}$ | 3.8±0.15$^{a}$ | 26.0±0.32$^{a}$ | 10.06±0.01$^{a}$ | 7.27±0.04$^{a}$ |
| Isomerization of galactose at different concentrations (% w/v) with 0.10 mol/mol-galactose of arginine | | | | | | | |
| 5 | 61.5±0.21$^{c}$ | 16.8±0.28$^{a}$ | 2.7±0.01 | 3.3±0.36$^{a}$ | 25.2±0.41$^{c}$ | 9.99±0.02$^{a}$ | 7.20±0.07$^{a}$ |
| 10 | 63.7±0.60$^{b}$ | 15.2±0.23$^{b}$ | 2.8±0.10 | 2.7±0.14$^{b}$ | 45.6±0.68$^{b}$ | 9.96±0.01$^{b}$ | 6.63±0.02$^{b}$ |
| 20 | 66.4±0.60$^{a}$ | 13.3±0.48$^{c}$ | 2.7±0.12 | 2.5±0.14$^{b}$ | 79.8±2.89$^{a}$ | 9.89±0.01$^{c}$ | 6.23±0.04$^{c}$ |

Values are expressed as mean ± standard deviation. Different letters in a same column indicate significant difference ($p < 0.05$). ns: not significant

## 2.6 Conclusions

In this study, for the first time, the isomerization of galactose to tagatose, a high-value rare sugar, using arginine as a catalyst was demonstrated under several conditions. The highest yield of tagatose of 16.8–17.3% was similar to other alkaline catalyzed isomerization of galactose. However, the highest productivity of 278 g/(L·h) found in this study was higher than those reported in the literature and showed the high potential of this method for tagatose production using this common food ingredient in a simple reaction system. Maillard reaction, which also inevitably occurred, could be suppressed to a relatively low extent which is favorable for further purification processes by performing the reaction at high temperature and short reaction time. Further study using a tubular reactor that is able to reach the desired temperature quicker should be conducted to precisely evaluate the kinetic parameters of reactions.

# CHAPTER 3
# Isomerization of lactose to lactulose in an aqueous solution containing arginine

In the present work, the isomerization of lactose (5%, w/v) to lactulose in an aqueous solution containing arginine (0.1 mol/mol lactose) with an initial pH of 9.80 was investigated. The consumption of lactose, and formation of lactulose and other monosaccharides (glucose and galactose) were monitored to evaluate the effects of reaction temperature (100, 110, and 120 °C) and time (0 - 20 min) on the isomerization and hydrolysis of lactose. The results showed that lactulose was formed during heating, and that the lactulose yield reached its maximum value more rapidly at higher temperature. The highest yield, approximately 26% w/w, was obtained after the reaction proceeded for 12 min at 120 °C. The progress of the Maillard reaction was monitored by measuring the absorbances at 280, 325, and 420 nm, and these parameters increased with both reaction temperature and time, whereas the pH gradually decreased. The present work demonstrated that lactose can be conveniently isomerized into its rare isomer using an environmentally friendly process.

## 3.1 Introduction

Lactose, a sugar naturally found in milk, has been converted into several functional food ingredients, such as lactulose, epilactose, galacto-oligosaccharide, and lactitol (Seki & Saito, 2012). Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is used to treat constipation, and has several health benefits as a prebiotic (Olano & Corzo, 2009; Schuster-Wolff-Bühring et al., 2010). Lactulose is obtained in small quantities from milk after thermal processing (Adachi & Patton, 1961), and has been investigated as an indicator of the heat treatment intensity of milk (de Oliveira Neves et al., 2018).

Several methods for the direct production of lactulose by the isomerization of lactose have been proposed (Karim & Aider, 2022). One method involves the conversion of the glucose unit in the disaccharide structure to a fructose unit through alkaline isomerization, and is known as Lobry de Bruyn-Alberda van Ekenstein

transformation (Schuster-Wolff-Bühring et al., 2010). Lactulose can also be produced enzymatically using galactose and fructose or lactose as substrates through transgalactosylation by β-galactosidase (Guerrero et al., 2011; Song et al., 2013a) or by β-galactosidase and glucose isomerase (Song et al., 2013b). In addition, cellobiose 2-epimerases can also effectively produce lactulose and epilactose from lactose (Jameson et al., 2021; Kim & Oh, 2012). However, enzymatic methods have a major drawback in terms of the cost of enzyme production. Therefore, a chemical process based on an alkaline catalyst is currently used for lactulose production (Aider & Halleux, 2007).

Besides the common bases used for the isomerization of sugars, such as sodium hydroxide (Dendene et al., 1994; Hajek et al., 2013; Hashemi & Ashtiani, 2010) which provided the maximum yield of 27.9% at pH 11 and 70°C (Hashemi & Ashtiani, 2010), other bases have been investigated for their use as catalysts in lactulose production. Formation of lactulose from lactose using ammonium carbonate provided 29.6% yield at 97 °C (Seo et al., 2016), whereas using carbonate-based catalysts, such as oyster shell powder and limestone, provided 18-21% yield (Paseephol et al., 2008). Alternatively, the isomerization of lactose in subcritical aqueous ethanol at high temperatures provided a maximum yield of 34% in 60% ethanol at 200 ℃ (Soisangwan, Gao, et al., 2017). Recently, Karim and Aider (2020) and Djouab and Aïder (2019) reported electroisomerization technologies for lactulose isomerization with the highest yields of 38 and 39.8%, respectively.

Arginine contains a guanidinium group, making it the most basic amino acid. It is readily available, and normally used as a nutritional additive. Although studies on the Maillard reaction of various sugars with amino acids, including arginine, have been published by several authors, the formation of ketose sugars from their aldose isomers has been mentioned in only a few studies. The potential of arginine as a catalyst for the production of rare ketose sugars from aldose sugars has been investigated for the isomerization of galactose to tagatose (Chapter 2) (Milasing et al., 2023) and ribose to ribulose (Khuwijitjaru & Adachi, 2023a). Nevertheless, investigations on the use of arginine as a catalyst for the preparation of rare sugars are limited.

Therefore, the objective of the present work was to determine whether arginine could directly catalyze the isomerization of disaccharide. Lactose was used as the

substrate for lactulose production at various temperatures and reaction times. Moreover, the potential use of lactose, which is significantly cheaper than galactose, as a raw material for the direct production of tagatose was also investigated.

## 3.2 Materials and methods

### 3.2.1 Reagent

Lactose monohydrate was purchased from KEMAUS (New South Wales, Australia). Lactulose (purity > 98.0%) was purchased from TCI (Tokyo, Japan). Galactose (> 99%), and glucose (> 99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Arginine (purity > 98%) was purchased from Merck (Darmstadt, Germany). Deionized water was used throughout the study.

### 3.2.2 Isomerization of lactose

Isomerization of lactose was performed in a screw-capped glass vial (total volume, 4 mL). A solution of lactose (5%, w/v) and arginine (0.1mol/mol lactose) was prepared in deionized water. The solution (3 mL) was then transferred to a glass vial. The vial was tightly closed, and heated in an aluminium block heater (Major Science, Taoyuan City, Taiwan) to the pre-determined temperatures (100, 110, and 120 °C). A lactose solution without arginine was used as a control at all temperatures. A type-K thermocouple was used to monitor the temperature of one vial containing the same solution. At 2, 4, 6, 8, 10, 12, 15, and 20 min, one vial was randomly taken, immediately cooled to room temperature using an ice-water bath, and then analyzed for chemical properties. All treatments were conducted in triplicates.

### 3.2.3 Sugar analyses

Lactose ($C_S$), lactulose ($C_P$), galactose, and glucose contents in the reaction solution were analyzed using a high-performance liquid chromatograph which consisted of an LC-20A pump and an RID-20A refractive index detector (Shimadzu,

Kyoto, Japan). COSMOSIL Sugar-D column (4.6 mm i.d. × 250 mm) with a guard column (4.6 mm i.d. × 10 mm) (Nacalai Tesque, Kyoto) was used for chromatographic separation with 80:20 v/v acetonitrile:water as a mobile phase at a flow rate of 1.0 mL/min. The column temperature was maintained at 30 °C using a column heater (Fortune Scientific, Bangkok, Thailand), and the injection volume was 20 µL. A calibration curve was constructed for each sugar using reference standards. Under the analytical conditions, galactose and glucose were co-eluted as a single peak, thus expressed as monosaccharide ($C_{mono}$). The mass ratio of each sugar to initial lactose ($C_{S0}$) was calculated.

### 3.2.4 Maillard reaction extent

To determine the extent of Maillard reaction during isomerization, the absorbances at 280, 325, and 420 nm were measured using a UV-vis spectrophotometer (Genesis 10s, Thermo Scientific, Waltham, MA, USA) to represent the early, intermediate, and final stages of the Maillard reaction, respectively (Echavarría et al., 2014).

## 3.3 Results and discussion

### 3.3.1 Appearances

Lactose isomerization was performed in a batch-type vial. As shown in Figure 1a, the temperatures of the reaction solutions increased rapidly in the first 2 - 4 min, and then gradually increased to the chosen temperatures within 10 min. Figure 3.1b shows changes in the appearance of the reaction solutions. The lactose solution samples without arginine appeared the same at all temperatures (only the samples at 120 °C are shown). It was clear that during the reaction, the brown color intensified with reaction time, and the intensification accelerated at higher temperatures. Since lactose, lactulose, galactose, and glucose are reducing sugars, they reacted with arginine via the Maillard reaction, leading to browning pigment formation. In addition, the thermal degradation of monosaccharides led to brown pigment formation (Woo et al., 2015). A brown color

after a prolonged reaction also appeared in the other base-catalyzed isomerization of sugars (Liu et al., 2014). Brown pigments are undesirable by-products of rare sugar production, and must be removed during downstream processing.

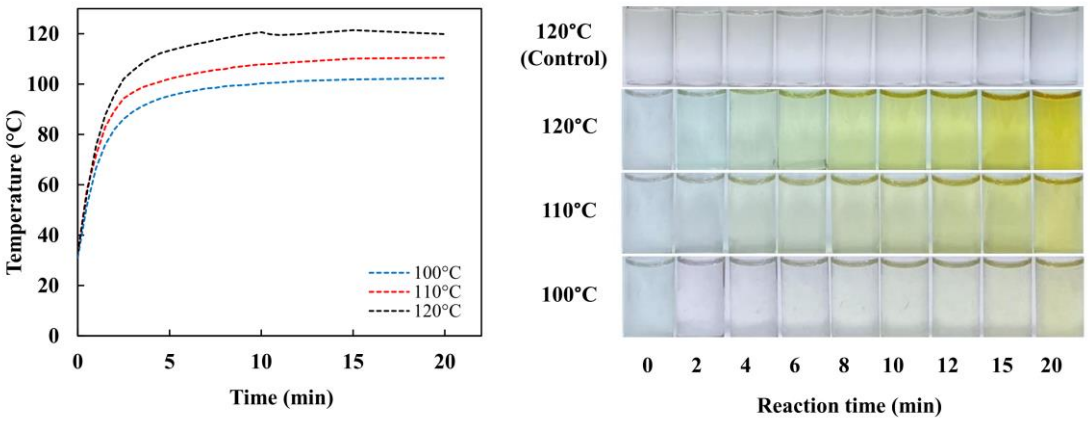



Figure 3.1 Temperature profiles of the reaction solutions containing 5% w/v lactose and 0.1 mol/mol lactose of arginine (a). Changes in appearances of treated solutions with time at various reaction temperatures (b)

### 3.3.2 Isomerization and hydrolysis of lactose

The changes in the lactose ($C_S/C_{S0}$), lactulose ($C_P/C_{S0}$), and monosaccharide (glucose and galactose) ($C_{mono}/C_{S0}$) contents with reaction time at various temperatures are shown in Figure 3.2 Lactose content gradually decreased with reaction time, suggesting that lactose either isomerized under alkaline conditions to form lactulose, reacted with arginine in the Maillard reaction, or simply hydrolyzed into monosaccharides under high temperature conditions. As the temperature increased, lactose was consumed to a greater extent. The final $C_S/C_{S0}$ values after 20 min at 100, 110, and 120 °C were 0.69, 0.69, and 0.51, respectively. Lactulose was not present in the initial solution, but was detected in the treated solution, and its content gradually increased with reaction time, and reached a plateau after a certain time, suggesting that the isomerization has reached equilibrium. At 120 °C, lactulose ratio reached a constant value after 12 min. The highest lactulose yields, $C_P/C_{S0}$ at 100, 110, and 120 °C were 0.23, 0.27, and 0.26, respectively. These values agreed with the reported yields obtained from isomerization using sodium hydroxide (Hajek et al., 2013; Hashemi & Ashtiani,

2010). Moreover, since only arginine was used in the present work to promote isomerization, this can be considered as a green process. Although cellobiose-2-epimerase can afford a considerably high lactulose yield (58%) from lactose, with a productivity of 204 g/(L·h) (Kim & Oh, 2012), the commercial preparation of the enzyme is expensive. In contrast, arginine is widely used as a food ingredient, and can be used for the isomerization of a wide variety of sugars (Khuwijitjaru & Adachi, 2023a; Milasing et al., 2023; Yang, Sherbahn, et al., 2016). Lactulose productivity at an initial lactose concentration of 5% w/v is only 67.5 g/(L·h). However, a previous study on galactose isomerization (Milasing et al., 2023) suggested that increasing the initial lactose concentration might improve the productivity. In the present work, no decrease in lactulose was observed during the reaction time of 20 min, indicating that lactulose degradation did not progress at a considerable rate. Many studies have reported a decrease in lactulose levels after long reaction times (Hashemi & Ashtiani, 2010; Soisangwan, Gao, et al., 2017). The monosaccharide (galactose and glucose) contents ($C_{mono}/C_{S0}$) slowly increased with reaction time; however, the highest values at 20 min were only 0.02 - 0.04. These monosaccharides might be the products of lactose hydrolysis. There was no change in the lactose concentration, and no lactulose was found in the samples without arginine at any temperatures; therefore, it can be concluded that thermal treatment alone did not result in the isomerization of lactose within the reaction time of 20 min used in the present work.

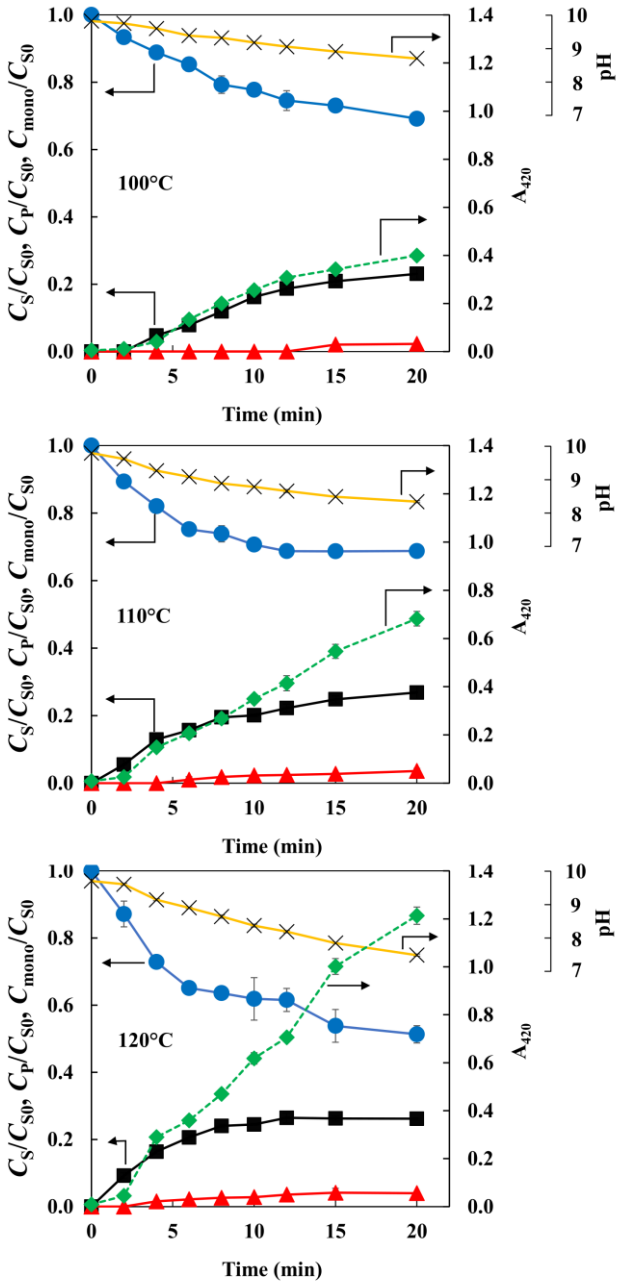



Figure 3.2 Changes in mass ratios of lactose ($C_S/C_S$, circle), ($C_P/C_{S0}$, square), monosaccharide ($C_{mono}/C_{S0}$, triangle), pH (cross), and absorbance at 420 nm (diamond) during heating of the reaction solution containing 5% w/v lactose and 0.1 mol/mol lactose of arginine

### 3.3.3 Degradation products and discoloration

Figure 3.2 also shows the changes in the pH and absorbance at 420 nm ($A_{420}$) of the treated solution at different reaction temperatures and times. The initial pH of the solution was approximately 9.80, which was attributed to the alkaline guanidinium group of arginine. During the reaction, pH continuously decreased with reaction time. At higher temperatures, the rates of pH decrease were higher, and the final pH values at 20 min were 8.71, 8.34, and 7.49 at 100, 110, and 120 °C, respectively. The decrease in pH indicated the formation of acidic products during the reaction. The dehydration and Maillard reaction of sugars can lead to the formation of formic, levulinic, lactic, and acetic acids (Aida et al., 2007; De Bruijn et al., 1987). $A_{420}$, which is a simple measurement for the final stage products of the Maillard reaction, increased with reaction time, and it was higher at higher temperatures. These results agreed with the previously mentioned changes in browning. At the highest lactulose yields of 100, 110, and 120 °C, the $A_{420}$ values were 0.40, 0.68, and 0.71, respectively. This suggested that the reaction temperature of 120 °C was more efficient as it provided the highest yield within a shorter time, and with slightly higher browning pigment content.

### 3.3.4 Relationship between pH and isomerization reaction

As shown in Figure 3.3, the changes in the lactose ($C_S/C_{S0}$) and lactulose ($C_P/C_{S0}$) ratios as functions of pH at different temperatures are almost the same. This suggested that the mechanisms of lactose consumption and lactulose formation are similar at different temperatures. Isomerization of lactose to lactulose in the presence of arginine may follow the Lobry de Bruyn-Alberda van Ekenstein transformation, which occurs in alkaline solutions. In aqueous solutions, arginine provides $OH^-$ ions by the protonation of the guanidinium group, which has a p*K*a of 12.5 or even higher (Fitch et al., 2015). The $OH^-$ ion reacts with the glucose moiety of lactose to form 1,2-enediol intermediate and converts it to a fructose molecule (Figure 3.4) (Wang, Wang, Lyu, Hua, et al., 2022).

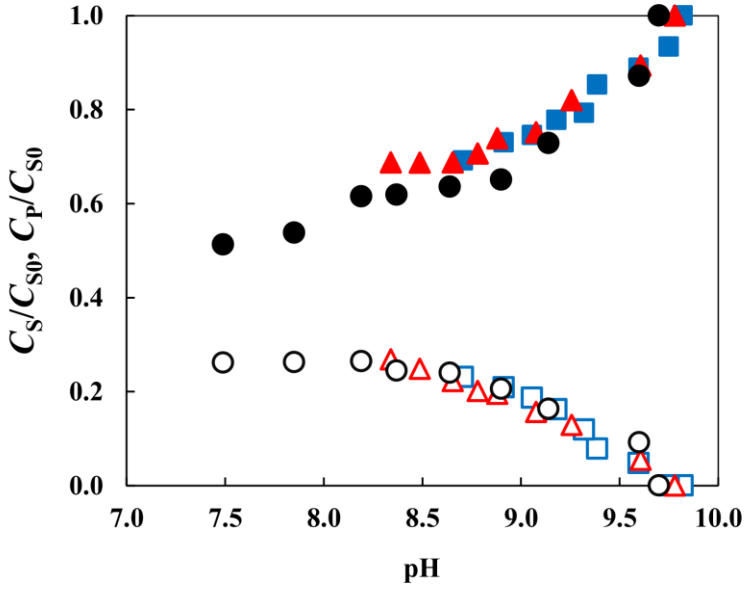



Figure 3.3 Changes in mass ratios of lactose ($C_S/C_{S0}$, closed symbols) and lactulose ($C_P/C_{S0}$, open symbols) with the solution pH during heating of the reaction solution containing 5% w/v lactose and 0.1 mol/mol lactose of arginine at 100 (square), 110 (triangle), and 120 °C (circle)

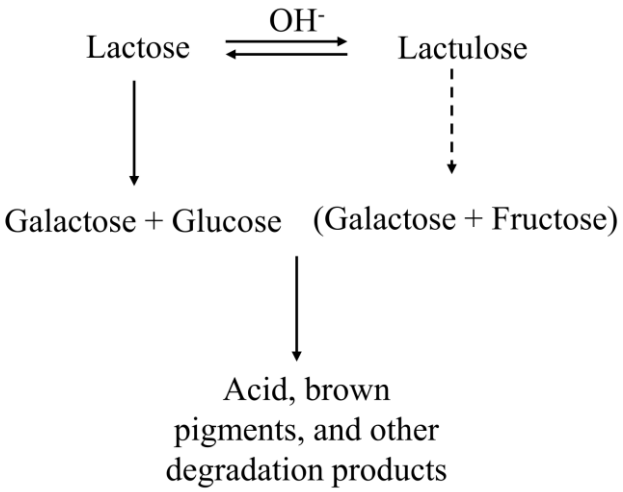



Figure 3.4 Reaction scheme for isomerization of lactose to lactulose, hydrolysis of lactulose, and degradation reactions occurring in arginine solutions. Dashed line represents negligible reaction

As shown in Figure 3.3, lactose content continued to decrease, whereas lactulose content reached a constant value of approximately 0.27 at pH 8.2. This suggested that lactose was consumed by other reactions, such as Maillard and hydrolysis reactions, as discussed in above sections. As reported in other studies, the prolongation of the reaction time also resulted in the degradation of lactulose (Dendene et al., 1994; Soisangwan, Gao, et al., 2017). However, during the 20-min reaction in the present work, a decrease in lactulose content was not observed.

### 3.3.5 Changes in Maillard reaction

Since arginine was used in the reaction, the Maillard reaction was unavoidable, and a lower extent of the reaction was preferred. The absorbances at 280, 325, and 420 nm were also monitored during the reaction (Echavarría et al., 2014). Figure 3.5 shows the relationship between $A_{325}$ and $A_{420}$ versus $A_{280}$. Both $A_{420}$ and $A_{325}$ increased almost linearly with $A_{280}$ for all the tested temperatures. This implied that the mechanisms and products of Maillard reaction at different temperatures were similar. The greater change in $A_{325}$ compared to $A_{420}$ might reflect the fact that $A_{325}$ represented intermediate products and $A_{420}$ represented the final products of the Maillard reaction. As shown by Liu et al. (2020), although arginine is the most basic amino acid, it resulted in fewer colored compounds during the Maillard reaction compared to lysine.

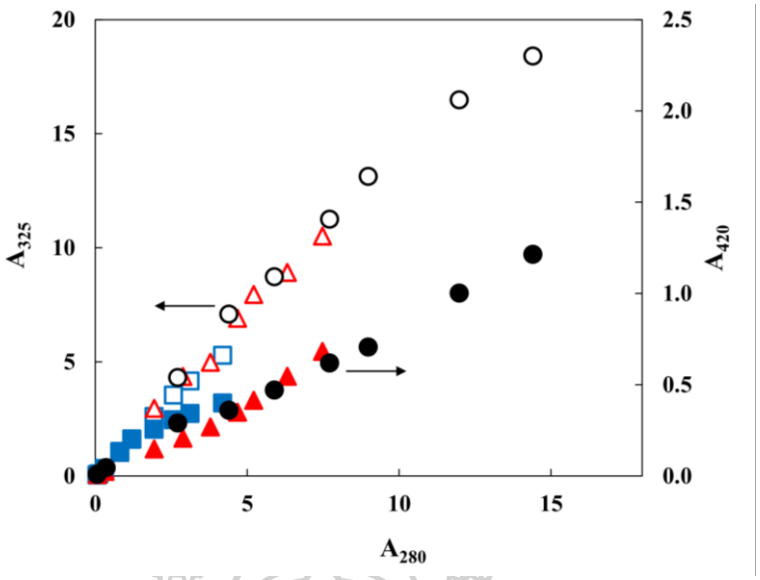



Figure 3.5 the relationships between absorbance at 325 nm ($A_{325}$, open symbols) and 420 nm ($A_{420}$, closed symbols) versus absorbance at 280 nm ($A_{280}$) of the solutions (b) during heating of the reaction solution containing 5% w/v lactose and 0.1 mol/mol lactose of arginine at 100 (square), 110 (triangle), and 120 °C (circle)

### 3.4 Conclusion

The present work demonstrated that lactose could be isomerized with arginine after heating in solution. The highest lactulose yield (27%) was comparable to that of a common base-catalyzed reaction. The Maillard reaction, which resulted in brown pigments, was unavoidable; however, by controlling the reaction temperature and time, optimum conditions with high lactulose yield and low browning pigment content could be obtained. Arginine is a common food ingredient, and its use as a catalyst for the isomerization of lactose to lactulose would be beneficial to the industry.

# CHAPTER 4
# Kinetic study of isomerization of galactose in arginine solution and the effect of ethanol addition

This work aimed to investigate the isomerization kinetic of galactose to tagatose in arginine solution at various temperatures and to study the effect of ethanol concentration on tagatose yield. A solution containing 5% w/v of galactose and 0.10 mol/mol-galactose was treated at 90-120 °C with different reaction time. Tagatose has reached the constant yield of approximately 17% at 90, 35, 15, and 7 min at 90, 100, 110, and 120 °C, respectively. Kinetic study showed that initial rate for tagatose formation was greatly increased with temperature. The activation energy was calculated to be 66.24 kJ/mol. Arginine degraded faster at higher temperatures. In addition, the effect of adding ethanol 5-40% w/w in galactose-arginine solution was investigated at 120 °C for 15 min. Increasing ethanol concentration to 10% showed slightly higher tagatose yield of 19% compared to 17.5% in water. However, ethanol 40% w/w, retarded the conversion of galactose and increased the tagatose yield significantly.

## 4.1 Introduction

In Chapter 2, arginine demonstrated significant potential as a catalyst for tagatose production, achieving a tagatose yield of 16.8–17.3%, comparable to other alkaline-catalyzed isomerization of galactose. Additionally, the highest productivity obtained was 278 g/(L·h), surpassing results reported for other alkaline-catalyzed processes and enzymatic methods.

Yang, Sherbahn, et al. (2016) reported the effect of reaction temperature (80–120 °C) on the rate constant for glucose isomerization by arginine and calculated an apparent activation energy was estimated to be approximately 44 ± 1.3 kJ/mol for the process. However, a kinetic study on the isomerization of galactose to tagatose in an arginine solution is still uncovered.

Moreover, in addition to chemo-catalysts, ethanol is an environmentally friendly solvent that acts as a catalyst in the isomerization of galactose to tagatose (Gao

et al., 2015c), as well as in the isomerization of glucose, fructose, mannose (Gao et al., 2015b), and other hexose sugars (Gao et al., 2015a) under green conditions known as subcritical aqueous conditions. It is expected that using ethanol as a co-catalyst with arginine may enhance the yield of tagatose.

In this Chapter, the objective is to investigate the kinetics of tagatose formation during the isomerization of galactose in an arginine solution across a range of temperatures and reaction times. Additionally, the study examines the degradation of arginine over extended heating periods to understand its stability and role as a catalyst in the isomerization process. the effect of ethanol on the isomerization of galactose in the presence of arginine to produce tagatose was also explored.

## 4.2 Materials and methods

### 4.2.1 Materials

D-Galactose (purity > 99%), and D-talose (purity > 99%), were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-tagatose (purity > 98.5%), and D-Sorbose (purity >98%) were purchased from TCI (Tokyo, Japan), L-arginine (purity > 98%) was purchased from Merck (Darmstadt, Germany). Ethanol (purity 99.9%), and acetonitrile (HPLC grade) were purchased from RCI Labscan (Bangkok, Thailand), and glycerin (99.5%) was purchased from Krungthepchemi (Bangkok).

### 4.2.2 Isomerization galactose to tagatose

The isomerization was performing in 2 mL screw-capped glass vial. A solution containing 5% w/v galactose and arginine 0.10 mol/mol-galactose was prepared using deionized water. Several vials were added with 1.5 mL of the solution, tightly closed and placed in a heating bath (Major Science, Taoyuan City, Taiwan) which contained 80% v/v glycerin as a heating medium. One vial was instated with a type-K thermocouple to measure the temperature of the mixture. The reaction was conducted at 90, 100, 110, and 120 °C. The reaction time for each temperature was 15, 30, 60, and 120 min, respectively. At decided time interval, one vial was removed from the bath and immediately cooled in ice water to stop the reaction. All isomerization experiments were performed in triplicate.

### 4.2.3 The effect of ethanol concentrations

Similarly to the method described in 4.2.2, the effect of ethanol concentration was investigated with the same setup. Solutions containing 5% w/v galactose and arginine 0.10 mol/mol-galactose were prepared using deionized water or aqueous ethanol at a concentration of 5, 10, 20, or 40% w/w, were treated at 120 °C for 15 min.

### 4.2.4 Sugar analysis

The concentration of sugars in the reaction mixture was quantified by a high-performance liquid chromatography (HPLC) equipped with an LC-20A pump (Shimadzu, Kyoto, Japan), a refractive index detector RID-20A (Shimadzu), and a COSMOIL® Sugar-D column (4.6 mm i.d. × 10 mm; Nacalai Tesque, Kyoto). 80% v/v acetonitrile was used as a mobile phase at a flow rate of 1.0 mL/min. The column was kept at 30 °C, and the sample volume injected was 20 µL.

### 4.2.5 Arginine analysis

The concentration of arginine remaining in the reaction mixture was determined using a high-performance liquid chromatography (HPLC) apply from Johnson et al. (2022). The system consisting of LC-20A pump, an SPD-M20A detector (Shimadzu), and a C-18 column (4.6 mm I.D. × 150 mm length; Inertsil® ODS-3, GL Science, Kyoto). The sample injected was 20 µL, and the mobile phase was 0.01M $H_3PO_4$, at flow rate 0.5 mL/min. Arginine was detected at 195 nm, and the concentration was calculated using a standard curve of pure arginine (5 -100 mg/L).

### 4.2.6 Determination of Maillard reaction products

Galactose and its isomeric products are reducing sugars that could react with amino acid to create coloring by the Maillard reaction. The treated solutions were measured for absorbances at 280 and 420 nm by a UV–vis spectrophotometer (Genesis

10s, Thermo scientific, Waltham, MA, USA). Samples that gave an absorbance higher than 1.0 were diluted by distilled water before reevaluation.

### 4.2.7 pH measurement

The pH of the reaction mixture was measured at room temperature using a pH meter (LAQUAtwin-pH-22, HORIBA Advanced Techno, Kyoto, Japan).

### 4.2.8 Statistical analysis

Analysis of variance (ANOVA) and significant differences between means from the triplicate experiment at a significant level of 0.05 by Duncan's multiple range test were performed using PASW Statistics for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

## 4.3 Results and discussion

### 4.3.1 Effects of temperature and time on isomerization of galactose

Figures 4.1 (a), (b), (c) and (d) show the changes over time of temperature of the reaction mixture, the remaining galactose, the yield of tagatose, talose, and sorbose, respectively. The temperature of the mixture rose from room temperature (~30 °C) and reached the target temperature within 5 min. Galactose concentration decreased rapidly during the first 10 min, then leveled off. It is believed that galactose decreased due to the isomerization in arginine solution with pH around 10.1 to tagatose via 1,2-enediol intermediate according to the LBAE transformation (Speck, 1958) and other degradation reactions (Kruse & Gawlik, 2003). After 15 min, the remaining galactose were of 81.5, 73.1, 67.9, and 61.7% at temperature of 90, 100, 110, and 120 °C respectively.

Tagatose is the main product of the isomerization as shown in Figure 4.1b and Table 4.1. Tagatose increased corresponded to the decreasing of galactose. Higher reaction temperatures increased the yield of tagatose and reach the constant values faster. No change in tagatose concentration after reaching certain value suggested that

no degradation of tagatose under the reaction conditions. Yields of tagatose were11.9, 15.7, 17.1, and 17.5% at 15 min at the reaction temperatures of 90, 100, 110, 120 °C, respectively. It should be noted that the yields of tagatose obtained in this system were slightly higher than those reported previously at all temperatures, with a reaction volume of 3 mL in a 4 mL glass vial, the mixture was heated using a block heater (Milasing et al., 2023). Higher yields of tagatose might be the result of the smaller size of the reaction vial and the heating medium, which provided a higher temperature increase of 10–17 °C in the first 2 min. This data suggests the rate of heating up promotes the isomerization of galactose to tagatose. It was reported that, using a batch reactor under subcritical treatment at 110 °C in arginine solution, galactose isomerized to yield tagatose at 16%. Another two rare sugars, talose and sorbose, were obtained from the reaction at low amounts (< 3%). Sorbose yields were slightly higher than talose yields at the reaction temperature 90-110 °C, but not at 120 °C (Table 4.1).

Initial pH of mixture solutions was 10.1 and the pH continuously decreased after the reaction (Table 4.1, Figure A9). Even tagatose yield became almost constant, pH still continued to decrease and it greatly decreased at higher temperatures.

In this study, HPLC measurement indicated that no 5-HMF in the solution after the reaction. This agreed with the study by Onishi et al. (2022) who reported that no detected levulinic acid, which is a rehydration product of 5-HMF. This suggests that 5-HMF is unlikely to form in arginine solution.

The plot between absorbances at 280 nm ($A_{280}$) against those at 420 nm ($A_{420}$) is shown in Figure A9b and it almost linearly correlate regardless to temperature and time similar to the results from the isomerization of lactose with arginine (Milasing et al., 2024).

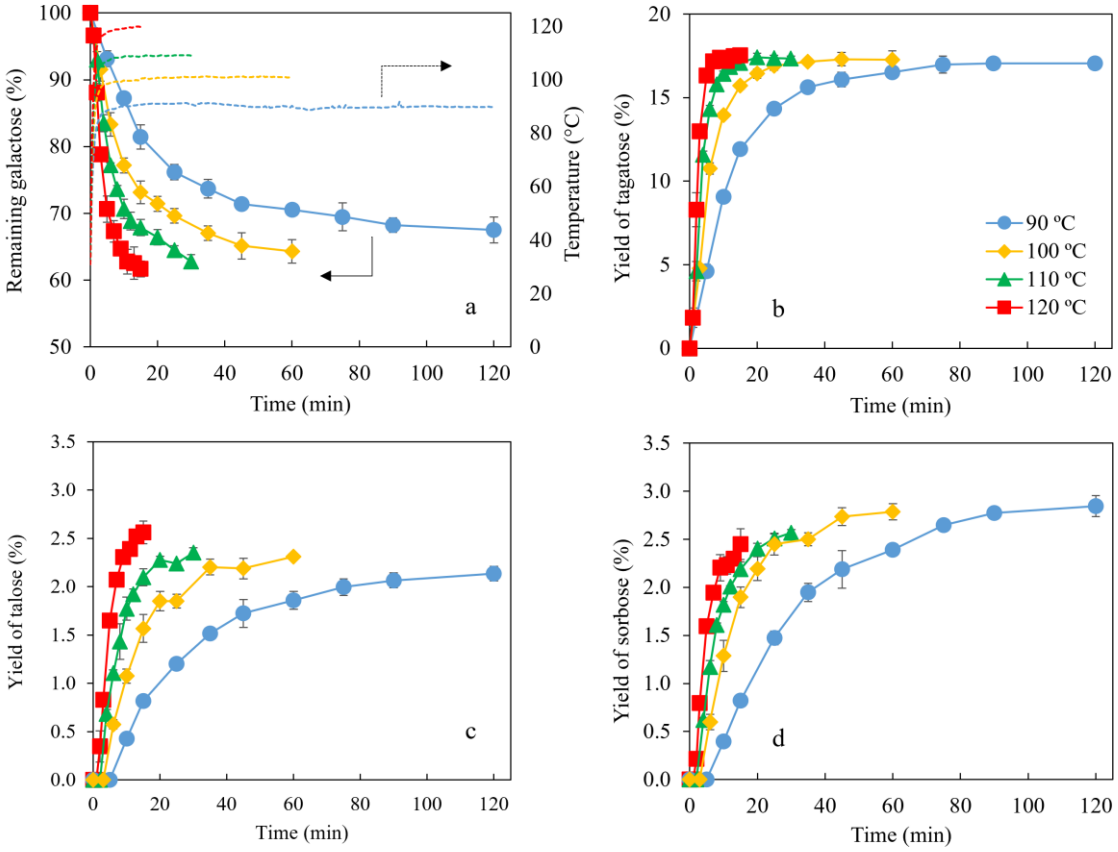



Figure 4.1 The isomerization of 5% w/v galactose with 0.10 mol/mol-galactose arginine at different temperature (circle, 90 °C, diamond, 100 °C, triangle, 110 °C, and, square, 120 °C) and reaction time

### 4.3.2 Relationship between pH of the treated solution with the yields of tagatose and $A_{420}$

The relationship between the remaining fraction of galactose, the yield of tagatose, and pH is shown in Figure 4.2. The yield of tagatose sharply increased and reached approximately 15% while the pH dropped to 9.5. However, the increase in tagatose yield slowed down and eventually stabilized when the pH dropped below 9.0. Kobayashi et al. (2023) also found that the yield of tagatose was almost constant after the pH decreased below approximately 9.0 for the treatment in arginine solution under subcritical water conditions. In contrast, the amount of galactose continuously decreased even when the pH fell below 9.0. Moreover, $A_{420}$ also continuously increased

with the decrease in pH. These results indicated that in arginine solution, galactose not only converted to its rare sugar but also to Maillard reaction products.

Table 4.1 Isomerization of 5% w/v galactose with 0.10 mol/mol-galactose arginine at different temperatures for 15 min

| Temperature (°C) | Remaining galactose (%) | Yield of tagatose (%) | Yield of talose (%) | Yield of sorbose (%) | pH |
|---|---|---|---|---|---|
| 90 | 81.4±1.8$^{a}$ | 11.9±0.1$^{d}$ | 0.8±0.0$^{d}$ | 0.8±0.0$^{d}$ | 9.7±0.0$^{a}$ |
| 100 | 73.1±1.7$^{b}$ | 15.7±0.2$^{c}$ | 1.6±0.1$^{c}$ | 1.9±0.1$^{c}$ | 9.2±0.0$^{b}$ |
| 110 | 67.9±1.2$^{c}$ | 17.1±0.3$^{b}$ | 2.1±0.1$^{b}$ | 2.2±0.1$^{b}$ | 8.7±0.0$^{c}$ |
| 120 | 61.7±1.2$^{d}$ | 17.5±0.2$^{a}$ | 2.6±0.1$^{a}$ | 2.4±0.2$^{a}$ | 7.4±0.1$^{d}$ |

Values are expressed as mean ± standard deviation, different letters in a same column indicate significant difference ($p < 0.05$). ns: not significant.

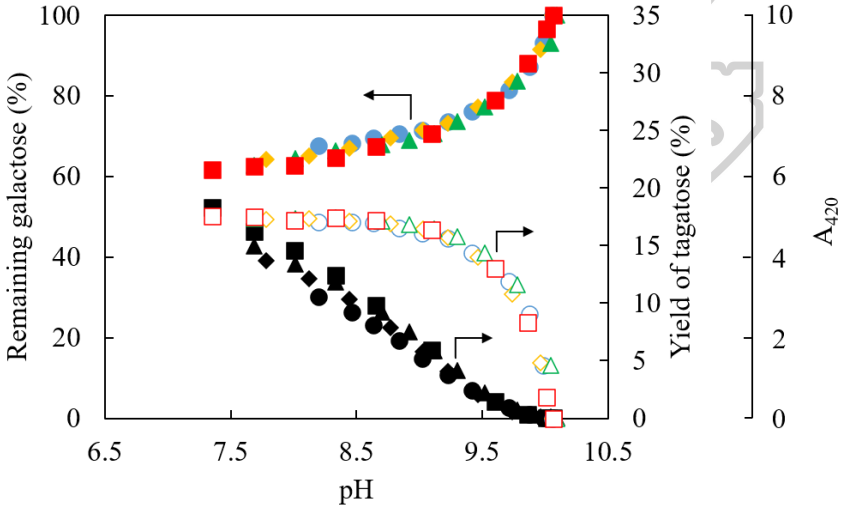



Figure 4.2 Changes in remaining galactose (color), yield of tagatose (white), and $A_{420}$ (black) against pH of the treated solution for the isomerization of 5% w/v galactose with 0.10 mol/mol-galactose arginine obtained at 90 °C (circle), 100 °C (diamond), 110 °C (triangle), and 120 °C (square)

### 4.3.3 Kinetic analysis of galactose isomerization

The maximum yield of tagatose at each reaction temperature are shown in Table 4.2. Tagatose yields of approximately 17.0-17.5% were obtained at the longest reaction time of each temperature. It was reported that reaction equilibrium shifted of glucose to formation fructose with increasing the temperature (Takasaki, 1967). However, no significant difference in the final yield of tagatose was found in this study. In KOH solution at pH 11.5, the yield of tagatose approximately 15% was obtained after 2 weeks at 25 °C (El Khadem et al., 1987).

The initial rates of tagatose formation ($r_0$), obtained from the slope of linear regression of the plot between tagatose concentration (mmol/L) and time (min) (Figure 4.3a), are shown in Table 4.2. The results clearly show that increasing reaction temperature increased $r_0$ values. The activation energy was calculated from the Arrhenius relationship as shown in Eq. (4.1) (Olano & Calvo, 1989). The Arrhenius plot is shown in Figure 4.3b and illustrated that the data fitted well with the model. The estimated apparent activation energy for tagatose formation was 66.24 kJ/mol. Troyano et al. (1992) reported the activation energy of tagatose formation during heat-treatment of milk at various temperatures (115–135 °C) of 115 kJ/mol.

$$\ln r_0 = -\left(\frac{Ea}{R}\right)\left(\frac{1}{T}\right) \qquad (4.1)$$

where $r_0$ is the initial rate of tagatose formation (mmol/L/min), Ea is activation energy, R is gas constant (8.314 J/mol/K), and T is temperature (K)

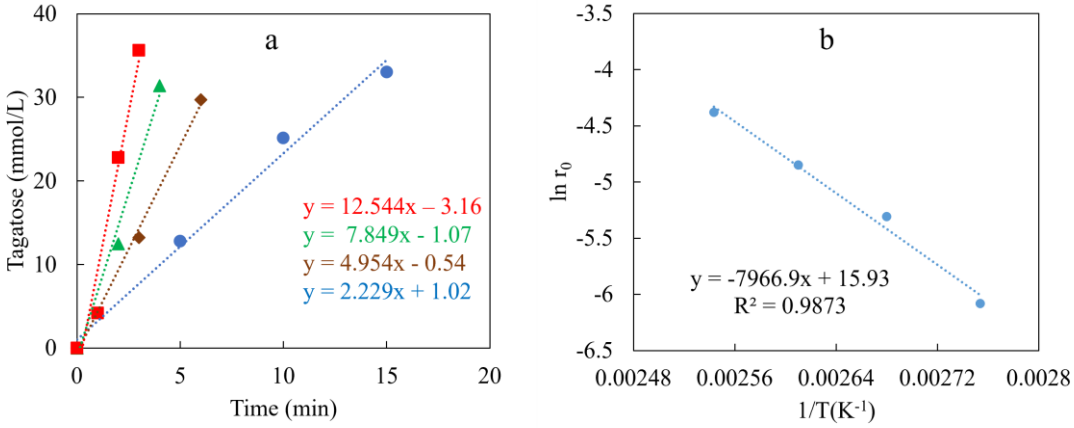



Figure 4.3 (a) Initial rate of tagatose formation (90 °C, circle; 100 °C, diamond; 110 °C, triangle; and 120 °C, square), (b) Arrhenius plot of the initial rate, $r_0$, for tagatose formation of the treated solution containing 5% w/v galactose with 0.10 mol/mol-galactose arginine

Table 4.2 Maximum yield, initial rate, and activation energy of tagatose formation. Reaction condition; 5% w/v galactose with 0.10 mol/mol-galactose arginine

| **Temperature (°C)** | **Maximum yield of tagatose (%)**[ns] | **Initial rate for tagatose formation, $r_0$ (mmol/L/min)** | **Activation energy of tagatose formation (kJ/mol)** |
|---|---|---|---|
| 90 | 17.0±0.3 | 2.23 | 66.24 |
| 100 | 17.3±0.4 | 4.95 | |
| 110 | 17.3±0.1 | 7.85 | |
| 120 | 17.5±0.2 | 12.54 | |

ns: not significant

### 4.3.4 Arginine degradation

Figure 4.4 shows the remaining amount of arginine in 5% w/v galactose after the isomerization at 90-120 °C. The results show that arginine depleted with heating time at all temperatures. Higher temperatures resulted in higher and faster degradation. At the same reaction time of 15 min, arginine remaining were 88.7, 79.2, 74.0, and 61.9% at 90, 100, 110, and 120 °C, respectively. Arginine degradation might cause by the Maillard reaction and decomposition by thermal process (Liu et al., 2020).

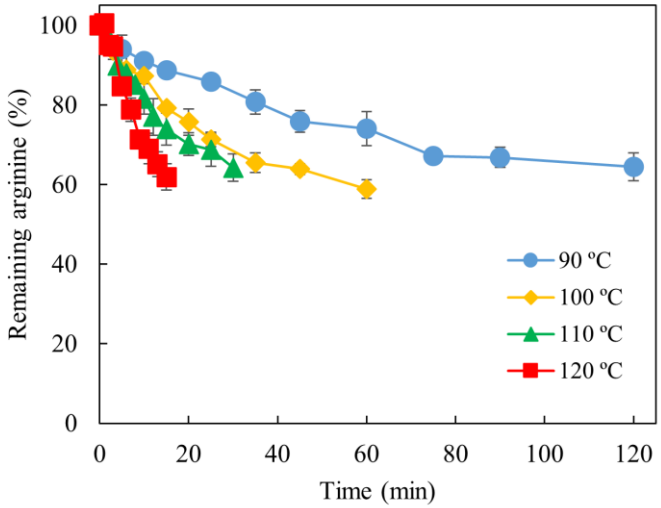



Figure 4.4 The remaining arginine after the isomerization of 5% w/v galactose with 0.10 mol/mol-galactose arginine at different temperatures (90 °C, circle; 100 °C, diamond; 110 °C, triangle; and 120 °C, square)

### 4.3.5 Effect of ethanol concentration

Ethanol is useful as green solvent for sugar isomerization reaction. In this study, ethanol at different concentrations was added to the galactose (5% w/v) and arginine (0.10 mol/mol-galactose) solution. The reaction temperature of 120 ºC and time of 15 min from section 4.3.4 was chosen due to the highest yield of tagatose with the shortest reaction time.

Figures 4.5a-d show the remaining galactose and the yield of tagatose, talose, sorbose and the temperature inside vial of the reaction. The results showed that

increasing ethanol concentration resulted in higher remaining galactose. At 15 min, the remaining galactose were 61.7, 64.0, 67.7, 68.4, and 73.2% for ethanol concentrations of 0, 5, 10, 20, and 40% w/w, respectively. As shown in Figure 4.5b, in the first 3 min, the yield of tagatose was almost the same for different ethanol concentrations, however, at longer reaction time the maximum yield of tagatose was higher (19.0%) when the ethanol concentration was increased to 10% w/w. However, when ethanol was increased to 20% w/w the yield of tagatose was almost the same for pure water. Moreover, at 40% w/w ethanol, the yield of tagatose was significantly lower than other conditions. Interestingly, Gao et al. (2015c) reported that in subcritical aqueous ethanol treatment, the isomerization of galactose significantly increased with ethanol concentration. The yield of talose was clearly lower than sorbose (Figures 4.5c, d). Moreover, lower yield of talose and sorbose were observed in conditions containing of ethanol. These results suggest that added ethanol suppress the isomerization of galactose to talose and sorbose.

### 4.3.6 pH and tagatose yield

It was reported that the isomerization by the Lobry de Bruyn-Alberda van Ekenstein transformation progressed well at a pH higher than 6.3 in the substrates pentose, hexose, and disaccharides as well (Onishi et al., 2022). Figure 4.6 shows the yield of tagatose with the pH of the solution. The results show that the yield of tagatose increased while the pH dropped at every ethanol concentration. Moreover, it was clearly shown that at a pH below 9.5 under 40% ethanol conditions, the yield of tagatose was lower than under other conditions. For the conditions of pure water and 5–20% ethanol, the yield of tagatose sharply increased when the pH dropped from 10 to 9.5 and hardly increased when the pH fell below 9.0. These results indicated that the acidic by-products were the inhibiting variable in the production of tagatose from galactose isomerization.

Figure 4.6b shows the plot between $A_{280}$ and $A_{420}$ of the treated solution at different ethanol concentrations. The results showed that $A_{420}$ were linearly increased along with $A_{280}$. $A_{280}$ was significantly higher than $A_{420}$ at all ethanol concentrations. In addition, the absorbance under 40% w/w ethanol conditions was higher than under the other conditions. These results indicated that a high concentration of ethanol suppressed isomerization and promoted the browning reaction.

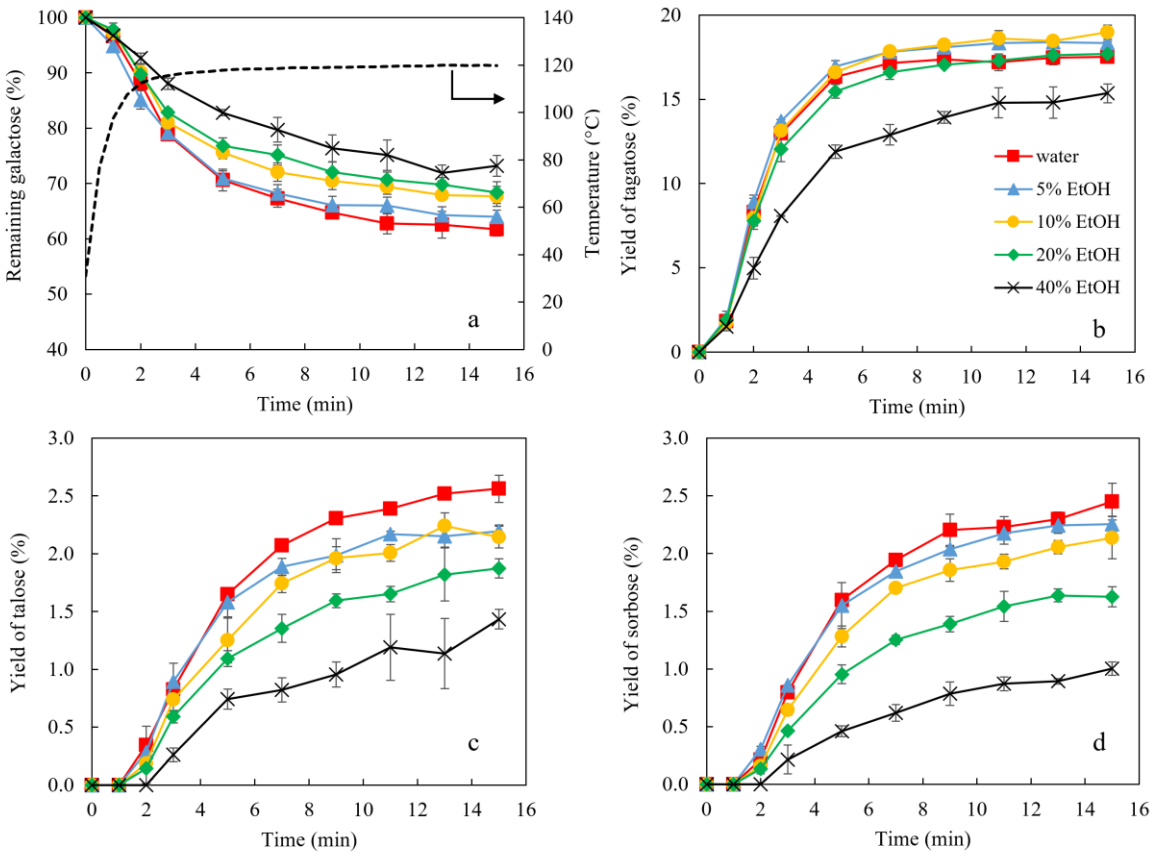



Figure 4.5 The isomerization of galactose 5% w/v with 0.10 mol/mol-galactose arginine at different ethanol concentrations (water (0%), square; 5%, triangle; 10%, circle; 20%, diamond; and 40% w/w, cross) at 120 °C for 15 min

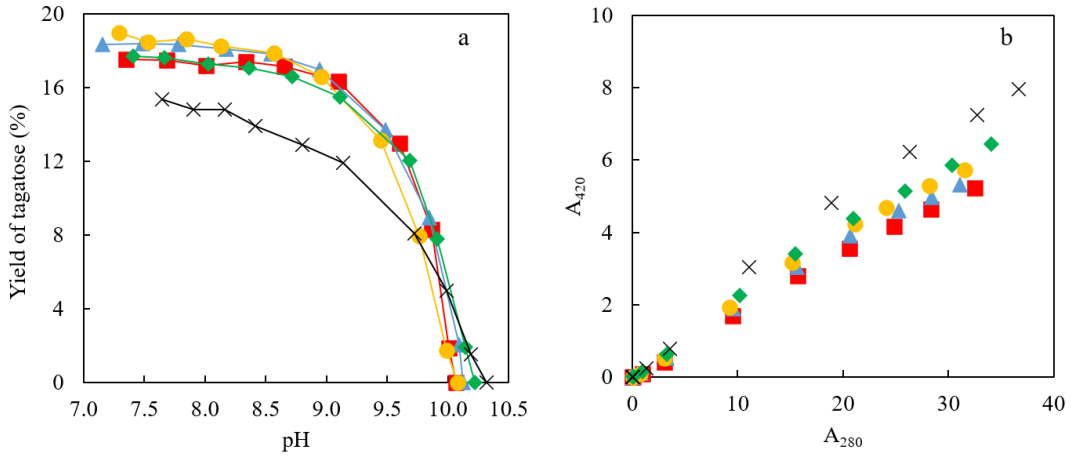



Figure 4.6 (a) Changes in yield of tagatose against pH of the treated solution (b) changes in $A_{420}$ vs $A_{280}$ of 5% w/v galactose with 0.10 mol/mol-galactose arginine at different ethanol concentration at 120 ºC for 15 min, the symbols are the same as in Figure 4.5

## 4.4 Conclusion

This study evaluates the isomerization of galactose into tagatose using arginine as an environmentally friendly catalyst under several conditions and at various temperatures. The highest yield of tagatose, 17.5%, was obtained at the highest temperature of 120 °C, corresponding to a decrease in the degradation of the substrate galactose and arginine with reaction time, which occurred much faster at higher temperatures. Talose and sorbose were observed, with yields of less than 3%. The Maillard reaction occurred with the same mechanism regardless of temperature and time. Adding ethanol to 10% w/w can improve the yield of tagatose to 19%. However, at 40% w/w ethanol, the remaining galactose was the highest, and the yield of the isomerization products was also lower.

# CHAPTER 5
# Enhanced catalytic activity of carbonate buffer for isomerization of galactose into tagatose

In this work, we systematically examined catalytic activity of CAPS at pH 10.4, carbonate at pH 10.4, triethylamine at pH 11.2, quinuclidine at pH 11.5, and L-arginine at pH 12.5 for isomerization D-galactose (Gal) to D-tagatose (Tag). The maximum yields of Tag were 15.0% with CAPS, 15.2% with carbonate, 19.3% with triethylamine, 19.6% with quinuclidine, and 18.1% with L-arginine. The rate of Tag formation with carbonate buffer was 3-8 times higher than with CAPS, despite the same pH. For catalysis by carbonate buffer, the reaction orders for hydroxide anions and carbonate species are approximately 1 and 0, respectively. *Operando* NMR studies of Gal-1-$^{13}$C isomerization in carbonate and CAPS buffers indicate similar tautomeric distributions in both buffers. Deuterium kinetic isotope effect demonstrated that carbonate facilitates isomerization through a proton transfer mechanism, with hydroxide anions acting as the catalytically active species whereas carbonate anions stabilize the enediolate anion and/or the transition state.

## 5.1 Introduction

Bases exhibit high catalytic activity for the isomerization of saccharides known as the Lobry de Bruyn-Alberda van Ekenstein transformation (Angyal, 2001; Delidovich, 2023; Delidovich & Palkovits, 2016a; Gao et al., 2024; Zhao et al., 2023). In the presence of bases, several consecutive and parallel reactions occur: Tag partly isomerizes into sorbose (Sorb), Gal and Tag can convert into talose (Tal), and the saccharides undergo degradation.

Structure-performance correlations of bases catalyzing the isomerization have been a long-standing question. It was demonstrated that the isomerization of glucose into fructose in the presence of buffers is an example of specific base catalysis, *i.e.* $OH^-$ rather than specific anions are the active catalytic species (Delidovich & Palkovits, 2016b; Michaelis & Rona, 1912). Interestingly, a few

results suggest the dependence of the reaction rate on the base structure for the isomerization of Gal into Tag. The isomerization rate of Gal catalyzed by the phosphate buffer, MOPS (3-(N-morpholino)propanesulfonic acid), and PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)) at the same pH 7 were different and dependent on the nature of the catalyst (Onishi et al., 2020). Moreover, the rates of isomerization of Gal into Tag and subsequent reactions were reported to depend on the concentration of the phosphate buffer – though different buffer concentrations resulted in somewhat different pH values of the reaction solution, which should have impacted the reaction rate (Onishi et al., 2024).

Chapters 2 and 4 demonstrated that the weakness of using a basic catalyst, such as arginine, is that the pH of the treated solution drops, preventing the yield of tagatose from increasing further. Using a buffer to resist changes in pH could help maintain an optimal environment for the reaction. This stabilization may lead to a higher and more consistent yield of tagatose.

In this work, we systematically explored the dependence of the catalytic activity for the isomerization of Gal into Tag, catalyzed by carbonate buffer, CAPS buffer (3-(cyclohexylamino)-1-propanesulfonic acid sodium salt), triethylamine, quinuclidine, and arginine. The isomerization rates were determined and molecular level insight was gained by measuring deuterium kinetic isotope effect using Gal-2-$d_1$ and through *operando* NMR spectroscopy.

## 5.2 Experimental

### 5.2.1 Chemicals

D-Galactose (98%) from Thermo scientific (Kandel, Germany), D-tagatose (99%), D-talose (97%), and CAPS from abcr (Karlsruhe, Germany) were used. D-Sorbose (98%) was purchased from J&K Scientific (Marbach, Germany). D-Glucose (≥99.5%), D-fructose (≥99%), quinuclidine, and triethylammonium chloride were purchased from Sigma-Aldrich (MO, USA). Sodium hydrogen carbonate was obtained from VWR chemicals (Leuven, Belgium), while L-arginine was purchased from Merck (Darmstadt, Germany). Sodium sulfate was obtained from Supelco (Darmstadt, Germany). D-Galactose-

1-$^{13}$C (99 atom%), D-galactose-2-$d_1$ (Gal-2-$d_1$) (98 atom%) from Omicron Biochemicals (IN, USA) were used. Deuterium oxide (99.9%) was purchased from Deutero (Kastellaun, Germany).

### 5.2.2 Catalytic tests

50 mL buffer solution was prepared with deionized water and the pH value was adjusted by a dropwise addition of 3 M NaOH or 3 M HCl. The buffer solution was transferred into a two-neck round bottom flask, equipped with a reflux condenser. The flask with the buffer solution was placed into a heated oil bath under stirring at 500 rpm. Gal (2.5 g) was dissolved in the thermostated buffer solution, and the reaction time was started (0 min). Aliquots of 2.5 mL were taken at different time lapses, and the reaction was stopped by cooling in an ice bath. The pH of the samples was measured using a pH meter (SI Analytics Titrator TitroLine® 7800, Mainz, Germany). The sample was 10 times diluted with deionized water for further analyses. The diluted samples were treated two times with ion-exchange resins to remove acidic by-products and neutralize. 10 mL of a diluted sample were stirred with 400 mg of Amberlyst® in $H^+$ form for 30 min, then with 1000 mg of Amberlite® anion exchange resin free base for 60 min. After the treatments, the solutions were filtered through a 0.22 μm nylon syringe filter (CHROMAFIL® Xtra PA-20/13, Düren, Germany).

To explore kinetic isotope effect (KIE), the experiments were carried out in a 20 mL 0.5 M carbonate or CAPS buffer solutions with 1% w/v of Gal or Gal-2-$d_1$ at pH 10.4 and 60 °C.

### 5.2.3 High-performance liquid chromatography (HPLC) analysis of carbohydrates

The reaction solutions were analyzed by HPLC by an Agilent Technologies 1200 system equipped with an RID detector. The sugars were separated by Sugar-D column (4.6 mm I.D. × 250 mm length, Nacalai Tesque, Kyoto, Japan) at 30 °C, and the 80:20 of acetonitrile:water was used as an eluent with flow rate of 1 mL/min.

### 5.2.4 UV measurement

UV spectra were recorded using a UV-vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

### 5.2.5 *Operando* nuclear magnetic resonance (NMR) spectroscopy

Carbonate and CAPS buffers (0.1 M) were prepared in $D_2O$. Gal-1-$^{13}C$ was dissolved in the buffers (36 mg, 0.2 mmol) and the pH was adjusted to 10.4 using NaOH. The NMR spectra were recorded every 15 min on a Bruker Avance III HD 400 MHz FT-NMR spectrometer (400.13 MHz for $^{1}H$, 100.6 MHz for $^{13}C$) either at 298 K or at 333 K, using a 5 mm broadband probehead with z-gradient. Spectra were measured using Bruker TOPSPIN 3.6 software and pulse programs from the Bruker library.

## 5.3 Results and discussion

### 5.3.1 Catalyst screening

We first screened soluble bases as catalysts for isomerization of Gal into Tag. The bases used were carbonate buffer ($pK_{a1}$ 10.4), CAPS buffer ($pK_a$ 10.4), TEA (triethylamine, $pK_a$ of the conjugate acid 11.2), quinuclidine ($pK_a$ of the conjugate acid 11.45), and Arg (L-arginine, $pK_a$ of the conjugate acid 12.5). The structures of the catalysts are provided in Table A1 in Appendix. The catalytic tests were performed with 5% w/v Gal and 0.5 M catalyst concentration at 60 °C. The solution pH was adjusted to $pK_a$ of the corresponding conjugate acid using NaOH to enable buffering effect and prevent pH drop during isomerization owing to acidic by-products. After dissolution of Gal, a slight pH decrease was observed in the amines solutions.

As Figure 5.1 a and b and Table 5.1 show, the rates of Gal transformation into Tag decrease in the following order: Arg ($pH_0$ 11.8) > TEA ($pH_0$ 11.1) ≈ carbonate buffer ($pH_0$ 10.4) ≈ quinuclidine ($pH_0$ 11.2) > CAPS ($pH_0$ 10.4). Unexpectedly, the isomerization rate with carbonate buffer was comparable to the initial rate of Tag accumulation with TEA and quiniclidine, even though the latter catalysts generated significantly higher First, soluble bases were screened

as catalysts for isomerization of Gal into Tag. The bases used were carbonate buffer (p$K_{a1}$ 10.4), CAPS buffer (p$K_a$ 10.4), TEA (triethylamine, p$K_a$ of the conjugate acid 11.2), quinuclidine (p$K_a$ of the conjugate acid 11.45), and Arg (L-arginine, p$K_a$ of the conjugate acid 12.5). The structures of the catalysts are provided in Table S1 in Appendix. The catalytic tests were performed with 5% w/v Gal and 0.5 M catalyst concentration at 60 °C. The solution pH was adjusted to p$K_a$ of the corresponding conjugate acid using NaOH to enable buffering effect and prevent pH drop during the isomerization owing to acidic by-products. After dissolution of Gal, a slight pH decrease was observed.

As shown in Figure 5.1a and b and Table 5.1, the rates of Gal transformation into Tag decrease in the following order: Arg ($pH_0$ 11.8) > TEA ($pH_0$ 11.1) ≈ carbonate buffer ($pH_0$ 10.4) ≈ quinuclidine ($pH_0$ 11.2) > CAPS ($pH_0$ 10.4). Unexpectedly, the isomerization rate in the presence of carbonate buffer was comparable to the initial rate of Tag accumulation with TEA and quiniclidine, even though the latter catalysts generated significantly higher pH values. It should be noted that in this work the isomerization rates observed after an induction period (Drabo et al., 2021; Toussaint & Delidovich, 2022) are reported.

The rates in the presence of Arg, TEA, quinuclidine, and CAPS buffer follow the general trend that the isomerization accelerates with increasing pH. Notably, the obtained maximal yield of Tag should be discussed. The isomerization of saccharides with chemo catalysts typically does not reach an equilibrium yield, but results in a steady-state yield owing to degradation reactions (Fischer, Drabo, & Delidovich, 2022). In general, the steady-state yield of Tag increases with the catalytic activity, being the highest for Arg (approximately 18%) and the lowest for CAPS buffer (approximately 6%). This observation aligns with the previously published higher steady-state yield in the presence of a strong base Arg compared to the weak base phosphate (Kobayashi et al., 2023). Selectivity for Tag decreases with the conversion of Gal (Figure 5.1c) owing to co-formation of Tal and Sorb (Figure A10 a, b) and degradation. The selectivity for Tag ranges between 40-60% at conversions of 5-25%. Figure 5.1d shows dependences of pH values on reaction time. CAPS and carbonate buffers exhibited stable pH values, whereas the pH value with TEA decreased from 11.1 to 10.7, with quinuclidine from

11.2 to 10.9, and with Arg from 11.8 to 11.1 after 90 min of reaction. The absorbance at 420 nm ($A_{420}$) was monitored over time for different catalysts. $A_{420}$ was increasing during the reaction as a result of caramelization reactions (Cämmerer et al., 1999). Particularly high $A_{420}$ was detected in the reaction catalyzed by Arg, likely owing to the Maillard reaction between Arg and the reducing sugars (Figure A10c).

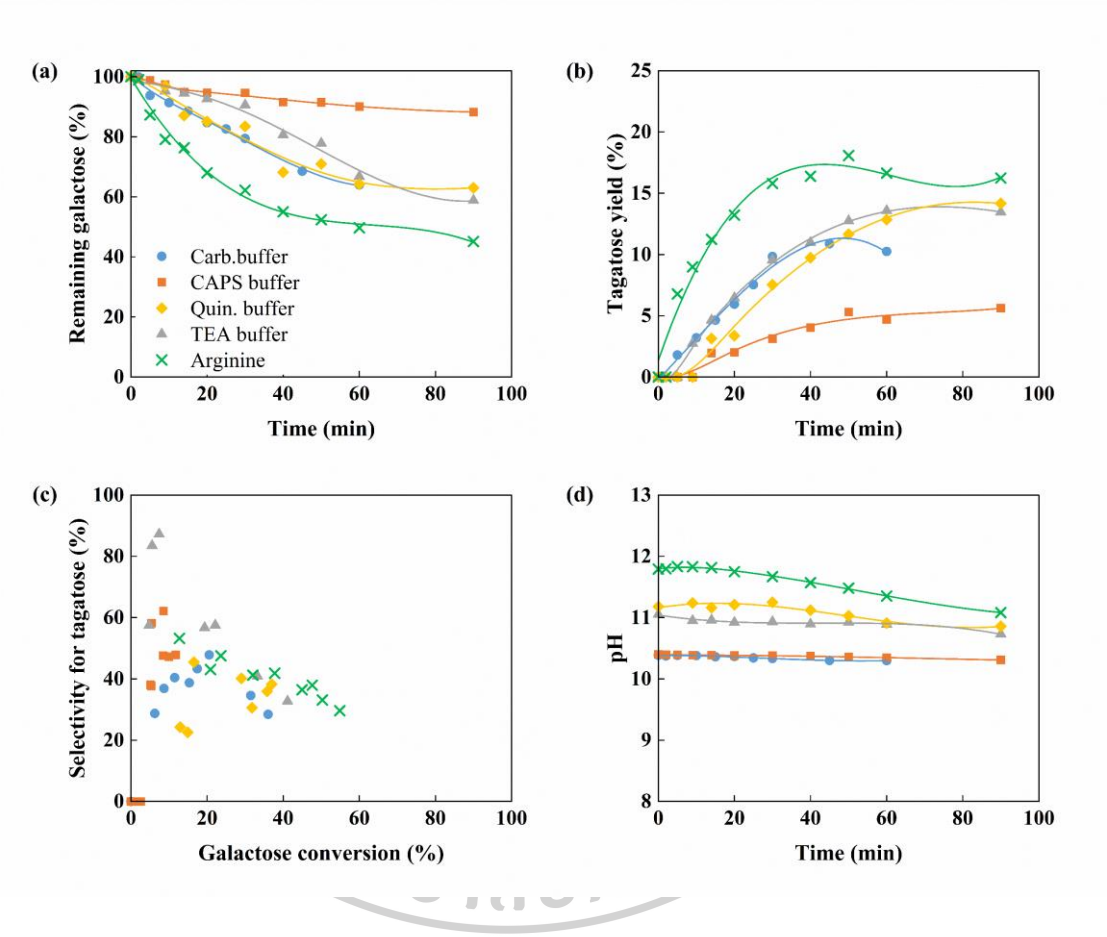



Figure 5.1 Results of Gal isomerization in the presence of the base catalysts, remaining galactose (a), tagatose yield (b), selectivity for tagatose-galactose conversion (c), and pH (d) plotted against time. Reaction conditions: 5% w/v Gal, 0.5 M base for carbonate, CAPS, TEA, and Arg, 0.1 M quinuclidine, $pH_0$ adjusted with NaOH, 60 °C

Next, temperature, pH, and concentration of the catalysts were systematically varied. This screening was performed for carbonate and CAPS buffers owing to their different catalytic activities despite similar p$K_a$ values. It was also of interest to compare the catalytic performance of TEA and

quinuclidine, which exhibit similar p$K_a$ values and comparable catalytic performance during the initial screening (Figure 5.1). The results of the experiments at different temperatures and pH values are summarized in Table 5.1, with corresponding graphs shown in Figure A11 and A12. For the tested catalysts, an increase in temperature improved the maximal yield of Tag. This can be explained by the positive enthalpy of isomerization, which suggests a higher equilibrium yield at higher temperature (Oh, 2007). At all tested temperatures, the rate of Tag formation was 3-8 times higher for catalysis by carbonate buffer compared to CAPS (entries 1-3 vs. 6-8 in Table 5.1). The rate of Tag formation in the presence of TEA was 1.1-2.4 times higher than for catalysis by quinuclidine (entries 12 and 13 vs. 17 and 18 in Table 5.1). Interestingly, Deshpande et al. reported higher catalytic activity of quinuclidine compared to TEA for isomerization of glucose (Glc) into fructose (Fru) (Deshpande et al., 2019).

We observed an acceleration in Tag formation with increasing of the pH value (Table 5.1). This result is expected, given the well-documented high catalytic activity of $OH^-$ for the isomerization reaction (Drabo et al., 2021; Toussaint & Delidovich, 2022). The maximal obtained yields of Tag were higher at higher pH values, consistent with previous studies that reported higher yields of Tag with phosphate catalyst at pH 12 (Wang et al., 2023; Zhao et al., 2023) than at pH 7 (Drabo & Delidovich, 2018; Kobayashi et al., 2023; Onishi et al., 2024; Onishi et al., 2021; Onishi et al., 2020). Higher pH values favor Tag accumulation over degradation, though degradation of Tag at harsh conditions (e.g. in the presence of quinuclidine at pH 12) was observed. Figure A13 shows the consumption of Gal and accumulation of Tag at different catalyst concentrations. In the explored concentration ranges (0.5 – 0.9 M for carbonate buffer, CAPS buffer, and TEA; 0.05 – 0.2 M for quinuclidine), the isomerization rate was hardly affected by the catalyst concentration. This supports previous reports highlighting the central role of specific base catalysis rather than general base catalysis in the sugar isomerization (Drabo et al., 2021; Toussaint & Delidovich, 2022).

### 5.3.2 Exploration of catalytic activity of carbonate buffer

The results of the catalyst screening summarized in Table 5.1 suggest a significantly higher rate of Tag accumulation – up to 8 times – in the presence of carbonate buffer than of CAPS buffer under the same reaction conditions, despite similar p$K_a$ values. We also tested carbonate and CAPS buffers for the isomerization of another substrate, Glc into Fru, and obtained similar results: the rate of Fru formation was 4.3 times higher with carbonate buffer than with CAPS buffer (Figure 5.2 and Table A2).

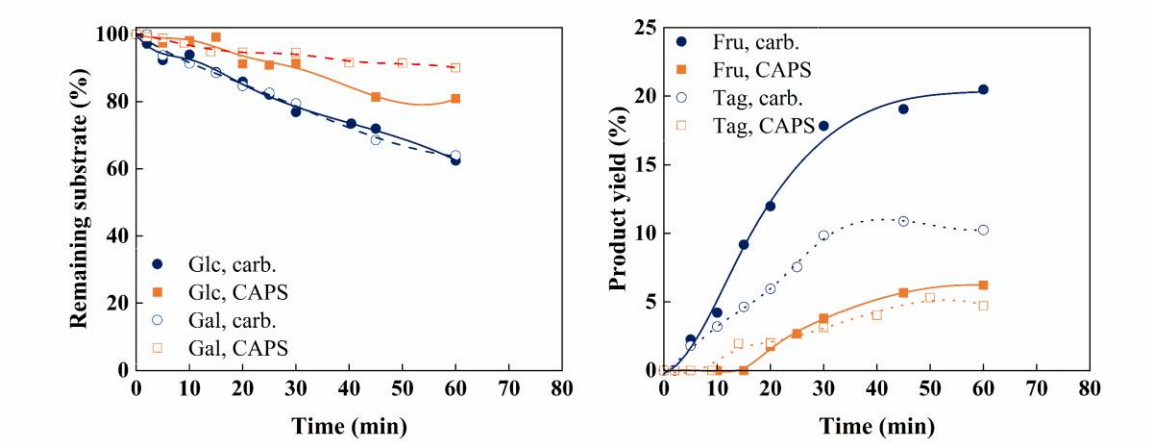



Figure 5.2 Results of Glc and Gal isomerization catalyzed by carbonate and CAPS buffers. Reaction conditions: 5% w/v Glc, Gal 0.5 M buffer at $pH_0$ 10.4, $pH_0$ adjusted with NaOH, 60 °C, 60 min

Table 5.1 Initial rates of Tag accumulation and maximal obtained yields of Tag. Reaction conditions: 5% w/v Gal, 0.5 M base for carbonate, CAPS, TEA, and Arg, 0.1 M quinuclidine, $pH_0$ adjusted with NaOH

| Entry | T, (°C) | $pH_0$ | Initial rates of Tag formation, (mmol/L/min) | Maximal yield of Tag, (%) |
|---|---|---|---|---|
| | | | *Carbonate* | |
| 1 | 50 | 10.4 | 0.27 | 5.9 |
| 2 | 60 | 10.4 | 0.70 | 10.9 |
| 3 | 70 | 10.2 | 5.46 | 14.6 |
| 4 | 60 | 9.9 | 0.35 | 5.6 |
| 5 | 60 | 11.1 | 1.44 | 15.2 |
| | | | *CAPS* | |
| 6 | 50 | 10.4 | -[a] | 0 |
| 7 | 60 | 10.4 | 0.26 | 5.6 |
| 8 | 70 | 10.4 | 0.69 | 15.0 |
| 9 | 60 | 10 | -[a] | 1.7 |
| 10 | 60 | 11 | 0.36 | 14.6 |
| | | | *TEA* | |
| 11 | 40 | 11.1 | -[a] | 2.2 |
| 12 | 50 | 11 | 0.34 | 10.3 |
| 13 | 60 | 11.1 | 0.74 | 13.6 |
| 14 | 60 | 10.3 | -[a] | 3.9 |
| 15 | 60 | 11.7 | 1.17 | 19.3 |
| | | | *Quinuclidine* | |
| 16 | 40 | 11.2 | -[a] | 0 |
| 17 | 50 | 11.2 | 0.14 | 9.4 |
| 18 | 60 | 11.2 | 0.67 | 14.2 |
| 19 | 60 | 10.8 | 0.50 | 14.1 |
| 20 | 60 | 11.6 | 0.88 | 19.6 |
| | | | *Arginine* | |
| 21 | 60 | 11.8 | 0.91 | 18.1 |

[a]The experiment was performed for 90 min, and the isomerization was too slow for measurement of the reaction rate

The dependences of the Tag formation rate on the concentrations of $OH^-$ and carbonate anions were examined in detail. First, catalytic activity of 0.5 M carbonate buffers with pH adjusted to a range of 10-11 was studied. The reaction order with respect to $OH^-$ close to unity (Figure 5.3a) was observed, as expected from the generally accepted reaction mechanism and the reaction network (De Wit et al., 1979; Drabo et al., 2021; Fischer, Drabo, Burow, et al., 2022; Toussaint & Delidovich, 2022). Additionally, the rate of Tag formation in carbonate buffers at pH 10.4 with total carbonate concentrations ranging from 0.1 to 1 M was investigated. The results shown in Figure 5.3b suggest that the isomerization rate is virtually independent of carbonate concentration.

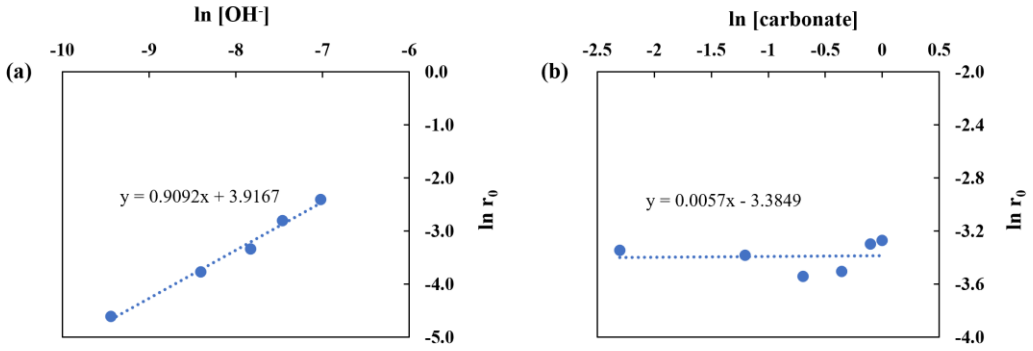



Figure 5.3 Initial rate of tagatose formation (ln $r_0$) (a) in 0.5 M carbonate buffer at different pH values (10 to 11) and (b) in carbonate buffer at different concentrations (0.1 M to 1 M) at pH 10.4. Reaction conditions: 5% w/v Gal, $pH_0$ adjusted with NaOH, 60 °C

Next, we focused on understanding the reasons for the acceleration of isomerization in the presence of carbonates. It was recently shown that ionic strength influences the rate of isomerization catalyzed by bases. This kinetic phenomenon known as salt effect, was explained by the dependence of observed kinetic constants on the thermodynamic ionization constants of saccharides,

which might vary with the nature and concentration of electrolytes (Fischer, Drabo, Burow, et al., 2022). A 0.5 M carbonate buffer generates an ionic strength of 1 M, whereas a 0.5 M CAPS buffer has an ionic strength of 0.25 M (Ellis & Morrison, 1982). $Na_2SO_4$ was added to the CAPS buffer reaction mixture to increase ionic strength, but the rates of Gal isomerization into Tag with and without sodium sulfate were identical (Figure A14). Thus, the salt effect did not account for the difference in catalytic activity between carbonate and CAPS buffers.

A monosaccharide exists in a solution as a mixture of furanose, pyranose, and open-chain forms, each exhibiting different reactivities for isomerization. It has been demonstrated that solvents (Drabo et al., 2023) or specific salts (Lin et al., 2024) can influence the thermodynamic equilibrium of these anomers, thus affecting the reaction rate and selectivity. In this work, *operando* $^{13}C$ NMR study of the isomerization of Gal-1-$^{13}C$ was performed using CAPS or carbonate buffer at pH 10.4 and $D_2O$ as a solvent. As shown in Figure 5.4, the resonances of galactofuranoses (GF) and galactopyranoses (GP) exhibit the same chemical shifts in both carbonate and CAPS buffers. Moreover, the resonances of the galactofuranoses manifest significant signal broadening, especially in carbonate buffer. Anomerization reaction presents a well-known example of general acid and base catalysis (Kaufmann et al., 2018; Pierce et al., 1985), and the NMR data suggest higher anomerization rates in carbonate than in CAPS buffer. The resonance of α-galactofuranose is not visible in spectra recorded in carbonate buffer owing to rapid anomerization. The ratios of (β-galactopyranose)/(α-galactopyranose) and (β-galactofuranose)/(α-galactopyranose + β-galactopyranose), calculated based on the areas of the corresponding resonances, were equal to 1.9 and 0.07, respectively, in both buffers (Figure 5.4). Moreover, the ratios do not vary during the experiment. The time-resolved NMR study suggests that the presence of carbonates and CAPS does not change the thermodynamic equilibrium of Gal anomerization.

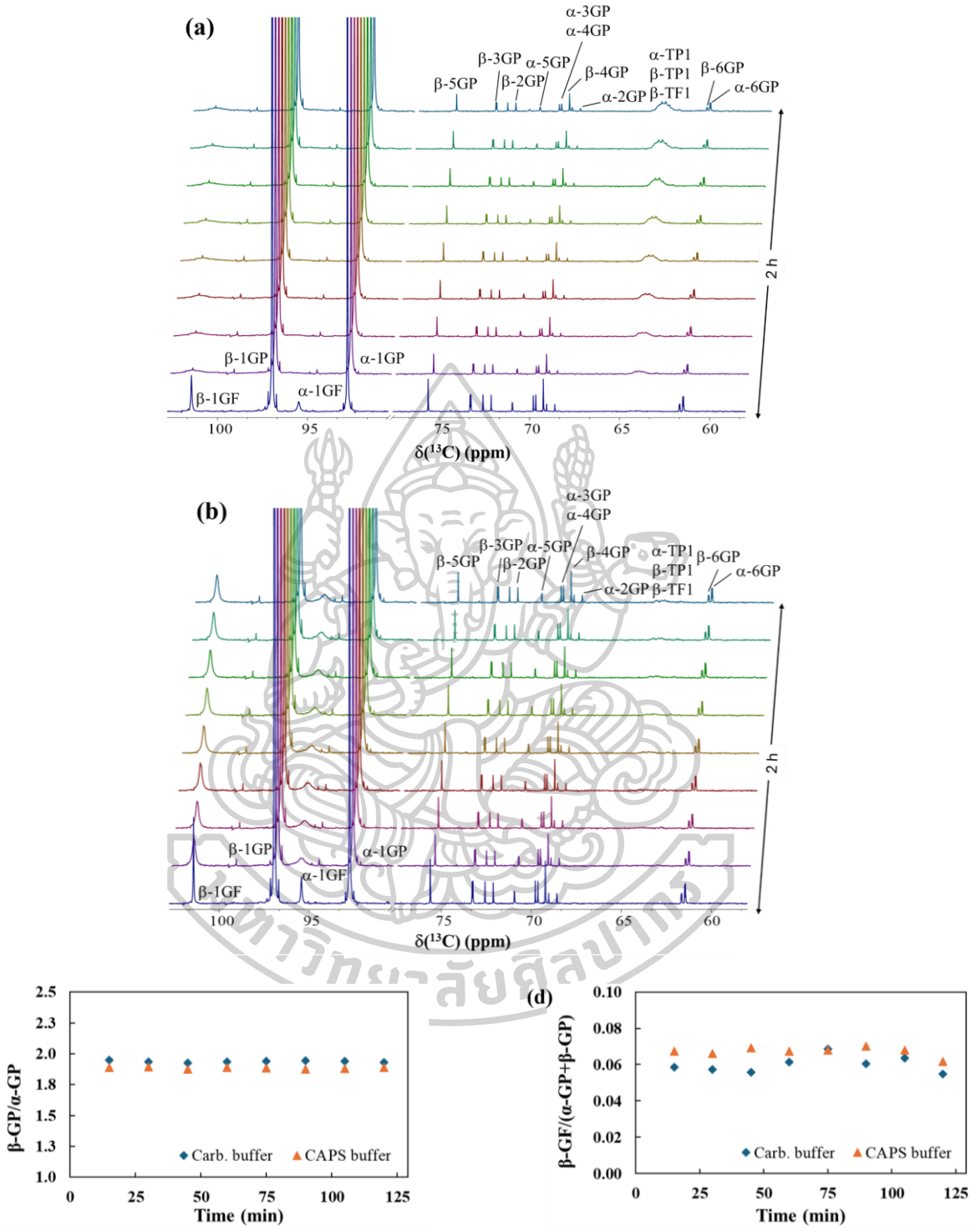



Figure 5.4 *Operando* NMR spectra showing Gal-1-$^{13}$C isomerization catalyzed by carbonate (a) and CAPS (b) buffers, anomeric ratio of β/α galactopyranose vs time (c), and anomeric ratio β-galactofuranose/(α-galactopyranose + β-galactopyranose) plotted

against time (d). Abbreviations: galactopyranose (GP), galactofuranose (GF), tagatopyranose (TP), and tagatofuranose (TF)

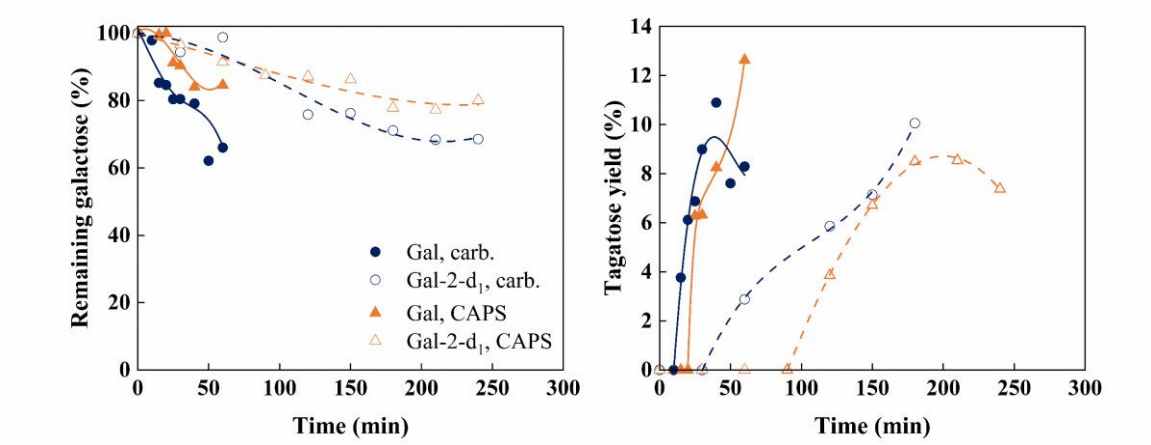
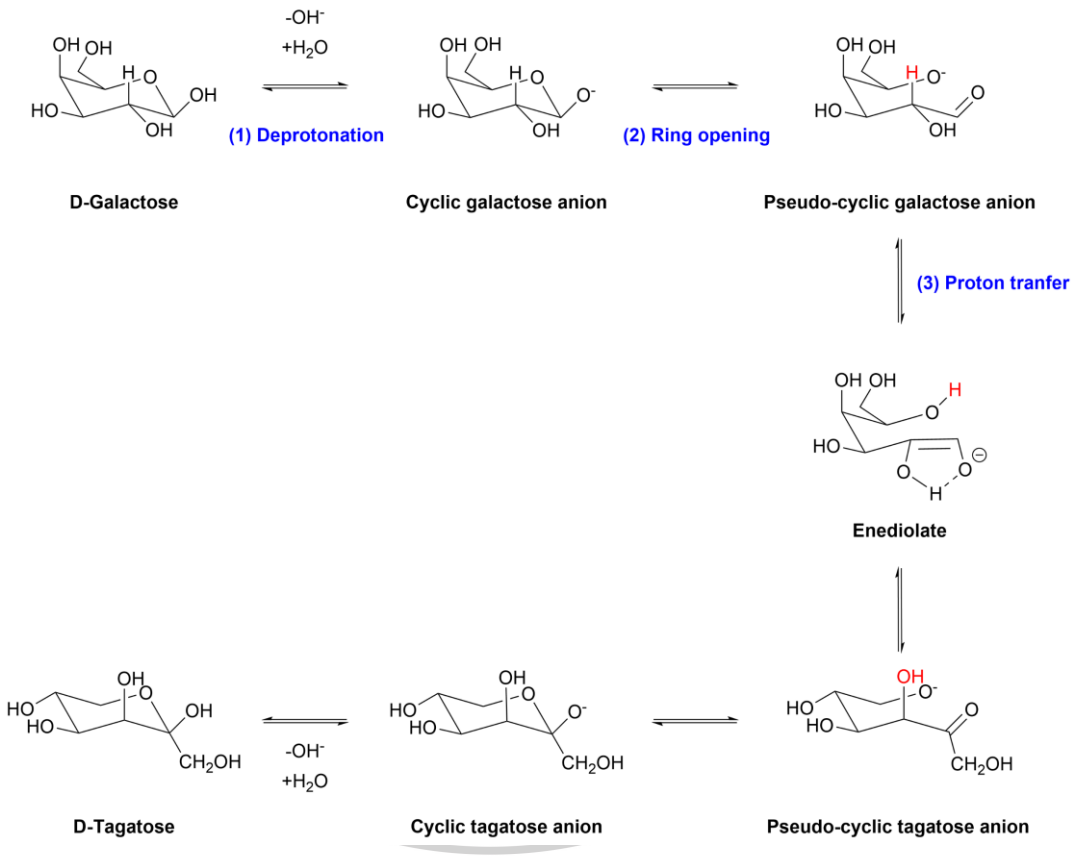



Figure 5.5 Results of isomerization of 1% w/v Gal and Gal-2-$d_1$ in 0.5 M of carbonate and CAPS buffers, $pH_0$ adjusted with NaOH to 10.4, 60 °C

Scheme 5.1 shows a generally accepted mechanism for the base-catalyzed isomerization of aldoses. This mechanism includes (1) deprotonation of an anomeric hydroxyl group, (2) ring opening of the anion, and (3) intermolecular proton transfer from C-2 to O-5. The third step is believed to be a rate-limiting step, as suggested by a few studies with aldoses deuterated (Carraher et al., 2015; Yabushita et al., 2019; Yang, Lan, et al., 2016; Yang, Sherbahn, et al., 2016) or tritiated (Isbell et al., 1971) at C-2 carbon atom. For isomerization of Glc, the kinetic isotope effect (KIE) was reported to range from *k*H/*k*D from 3.1 to 3.8. To our knowledge, no KIE studies for deuterated Gal have been reported yet. The isomerization rates of Gal and Gal-2-$d_1$ in CAPS and carbonate buffers were examined (Figure 5.5). The KIE values were determined by comparing the rates of Tag formation for deuterated and not deuterated substrates. The KIE values were $k_H/k_D$ = 2.5 for CAPS buffer and $k_H/k_D$ = 5.0 for carbonate buffer. The large KIE values suggest that the cleavage of the C-H bond at C2 is a rate-limiting step for catalysis by both buffers, consistent with the mechanism in Scheme 5.1. However, different KIE values indicate different reaction mechanisms and/or transition states in CAPS and carbonate buffers. A change in the reaction mechanism, with carbonate

anions acting as a general base, would result in a first order reaction with respect to carbonates. This was not observed, and the reaction order with respect to carbonates was approximately 0 (Figure 5.3a), excluding the role of carbonates as a general base. Stabilization of a transition state and/or the enediolate anion by carbonate presents an alternative explanation.




Scheme 5.1 Mechanism of base-catalyzed isomerization of Gal into Tag

## 5.4 Conclusion

The obtained data provide a solid foundation for developing both a catalyst and a catalytic process for isomerizing Gal into Tag. The isomerization with five bases revealed general trends: increasing pH and temperature improves yield of Tag. Typically, pH correlates with the reaction rate, but the carbonate buffer is significantly more catalytically active than CAPS buffer at the same pH value. Investigations with

carbonate buffer suggest that hydroxide anions are the active catalytic species. The deuterium kinetic isotope effect indicates cleavage of the C-H bond at C2 atom of Gal is the rate-limiting step for both CAPS and carbonate buffers, consistent with a proton-transfer mechanism involving an enediolate anion intermediate. The different KIE values observed for CAPS and carbonate buffers (2.5 vs. 5.0) suggest stabilization of the transition state and/or the enediolate anion by the carbonate species. Further experimental and computational studies will help elucidate the reasons for the promoting effect of carbonate species.

# CHAPTER 6
# Summary of findings and conclusions

This study revealed that arginine is a useful ‘green’ catalyst for tagatose production from the isomerization of galactose under several reaction conditions. A higher yield of tagatose was obtained at higher reaction temperatures. In addition, increasing arginine concentration results in an increased yield of tagatose and also promotes the Maillard reaction. However, increasing galactose concentration significantly increased the productivity of tagatose.

Moreover, the isomerization of the disaccharide lactose to directly produce tagatose, catalyzed by arginine, was investigated. However, the rate of lactose isomerization to lactulose was higher than that of its hydrolysis. Increasing temperature results in an increase in the yield of lactulose.

The kinetic study of galactose isomerization to tagatose, catalyzed by arginine, showed that higher temperatures led to a higher initial rate of tagatose formation. Arginine degraded faster at higher temperatures, which was associated with the degradation of galactose. Ethanol acts as a co-catalyst with arginine to improve the yield of tagatose, promoting both isomerization and the Maillard reaction.

The carbonate buffer demonstrated outstanding catalytic activity for the isomerization of galactose to tagatose. High pH and temperature yielded the maximum yield of tagatose. The results suggest that hydroxide anions are the active catalysts in the carbonate buffer. *Operando* NMR suggests similar chemical shifts in carbonate and CAPS buffer. The deuterium kinetic isotope effect demonstrated that carbonate facilitates isomerization through a proton transfer mechanism, with hydroxide anions acting as the catalytically active species, while carbonate anions stabilize the enediolate anion and/or the transition state.

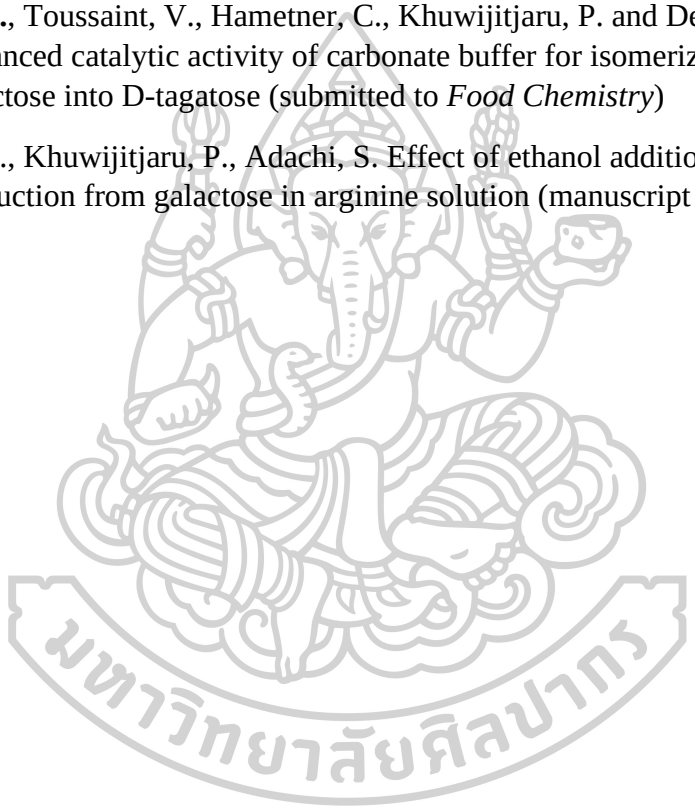
# LIST OF PUBLICATIONS

**Milasing, N.**, Khuwijitjaru, P., Adachi, S. (2023) Isomerization of galactose to tagatose using arginine as a green catalyst. *Food Chemistry*, 398, 133858. doi: 10.1016/j.foodchem.2022.133858

**Milasing, N.**, Amornrattanachart, T., Khuwijitjaru. P, Adachi, S. (2024) Isomerization of lactose to lactulose in an aqueous solution containing arginine. *International Food Research Journal* 31(1): 80 – 86. doi:10.47836/ifrj.31.1.07

**Milasing, N.**, Toussaint, V., Hametner, C., Khuwijitjaru, P. and Delidovich, I. Enhanced catalytic activity of carbonate buffer for isomerization of D-galactose into D-tagatose (submitted to *Food Chemistry*)

**Milasing, N**., Khuwijitjaru, P., Adachi, S. Effect of ethanol addition on tagatose production from galactose in arginine solution (manuscript in preparation)



# REFERENCES


Adachi, S., Khuwijitjaru, P., & Kobayashi, T. (2022). Continuous production of maltulose from maltose in a pressurized hot phosphate buffer. *Japan Journal of Food Engineering*, *23*(2), 63-69. https://doi.org/10.11301/jsfe.22608

Adachi, S., Miyagawa, Y., Khuwijitjaru, P., & Kobayashi, T. (2021). Isomerization of maltose to maltulose in a pressurized hot phosphate buffer. *Biocatalysis and Agricultural Biotechnology*, *37*, 102164. https://doi.org/10.1016/j.bcab.2021.102164

Adachi, S., & Patton, S. (1961). Presence and significance of lactulose in milk products: A review1. *Journal of Dairy Science*, *44*(8), 1375-1393. https://doi.org/10.3168/jds.S0022-0302(61)89899-8

Aida, T. M., Sato, Y., Watanabe, M., Tajima, K., Nonaka, T., Hattori, H., & Arai, K. (2007). Dehydration of D-glucose in high temperature water at pressures up to 80MPa. *The Journal of Supercritical Fluids*, *40*(3), 381-388. https://doi.org/10.1016/j.supflu.2006.07.027

Aider, M., & Halleux, D. d. (2007). Isomerization of lactose and lactulose production: review. *Trends in Food Science & Technology*, *18*(7), 356-364. https://doi.org/10.1016/j.tifs.2007.03.005

Angyal, S. J. (2001). The Lobry de Bruyn-Alberda van Ekenstein transformation and related reactions. In A. E. Stütz (Ed.), *Glycoscience: Epimerisation, Isomerisation and Rearrangement Reactions of Carbohydrates* (pp. 1-14). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-44422-X_1

Armstrong, L. M., Luecke, K. J., & Bell, L. N. (2009). Consumer evaluation of bakery product flavour as affected by incorporating the prebiotic tagatose. *International Journal of Food Science & Technology*, *44*(4), 815-819. https://doi.org/10.1111/j.1365-2621.2009.01909.x

Ashoor, S. H., & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science*, *49*(4), 1206-1207. https://doi.org/10.1111/j.1365-2621.1984.tb10432.x

Beadle, J. R., Saunders, J. P., & Wajda, J., Thomas J. (1992). *Process for manufacturing tagatose* (US Patent No. US5078796).

Beadle, J. R., Saunders, J. P., & Wajda Jr., T. J. (1989). *Process for manufacturing tagatose* (US Patent No. US5002612A).

Bertelsen, H., Jensen, B. B., & Buemann, B. (1999). D-Tagatose - a novel low-calorie bulk sweetener with prebiotic properties. In A. Corti (Ed.), *Low-Calories Sweeteners: Present and Future* (pp. 98-109). Karger.

Bober, J. R., & Nair, N. U. (2019). Galactose to tagatose isomerization at moderate temperatures with high conversion and productivity. *Nature Communications*, *10*(1), 4548. https://doi.org/10.1038/s41467-019-12497-8

Cämmerer, B., Wedzicha, B. L., & Kroh, L. W. (1999). Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. *European Food Research and Technology*, *209*(3), 261-265. https://doi.org/10.1007/s002170050490

Carraher, J. M., Fleitman, C. N., & Tessonnier, J.-P. (2015). Kinetic and mechanistic study of glucose isomerization using homogeneous organic Brønsted base

catalysts in water. *ACS Catalysis*, *5*(6), 3162-3173. https://doi.org/10.1021/acscatal.5b00316

Damodaran, S., & Parkin, K. L. (2017). *Fennema's food chemistry*. CRC Press. https://books.google.co.th/books?id=KTclDwAAQBAJ

De Bruijn, J. M., Kieboom, A. P. G., & van Bekkum, H. (1987). Alkaline degradation of monosaccharides part VII. A mechanistic picture. *Starch - Stärke*, *39*(1), 23-28. https://doi.org/10.1002/star.19870390107

de Oliveira Neves, L. N., Marques, R., da Silva, P. H. F., & de Oliveira, M. A. L. (2018). Lactulose determination in UHT milk by CZE-UV with indirect detection. *Food Chemistry*, *258*, 337-342. https://doi.org/10.1016/j.foodchem.2018.03.069

De Wit, G., Kieboom, A. P. G., & van Bekkum, H. (1979). Enolisation and isomerisation of monosaccharides in aqueous, alkaline solution. *Carbohydrate Research*, *74*(1), 157-175. https://doi.org/10.1016/S0008-6215(00)84773-4

Delgado-Andrade, C., Seiquer, I., Haro, A., Castellano, R., & Navarro, M. P. (2010). Development of the Maillard reaction in foods cooked by different techniques. Intake of Maillard-derived compounds. *Food Chemistry*, *122*(1), 145-153. https://doi.org/10.1016/j.foodchem.2010.02.031

Delidovich, I. (2021). Recent progress in base-catalyzed isomerization of D-glucose into D-fructose. *Current Opinion in Green and Sustainable Chemistry*, *27*, 100414. https://doi.org/10.1016/j.cogsc.2020.100414

Delidovich, I. (2023). Toward understanding base-catalyzed isomerization of saccharides. *ACS Catalysis*, *13*(4), 2250-2267. https://doi.org/10.1021/acscatal.2c04786

Delidovich, I., & Palkovits, R. (2016a). Catalytic isomerization of biomass-derived aldoses: A review. *ChemSusChem*, *9*(6), 547-561. https://doi.org/10.1002/cssc.201501577

Delidovich, I., & Palkovits, R. (2016b). Fructose production via extraction-assisted isomerization of glucose catalyzed by phosphates. *Green Chemistry*, *18*(21), 5822-5830. https://doi.org/10.1039/C6GC01712F

Dendene, K., Guihard, L., Nicolas, S., & Bariou, B. (1994). Kinetics of lactose isomerisation to lactulose in an alkaline medium. *Journal of Chemical Technology & Biotechnology*, *61*(1), 37-42. https://doi.org/10.1002/jctb.280610106

Deshpande, N., Cho, E. H., Spanos, A. P., Lin, L.-C., & Brunelli, N. A. (2019). Tuning molecular structure of tertiary amine catalysts for glucose isomerization. *Journal of Catalysis*, *372*, 119-127. https://doi.org/10.1016/j.jcat.2019.02.025

Djouab, A., & Aïder, M. (2019). Whey permeate integral valorisation via in situ conversion of lactose into lactulose in an electro-activation reactor modulated by anion and cation exchange membranes. *International Dairy Journal*, *89*, 6-20. https://doi.org/10.1016/j.idairyj.2018.07.019

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

Drabo, P., Fischer, M., Emondts, M., Hamm, J., Engelke, M., Simonis, M., Qi, L., Scott, S. L., Palkovits, R., & Delidovich, I. (2023). Solvent effects on catalytic activity

and selectivity in amine-catalyzed D-fructose isomerization. *Journal of Catalysis*, *418*, 13-21. https://doi.org/10.1016/j.jcat.2022.12.029

Drabo, P., Fischer, M., Toussaint, V., Flecken, F., Palkovits, R., & Delidovich, I. (2021). What are the catalytically active species for aqueous-phase isomerization of D-glucose into D-fructose in the presence of alkaline earth metal (hydr)oxides? *Journal of Catalysis 402*, 315-324. https://doi.org/10.1016/j.jcat.2021.08.036

Echavarría, A. P., Pagán, J., & Ibarz, A. (2014). Kinetics of color development of melanoidins formed from fructose/amino acid model systems. *Food Science and Technology International*, *20*(2), 119-126. https://doi.org/10.1177/1082013213476071

Ekeberg, D., Morgenlie, S., & Stenstrøm, Y. (2007). Aldose–ketose interconversion in pyridine in the presence of aluminium oxide. *Carbohydrate Research*, *342*(14), 1992-1997. https://doi.org/10.1016/j.carres.2007.05.033

El Khadem, H. S., Ennifar, S., & Isbell, H. S. (1987). Contribution of the reaction pathways involved in the isomerization of monosaccharides by alkali. *Carbohydrate Research*, *169*, 13-21. https://doi.org/10.1016/0008-6215(87)80238-0

El Khadem, H. S., Ennifar, S., & Isbell, H. S. (1989). Evidence of stable hydrogen-bonded ions during isomerization of hexoses in alkali. *Carbohydrate Research*, *185*(1), 51-59. https://doi.org/10.1016/0008-6215(89)84020-0

Ellis, K. J., & Morrison, J. F. (1982). [23] Buffers of constant ionic strength for studying pH-dependent processes. In D. L. Purich (Ed.), *Methods in Enzymology* (Vol. 87, pp. 405-426). Academic Press. https://doi.org/10.1016/S0076-6879(82)87025-0

Fischer, M., Drabo, P., Burow, L., & Delidovich, I. (2022). Kinetic salt effect on base-catalyzed isomerization of D-glucose into D-fructose. *Chempluschem*, *87*(12), e202200389. https://doi.org/10.1002/cplu.202200389

Fischer, M., Drabo, P., & Delidovich, I. (2022). Study of base-catalyzed isomerization of D-glucose with a focus on reaction kinetics. *Reaction Kinetics, Mechanisms and Catalysis*, *135*(5), 2357-2377. https://doi.org/10.1007/s11144-022-02277-9

Fitch, C. A., Platzer, G., Okon, M., Garcia-Moreno, B. E., & McIntosh, L. P. (2015). Arginine: Its p*K*a value revisited. *Protein Science*, *24*(5), 752-761. https://doi.org/10.1002/pro.2647

Frank, M., Miethchen, R., & Degenring, D. (1999). D-Tagatose derivatives from D-fructose by a facile epimerisation procedure. *Carbohydrate Research*, *318*(1), 167-170. https://doi.org/10.1016/S0008-6215(99)00090-7

Gao, D.-M., Kobayashi, T., & Adachi, S. (2015a). Kinetic effect of alcohols on hexose isomerization under subcritical aqueous conditions. *Chemical Engineering Research and Design*, *104*, 723-729. https://doi.org/10.1016/j.cherd.2015.10.018

Gao, D. M., Kobayashi, T., & Adachi, S. (2015b). Kinetic analysis for the isomerization of glucose, fructose, and mannose in subcritical aqueous ethanol. *Bioscience, Biotechnology, and Biochemistry*, *79*(6), 1005-1010. https://doi.org/10.1080/09168451.2014.1003129

Gao, D. M., Kobayashi, T., & Adachi, S. (2015c). 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

Gao, D. M., Kobayashi, T., & Adachi, S. (2016). Production of keto-disaccharides from aldo-disaccharides in subcritical aqueous ethanol. *Bioscience, Biotechnology and Biochemistry*, *80*(5), 998-1005. https://doi.org/10.1080/09168451.2015.1127135

Gao, D. M., Zhang, X., Liu, H., Fujino, H., Lei, T., Sun, F., Zhu, J., & Huhe, T. (2024). Critical approaches in the catalytic transformation of sugar isomerization and epimerization after Fischer－History, challenges, and prospects. *Green Energy & Environment* *9*(3), 435-453. https://doi.org/10.1016/j.gee.2023.02.003

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*.

Guerrero, C., Vera, C., Plou, F., & Illanes, A. (2011). Influence of reaction conditions on the selectivity of the synthesis of lactulose with microbial β-galactosidases. *Journal of Molecular Catalysis B: Enzymatic*, *72*(3), 206-212. https://doi.org/10.1016/j.molcatb.2011.06.007

Hajek, J., Murzin, D. Y., Salmi, T., & Mikkola, J.-P. (2013). Interconversion of lactose to lactulose in alkaline environment: Comparison of different catalysis concepts. *Topics in Catalysis*, *56*(9), 839-845. https://doi.org/10.1007/s11244-013-0044-z

Hashemi, S. A., & Ashtiani, F. Z. (2010). The isomerization kinetics of lactose to lactulose in the presence of sodium hydroxide at constant and variable pH. *Food and Bioproducts Processing*, *88*(2), 181-187. https://doi.org/10.1016/j.fbp.2009.11.001

Health-Canada. (2024). *Novel food information: D-tagatose*. From https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/d-tagatose.html

Isbell, H. S., Linek, K., & Hepner, K. E. (1971). Transformations of sugars in alkaline solutions: part II. Primary rates of enolization. *Carbohydrate Research*, *19*(3), 319-327. https://doi.org/10.1016/S0008-6215(00)86162-5

Jameson, J. K., Mathiesen, G., Pope, P. B., Westereng, B., & La Rosa, S. L. (2021). Biochemical characterization of two cellobiose 2-epimerases and application for efficient production of lactulose and epilactose. *Current Research in Biotechnology*, *3*, 57-64. https://doi.org/10.1016/j.crbiot.2021.02.003

Johnson, J. B., Ohri, B., Walsh, K. B., & Naiker, M. (2022). A simple isocratic HPLC–UV method for the simultaneous determination of citrulline and arginine in australian cucurbits and other fruits. *Food Analytical Methods*, *15*(1), 104-114. https://doi.org/10.1007/s12161-021-02110-4

Karim, A., & Aider, M. (2020). Sustainable electroisomerization of lactose into lactulose and comparison with the chemical isomerization at equivalent solution alkalinity. *ACS Omega*, *5*(5), 2318-2333. https://doi.org/10.1021/acsomega.9b03705

Karim, A., & Aider, M. (2022). Production of prebiotic lactulose through isomerisation of lactose as a part of integrated approach through whey and whey permeate complete valorisation: A review. *International Dairy Journal*, *126*, 105249. https://doi.org/10.1016/j.idairyj.2021.105249

Kaufmann, M., Krüger, S., Mügge, C., & Kroh, L. W. (2018). General acid/base catalysis of sugar anomerization. *Food Chemistry*, *265*, 216-221. https://doi.org/10.1016/j.foodchem.2018.05.101

Khuwijitjaru, P., & Adachi, S. (2023a). Arginine-catalyzed isomerization of ribose to ribulose. *Process Biochemistry*, *130*, 434-439. https://doi.org/10.1016/j.procbio.2023.05.003

Khuwijitjaru, P., & Adachi, S. (2023b). Isomerization of ribose to ribulose using basic amino acids as a catalyst. *Food Science and Technology Research*, *29*(3), 231-236. https://doi.org/10.3136/fstr.FSTR-D-22-00215

Khuwijitjaru, P., Milasing, N., & Adachi, S. (2018). Production of D-tagatose: A review with emphasis on subcritical fluid treatment. *Science, Engineering and Health Studies*, *12*(3), 159-167.

Kim, B.-C., Lee, Y.-H., Lee, H.-S., Lee, D.-W., Choe, E.-A., & Pyun, Y.-R. (2002). Cloning, expression and characterization of L-arabinose isomerase from *Thermotoga neapolitana*: bioconversion of D-galactose to D-tagatose using the enzyme. *FEMS Microbiology Letters*, *212*(1), 121-126. https://doi.org/10.1111/j.1574-6968.2002.tb11254.x

Kim, H. J., Ryu, S. A., Kim, P., & Oh, D. K. (2003). A feasible enzymatic process for D-tagatose production by an immobilized thermostable L-arabinose isomerase in a packed-bed bioreactor. *Biotechnol Prog*, *19*(2), 400-404. https://doi.org/10.1021/bp025675f

Kim, J.-S., & Lee, Y.-S. (2008). Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as result of the Maillard reaction. *Food Chemistry*, *108*(2), 582-592. https://doi.org/10.1016/j.foodchem.2007.11.014

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

Kim, Y. S., & Oh, D. K. (2012). Lactulose production from lactose as a single substrate by a thermostable cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus*. *Bioresource Technology*, *104*, 668-672. https://doi.org/10.1016/j.biortech.2011.11.016

Kobayashi, T., Khuwijitjaru, P., & Adachi, S. (2023). Isomerization and epimerization of glucose and galactose in arginine solution and phosphate buffer under subcritical fluid conditions. *Bioscience, Biotechnology, and Biochemistry*, *87*(7), 758-764. https://doi.org/10.1093/bbb/zbad047

Körner, P. (2021). Hydrothermal degradation of amino acids. *ChemSusChem*, *14*(22), 4947-4957. https://doi.org/10.1002/cssc.202101487

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

Lamberts, L., Rombouts, I., & Delcour, J. A. (2008). Study of nonenzymic browning in α-amino acid and γ-aminobutyric acid/sugar model systems. *Food Chemistry*, *111*(3), 738-744. https://doi.org/10.1016/j.foodchem.2008.04.051

Lamothe, L. M., Lê, K.-A., Samra, R. A., Roger, O., Green, H., & Macé, K. (2019). The scientific basis for healthful carbohydrate profile. *Critical Reviews in Food Science and Nutrition*, *59*(7), 1058-1070. https://doi.org/10.1080/10408398.2017.1392287

Lim, B.-C., Kim, H.-J., & Oh, D.-K. (2007). High production of D-tagatose by the addition of boric acid. *Biotechnology Progress*, *23*(4), 824-828. https://doi.org/10.1021/bp070056y

Lin, C., Shi, Y., Xu, L., Wang, Z., Zhao, L., Wu, H., Cao, F., & Wei, P. (2024). Insight into the alkaline earth metal salt promotion for alkali-catalyzed glucose isomerization. *Catalysis Science & Technology*, *14*(3), 718-727. https://doi.org/10.1039/D3CY01241G

Lipinski, G. W. (2006). Reduced-calorie sweeteners and caloric alternatives. In (pp. 252-280). https://doi.org/10.1533/9781845691646.2.252

Liu, C., Carraher, J. M., Swedberg, J. L., Herndon, C. R., Fleitman, C. N., & Tessonnier, J.-P. (2014). Selective base-catalyzed isomerization of glucose to fructose. *ACS Catalysis*, *4*(12), 4295-4298. https://doi.org/10.1021/cs501197w

Liu, H. M., Han, Y. F., Wang, N. N., Zheng, Y. Z., & Wang, X. D. (2020). Formation and antioxidant activity of Maillard reaction products derived from different sugar-amino acid aqueous model systems of sesame soasting. *Journal of Oleo Science*, *69*(4), 391-401. https://doi.org/10.5650/jos.ess19336

Lü, X., & Saka, S. (2012). New insights on monosaccharides' isomerization, dehydration and fragmentation in hot-compressed water. *The Journal of Supercritical Fluids*, *61*, 146-156. https://doi.org/https://doi.org/10.1016/j.supflu.2011.09.005

Marcus, J. B. (2013). Chapter 4 - Carbohydrate basics: sugars, starches and fibers in foods and health: Healthy carbohydrate choices, roles and applications in nutrition, food science and the culinary arts. In J. B. Marcus (Ed.), *Culinary Nutrition* (pp. 149-187). Academic Press. https://doi.org/10.1016/B978-0-12-391882-6.00004-2

Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*, *11*(9), 364-373. https://doi.org/10.1016/S0924-2244(01)00022-X

Michaelis, L., & Rona, P. (1912). Über die umlagerung der glucose bei alkalischer reaktion, ein beitragzur theorieder katalyse. *Biochem. Z.*, *47*, 447 (in German).

Milasing, N., Amornrattanachart, T., Khuwijitjaru, P., & Adachi, S. (2024). Isomerisation of lactose to lactulose in an aqueous solution containing arginine. *International Food Research Journal*, *31*, 80-86. https://doi.org/10.47836/ifrj.31.1.07

Milasing, N., Khuwijitjaru, P., & Adachi, S. (2023). Isomerization of galactose to tagatose using arginine as a green catalyst. *Food Chemistry*, *398*, 133858. https://doi.org/10.1016/j.foodchem.2022.133858

O'Brien-Nabors, L. (2012). *Alternative sweeteners* (4 ed.). Taylor & Francis. https://doi.org/10.1201/b11242

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

Oh, D.-K., Kim, H.-J., Ryu, S.-A., Rho, H.-J., & Kim, P. (2001). Development of an immobilization method of L-arabinose isomerase for industrial production of tagatose. *Biotechnology Letters*, *23*(22), 1859-1862. https://doi.org/10.1023/A:1012730522288

Olano, A., & Calvo, M. M. (1989). Kinetics of lactulose, galactose and epilactose formation during heat-treatment of milk. *Food Chemistry*, *34*(4), 239-248. https://doi.org/https://doi.org/10.1016/0308-8146(89)90101-5

Olano, A., & Corzo, N. (2009). Lactulose as a food ingredient. *Journal of the Science of Food and Agriculture*, *89*(12), 1987-1990. https://doi.org/10.1002/jsfa.3694

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

Onishi, Y., Adachi, S., Tani, F., & Kobayashi, T. (2024). Effect of phosphate buffer concentration on the isomerization of galactose to rare sugars under subcritical water conditions. *Food Chemistry*, *434*, 137432. https://doi.org/10.1016/j.foodchem.2023.137432

Onishi, Y., Furushiro, Y., Adachi, S., & Kobayashi, T. (2021). Isomerization and epimerization of galactose to tagatose and talose in a phosphate buffer containing organic solvents under subcritical water conditions. *Industrial & Engineering Chemistry Research*, *60*(14), 5084-5089. https://doi.org/10.1021/acs.iecr.1c00682

Onishi, Y., Furushiro, Y., Hirayama, Y., Adachi, S., & Kobayashi, T. (2020). Production of tagatose and talose through isomerization of galactose in a buffer solution under subcritical water conditions. *Carbohydrate Research*, *493*, 108031. https://doi.org/10.1016/j.carres.2020.108031

Paseephol, T., Small, D. M., & Sherkat, F. (2008). Lactulose production from milk concentration permeate using calcium carbonate-based catalysts. *Food Chemistry*, *111*(2), 283-290. https://doi.org/10.1016/j.foodchem.2008.03.051

Pierce, J., Serianni, A. S., & Barker, R. (1985). Anomerization of furanose sugars and sugar phosphates. *Journal of the American Chemical Society*, *107*(8), 2448-2456. https://doi.org/10.1021/ja00294a041

Renn, P. T., & Sathe, S. K. (1997). Effects of pH, temperature, and reactant molar ratio on L-leucine and D-glucose Maillard browning reaction in an aqueous system. *Journal of Agricultural and Food Chemistry*, *45*(10), 3782-3787. https://doi.org/10.1021/jf9608231

Schuster-Wolff-Bühring, R., Fischer, L., & Hinrichs, J. (2010). Production and physiological action of the disaccharide lactulose. *International Dairy Journal*, *20*(11), 731-741. https://doi.org/10.1016/j.idairyj.2010.05.004

Seki, N., & Saito, H. (2012). Lactose as a source for lactulose and other functional lactose derivatives. *International Dairy Journal*, *22*(2), 110-115. https://doi.org/10.1016/j.idairyj.2011.09.016

Seo, Y. H., Sung, M., & Han, J.-I. (2016). Lactulose production from cheese whey using recyclable catalyst ammonium carbonate. *Food Chemistry*, *197*, 664-669. https://doi.org/10.1016/j.foodchem.2015.10.078

Skytte, U. P. (2006). Tagatose. In H. Mitchell (Ed.), *Sweeteners and Sugar Alternatives in Food Technology* (pp. 262-294). Blackwell Publishing. https://doi.org/10.1002/9780470996003.ch14

Soisangwan, N., Gao, D.-M., Kobayashi, T., Khuwijitjaru, P., & Adachi, S. (2017). Production of lactulose from lactose in subcritical aqueous ethanol. *Journal of Food Process Engineering*, *40*(2), e12413. https://doi.org/10.1111/jfpe.12413

Soisangwan, N., Gao, D. M., Kobayashi, T., Khuwijitjaru, P., & Adachi, S. (2016). Kinetic analysis for the isomerization of cellobiose to cellobiulose in subcritical aqueous ethanol. *Carbohydrate Research*, *433*, 67-72. https://doi.org/10.1016/j.carres.2016.07.015

Soisangwan, N., Khuwijitjaru, P., Kobayashi, T., & Adachi, S. (2017). Kinetic analysis of lactulose production from lactose in subcritical aqueous ethanol. *Food Science and Technology Research*, *23*(1), 45-49. https://doi.org/10.3136/fstr.23.45

Song, Y. S., Lee, H. U., Park, C., & Kim, S. W. (2013a). Batch and continuous synthesis of lactulose from whey lactose by immobilized β-galactosidase. *Food Chemistry*, *136*(2), 689-694. https://doi.org/10.1016/j.foodchem.2012.08.074

Song, Y. S., Lee, H. U., Park, C., & Kim, S. W. (2013b). Optimization of lactulose synthesis from whey lactose by immobilized β-galactosidase and glucose isomerase. *Carbohydrate Research*, *369*, 1-5. https://doi.org/10.1016/j.carres.2013.01.002

Speck, J. C., Jr. (1958). The Lobry de Bruyn-Alberda van Ekenstein transformation. *Advances in Carbohydrate Chemistry*, *13*, 63-103. https://doi.org/10.1016/s0096-5332(08)60352-5

Takasaki, Y. (1967). Kinetic and equilibrium studies on D-glucose-D-fructose isomerization catalyzed by glucose isomerase from *Streptomyces* sp. *Agricultural and Biological Chemistry*, *31*(3), 309-313. https://doi.org/10.1080/00021369.1967.10858809

Toussaint, V., & Delidovich, I. (2022). Revealing the contributions of homogeneous and heterogeneous catalysis to isomerization of D-glucose into D-fructose in the presence of basic salts with low solubility. *Catalysis Science & Technology*, *12*(13), 4118-4127. https://doi.org/10.1039/D2CY00551D

Troyano, E., Martinez-Castro, I., & Olano, A. (1992). Kinetics of galactose and tagatose formation during heat-treatment of milk. *Food Chemistry*, *45*(1), 41-43. https://doi.org/10.1016/0308-8146(92)90010-Y

Vera, C., & Illanes, A. (2016). Chapter 3 - Lactose-derived nondigestible oligosaccharides and other high added-value products. In A. Illanes, C. Guerrero, C. Vera, L. Wilson, R. Conejeros, & F. Scott (Eds.), *Lactose-Derived Prebiotics* (pp. 87-110). Academic Press. https://doi.org/10.1016/B978-0-12-802724-0.00003-2

Wang, G., Lyu, X., Wang, L., Wang, M., & Yang, R. (2023). Highly efficient production and simultaneous purification of D-tagatose through one-pot extraction-assisted isomerization of D-galactose. *Food Chemistry: X*, *20*, 100928. https://doi.org/10.1016/j.fochx.2023.100928

Wang, M., Wang, L., Lyu, X., Hua, X., Goddard, J. M., & Yang, R. (2022). Lactulose production from lactose isomerization by chemo-catalysts and enzymes: Current status and future perspectives. *Biotechnology Advances*, *60*, 108021. https://doi.org/10.1016/j.biotechadv.2022.108021

Wang, Z., Wang, M., Lyu, X., Wang, C., Tong, Y., Hua, X., & Yang, R. (2022). Recycling preparation of high-purity tagatose from galactose using one-pot boronate affinity adsorbent-based adsorption-assisted isomerization and simultaneous purification. *Chemical Engineering Journal*, *446*, 137089. https://doi.org/10.1016/j.cej.2022.137089

Woo, K. S., Kim, H. Y., Hwang, I. G., Lee, S. H., & Jeong, H. S. (2015). Characteristics of the thermal degradation of glucose and maltose solutions. *Preventive Nutrition and Food Science*, *20*(2), 102-109. https://doi.org/10.3746/pnf.2015.20.2.102

Xu, B., Jacobs, M. I., Kostko, O., & Ahmed, M. (2017). Guanidinium group remains protonated in a strongly basic arginine solution. *ChemPhysChem*, *18*(12), 1503-1506. https://doi.org/10.1002/cphc.201700197

Yabushita, M., Shibayama, N., Nakajima, K., & Fukuoka, A. (2019). Selective glucose-to-fructose isomerization in ethanol catalyzed by hydrotalcites. *ACS Catalysis*, *9*(3), 2101-2109. https://doi.org/10.1021/acscatal.8b05145

Yang, Q., Lan, W., & Runge, T. (2016). Salt-promoted glucose aqueous isomerization catalyzed by heterogeneous organic base. *ACS Sustainable Chemistry & Engineering*, *4*(9), 4850-4858. https://doi.org/10.1021/acssuschemeng.6b01132

Yang, Q., Sherbahn, M., & Runge, T. (2016). Basic amino acids as green catalysts for isomerization of glucose to fructose in water. *ACS Sustainable Chemistry & Engineering*, *4*(6), 3526-3534. https://doi.org/10.1021/acssuschemeng.6b00587

Yoshida, H., Yamada, M., Nishitani, T., Takada, G., Izumori, K., & Kamitori, S. (2007). Crystal structures of D-tagatose 3-epimerase from *Pseudomonas cichorii* and its complexes with D-tagatose and D-fructose. *Journal of Molecular Biology*, *374*(2), 443-453. https://doi.org/10.1016/j.jmb.2007.09.033

Zhang, G., An, Y., Parvez, A., Zabed, H. M., Yun, J., & Qi, X. (2020). Exploring a highly D-galactose specific L-arabinose isomerase from *Bifidobacterium adolescentis* for D-tagatose production. *Frontiers in Bioengineering and Biotechnology*, *8*, 377. https://doi.org/10.3389/fbioe.2020.00377

Zhao, J., Wang, Z., Jin, Q., Feng, D., & Lee, J. (2023). Isomerization of galactose to tagatose: Recent advances in non-enzymatic isomerization. *Journal of Agricultural and Food Chemistry*, *71*(10), 4228-4234. https://doi.org/10.1021/acs.jafc.3c00095

# APPENDIX

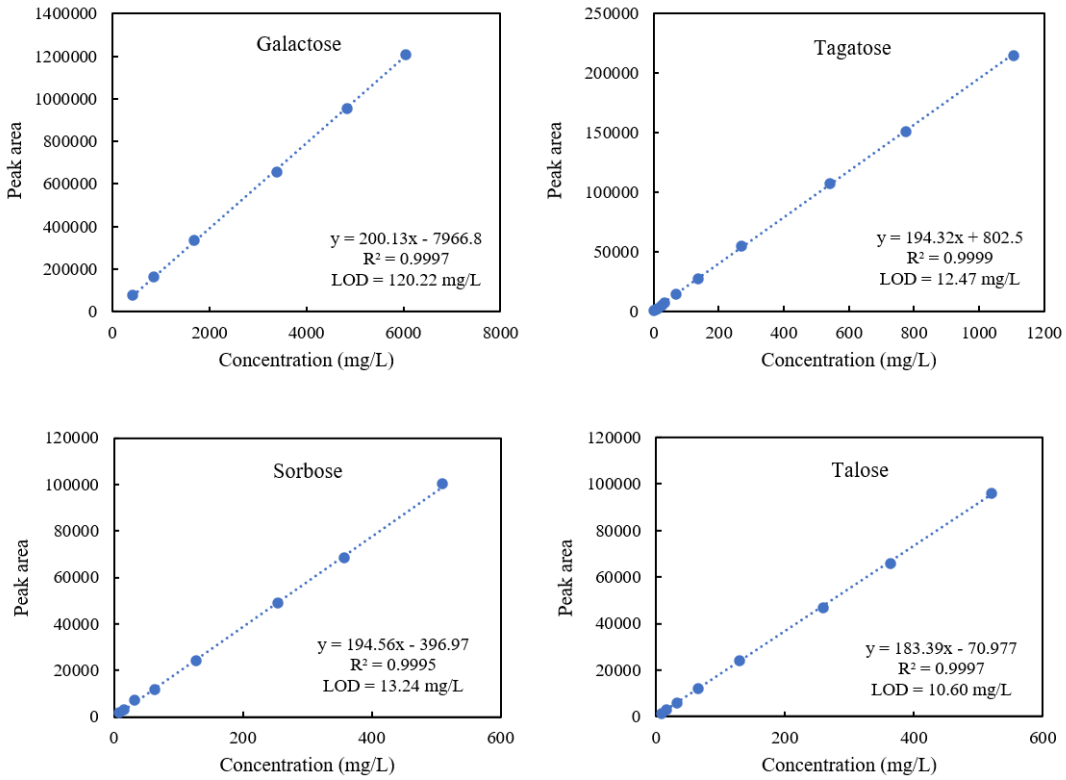



Figure A1 Calibration curves for galactose, tagatose, sorbose, and talose determination. Limit of detection (LOD) were estimated from the signal at $y_B + 3s_{y/x}$; where $y_B$ is y-axis intercept of the regression line and $s_{y/x}$ is standard error of the regression

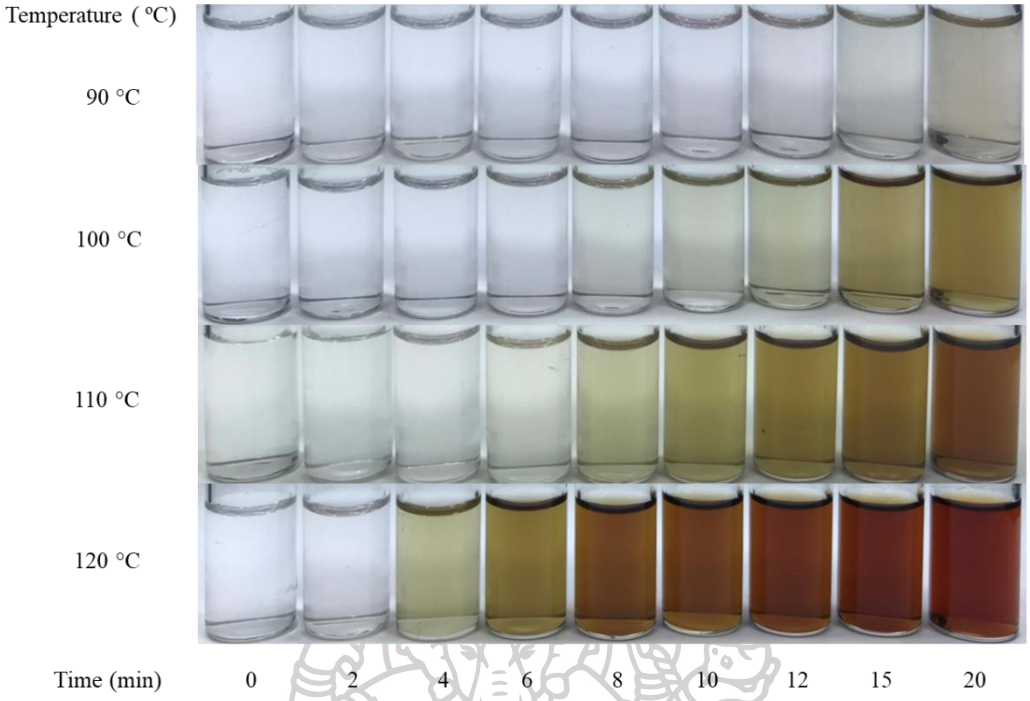



Figure A2 Changes in the appearance of the treated solution containing 5% w/v galactose and arginine of 0.10 mol/mol-galactose at different temperatures

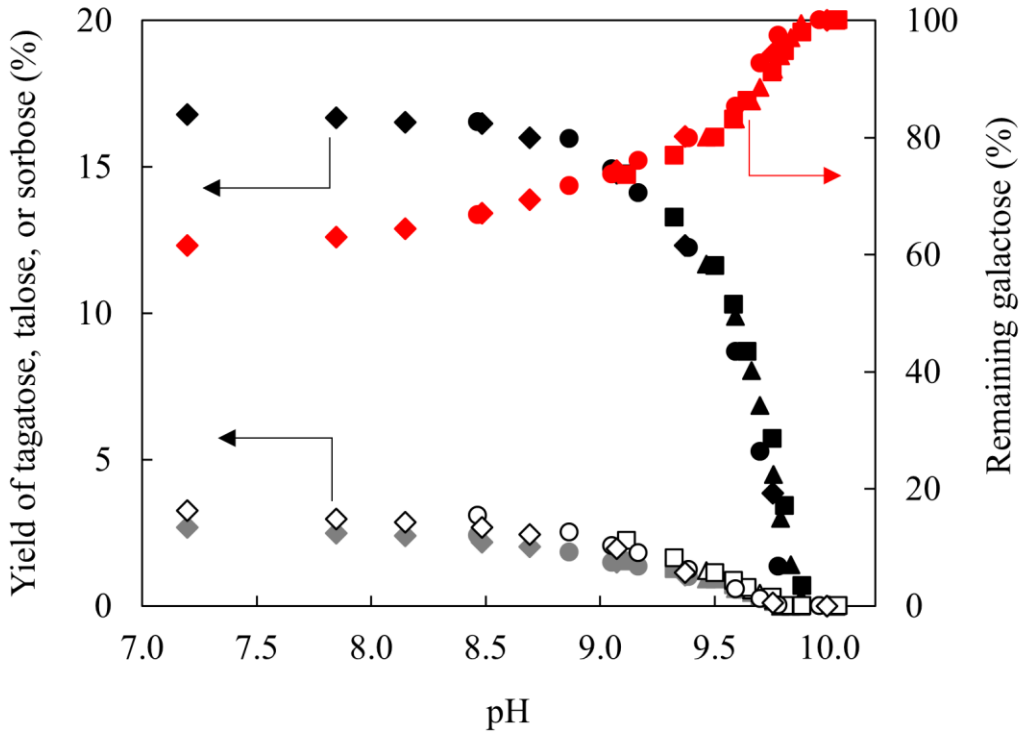



Figure A3 Changes in yields of tagatose (black), talose (grey), sorbose (white) (%), and remaining galactose (red) (%) with pH of the mixture during the treatment of 5% w/v galactose with arginine of 0.10 mol/mol-galactose at 90–120 °C, 0–20 min. Triangle, square, circle, and diamond symbols represent the results obtained at 90, 100, 110, and 120 °C, respectively

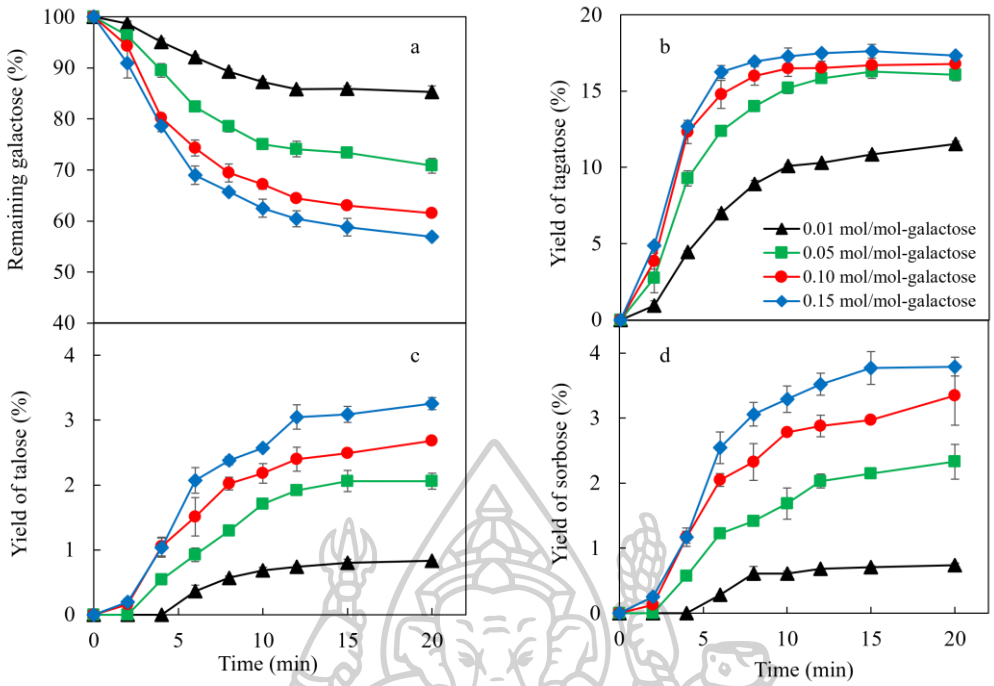



Figure A4 Isomerization of galactose (5% w/v) using arginine at different concentrations (0.01−0.15 mol/mol-galactose) as a catalyst: (a) remaining galactose, (b) yield of tagatose, (c) yield of talose, and (d) yield of sorbose at 120 °C

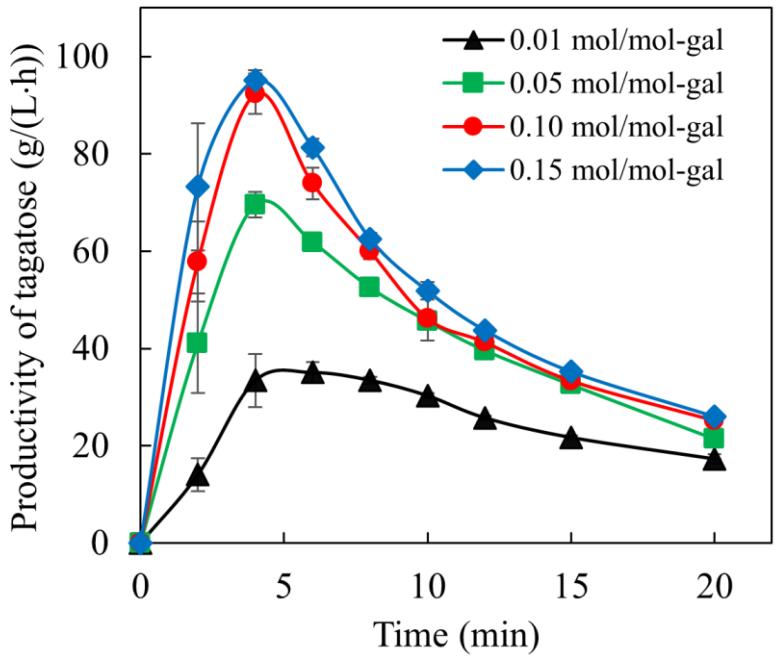



Figure A5 Productivity of tagatose of the treated solution containing 5% w/v galactose with different arginine concentrations (0.01–0.15 mol/mol-galactose) at 120 °C

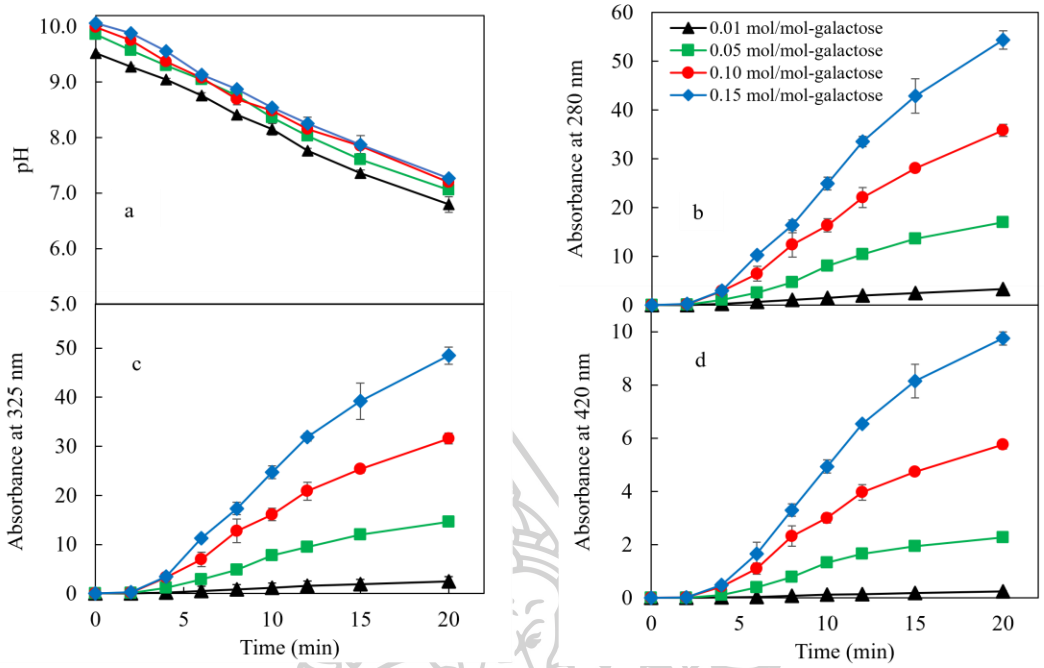



Figure A6 (a) pH; the absorbance values at (b) 280, (c) 325, and (d) 420 nm of the treated solution containing 5% w/v galactose with different arginine concentrations (0.01−0.15 mol/mol-galactose) at 120 °C

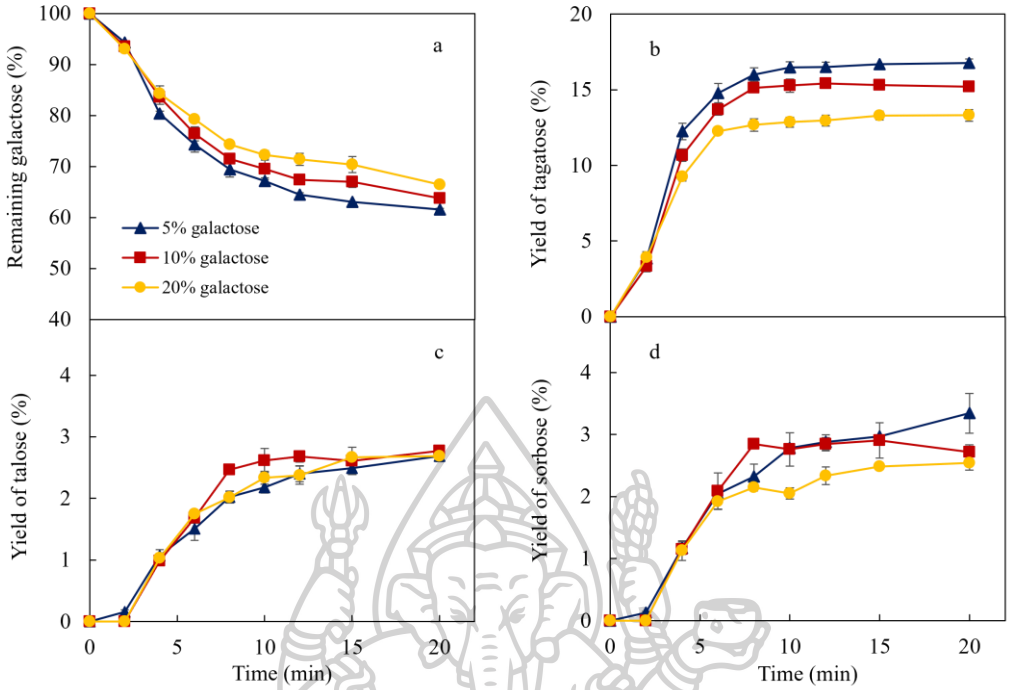



Figure A7 Isomerization of galactose at different initial concentrations (5−20% w/v) using arginine (0.10 mol/mol-galactose) as a catalyst: (a) remaining galactose, (b) yield of tagatose, (c) yield of talose, and (d) yield of sorbose at 120 °C

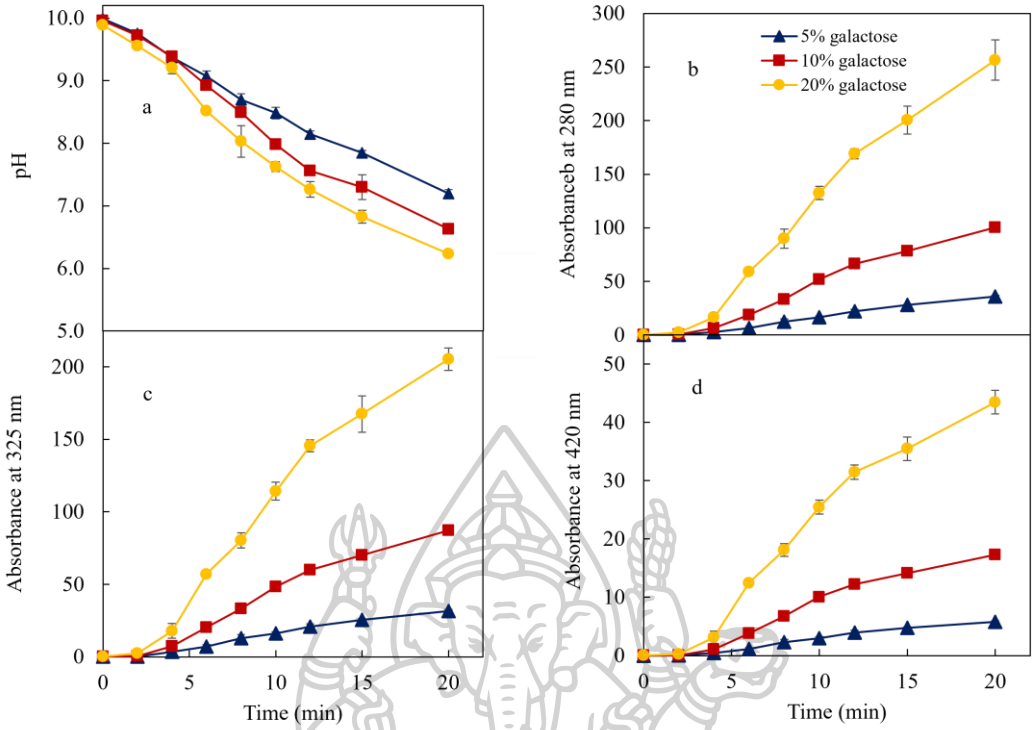



Figure A8 (a) pH; the absorbance values at (b) 280, (c) 325, and (d) 420 nm of the treated solution containing different galactose concentrations (5−20% w/v) with arginine of 0.10 mol/mol-galactose at 120 °C

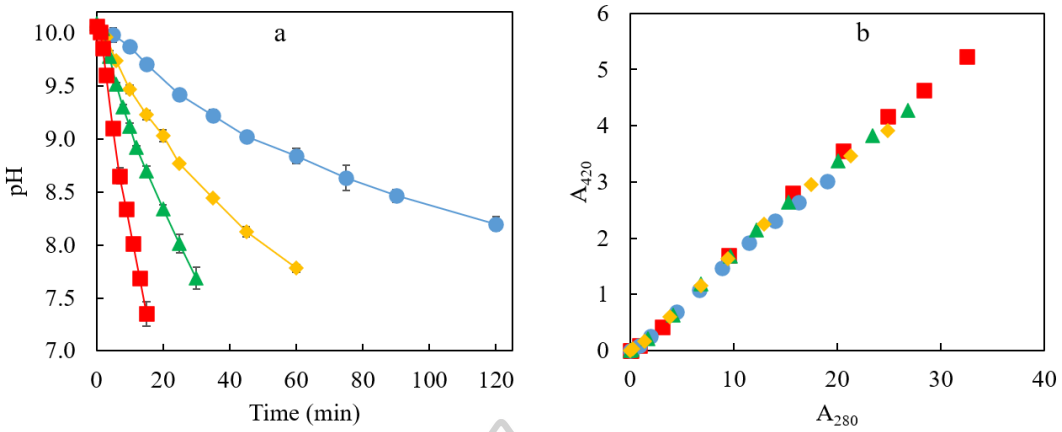
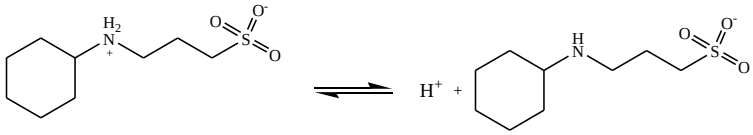
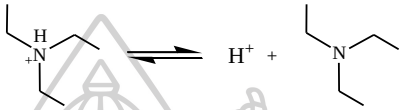
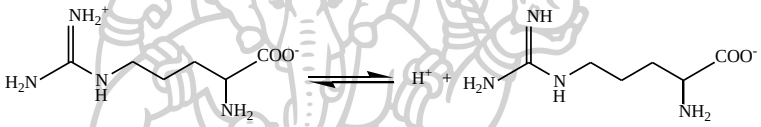



Figure A9 (a) pH, (b) the absorbance at 280 *vs* at 420 of the treated solution containing 5% w/v galactose with arginine of 0.10 mol/mol-galactose at different temperature and time

Table A1 Base catalysts used in this work and the p*K*a values of the conjugate acids

| **Catalyst** | **Dissociation equation** | **p$K_a$** |
|---|---|---|
| Carbonate buffer | $HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$ | 10.4 |
| CAPS buffer |  | 10.4 |
| TEA (triethylamine) |  | 11.2 |
| Quinuclidine |  | 11.5 |
| Arginine |  | 12.5 |

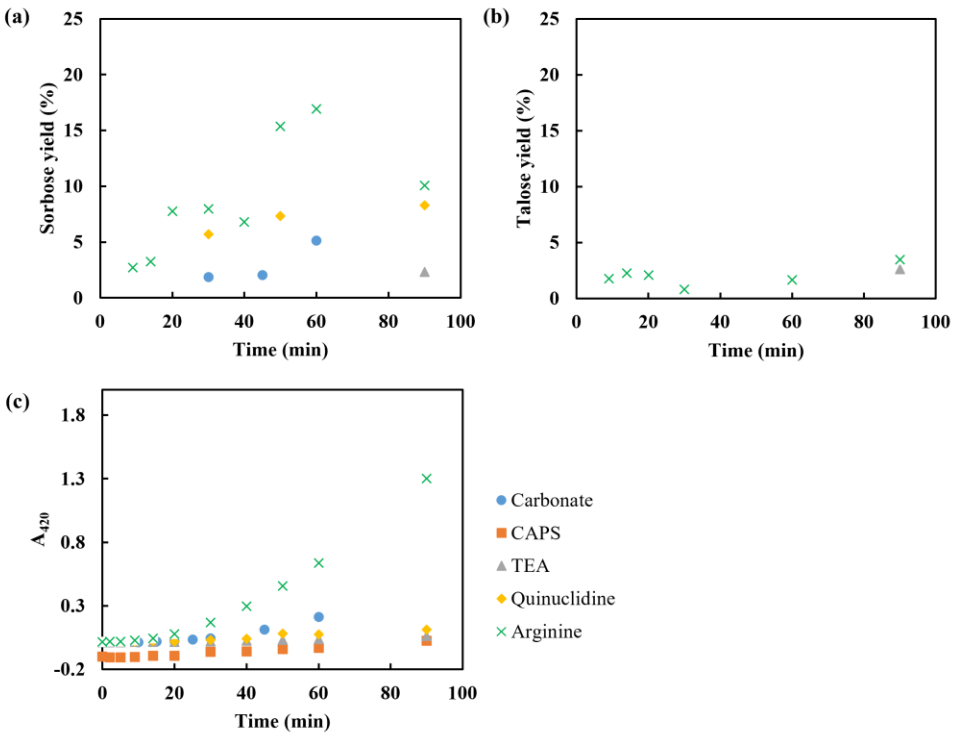



Figure A10 Yields of Sorb (a) and Tal (b) as well as absorbance at 420 nm (c) for isomerization in the presence of the bases. Reaction conditions: 5% w/v Gal, 0.5 M base for carbonate ($pH_0$ 10.4), CAPS ($pH_0$ 10.4), TEA ($pH_0$ 11.1), and Arg ($pH_0$ 11.8), 0.1 M quinuclidine ($pH_0$ 11.2), $pH_0$ adjusted with NaOH, 60 °C

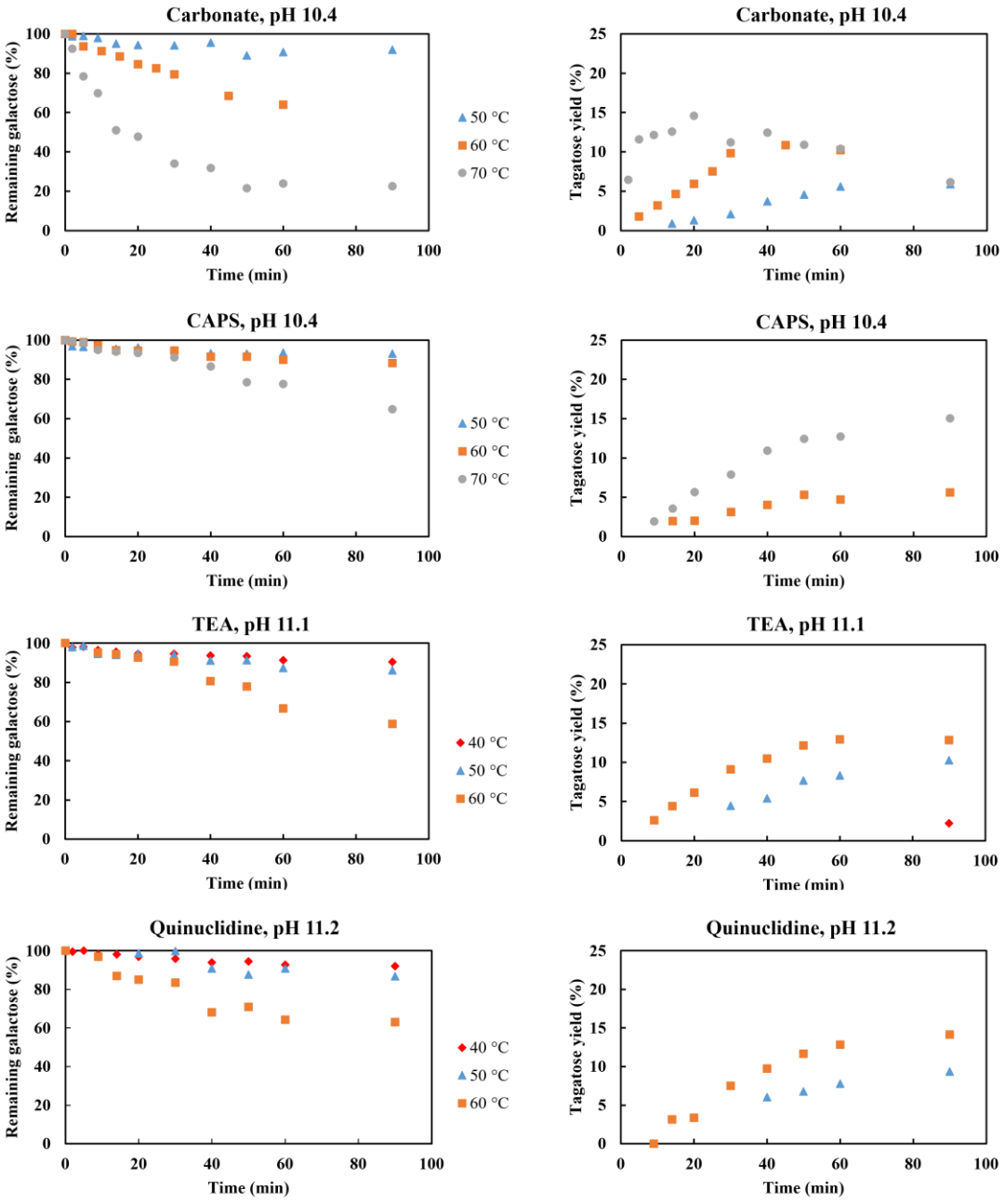



Figure A11 Results of Gal isomerization at different temperatures in the presence of the base catalysts. Reaction conditions: 5% w/v Gal, 0.5 M base for carbonate ($pH_0$ 10.4), CAPS ($pH_0$ 10.4), TEA ($pH_0$ 11.1), 0.1 M quinuclidine ($pH_0$ 11.2), $pH_0$ adjusted with NaOH

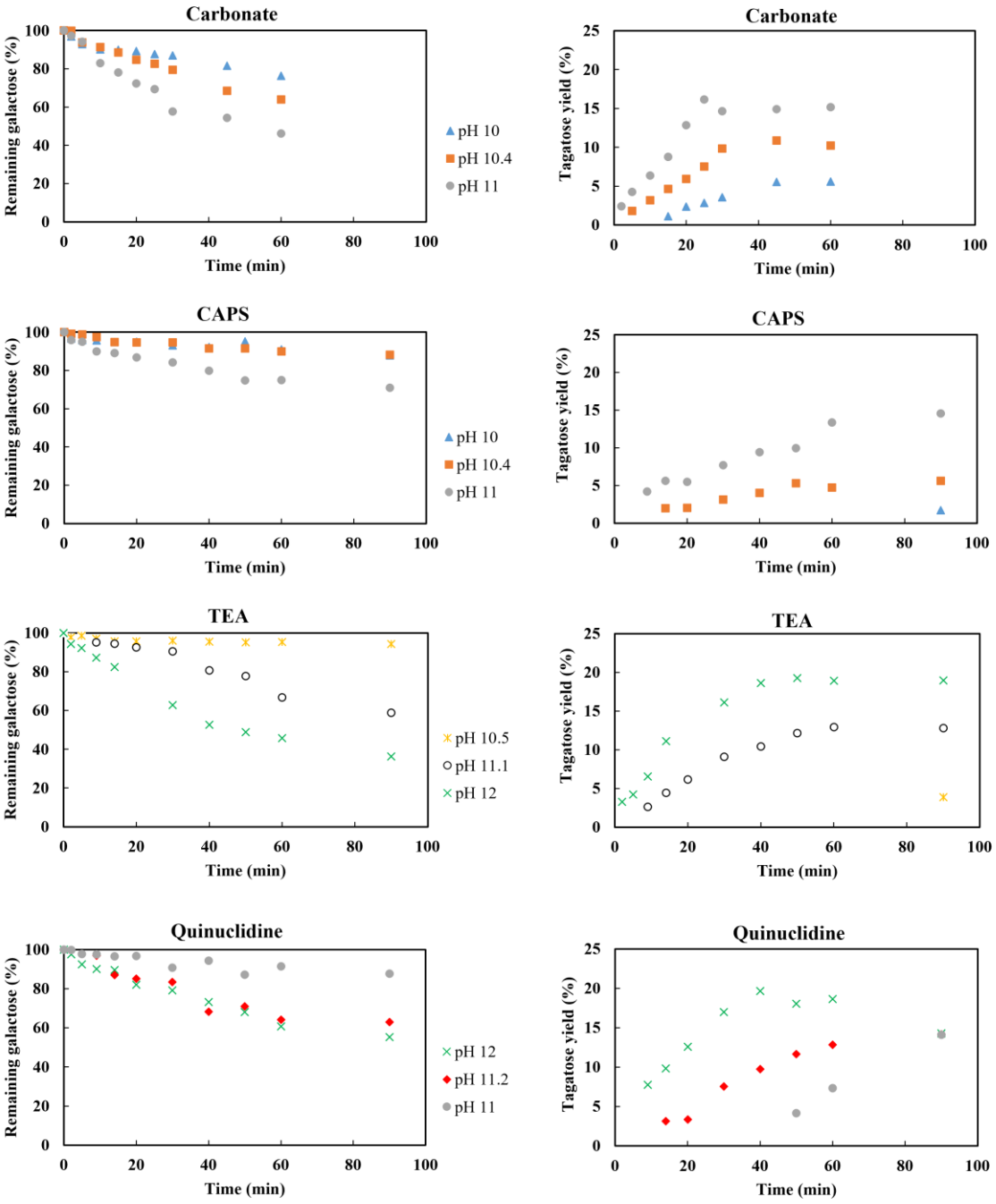



Figure A12 Results of Gal isomerization at different pH values in the presence of the base catalysts. Reaction conditions: 5% w/v Gal, 0.5 M base for carbonate, CAPS, TEA, and Arg; 0.1 M quinuclidine, $pH_0$ adjusted with NaOH, 60 °C

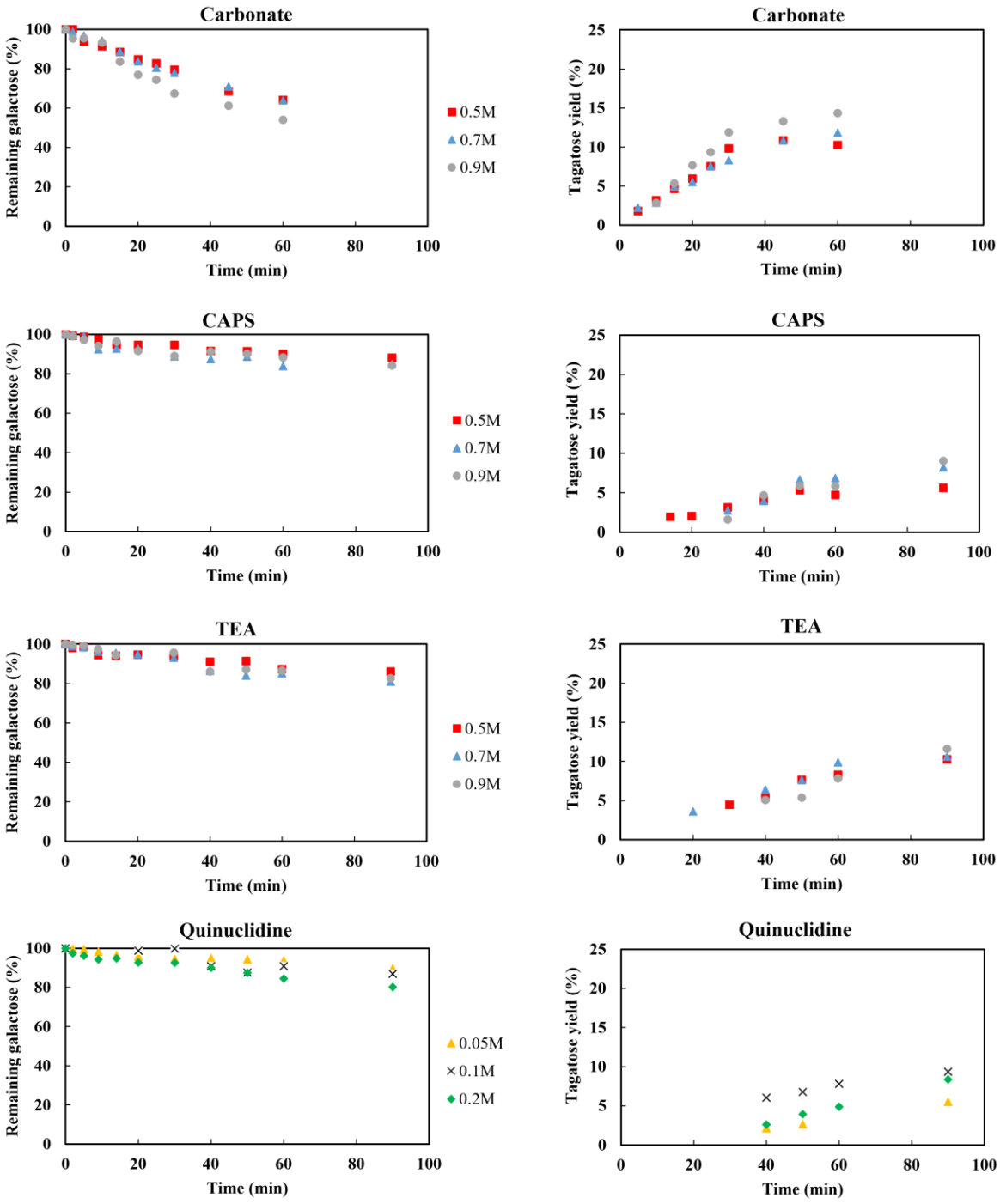
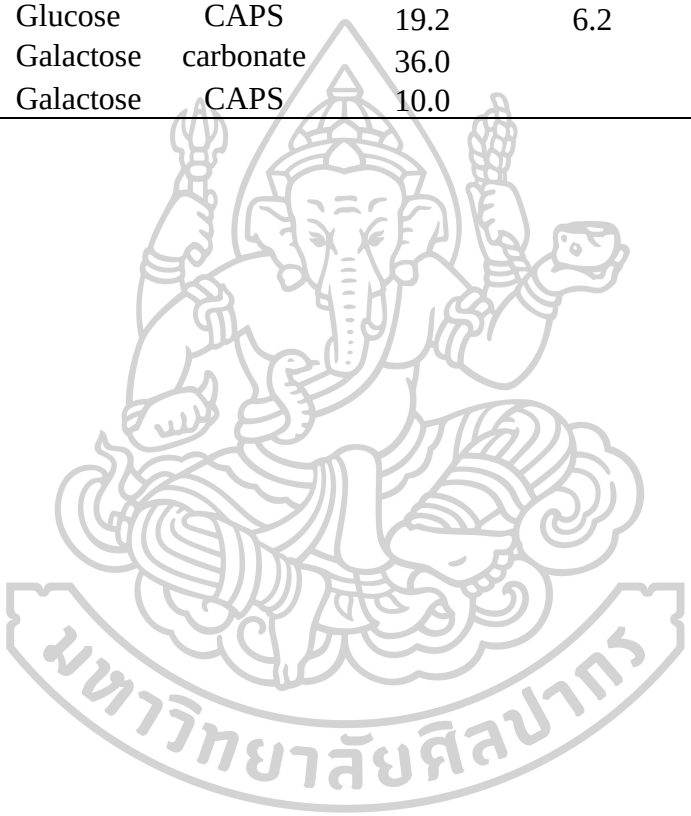



Figure A13 Results of Gal isomerization at different catalyst concentrations in the presence of the bases. Reaction conditions: 5% w/v Gal, carbonate at $pH_0$ 10.4, CAPS at $pH_0$ 10.4, 60 °C, TEA at $pH_0$ 11.1, quinuclidine at $pH_0$ 11.2, $pH_0$ adjusted with NaOH, 50 °C

Table A2 Results of Glc and Gal isomerization catalyzed by carbonate and CAPS buffers. Reaction conditions: 5% w/v Glc or Gal, 0.5 M buffer at $pH_0$ 10.4, $pH_0$ adjusted with NaOH, 60 °C, 60 min

| **Entry** | **Substrate** | **Buffer** | **Substrate conversion (%)** | **Fructose yield (%)** | **Tagatose yield (%)** | **$pH_{fin}$** |
|---|---|---|---|---|---|---|
| 1 | Glucose | carbonate | 37.6 | 20.5 | | 10.3 |
| 2 | Glucose | CAPS | 19.2 | 6.2 | | 10.4 |
| 3 | Galactose | carbonate | 36.0 | | 10.2 | 10.3 |
| 4 | Galactose | CAPS | 10.0 | | 4.7 | 10.4 |



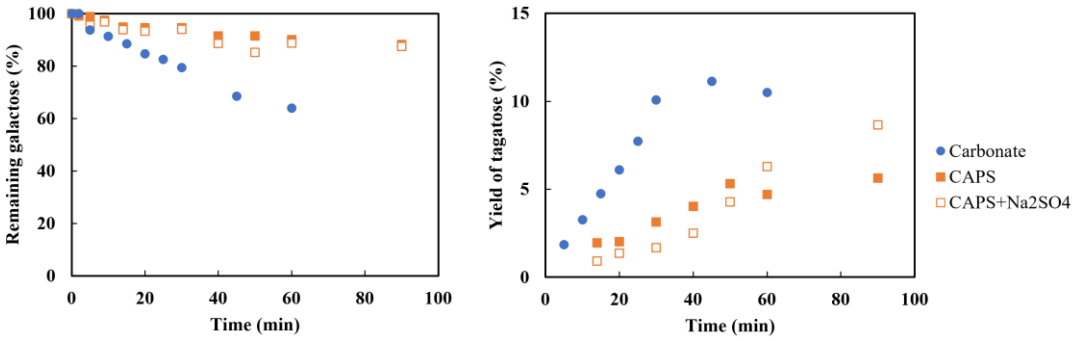



Figure A14 Results of Gal isomerization catalyzed by carbonate buffer or by CAPS buffer with and without $Na_2SO_4$. Reaction conditions: 5% w/v Gal, 0.5 M buffer at $pH_0$ 10.4, $pH_0$ adjusted with NaOH, 60 °C

# VITA

**NAME** Neeranuch Milasing

**INSTITUTIONS ATTENDED** 2015: B.Sc. (Food Technology), First-Class Honours
Rangsit University, Thailand

**PUBLICATION**

Journal

1) Milasing, N., Khuwijitjaru, P., & Adachi, S. (2023). Isomerization of galactose to tagatose using arginine as a green catalyst. Food Chemistry, 398, 133858.

2) Milasing, N., Amornrattanachart, T., Khuwijitjaru, P., & Adachi, S. (2024). Isomerisation of lactose to lactulose in an aqueous solution containing arginine. International Food Research Journal, 31, 80-86.

3) Milasing, N., Toussaint, V., Hametner, C., Khuwijitjaru, P. and Delidovich, I. Enhanced catalytic activity of carbonate buffer for isomerization of D-galactose into D-tagatose (submitted to Food Chemistry)

4) Milasing, N., Khuwijitjaru, P., Adachi, S. Effect of ethanol addition on tagatose production from galactose in arginine solution (manuscript in preparation)

5) Khuwijitjaru, P., Milasing, N., Adachi, S. (2018) Production of D-tagatose: A review with emphasis on subcritical fluid treatment. Science, Engineering and Health Studies 12(3): 159-167.

6) Limsangouan, N., Milasing, N., Thongngam, M., Khuwijitjaru, P. and Jittanit, W. (2019) Physical and chemical properties, antioxidant capacity and total phenolic content of tamarind (Tamarindus indica) seed xyloglucan component extracted using subcritical water. Journal of Food Processing and Preservation. e14146.

7) Klinchongkon, K., Intim, B., Milasing, N., Khuwijitjaru, P. (2022) Effect of ethanol concentration and temperature on solubility of fructose. Food Science and Technology Research 28 (1), 105–109.

Conferences

1) Milasing, N., and Khuwijitjaru, P. Production of tagatose from galactose by subcritical aqueous ethanol. Food Innovation Asia Conference 2020 (FIAC 2020)

2) Milasing, N., Sriisaranusorn, N., Klinchongkon, K., and Khuwijitjaru, P. Separation of psicose from common sugars using cation-exchange resin column chromatography. Food Innovation Asia Conference 2021 (FIAC 2021)

**AWARD RECEIVED** Honorable Mention Graduate Student Poster Competition Award, Food Innovation Asia Conference 2021 (FIAC 2021)