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Isomerisation of lactose to lactulose in an aqueous solution containing arginine

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Abstract

In the present work, the isomerisation 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 isomerisation 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 isomerised into its rare isomer using an environmentally friendly process.

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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 and Saito, 2012). Lactulose (4-O-β-$_D$-galactopyranosyl-$_D$-fructose) is used to treat constipation, and has several health benefits as a prebiotic (Olano and Corzo, 2009; Schuster-Wolff-Bühring *et al*., 2010). Lactulose is obtained in small quantities from milk after thermal processing (Adachi and 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 isomerisation of lactose have been proposed (Karim and Aider, 2022). One method involves the conversion of the glucose unit in the disaccharide structure to a fructose unit through alkaline isomerisation, 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 (Kim and Oh, 2012; Jameson *et al*., 2021). 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 and de Halleux, 2007).

Besides the common bases used for the isomerisation of sugars, such as sodium hydroxide (Dendene *et al*., 1994; Hashemi and Ashtiani, 2010; Hajek *et al*., 2013) which provided the maximum yield of 27.9% at pH 11 and 70°C (Hashemi and 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 -

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21% yield (Paseephol *et al*., 2008). Alternatively, the isomerisation of lactose in subcritical aqueous ethanol at high temperatures provided a maximum yield of 34% in 60% ethanol at 200°C (Soisangwan *et al*., 2017). Recently, Karim and Aider (2020) and Djouab and Aïder (2019) reported electroisomerisation technologies for lactulose isomerisation with the highest yields of 38 and 39.8%, respectively.

Recently, however, a "green" method for sugar isomerisation is gaining research interest. Yang *et al*. (2016) reported that glucose could be efficiently isomerised to fructose in an arginine solution. 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 isomerisation of galactose to tagatose (Milasing *et al*., 2023) and ribose to ribulose (Khuwijitjaru and Adachi, 2023). 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 catalyse the isomerisation of disaccharide. Lactose was used as the substrate for lactulose production at various temperatures and reaction times.

Materials and methods

Reagent

Lactose monohydrate was purchased from KEMAUS (New South Wales, Australia). Lactulose (purity > 98.0%) was purchased from TCI (Tokyo, Japan). Galactose (> 99%), glucose (> 99.5%), and 5-hydroxymethyl-2-furaldehyde (5-HMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Arginine (purity > 98%) was purchased from Merck (Darmstadt, Germany). Deionised water was used throughout the study.

Isomerisation of lactose

Isomerisation of lactose was performed in a screw-capped glass vial (total volume, 4 mL). A solution of lactose (5%, w/v) and arginine (0.1 mol/mol lactose) was prepared in deionised 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 analysed for chemical properties. All treatments were conducted in triplicates.

Sugar analyses

Lactose (C_S), lactulose (C_P), galactose, and glucose contents in the reaction solution were analysed 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.

Maillard reaction extent

To determine the extent of Maillard reaction during isomerisation, 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). In addition, 5-HMF, which is a well-known intermediate compound of the Maillard reaction of hexose sugar (Damodaran and Parkin, 2017) and dehydration product of monosaccharides (Istasse and Richel, 2020), was also quantitatively determined from the absorbance at 285 nm (de Andrade *et al*., 2017) using a standard curve of reference standard.

Results and discussion

Appearances

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

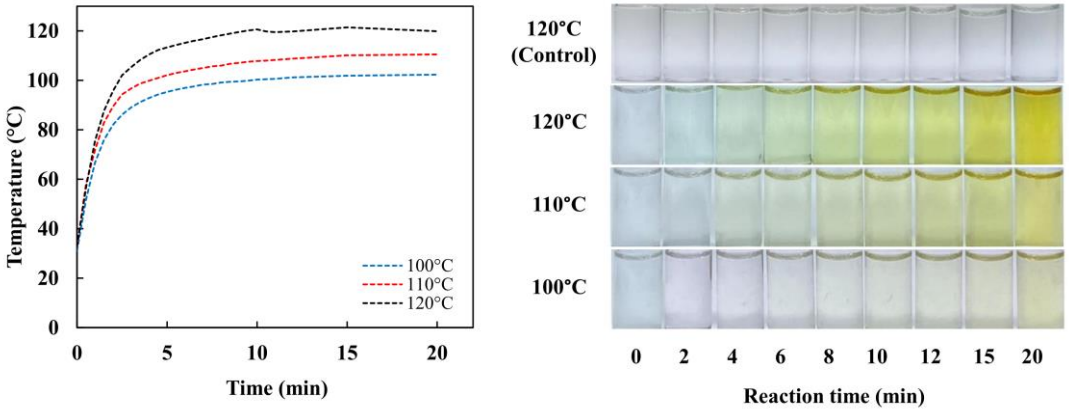

Figure 1. Temperature profiles of the reaction solutions containing 5% (w/v) lactose and 0.1 mol/mol lactose of arginine **(a)**. Changes in appearances of treated solutions with time at various reaction temperatures **(b)**.

Isomerisation 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 2. Lactose content gradually decreased with reaction time, suggesting that lactose either isomerised under alkaline conditions to form lactulose, reacted with arginine in the Maillard reaction, or simply hydrolysed 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 isomerisation 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 isomerisation using sodium hydroxide (Hashemi and Ashtiani, 2010; Hajek *et al*., 2013). Moreover, since only arginine was used in the present work to promote isomerisation, 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 and 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 isomerisation of a wide variety of sugars (Khuwijitjaru *et al*., 2023; Milasing *et al*., 2023; Yang *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 isomerisation (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 and Ashtiani, 2010; Soisangwan *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 isomerisation of lactose within the reaction time of 20 min used in the present work.

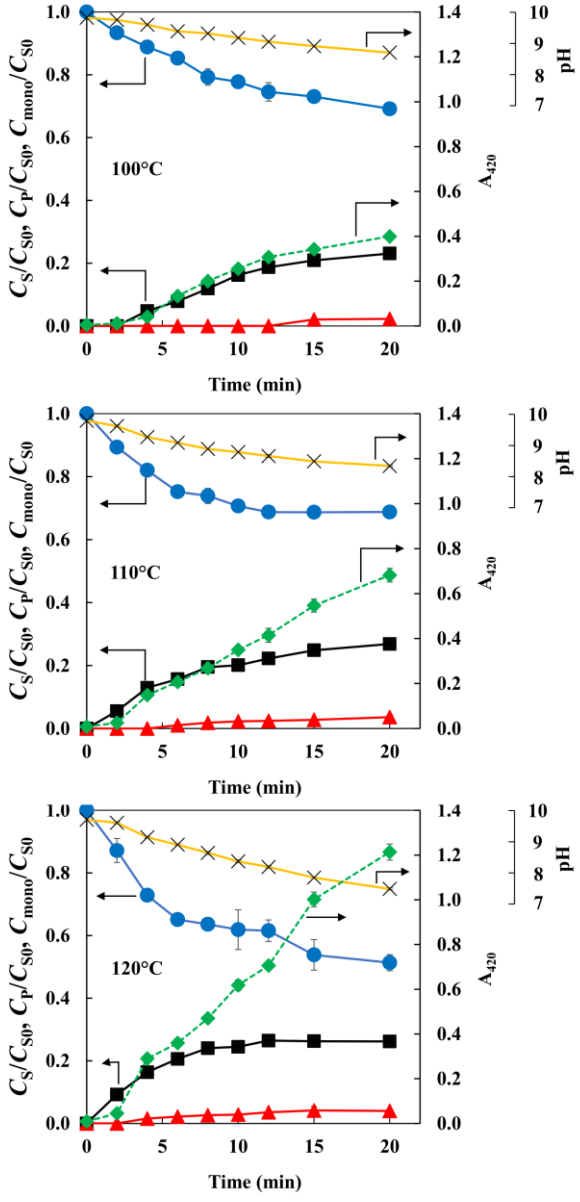

Figure 2. Changes in mass ratios of lactose (C_S/C_{S0}, ●), lactulose (C_P/C_{S0}, ■), monosaccharide (C_{mono}/C_{S0}, ▲), pH (×), and absorbance at 420 nm (◆) during heating of the reaction solution containing 5% (w/v) lactose and 0.1 mol/mol lactose of arginine.

Degradation products and discoloration

Figure 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 (De Bruijn *et al.*, 1987; Aida *et al.*, 2007). 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.

Relationship between pH and isomerisation reaction

As shown in Figure 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.

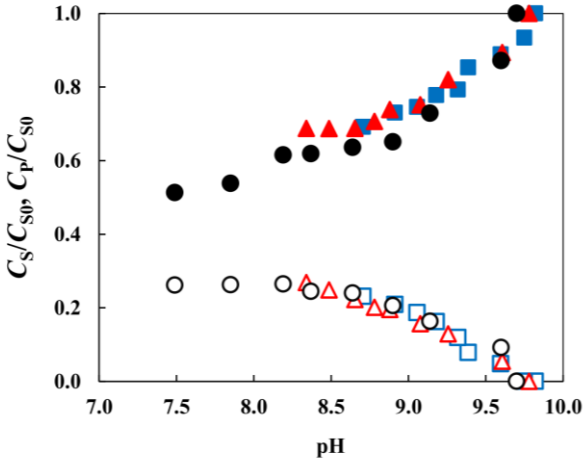

Figure 3. Changes in mass ratios of lactose (C_S/C_{S0}, closed symbols) and lactulose (C_P/C_{S0}, open symbols) with the solution pH during heating of the reaction solution containing 5% (w/v) lactose and 0.1 mol/mol lactose of arginine at 100 (■, □), 110 (▲, △), and 120°C (●, ○).

This suggested that the mechanisms of lactose consumption and lactulose formation are similar at different temperatures. Isomerisation 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 pK_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 4) (Wang *et al*., 2022).

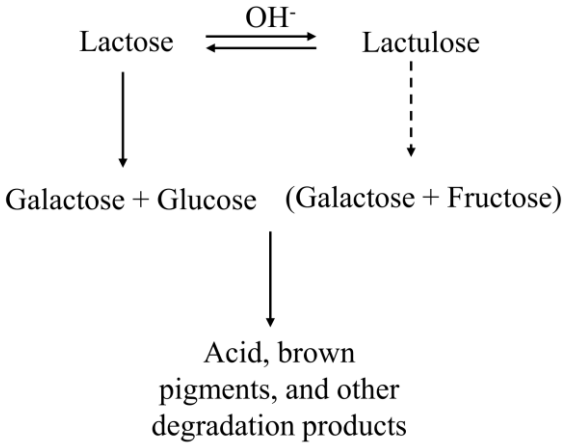

Figure 4. Reaction scheme for isomerisation of lactose to lactulose, hydrolysis of lactulose, and degradation reactions occurring in arginine solutions. Dashed line represents negligible reaction.

As shown in Figure 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 *et al*., 2017). However, during the 20-min reaction in the present work, a decrease in lactulose content was not observed.

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. As shown in Figure 5a, the 5-HMF content, measured by an absorbance at 285 nm, continuously increased with reaction time at all temperatures, and the values were higher at higher temperatures. The absorbances at 280, 325, and 420 nm were also monitored during the reaction (Echavarría *et al*., 2014). Figure 5b 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 coloured compounds during the Maillard reaction compared to lysine.

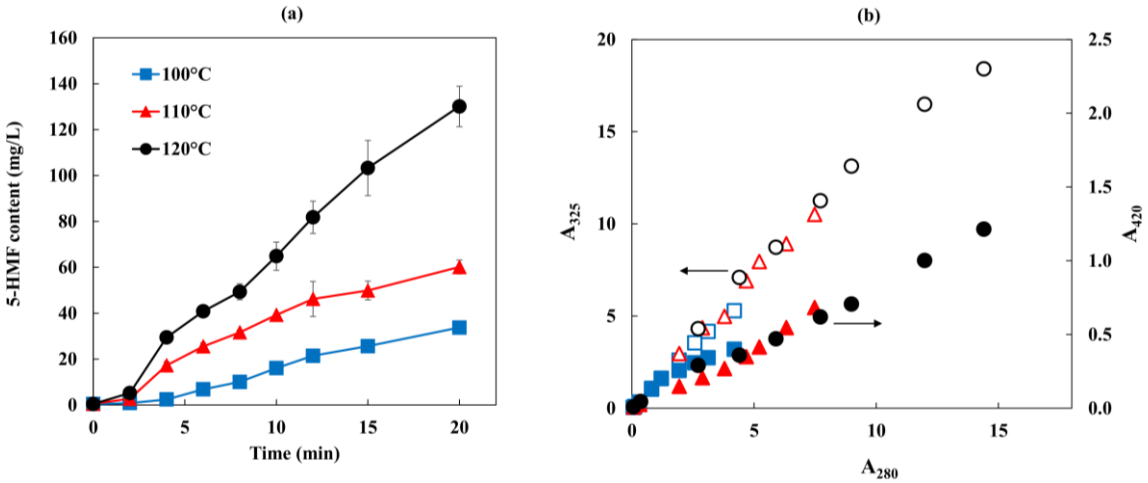

Figure 5. Changes in the 5-HMF content (closed symbols) with time **(a)** and relationships between absorbance at 325 nm (A_{325}, open symbols) and 420 nm (A_{420}, closed symbols) versus absorbance at 280 nm (A_{280}) of the solutions **(b)** during heating of the reaction solution containing 5% (w/v) lactose and 0.1 mol/mol lactose of arginine at 100 (■, □), 110 (▲, △), and 120°C (●, ○).

Conclusion

The present work demonstrated that lactose could be isomerised with arginine after heating in solution. The highest lactulose yield (27%) was comparable to that of a common base-catalysed 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 isomerisation of lactose to lactulose would be beneficial to the industry.

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