

1 Isomerization of maltose to maltulose under microwave heating using
2 uncalcined scallop shell powder

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Abstract

Uncalcined scallop shell powder, consisting mainly of calcium carbonate, and an aqueous maltose solution were placed in a pressure-resistant vessel and heated in a domestic microwave oven to isomerize maltose to maltulose. When a maltose solution (50 mL) was heated at a power of 700 W, a high maltulose yield of approximately 30% was achieved in as short a time as 105 s. As the reaction progressed, the shell powder dissolved and neutralized acidic by-products to suppress the decrease in pH, keeping the pH above 7, where the isomerization by Lobry de Bruyn-Alberda van Ekenstein transformation proceeded. The average selectivity for isomerization of maltose to maltulose under various reaction conditions was 0.822. This would be because the shell powder kept the pH of the reaction solution in the range of 7 to 9 and suppressed the progression of side reactions. The coloration of the reaction solution increased rapidly when the yield of maltulose approached its maximum value. This indicated that if the reaction were to be stopped when the yield of maltulose was close to its maximum value, a high yield of maltulose solution with low coloration could be obtained.

Keywords: Isomerization • Maltose • Maltulose • Microwave heating • Shell powder

1. Introduction

Maltulose (4-*O*- α -D-glucopyranosyl-D-fructofuranose) is a disaccharide consisting of a glucose molecule and a fructose molecule linked via an α -1,4 glycosidic bond. It is rarely found in nature, but honey contains maltulose at about 1-3% of its total sugar (de la Fuente et al., 2011; Mateo & Bosch-Reig, 1997). However, maltulose has been found to account for up to 5.6% of the total sugar in saccharified starch obtained by autoclave liquefaction at 121-143°C and pH 4.5-6.5 (Dias & Panchal, 1987). Apart from its sweetness of 31% compared with sucrose (Ruiz-Acetiuno et al., 2018), other physical properties and physiological functions of maltulose as a food additive have not been fully elucidated. The lack of an efficient mass-production method for maltulose has made it difficult to evaluate the functions of maltulose and to develop applications for this substance. Therefore, it is necessary to establish an efficient mass-production method for maltulose to determine its physicochemical properties and identify opportunities for its use in various industries.

Brands and van Boekel (2003) reported that maltulose could be produced by heating maltose with casein in a phosphate buffer of pH 6.8 and at 120°C. Maltose is isomerized to maltulose through an enediol intermediate via the well-known Lobry de Bruyn-Alberda van Ekenstein (LBAE) transformation (Sowden & Schaffer, 1952; Delidovich, 2023). We have also reported the formation of maltulose from maltose in phosphate buffer (pH 7.0) under subcritical water conditions, with a maximum yield of approximately 20% (Adachi et al., 2022; 2021).

Microwave heating is a rapid heating method that is particularly useful in aqueous systems because it is based on the motion of water molecules and charged ions due to the action of electromagnetic waves with frequencies in the range of 0.3 to 300 GHz (Meda et al., 2017). Microwave heating has been used to accelerate several types of chemical reactions and is recognized to be a useful heating method in green chemistry (Bassyouni et al., 2012). Microwave heating has also been shown to be more energy efficient than conventional heating, especially for short reaction times (Moseley & Woodman, 2009). For example, Nooshkam and Madadlou (2016) reported that lactose could be isomerized to lactulose in just 60 s in a

solution adjusted to pH 9.0 with sodium hydroxide.

Recently, we investigated the potential use of arginine, a basic amino acid widely used as a food additive to produce rare sugars from common monosaccharides at high temperatures (Khuwijitjaru & Adachi, 2023a; 2023b; Khuwijitjaru et al., 2023; Kobayashi et al., 2023; Milasing et al., 2023). We performed isomerization of maltose dissolved in arginine solution at 110°C and achieved a maximum maltulose yield of 30% (Khuwijitjaru et al., 2024). However, brown pigments were inevitably formed due to the Maillard reaction. Li et al. (2018) showed that microwave heating of glucose in an alkaline solution (pH 10.65) resulted in greater glucose degradation and a decrease in pH, causing an increase in absorbance at 420 nm. We have also investigated the effects of applied electrical energy on the isomerization behavior of maltose (Khuwijitjaru et al., 2024).

Low-cost, calcium carbonate-based natural materials, such as egg shells and crab shells, have previously been used for the isomerization of sugars (Montilla et al., 2005; Shen et al., 2019). Scallop shells are an abundant by-product of scallop meat processing. The main component of shells is calcium carbonate, some of which is calcined and converted to calcium oxide (quicklime) (Hosoda et al., 1996), which is used as a soil conditioner. Calcium carbonate is only slightly soluble in water at room temperature (0.1521 mmol/kg), and the aqueous solution is slightly alkaline (Zhao et al., 2015). The isomerization of saccharides under alkaline conditions produces acidic substances as by-products, which lower the pH and prevent the isomerization from proceeding (Onishi et al., 2022). It is anticipated that calcium carbonate, as the main component of shells, would neutralize the acidic substances formed during the isomerization process and suppress the decrease in pH. In this study, we used uncalcined scallop shell powder for isomerizing maltose to maltulose under microwave heating and investigated the effects of operating conditions on the yield of maltulose, pH decrease, and coloration. The concentrations of calcium ions that leached from the shell powder were also measured.

2. Materials and Methods

2.1. Materials

D-Maltose monohydrate (Wako special grade; hereafter referred to simply as maltose), acetonitrile (HPLC grade), and distilled water (HPLC grade) were purchased from FUJIFILM Wako Pure Chemicals Corp. (Osaka, Japan). Shell powder 120 (scallop shell, uncalcined) is a product of Furusato Bussan (Aomori, Japan), and is referred to hereafter as shell powder.

2.2. Isomerization of maltose by microwave heating using shell powder

To investigate the effect of microwave oven power on the isomerization of maltose to maltulose, 50 mL of 0.2 mol/L maltose solution and 0.5 g of shell powder were placed in a perfluoroalkoxy alkane (PFA) pressure-resistant vessel with a relief valve (120 mL volume, pressure resistance 0.7 MPa; Flon Industries, Tokyo, Japan). The microwave heating was carried out at a constant power of 200, 500, or 700 W in a domestic microwave oven (E-701S, Yuasa Primus, Osaka, Japan) with rotating the vessel at 10 rpm.

After a predetermined time, the vessel was removed from the oven and immediately cooled to room temperature in an ice water bath. The reaction solution was filtered through a membrane filter (0.2-μm pore size, DISMIC-28CP, Toyo Roshi, Tokyo, Japan). The concentrations of remaining maltose and formed maltulose, the pH, and the calcium ion concentration in the clear reaction solution after removal of the shell powder were measured. The absorbance at 280 and 420 nm was measured as an indicator of the coloration of the reaction solution. Each of these items were measured in all of the experiments conducted in this study.

During the heating, the inner temperature of the vessel was expressed as follows:

$$T = T_0 + \frac{f_E P}{UA} \left[1 - \exp \left(-\frac{UA}{c_p w_0} t \right) \right] \quad (1)$$

where, T_0 and T are the temperatures [°C] of water before and after microwave heating at time t [s], f_E is the energy efficiency during microwave heating (dimensionless), P is the output power of microwave

[W], U is the overall heat transfer coefficient [$\text{W}/(\text{m}^2\cdot\text{K})$], A is the heat transfer area [m^2]. c_p is the heat capacity of water [$\text{J}/(\text{g}\cdot^\circ\text{C})$] (assumed to be $4.2 \text{ J}/(\text{g}\cdot^\circ\text{C})$), w_0 is the weight of the liquid [g] (Khuwijitjaru et al., 2024). Based on the equation (1), when heated at the highest power (700 W) for 105 s, the maximum temperature of the reaction mixture would reach about 160°C . Similar calculations show that at 500 W for 180 s, the temperature would be 158°C ; and at 200 W for 600 s, 133°C . In addition, temperature changes under other conditions have been previously reported (Khuwijitjaru et al., 2024).

2.3. Effect of microwave oven power on the isomerization of maltose

The microwave oven used had three power levels: 200, 500, and 700 W. The microwave was used to heat a 50-mL maltose solution of concentration 0.2 mol/L at the different power levels to examine the effect of power on the reaction efficiency. The longest heating times were 600, 190, and 105 s at 200, 500, and 700 W, respectively. These times were determined to be the longest times within which the relief valve of the pressure-resistant vessel would not be released.

2.4. Effect of the quantity of shell powder

To examine the effect of the quantity of shell powder, the maltose concentration was fixed at 0.2 mol/L and its volume was kept constant at 50 mL. Then, 0, 0.02, 0.05, 0.1, 0.3, 0.5, or 1.0 g of shell powder was added, and microwave heating was performed at a constant power of 700 W for 30 to 105 s. The ratio of shell powder to maltose ranged from 0 to 100 g-shell:mol-maltose.

2.5. Effect of the volume of substrate solution

To determine the effect of the volume of substrate solution, 50, 75, or 100 mL of 0.2 mol/L maltose solution was added to a pressure-resistant vessel. Then, 0.5, 0.75, or 1.0 g of shell powder was added so that the ratio of maltose to shell powder remained constant, i.e., 50 g-shell powder:mol-maltose; microwave heating was then performed at 700 W. The longest heating times were 105, 140, and 170 s for 50, 75, and

100 mL of maltose solution, respectively. Longer heating times would have resulted in higher temperatures and thus higher pressures in the vessel, causing the relief valve open and release the liquid. Therefore, in subsequent experiments with 50 mL of maltose solution, the longest heating time was 105 s, regardless of the maltose concentration.

2.6. Effect of maltose concentration at a constant quantity of shell powder

The volume of maltose solution and the quantity of shell powder in the pressure-resistant vessel were kept constant at 50 mL and 0.5 g, respectively, and the concentration of maltose was changed to 0.1, 0.2, 0.5, or 1.0 mol/L. Microwave heating was then conducted at 700 W for 105 s to examine the effect of maltose concentration at a constant quantity of shell powder.

2.7. Effect of maltose concentration at a constant ratio of the quantity of shell powder to the volume of maltose solution

To obtain a constant ratio of 50 g-shell powder:mol-maltose, 50 mL of 0.1, 0.2, 0.5, or 1.0 mol/L maltose solution and 0.25, 0.5, 1.25, or 2.5 g shell powder were placed in a pressure-resistant vessel and microwave-heated at 700 W for up to 105 s.

2.8. Analysis

A high-performance liquid chromatography (HPLC) system consisting of a pump (LC-10AD_{VP}, Shimadzu Corp., Kyoto, Japan), a COSMOSIL Sugar-D column (4.6-mm internal diameter (i.d.) × 250 mm, Nacalai Tesque, Kyoto, Japan), a differential refractometer detector (Shodex RI-101, Resonac, Tokyo, Japan), and a recorder (Chromatopac C-R8A, Shimadzu Corp.) was used to measure the concentrations of remaining maltose and produced maltulose in the reaction solution. A 5-μL sample of the reaction solution was applied to the column using a micro syringe (701N, Hamilton, Reno, NV, USA) and eluted with acetonitrile:water (80:20 volume ratio) at a flow rate of 1.0 mL/min.

The pH and calcium ion concentration of the reaction solution were measured using a LAQUAtwin pH meter and a LAQUAtwin calcium ion meter (both from Horiba Corp., Kyoto, Japan), respectively. The absorbance of the reaction solution at 280 and 420 nm was measured using a UV-visible spectrophotometer (UV-1280, Shimadzu Corp.), as an indicator of the coloration of the reaction solution. All analyses were performed at room temperature.

3. Results and Discussion

3.1. Effect of the power of the microwave oven on the isomerization

Isomerization of maltose was observed upon microwave heating of maltose solution. Figure S1 shows the typical HPLC chromatogram for the treated solution obtained by 700 W for 105 s at a constant initial maltose concentration of 0.2 mol/L and volume of 50 mL. Maltulose showed the same HPLC elution behavior (RT=14.5 min) as in the previous study, and no discrepancies were observed (Gao et al., 2016). In addition, this peak was already identified as maltulose by NMR. In parallel with the formation of maltulose, the release of glucose was observed as a degradation product (RT=9.2 min). However, glucose is not a rare sugar and is not discussed in detail here.

Figure 1(a) shows the changes over time in the fraction of remaining maltose and the yield of maltulose when microwave heating was operated at 200, 500, or 700 W. At each of the power levels, the fraction of remaining maltose and yield of produced maltulose did not change significantly immediately after heating (within 30 s). This may have been because the temperature of the reaction solution only slightly increased and the reaction barely progressed. As the heating time became longer, the temperature of the reaction solution increased, resulting in a rapid decrease in the fraction of remaining maltose and a rapid increase in the yield of maltulose. Thus, the yield of maltulose increased in response to the decrease in maltose. The changes in the fraction of remaining maltose and the yield of maltulose occurred more rapidly at higher power. However, at the end of the reaction, the fraction of remaining maltose and the yield of maltulose were

approximately 65% and 30%, respectively, at any power. The yield of maltulose was almost the same as the yield of maltulose (~30%) when arginine was added (Khwijitjaru et al., 2024), but slightly higher than in phosphate buffer (27%) (Onishi, 2022).

Changes over time in pH and calcium ion concentration are shown in Fig. 1(b). Figure 1(c) shows the changes over time in the absorbance at 280 and 420 nm, A_{280} and A_{420} , respectively. The pH decreased at

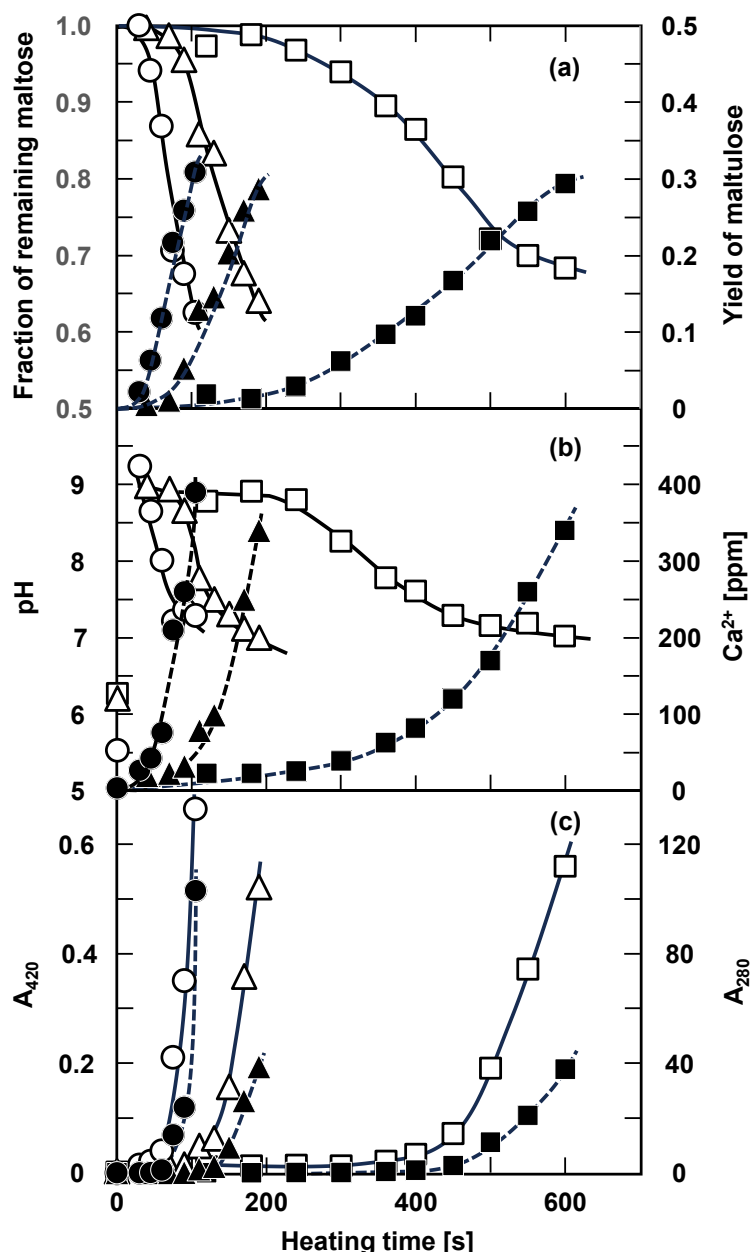


Fig. 1 Effects of the applied power on the changes over time: (a) The fraction of remaining maltose C_S/C_{S0} (open symbols) and the yield of maltulose C_P/C_{S0} (closed symbols), (b) pH (open symbols) and calcium ion (Ca^{2+}) concentration (closed symbols), (c) absorbance at 280 and 420 nm (A_{280} (open symbols) and A_{420} (closed symbols)) for 0.2 mol/L maltose (50 mL) with 0.5 g shell powder at microwave heating powers of 200 W ($\square$, $\blacksquare$), 500 W ($\triangle$, $\blacktriangle$), and 700 W ($\circ$, $\bullet$).

differing rates depending on the applied power, but in all cases the pH approached approximately 7. The calcium ion concentration increased rapidly with increasing heating time, with the changes in A_{280} and A_{420} showing a similar trend. These results suggest that calcium carbonate, the main component of shells, gradually dissolved and then neutralized the acidic substances produced by the side reactions, thereby preventing a large decrease in pH and enabling a high maltulose yield of approximately 30% to be achieved. As the reaction time increased, coloration reactions such as caramelization (Mori & Ito, 2004) became more pronounced. However, the absorbance, A_{280} and A_{420} , in this study was smaller than that with arginine (Khwijitjaru et al., 2024), suggesting that coloration is less likely to occur. There is also a review on the isomerization of lactose using strong bases such as calcium hydroxide (Aider et al., 2007). In spite of the higher lactulose yields in these cases, strong alkaline conditions accelerate the decomposition of sugars, resulting in pronounced coloration. These facts suggest the advantages of using shell powder, a difficult-to-solubilize weak base, for the isomerization. In addition, microwave heating at 700 W had a shorter reaction time, i.e., higher productivity, therefore the subsequent studies were conducted at 700 W.

3.2. Effect of the quantity of shell powder

Figure 2(a) shows the changes over time in the fraction of remaining maltose and the yield of maltulose when shell powder (0–1.0 g) was added to 50 mL of 0.2 mol/L maltose and microwaved at 700 W. The changes over time in pH and calcium ion concentration and in A_{280} and A_{420} are shown in Figs. 2(b) and 2(c), respectively. The reaction was faster when the quantity of shell powder was higher, suggesting that the increased surface area of the shell powder contributed to the acceleration of the reaction. On the other hand, the change in pH was virtually independent of the quantity of shell powder and is represented by a single curve in Fig. 2(b). The quantity of shell powder had little effect on the pH because neutralization occurred rapidly, and was sufficient to neutralize the acidic substances formed. Conversely, the calcium ion concentration and A_{280} and A_{420} (coloration) tended to increase with the quantity of shell powder. This is related to the fact that the reaction proceeded more easily when there was a larger quantity of shell powder,

suggesting that the side reactions were also accelerated when there was a greater quantity of shell powder. As 0.5 and 1.0 g of shell powder showed almost the same change over time, 0.5 g of shell powder per 50 mL of maltose solution was used as the standard condition in the subsequent experiments.

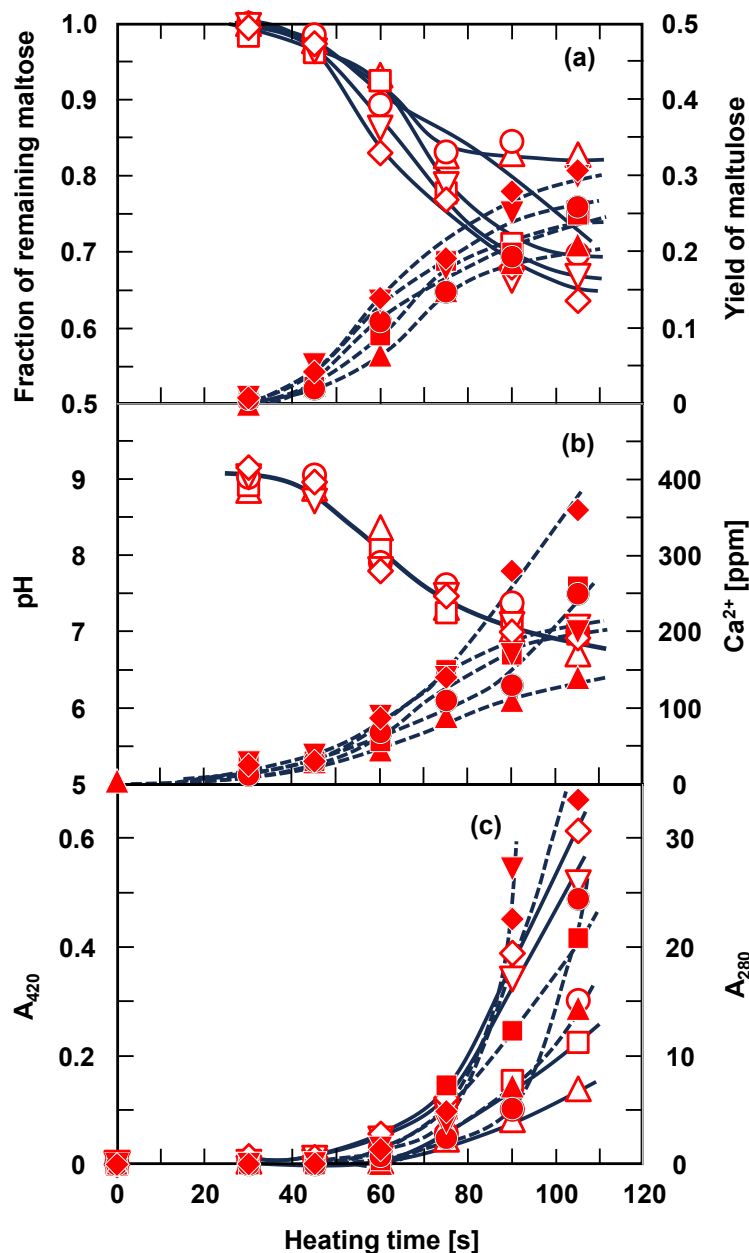


Fig. 2 Effects of the quantities of shell powder on the changes over time: (a) The fraction of remaining maltose C_S/C_{S0} (open symbols) and maltose yield C_P/C_{S0} (closed symbols), (b) pH (open symbols) and calcium ion (Ca^{2+}) concentration (closed symbols), (c) absorbance at 280 and 420 nm (A_{280} (open symbols) and A_{420} (closed symbols)) for 50 mL of 0.2 mol/L maltose. Various quantities of shell powder were added: 0.02 g ($\triangle$, $\blacktriangle$), 0.05 g ($\square$, $\blacksquare$), 0.1 g ($\circ$, $\bullet$), 0.3 g (∇ , $\blacktriangledown$), and 1.0 g ($\diamond$, $\blacklozenge$).

3.3. Effect of the volume of substrate solution

Microwave heating was performed at a constant power of 700 W with 50–100 mL of 0.2 mol/L maltose solution and 0.5–1.0 g of shell powder to maintain a constant shell powder:maltose ratio of 50 g-shell powder:mol-maltose. Figure 3 shows the changes over time in the fraction of remaining maltose, yield of maltulose, pH, calcium ion concentration, and A_{280} and A_{420} . As the applied power was constant at 700 W,

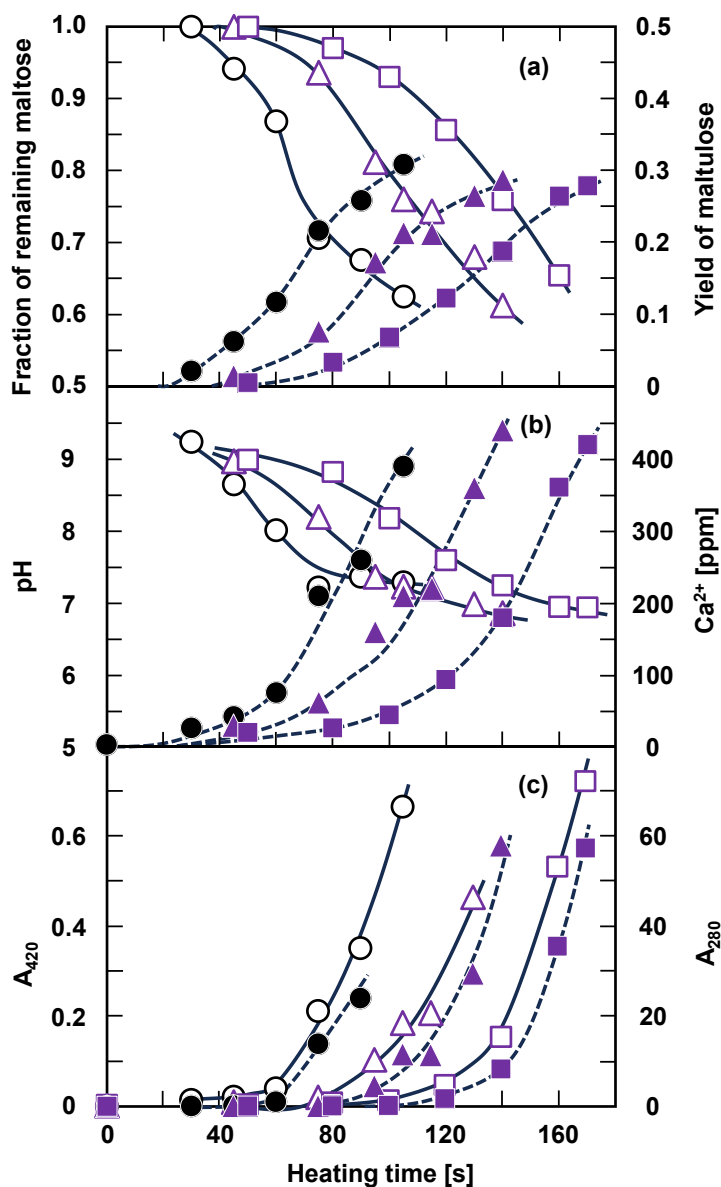


Fig. 3 Effects of the solution volume on the changes over time: (a) The fraction of remaining maltose C_s/C_{S0} (open symbols) and the yield of maltulose C_p/C_{S0} (closed symbols), (b) pH (open symbols) and calcium ion (Ca^{2+}) concentration (closed symbols), (c) absorbance at 280 and 420 nm (A_{280} (open symbols) and A_{420} (closed symbols)) for 0.2 mol/L maltose of volumes 50 mL ($\circ$, $\bullet$), 75 mL ($\triangle$, $\blacktriangle$), and 100 mL ($\square$, $\blacksquare$) during microwave heating at 700 W. The ratio of shell powder to maltose was fixed at 50 mg-shell powder:mol-maltose.

231 the temperature of the reaction solution increased more slowly as the volume of the substrate solution
 232 increased, and the reaction took longer to proceed. The pH gradually decreased with heating time,
 233 approaching approximately pH 7. The calcium ion concentration increased to approximately 400 ppm with
 234 heating time. These results suggest that the high maltulose yield of approximately 30% was achieved because
 235 some of the shell powder dissolved as the reaction progressed, as intended, neutralizing the acidic by-products

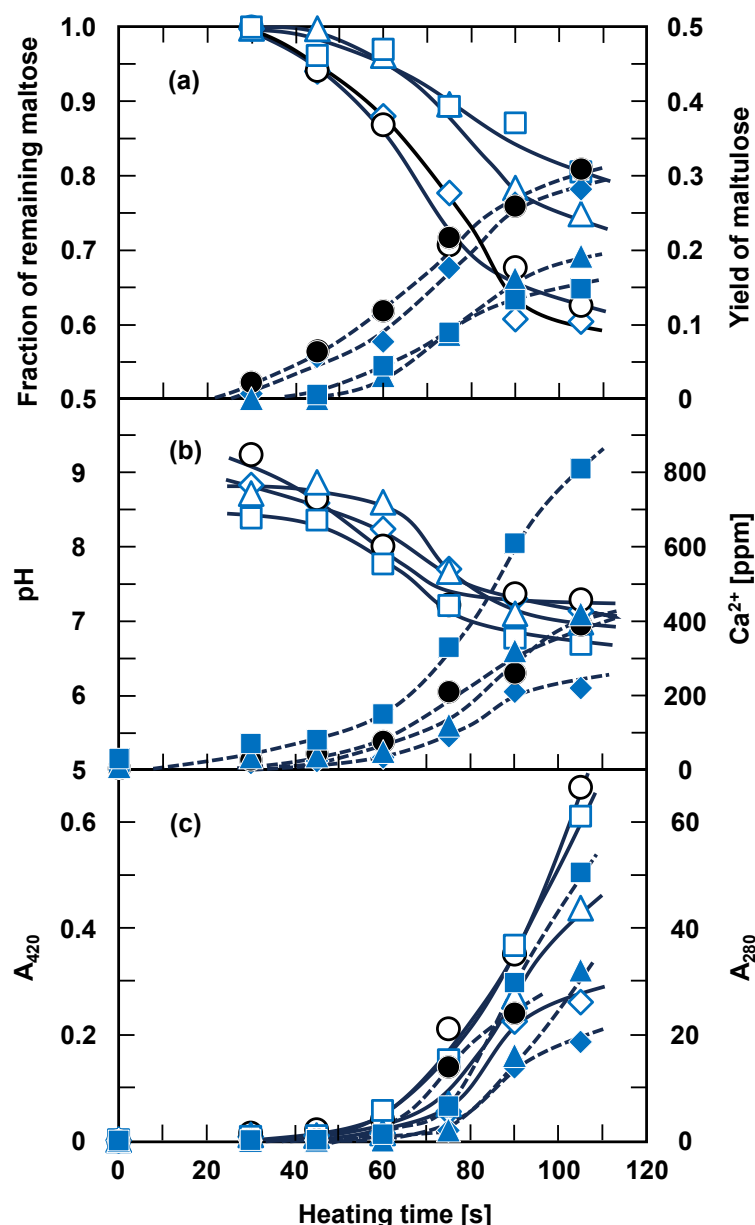


Fig. 4 Effects of the initial maltose concentration on the changes over time with a constant quantity of shell powder: (a) The fraction of remaining maltose C_S/C_{S0} (open symbols) and the yield of maltulose C_P/C_{S0} (closed symbols), (b) pH (open symbols) and calcium ion (Ca^{2+}) concentration (closed symbols), (c) absorbance at 280 and 420 nm (A_{280} (open symbols) and A_{420} (closed symbols)) during microwave heating at 700 W for 50 mL of concentrations of 0.1 mol/L ($\diamond$, $\blacklozenge$), 0.2 mol/L ($\circ$, $\bullet$), 0.5 mol/L ($\triangle$, $\blacktriangle$), and 1.0 mol/L ($\square$, $\blacksquare$) maltose. The quantity of shell powder was 0.5 g.

and preventing a rapid decrease in pH. The absorbance (coloration) of the reaction solution at 280 and 420 nm increased markedly during the latter half of the reaction, as the temperature of the reaction solution increased.

3.4. Effect of maltose concentration with a constant quantity of shell powder

The quantities of maltose solution and shell powder in the pressure-resistant vessel were kept constant at 50 mL and 0.5 g, respectively, and the concentration of maltose was changed to 0.1–1.0 mol/L. Figure 4 shows the changes over time in the fraction of remaining maltose, yield of maltulose, pH, calcium ion concentration, and A_{280} and A_{420} , when microwave heating was conducted for 105 s at 700 W. As mentioned above, the longest reaction time was fixed to 105 s because the relief valve operates if the internal pressure of the reaction vessel increases to more than 0.7 MPa, causing the reaction solution to spurt out. Therefore, the reaction time was insufficient for the initial maltose concentrations of 0.5 and 1.0 mol/L, resulting in a higher fraction of remaining maltose and lower yield of maltulose than for the initial maltose concentrations of 0.1 or 0.2 mol/L.

3.5. Effect of maltose concentration at a constant ratio of the quantity of shell powder to the volume of maltose solution

Maltose solution (0.1–1.0 mol/L, 50 mL) and shell powder (0.25–2.5 g) were placed in the vessel to give a ratio of the quantity of shell powder to maltose of 50 g-shell powder:mol-maltose. Figure 5 shows the changes over time in the fraction of remaining maltose, yield of maltulose, pH, calcium ion concentration, and A_{280} and A_{420} , during microwave heating to 105 s at 700 W.

In the previous subsection, the quantity of shell powder was constant at 0.5 g/50 mL, regardless of the maltose concentration, but here the quantity of shell powder added was increased in proportion to the initial maltose concentration, resulting in a larger decrease in the fraction of remaining maltose and higher yield of maltulose when the initial maltose concentration was 0.5 or 1.0 mol/L than in the previous subsection. The

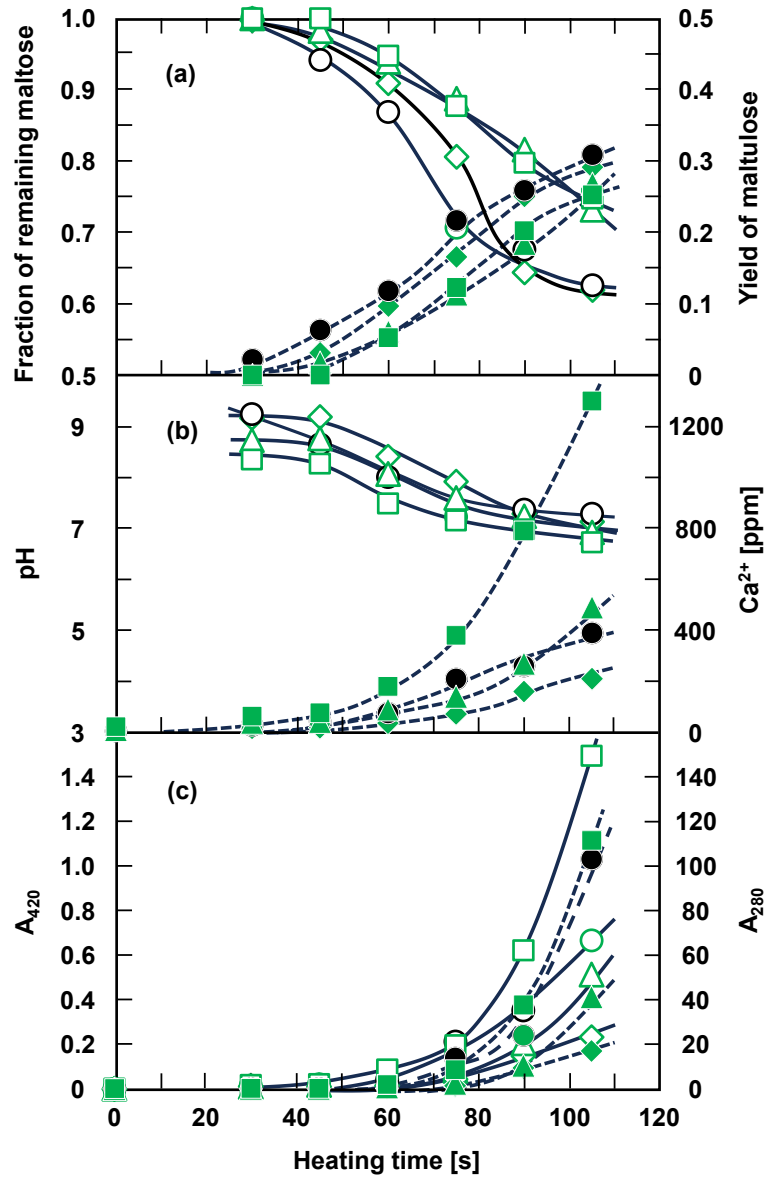


Fig. 5 Effects of the initial maltose concentration on the changes over time at a constant ratio of the quantity of shell powder to the volume of maltose solution: (a) The fraction of remaining maltose C_s/C_{s0} (open symbols) and the yield of maltulose C_P/C_{s0} (closed symbols), (b) pH (open symbols) and calcium ion (Ca^{2+}) concentration (closed symbols), (c) absorbance at 280 and 420 nm (A_{280} (open symbols) and A_{420} (closed symbols)) during microwave heating at 700 W for 50 mL of concentrations of 0.1 mol/L ($\diamond$, $\blacklozenge$), 0.2 mol/L ($\circ$, $\bullet$), 0.5 mol/L ($\triangle$, $\blacktriangle$), and 1.0 mol/L ($\square$, $\blacksquare$) maltose. The ratio of shell powder to maltose was fixed at 50 mg-shell powder:mol-maltose.

trend in the pH change was similar to that shown in Fig. 4(b). Although the trend in the change in calcium ion concentration was also similar to that shown in Fig. 4(b), the calcium ion concentration was higher when the initial maltose concentration was higher due to the larger quantity of shell powder. Similarly, A_{280} and A_{420} were higher when the quantity of shell powder was constant at 0.5 g. The increase in the quantity of

shell powder promoted the coloration of the reaction solution.

3.6. Selectivity in the isomerization of maltose to maltulose

The relationships between the conversion of maltose and the yield of maltulose under the various conditions shown in Figs. 1(a) through 5(a) are summarized in a single graph in Fig. 6. Although there is some variation, all the plots lie on approximately one straight line passing through the origin, and the selectivity of isomerization of maltose to maltulose, calculated from the slope, was 0.822. This means that approximately 18% of maltose was converted to by-products other than the target product, maltulose. We previously reported that the selectivities in the isomerization of maltose to maltulose in subcritical aqueous ethanol (Gao et al., 2016) and subcritical phosphate buffer (Adachi et al., 2021) were approximately 0.37 and 0.65, respectively, depending on the operating conditions. The isomerization by LBAE transformation proceeded only slightly at a pH of 6.3 (Onishi et al., 2020). In the present study, the pH of the reaction solution

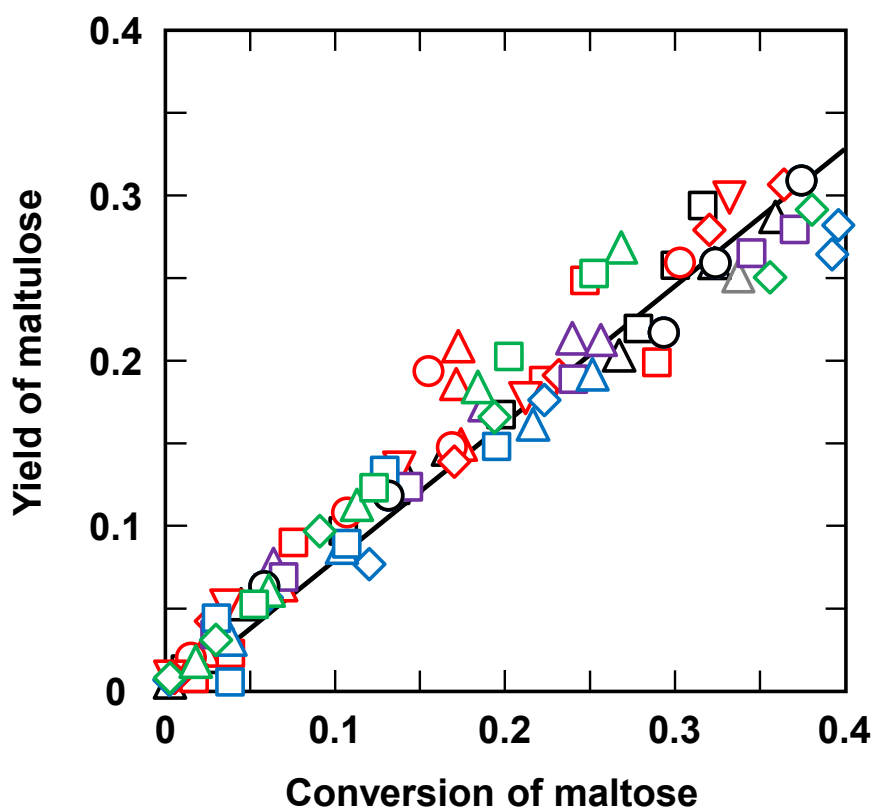


Fig. 6 Selectivity in the isomerization of maltose to maltulose. $1 - C_S/C_{S0}$ is the conversion of maltose and C_P/C_{S0} is the yield of maltulose. The symbols are the same as in Figs. 1 through 5.

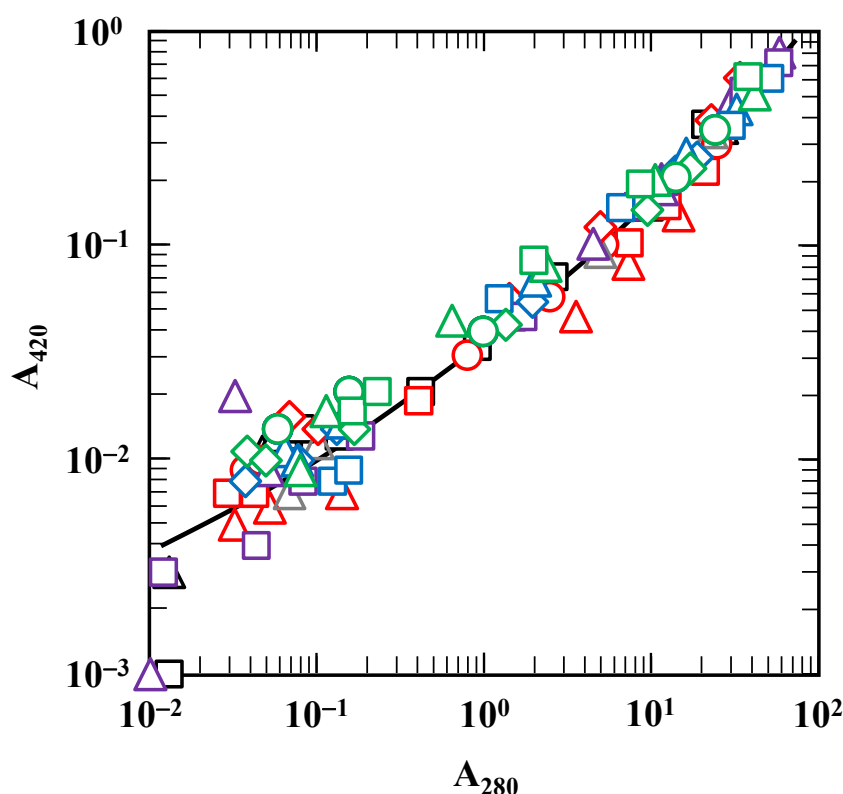


Fig. 7 Relationship between absorbance at 280 and 420 nm during microwave heating for the isomerization of maltose to maltulose under all of the conditions. The symbols are the same as in Figs. 1 through 5.

was maintained above 7 because the acidic substances formed by the side reactions were neutralized by the shell powder, which contributed to the high yield of maltulose and consequently the high selectivity. Another possible reason for the high selectivity is the greater stability of the intermediate, enediol, formed during the LBAE transformation, which may prevent the formation of organic acids from progressing.

3.7. Relationship between absorbance at 280 and 420 nm

Figure 7 summarizes the relationship between A_{280} and A_{420} of the reaction solution under the various conditions shown in Figs. 1(c) through 5(c). The plots under all the conditions could almost be represented by a single curve. This suggests that the same reaction mechanism is responsible for the formation of colored substances regardless of the initial maltose concentration or the quantity of shell powder.

3.8. Relationship between pH decrease and coloration

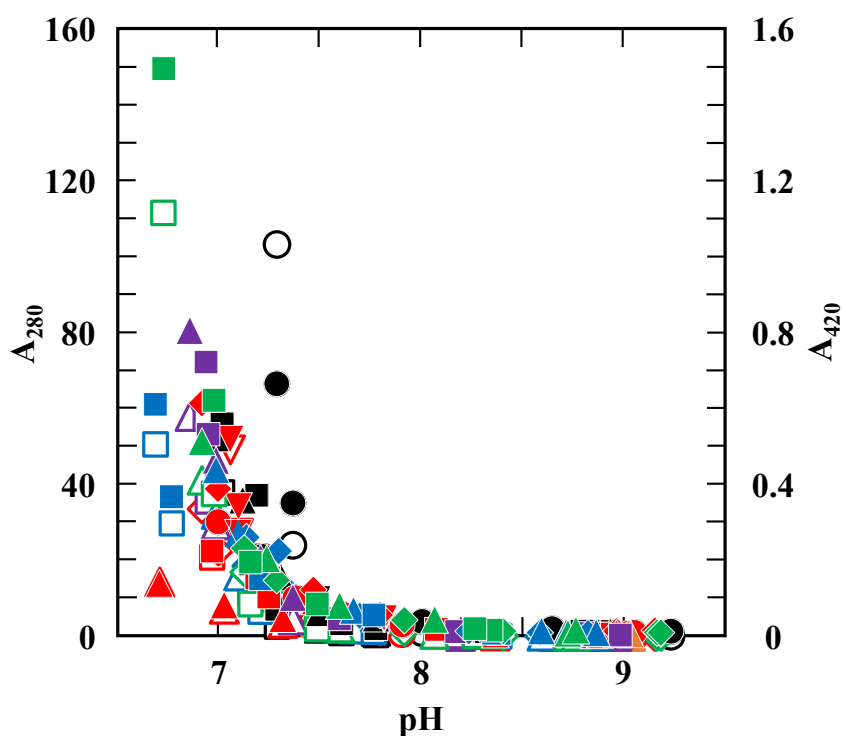


Fig. 8 Changes in absorbance at 280 and 420 nm during pH decrease in microwave heating for the isomerization of maltose to maltulose under all of the conditions. The symbols are the same as in Figs. 1 through 5.

Figure 8 shows the relationships between pH and A_{280} and A_{420} , which are indicators of coloration, reflecting the formation of by-products under the various conditions shown in Figs. 1 (a, c) through 5 (a, c). Under all reaction conditions, both A_{280} and A_{420} only increased slightly until the pH decreased to 7.5, but both A_{280} and A_{420} significantly increased when the pH decreased below 7.5. This was due to the predominance of the formation of HMF, which tends to proceed under acidic conditions, i.e., as pH decreases, A_{280} increases, which is close to the absorption peak of HMF. Furthermore, as a portion of HMF is polymerized to caramel and humin, A_{420} also increases significantly (van Zandvoort et al., 2013; Yalayan et al., 1985).

3.9. Factors affecting calcium ion concentration

As described above, under all the conditions, an increase in calcium ion concentration was observed from the initial stages of the reaction. During the latter half of the reaction, as the temperature of the reaction solution increased, the calcium ion concentration increased rapidly. Figure 9(a) shows the relationship

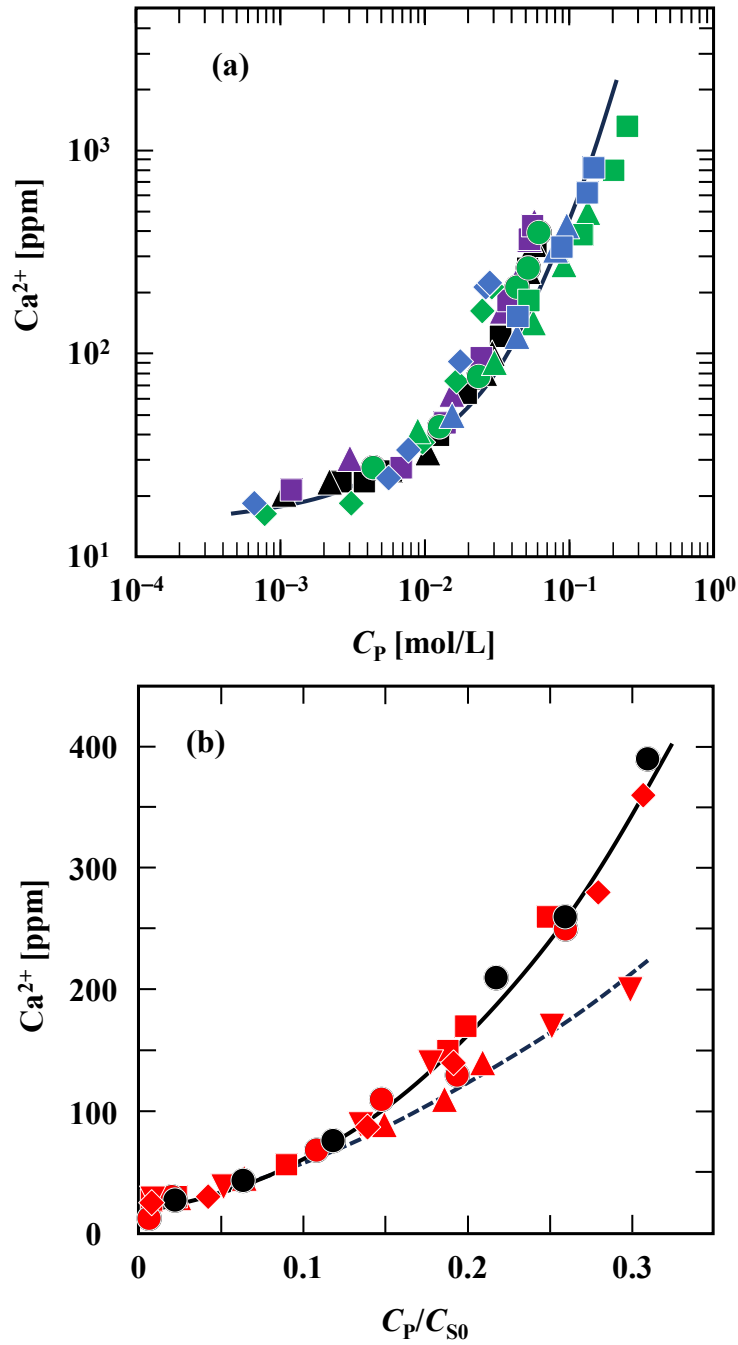


Fig. 9 (a) Relationship between maltulose concentration C_P and calcium concentration Ca^{2+} at a constant concentration of shell powder of 10 g/L and (b) that between the yield of maltulose C_P/C_{S0} and calcium concentration Ca^{2+} when the initial maltulose concentration was constant at 0.2 mol/L and the quantity of shell powder was varied. The symbols are the same as in Figs. 1 through 5.

between the maltulose concentration and calcium concentration when maltulose was isomerized to maltulose by microwave heating at a shell powder concentration of 10 g/L. The calcium concentration increased as the maltulose concentration increased. In particular, the calcium concentration increased markedly in the region where the maltulose concentration increased during the latter half of the reaction. This was partly due to the

dissolution of the shell powder, leading to the neutralization of the acidic substances that were formed during the latter half of the reaction. In addition, it was reported that maltulose (a keto-disaccharide) interacts more readily via ligand exchange with calcium ions than maltose (an aldo-disaccharide) does (Angyal, 1989). This means that maltulose tends to form complexes with calcium. As a result, a new reaction equilibrium is formed between maltulose and the maltulose-calcium complex. This equilibrium may shift the equilibrium in the isomerization reaction toward the formation of maltulose.

The slope of the plot increased with increasing maltulose concentration. However, the plots did not become vertical. As shown in Fig. 8, the pH did not fall below 6.3 in any of the cases examined. These results suggest that the yield of maltulose could be further improved if the reaction were to be continued at a higher temperature. In the present reaction system, a longer reaction time was not possible due to the limitations of the reaction vessel, but the yield could be improved if there were a means by which to extend the reaction time.

Figure 9(b) shows the relationship between the yield of maltulose and calcium concentration when the initial maltose concentration was kept constant at 0.2 mol/L and the concentration of shell powder was changed. The calcium ion concentration tended to be slightly lower when the shell powder concentration was low, but the calcium concentration increased with the formation of maltulose.

4. Conclusions

When calcium carbonate is dispersed in water, it is slightly alkaline. The main component of scallop shells is calcium carbonate. Here, we used microwave heating, which can rapidly increase the temperature of an aqueous solution, and attempted to isomerize maltose, an aldo-disaccharide, to its corresponding keto-disaccharide, maltulose, using uncalcined scallop shell powder. Acidic substances formed as by-products during the isomerization process were neutralized by the calcium carbonate in the shell powder, and the pH of the solution during the reaction was kept above 7, allowing isomerization by LBAE transformation to proceed. A high yield of maltulose of approximately 30% was achieved in a very short reaction time of 105

s when 50 mL of substrate solution was heated at 700 W, except when the maltose concentration was high and the shell powder concentration was low. The selectivity in the isomerization of maltose to maltulose was as high as 0.822 under all conditions. In addition, calcium ions were leached from the shell powder during the formation of maltulose, and the reaction solution contained a high concentration of calcium ions. This reaction system could be used for the isomerization of other saccharides and represents a promising approach to producing functional saccharides (rare sugars) containing calcium ions at a high concentration.

Author Contribution

Takashi Kobayashi: conceptualization, project management, supervision, writing, methodology. Yoshiyuki Watanabe: review, proofreading, and editing. Pramote Khuwijitjaru: review and editing. Shuji Adachi: original draft writing, review, and laboratory practice. All authors read and approved the final manuscript.

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Data availability

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

Declaration of competing interest

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

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