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Microwave-Assisted Isomerization of Glucose to Fructose.

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ABSTRACT

D-Glucose isomerized to D-fructose in the yield of c.a. 7% in an aqueous solution (1mM) under Microwave irradiation (2.45GHz, 350W) for 180 sec. The reaction solution was analyzed by High-performance Liquid Chromatography and a colorimetric method using enzymes to show the decrease of D-glucose and the formation of D-fructose.

Keywords: Microwave, Isomerization, Glucose, Fructose

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INTRODUCTION

Microwave-assisted reactions in the fields of food science have been under investigation for the past 60 years [1]. More recently, studies related to organic syntheses have also been reported [1]. Microwaves induce localized heating in a small part of a reaction system and facilitate many types of reactions, different from other thermal reactions [1, 2].Microwaves actually accelerate the rotation of molecules, and energy is transmitted between the molecules. Generally, such a process of the energy transmission seems to stabilize the transition states possessing dipolar form [2].

Microwave-assisted reactions have been applied to the chemistry of carbohydrates [1, 3–9].Richard and Paquot classify the microwave-assisted reactions of saccharides into two categories: reactions involving hydroxyl groups for the production of novel entities (category 1), and dehydration reactions leading to the formation of furfural and related platform molecules (category 2) [4]. It has been reported that, under microwave irradiation, thermal hydrolysis of a disaccharide (maltose) is accelerated [6], and the decomposition of monosaccharides to 5-hydroxyfurfural or furfural takes place [7, 8].

However, not many research papers on the isomerization of saccharides have been published [1, 3, 8–9]. This report describes the isomerization of glucose (D-glucose) to fructose (D-fructose) in water under microwave irradiation.

Experimental

Chemicals

D-Glucose, D-maltose, and D-fructose were purchased from Kanto Chemical Co. (Tokyo, Japan). Acetonitrile was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Microwave irradiation

The microwave generator used for the experiments was a UMB-1736 (TsannKuen, Tokyo, Japan). Microwave irradiation (2.45 GHz, 350 W) was carried out in glass vials (height 6.3cm,I.D. 2.6cm) containing aqueous solutions (4 mL) of D-glucose and D-fructose(1 mg/mL), separately, every 10 sec for 10–60 sec and every 30 sec for 60–180 sec. The vials were positioned at the center of the turntable. The reaction temperature changed from 25up to 58°C for 180 sec irradiation.

HPLC (High –Performance Liquid Chromatography) analysis

HPLC analyses were carried out using aShi madzu (Kyoto, Japan) HPLC system composed of a LC-20AT pump, CBM-20A communication bus module, SIL-20A autosampler, CTO-20A column oven, SPD-20AV UV/Vis detector, and RID-10A refractive index detector. A Shodex NH_2P-50 4D (150 × 4.6mm I.D.)HPLC separation column was used.

Colorimetric analysis of the reaction solution by means of an enzymatic method

An enzymatic quantification kit (F-kit: cat No. 716260) for the analyses of fructose and glucose was purchased from J. K. International (Tokyo, Japan). D-Glucose reacts with adenosine-5′-triphosphate (ATP)in the presence of hexokinase to afford D-glucose-6-phosphate (G-6-P) and adenosine-5′-diphosphate (ADP).The resulting G-6-P continuously reacts with nicotinamideadenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to afford reduced nicotinamideadenine dinucleotide phosphate (NADPH). The two reactions give rise to an increase in ultraviolet (UV) absorption at 340nm due to NADPH, which is equal to the initial quantity of free D-glucose. D-Fructose reacts with ATP in the presence of hexokinase to afford D-fructose-6-phosphate (F-6-P) and ADP. The resulting F-6-Preacts in the presence of phosphoglucose isomerase (PGI) to afford G-6-P, which also reacts with NADP, catalyzed by G6P-DH, to afford NADPH. The second increase in UV absorption at 340nm is due to NADPH; it isequal to the initial quantity of free D-fructose.The determination of D-glucose and D-fructose can be carried out continuously by monitoring the UV absorption at 340nm.

A sample solution (0.1 mL) containing D-glucose or D-fructose was mixed with pure water (1.9mL) and reagent solution I (1.0mL) taken from the stock solution (45mL, pH 7.6) containing triethanolamine buffer, NADP (110mg), ATP (260mg) and a little amount of magnesium sulfate as a stabilizer. UV absorption (E_1) of a blank sample of pure water (2.0mL) and solution I (1.0mL) was recorded at 340nm. UV absorption (E_1) of a D-glucose sample after 3 min reaction at 37°C was also recorded under the same conditions. Then, solution II (0.02mL), containing hexokinase and G6P–GH, was added to the blank and D-glucose sample. After 15min reaction at 37°C, the UV absorbance (E2) at 340nm was recorded for both solutions. The difference between E_2 and E_1 is proportional to the concentration of D-glucose in the sample solution.

To the resulting blank solution and to the sample solution was further added 0.020mL solution III. After 15min reaction at 37°C, the UV absorption at 340nm was recorded as E_3 for the blank and the reaction solution. The difference between E_2 and E_3 is proportional to the concentration of D-fructose in the sample solution.

Heating the sample solutions in an aluminum heating bath

As control experiments, sealed glass vials (height 6.3 cm, I.D. 2.6 cm) containing aqueous solutions (4 mL) of D-glucose and D-fructose (1 mg/mL), separately, in an aluminum heating bath (MG-2200, EYELA, Tokyo, Japan) at 100°C for 30 min.

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RESULTS AND DISCUSSION

HPLC of glucose solutions irradiated by microwaves

The formation of D-fructose from D-glucose was observed in the HPLC chromatograms, as shown in Fig. 1. The peak of D-fructose on the chromatogram after application of 180 sec microwave irradiation to a 4 mL aqueous solution containing D-glucose (1mg/mL)is observed, but not the peak of D-mannose, which is an isomer of D-glucose.

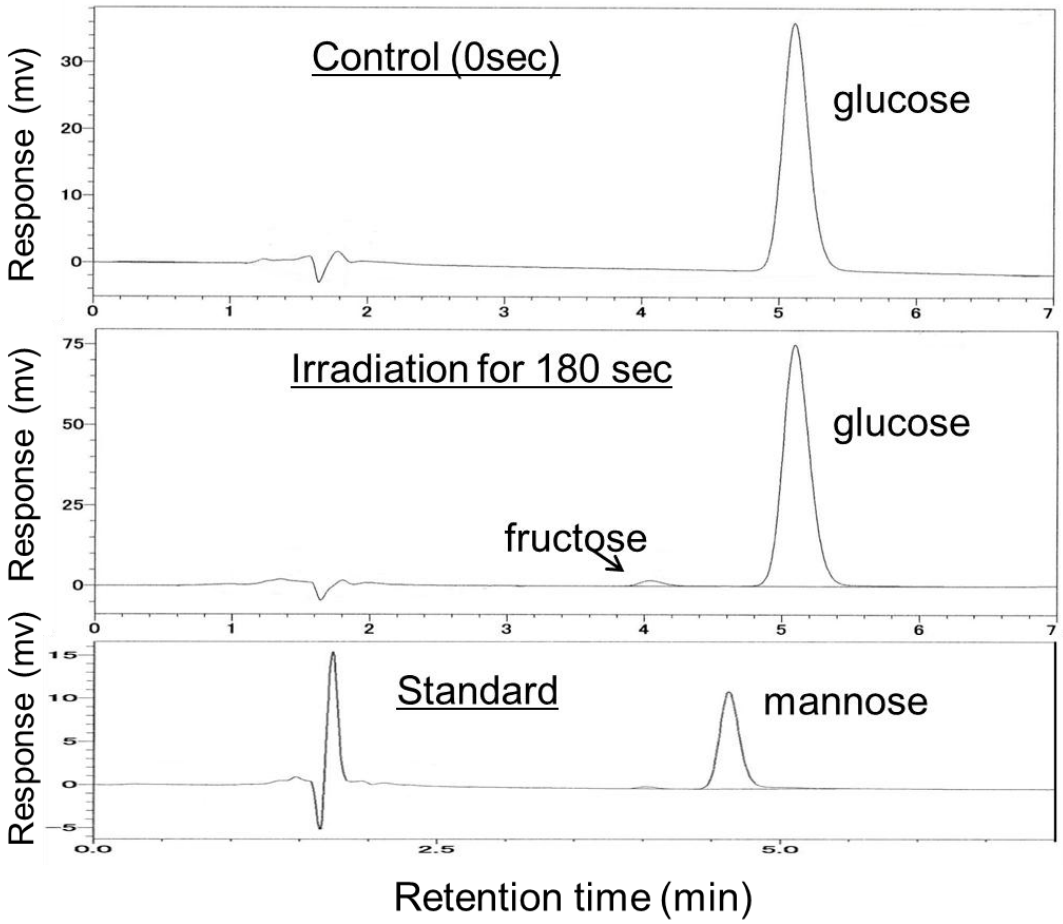

Figure 1: Typical HPLC chromatograms of standard monosaccharides (D-glucose and D-mannose) and the reaction solution of glucose irradiated by microwaves (2.45GHz, 350W) for 180 sec.

This result suggests that the isomerization of glucose to mannose does not proceed under microwave irradiation, as in the reaction of the Lobry de Bruyn–Alberda van Ekenstein transformation reported in the literature [10, 11]. See Fig. 2.

The temperature of the reaction solutions rapidly increased to 66–84°C. The sample solutions released water vapor during the reaction and became concentrated solutions. These were analyzed by HPLC. The same experiments were carried out three times. Fig. 3 shows the average values for the formation of fructose over time and the standard deviation. The

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recovery of glucose and the yield of fructose were calculated on the basis of the initial solution volume (4mL, containing 4.0gD-glucose).

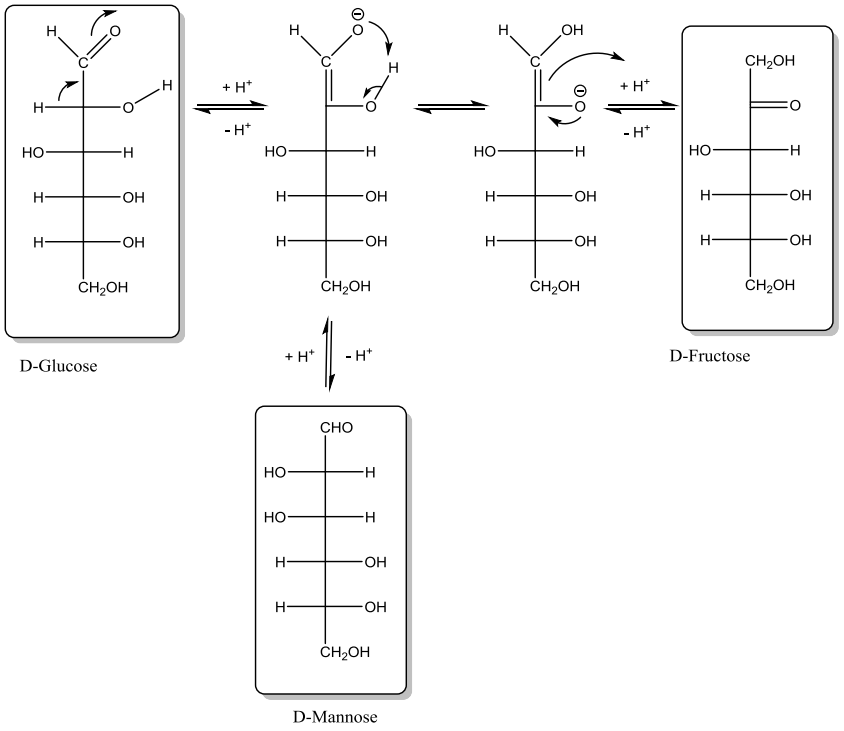

Figure 2: Reaction mechanism of isomerization of D-glucose under basic conditions [10, 11].

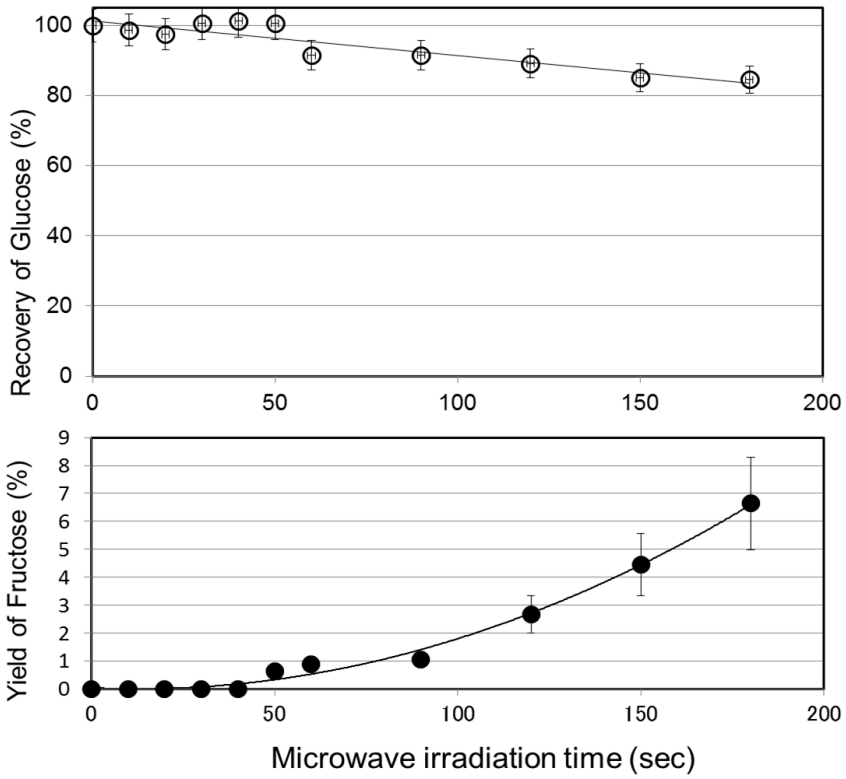

Figure 3: Formation of D-fructose from D-glucose solution irradiated by microwaves (2.45GHz, 350W).

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As the reaction for 180 sec released 2.10g water, the concentration in Fig.2was calculated by multiplying the experimental data with (4.0–2.10) / 4.0. Each concentration was calculated by multiplying the experimental data with (4.0–Dec) / 4.0, where Dec is the weight decrease from each initial reaction solution. Glucose decreased with time, to give 85±5% recovery after 180 sec of microwave irradiation. Fructose gradually increased with time, to yield 6.6±1.7%.

Although the control experiments for the same saccharides (D-glucose, D-fructose) was carried out in an aluminum heat bath, neither the decrease nor the increase of saccharides was observed.

To check the reaction of fructose to glucose, the solutions of D-fructose were microwave irradiated. The resulting HPLC chromatograms did not show the peak of D-glucose but the recovery of D-fructose, as shown in Fig. 4.

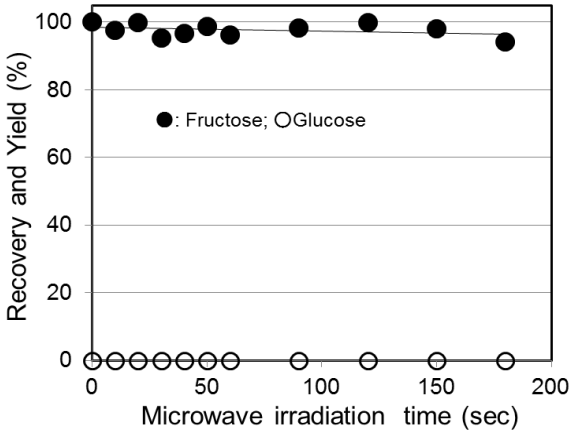

Figure 4: Microwave irradiationofasolution (4mL) containing fructose (1mg/mL).

Colorimetric analysis of D-glucose and D-fructose using an enzymatic method

D-glucose and D-fructose werefurther quantified by means of a colorimetric analysis using enzymatic reactions. Results are tabulated in Table 1.

Table 1: Colorimetric analysis of D-glucose and D-fructose using enzymatic reactions.

Sample / Blank	E_1	E_2	E_3	ΔE_{glu}	ΔE_{fru}	C_{glu}(g/L) (Yield %)	C_{fru}(g/L) (Yield %)
Blank	0.137	0.140	0.138	–	–	–	–
30sec irradiation	0.137	1.251	1.249	1.111	–0.003	0.960 (96)	0.00 (0)
60sec irradiation	0.132	1.158	1.179	1.023	0.023	0.884 (69)	0.020 (1.6)
120sec irradiation	0.131	1.190	1.213	1.056	0.025	0.912 (50)	0.022 (1.2)

E_1, E_2, E_3: UV absorption at 340nm; $\Delta E_{glu} = (E_2 - E_1)_{sample} - (E_2 - E_1)_{blank}$;
$\Delta E_{fru} = (E_3 - E_2)_{sample} - (E_3 - E_2)_{blank}$; $C_{glu} = 0.864 \times \Delta E_{glu}$ (g/L); $C_{fru} = 0.869 \times \Delta E_{fru}$ (g/L)

The recovery of D-glucose and the yield of D-fructose were calculated using the remaining weight of the reaction solution after microwave irradiation. The remaining weight was 4.0 g at 30 sec,3.1 g at 60 sec, and 2.2 g at 120 sec. Table 1 clearly shows the formation of D-fructose from D-glucose by microwave irradiation. Furthermore, the yields determined by the colorimetric method are similar to the yields (0.6-2.7%) obtained by HPLC.

CONCLUSION

This research shows that the microwave-assisted reaction of D-glucose affords D-fructose in a yield of ca. 7% after 180 sec reaction without bases and subcritical water [8]. The detailed mechanism of the isomerization of D-glucose to D-fructose should be clarified in further research.

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