Isomerization of Glucose to Fructose

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Introduction

In the previous paper, one of the authors described "A Survey on the Dextrose Industry in Japan and its Future" in the 1967 issue. As shown in the earlier paper, the supply and demand situation of starch in this country was drastically changed since 1963–64.

In the later 1950's, the Japanese Government had a big problem to eliminate the big stock of the over-produced sweet potato starch. Therefore, extensive research works had been undertaken to look for new uses of the starch.

As the results of these efforts, a complete enzymic saccharification method to produce dextrose from starch had been established as the first technology in the world.

Following this achievement, further study on the utilization of dextrose as a sweetener was proceeded. Dextrose has less sweetness compared with sucrose. In order to increase the sweetness of dextrose, glucose isomerization to fructose, sweetest sugar, has been conducted in this Institute and other establishments.

Two isomerization methods are being discussed in the following chapter. One is an enzymic isomerization using glucose isomerase and the other is a chemical method by the improved alkaline method.

Enzymatic isomerization of glucose

Some strains of *Streptomyces* species have been found to produce isomerase which catalyzed a conversion of glucose to fructose[1),2)]. The enzyme from Streptomyces with high yield, heat-tolerance and strong activity had superiority to other microbial isomerases and these characteristics brought a promise of industrial production of isomerized sugar.

Streptomyces phaeochromogenus was propagated in the medium containing the following materials: Carbon source, 1 g; pepton 1 g; meat extract 0.5 g; yeast extract 0.25 g; NaCl 0.5 g; $MgSO_4 7H_2O$ 0.05 g; in 100 ml and pH 7.0 After being shaken for 24 hours at 29 to 30°C, cells were harvested by centrifuge.

The enzyme glucose isomerase was produced in the cell when the cell assimilated D-xylose and glycerin as carbon source, though D-xylose was far more effective.

Other sugars were not effective for the enzyme production. To obtain the enzyme with high activity, however, it was not necessary to use only xylose as the carbon source throughout the propagation period. On the contrary, as shown in Fig. 1, more active enzyme was obtained with the medium containing xylose-glucose mixture.

From the viewpoint of industrial production of the enzyme, various raw materials such as corn hull, corn cob, cotton seed, wheat bran, baggasse as xylose source and corn steep liquor, wheat bran extract, fish meal, etc., as nitrogen source will be available.

The isomerase from *Streptomyces* required magnesium for the maximal activity and the addition of cobaltous ion remarkably intensified the heat tolerance of the enzyme.

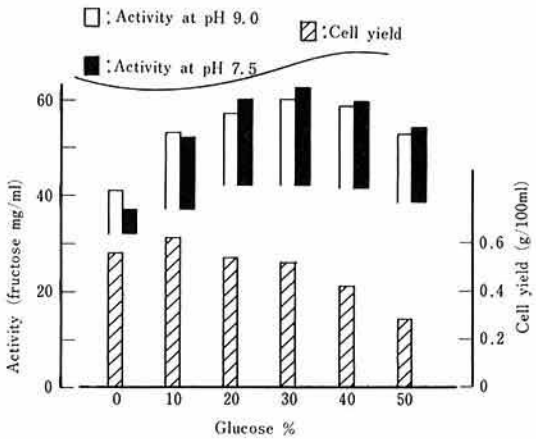


Fig. 1. Effect of additional glucose

Moreover, supplement of the propagation medium with very small amount of cobaltous ion such as 10^{-3}M, resulted in the production of more heat tolerate and active enzyme[3].

As seen in Fig. 2, the enzyme reacted effectively at a rather high temperature. Therefore, it was also possible to use intact cell itself as enzyme at the temperature over 60°C represssing other metabolic enzymes.

The enzyme prepared from the cell grown in the presence of cobaltous ion had rather wide active pH range from 7.0 to 9.5. Mutual conversion between glucose and fructose was investigated, starting with either sugar as substrate, and at equilibrium fructose was found to constitute approximately 50 per cent of the total sugar.

Even in a concentrated solution such as 4 moles/1, the enzyme was not inhibited by the substrate glucose. The isomerization rate of 50 per cent (w/w) glucose solution by the cell with the standard enzyme activity is shown in Table 1.

Table 1. Isomerization in a concentrated glucose solution

Cell added, % (as dry matter)	Fructose, % in total sugar	
	24 hr.	48 hr.
0.5	37.2	47.1
1.0	41.6	51.0

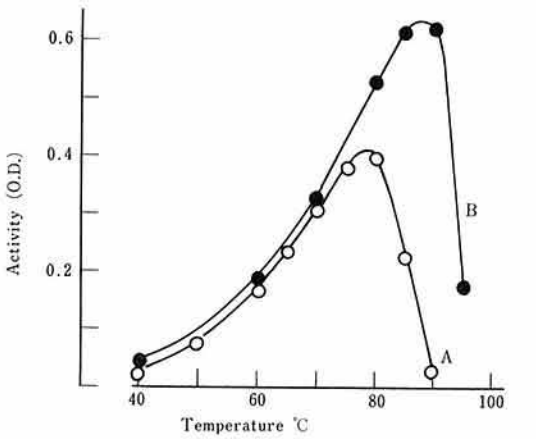
Reaction mixture: glucose 50% (w/w); phosphate buffer pH 7.5, 0.05 M; $MgSO_4$, 0.02 M and resting cell.

About a half amount of glucose was converted into fructose within 48 hours at 60°C by the addition of the cell in an amount of 0.5 per cent (as dry matter) of the glucose amount.

Dehydration of active cell was effectively achieved by natural evaporation at room temperature or by precipitation with chilled aceton and the enzyme activity in the dried cell was maintained without great loss even after the storage of 8 weeks[4].

Freeze dehydration was the most effective method in keeping the activity and moreover, the cell dried by this method could be reused in following several reactions, because of little release of the enzyme from the cell during the isomerization process.

DEAE Sephadex was found to be a rather strong absorbent for the isomerase. The enzyme was not released from the Enzyme-DEAE Sephadex complex by KCl solution of the concentration below 0.4 M. A continuous isomerization method could be carried out using 30 per cent (w/w) glucose solution



A: Without Co^{++}; Protein, 1.93mg/ml of reaction mixture.
B: With 10^{-3}M Co^{++}; Protein, 1.72mg/ml of reaction mixture

Fig. 2. Effect of temperature

and the column of Enzyme-DEAE Sephadex complex[5].

Chemical isomerization of glucose

Since 1895, there have been many investigators who worked on sugar isomerization in alkaline solution. This reaction was named Lobry de Bruyn — Alberda van Ekenstein transformation after the names of the discoverer of the phenomena.

Many excellent works have been done by various groups of scientists for the elucidation of reaction mechanism and its kinetics. However, practical industrial application had never been successful for the purpose of sweetness fortification of glucose.

The main problems was unstableness of sugar in warm alkaline solution. This character caused sugar destruction to color substances and organic acids during the course of reaction.

An extensive research had been conducted in this laboratory on the basic reaction conditions of alkaline glucose isomerization under the condition of various pH value, concentration of glucose and catalysts, varieties of catalysts and temperatures[6),7),8),9)].

Kinetic studies on the reaction velocity of glucose isomerization to fructose, destruction of glucose and fructose in alkaline solution, gave us the theoretical answer.

If we used high temperature reaction condition and stopped reaction in a very short time where velocity of glucose isomerization exceeded much higher than that of sugar destruction, theoretically we should get pretty high percentage of fructose formation with a very low sugar destruction.

These observations suggested that if we used just an initial stage of the reaction, 35 per cent of glucose could be isomerized to fructose with only one to two per cent of sugar destruction to color substances and organic acids.

This sugar loss was unimaginably low compared with that of the earlier studies where they had reported about the 20 to 30 per cent of sugar degradation at low temperature reaction.

This was absolutely proved both in laboratory and pilot plant scale experiments[10),11),12)].

The following data are one of the experimental results.

Table 2. Results of pilot plant of continuous isomerization

Condition	
Conc. of glucose	40-65% (w/v)
Conc. of NaOH	0.8-1.0%
Temperature	70-90°C
Time	5-75 min.
Results	
Degree of production of fructose	33-35%
Degree of degradation of glucose	1-3%
Specific conductivity of saccharified solutions purified by ion-exchange resins	$3\times10^6\,\Omega$cm
Products	
Colorless and clear	
Sweetness like invert sugar	

These were obtained from the experiments which had been conducted by the pilot plant equipment of a continuous flow system.

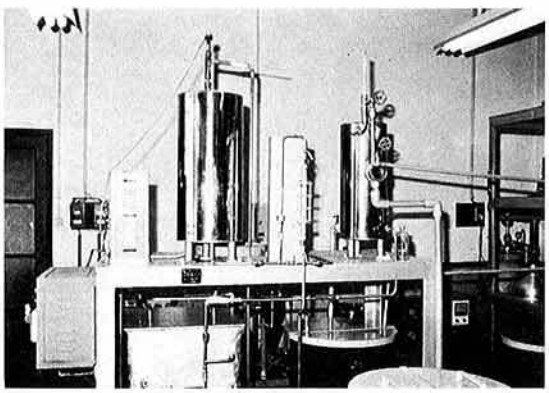


Fig. 3. Continuous flow system pilot plant for chemical isomerization

Since the success in the process of both enzymic and chemical isomerization of glucose, many possible uses of this isomerized sugar had been discussed in many food industries.

Now, the utilization of the isomerized syrup has been developed in many food industries—beverages, bread, Tsukudani, syrup for canned fruits, etc.

Also, syrup is expected to be used as a liquid type—sweetener in various industries.

References

1) Tsumura, N. & Sato, T.: Enzymatic conversion of D-glucose to D-fructose. VI. Properties of the enzyme from *Streptomyces phaeochromogenus*. *Agr. Biol. Chem.* **29**, 1129 (1965).
2) Tsumura, N.: et al. Enzymatic conversion of D-glucose to D-fructose. VII. On the constituents of cultivation medium for *Streptomyces phaeochromogenus*. *J. Ferm. Assoc., Japan*, **23**, 32 (1965).
3) Tsumura, N., Hagi, M. & Sato, T.: Enzymatic conversion of D-glucose to D-fructose. VIII. Propagation of *Streptomyces phaeochromogenys* in the presence of cobaltous ion. *Agr. Biol. Chem.* **31**, 902 (1967).
4) Tsumura, N., Hagi, M. & Sato, T.: Enzymatic conversion of D-glucose to D-fructose. IX. Dehydration and preservation of the cell as enzyme source. *Agr. Biol. Chem.* **31**, 908 (1967).
5) Tsumura, N. & Ishikawa, M.: A continuous glucose isomerization method by an adsorbed enzyme column. *J. Food Sci. Technol.* **14**, 539 (1967).
6) Kainuma, K. & Suzuki, S.: Studies on the isomerization of dextrose into fructose. I. Isomerization with sodium hydroxide. *J. Agr. Chem. Soc. Japan*, **38**, 556 (1964).
7) Kainuma, K., Tadokoro, K. & Suzuki. S.: Studies on the isomerization of dextrose into fructose. II. Isomerization with various kinds of alkali reagents. *J. Agr. Chem. Soc. Japan*, **40**, 35 (1966).
8) Kainuma, K., Tadokoro, K. & Suzuki, S.: Studies on the isomerization of dextrose into fructose. III. Effects of cations on the isomerization of dextrose. *J. Agr. Chem. Soc. Japan*, **42**, 173 (1968).
9) Kainuma, K. & Suzuki, S.: Isomerization of dextrose into fructose by the alkali method. I. Basical conditions of isomerization with several kinds of alkali reagents. *Die Stärke*, **18**, 135 (1966).
10) Kainuma, K. & Suzuki, S.: Isomerization of dextrose into fructose by the alkali method. II. Design and installation of the pilot plant of the continuous isomerization and determination of its operating conditions. *Die Stärke*, **19**, 60 (1967).
11) Kainuma, K. & Suzuki, S.: Isomerization of dextrose into fructose by the alkali method. III. Isomerization with the pilot plant of the continuous flow system. *Die Stärke*, **19**, 66 (1967).
12) Suzuki, S.: Starch hydrolyzing industry in Japan: The progress of the past decade and future prospects. *J. Jap. Soc. Starch Sci.*, **17**, 155 (1969).