CHARACTERISTICS OF IMMOBILIZED GLUCOSE ISOMERASE

G.F. Hutasoit
Indonesian Sugar Research Institute, Pasuruan, Indonesia

Key words: Molasses, liquid sugar, glucose isomerase, immobilization

ABSTRACT

The production of a liquid sugar with a high fructose content from molasses may be achieved by separating glucose from fructose and converting the separated glucose to fructose by glucose isomerase (GI). The GI could be used efficiently when in the form of immobilization. Ion exchanger DEAE-cellulose support was better than DEAE-sephadex A-50, in terms of the amount and activity of immobilized GI. The slower the flow rate and the higher concentration of glucose substrate, the greater the amount of fructose formed in continuous isomerization. The amount of fructose formed in a batch system was higher than the continuous system for approximately the same duration of reaction.

INTRODUCTION

The process for manufacturing liquid sugar from cane molasses may require glucose isomerase (GI) to increase its sweetness. The activity and stability of GI from Bacillus stearothermophilus were optimized through determination of its characteristics (Hutasoit2). The production of GI in sugar industries requires an efficient and careful treatment since it is a sensitive product. One of the ways to achieve this requirement is to use GI in an immobilized form. The advantages of immobilized GI are to reduce the purification cost of the product, and the possibility of a continuous process to reduce processing time and labour, and to reduce physical losses of GI. Enzymes have been immobilized by many methods but only a few are commercially applicable because of high activity reduction. The objective of this study was to characterise the properties of immobilized GI on an ion exchanger.

MATERIALS AND METHODS

GI was extracted from Bacillus stearothermophilus and purified by heating, fractionation and batch ion exchange chromatography (Hutasoit3). It was added to prepared DEAE-sephadex A-50 and DEAE-cellulose supports at the rate of 20 ml GI solution per g dry gel of the supports. The protein content of the GI solution was 12.93 mg/ml and activity was 9.06 EU/ml. The suspension was shaken for two hours and filtered. The protein which was not bound in the ion exchanger was washed out by using 0.05 M Tris buffer (pH 7.0) until protein in the filtrate was no longer detectable. The protein content and GI activity of the washings were determined.

The amount of protein bound in the ion exchanger was computed as the difference between the amount of protein added and the amount of protein washed out. The activity of bound GI was computed by the difference between activity of the GI added and that of the filtrate washed out.

For batch isomerization the substrate solution was a mixture of 0.5 M glucose and 0.01 M CaCl₂·6H₂O in distilled water, and the pH was adjusted to 7.0 with 1.0 M Na₂CO₃. Batch isomerization was effected by adding 20 ml substrate solution to immobilized GI. The reaction mixture was incubated at 60°C in a shaker (200 rpm) for 10 minutes. The reaction mixture was filtered and the protein and fructose contents of the supernatant were determined. This method was repeated five times.
For continuous isomerization a glass column (1 X 30 cm) fitted with a water jacket for temperature control was used. The lower part of the column was packed with shallow glass wool and glass beads; then with the immobilized GI and finally with another layer of glass wool and glass beads. A packed column of immobilized GI was operated at 60°C. Glucose at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 M was dissolved in distilled water, containing 0.01 M CoCl₂·6H₂O and adjusted to pH 7.0 with 1.0 M Na₂CO₃. These glucose solutions were used as substrates for isomerization whilst flowing through the glass column. The flow rate of the substrate was varied for every concentration of glucose solution at the rate of 30, 40, 50 and 60 ml per hour. The amount of fructose formed was analysed. The Km and Vmax values were determined from the Eadie-Scatchard plot of the Michaelis-Menten equation.

Protein and fructose contents were determined by the Folin-Lowry and modified cysteine-carbazole methods, respectively. One unit of GI (EU) was defined as that amount of GI which would produce one micromole of fructose per minute under the assay conditions described.

RESULTS AND DISCUSSION

Immobilization

The addition of GI to each of the ion exchanger supports was 258.60 mg protein per g support with an activity of 181.20 EU per g support. The bound or immobilised GI on DEAE-sephadex A-50 was 162.32 mg protein per g support with an activity of 122.28 EU per g support. The immobilised GI on DEAE-cellulose was 194.90 mg protein per g support with an activity of 140.59 EU per g support.

The amount of immobilized GI on DEAE-cellulose was higher than on DEAE-sephadex A-50, as was its activity. This may have been because the available capacity of DEAE-cellulose was higher than that of DEAE-sephadex A-50.

Batch isomerization

The average activity of immobilized GI on DEAE-cellulose (98.52 EU per g support) was higher than that on DEAE-sephadex A-50 (53.84 EU per g support). Statistical analysis showed that the activity was significantly different. The difference in activities was caused by the difference in the amount and activity of immobilized GI on each ion exchanger support.

Continuous isomerization

Immobilized GI on DEAE-cellulose was chosen for continuous isomerization since it had a higher amount and more activity than DEAE-Sephadex A-50. The effect of glucose concentration and flow rate on fructose formed is shown in Fig 1. The analysis of variance of the effect of glucose concentration and flow rate is given in Table 1. Glucose concentration and flow rate significantly affected fructose formation. The slower the flow rate, the greater the amount of fructose formed since the isomerization reaction was longer. The higher the glucose concentration the greater the amount of fructose formed at the same flow rate. This may be because GI catalyzes the reversible reaction.

Both flow rate and glucose concentration should be considered for commercial application because a higher glucose concentration will increase the viscosity of the substrate solution and affect flow rate. To reduce unproductive time, the flow rate should be determined such that the reaction time of isomerization will not be longer than the
Figure 1. Effect of glucose concentration and flow rate on fructose formed.

Table 1 - Analysis of variance of the effect of glucose concentration and flow rate of fructose formed during continuous isomerization.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Fcalc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>1</td>
<td>71.306</td>
<td>23.769</td>
<td>0.93ns</td>
</tr>
<tr>
<td>Treatment</td>
<td>19</td>
<td>4857.854</td>
<td>255.677</td>
<td>9.97**</td>
</tr>
<tr>
<td>Glucose concentration (A)</td>
<td>4</td>
<td>778.045</td>
<td>194.511</td>
<td>7.58**</td>
</tr>
<tr>
<td>Flow rate (B)</td>
<td>3</td>
<td>3916.685</td>
<td>1305.562</td>
<td>50.90**</td>
</tr>
<tr>
<td>A X B</td>
<td>12</td>
<td>163.124</td>
<td>13.594</td>
<td>0.53ns</td>
</tr>
<tr>
<td>Error</td>
<td>57</td>
<td>1461.989</td>
<td>25.648</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>6391.149</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

time required to isomerize about 50% of the glucose, since the conversion of glucose to fructose by GI is not much higher than 50% (reversible reaction).
The Michaelis constant (Km) was computed from the Eadie-Scatchard plot of the ratio of the rate of reaction and substrate concentration (v/[s]) versus the rate of reaction (v) as shown in Fig 2. The rate of reaction (v) was determined from the slope of the plot of fructose formed (micromole) versus the residence time (minutes) of the substrate so-
solution in the column. The residence time was computed by dividing void volumes (11.5 ml) by flow rate (ml/hour). The residence times were 12, 14, 17 and 23 minutes at flow rates of 60, 50, 40 and 30 ml/hour, respectively. The Km and Vmax values were 0.45 M and 4.43 micromole per minute, respectively. These values were different from those obtained with free Gl and the difference could be attributed to the difference in pH and temperature, and to a probable alteration of the Gl active site upon binding in the ion exchanger (Hutasoit3).

Figure 2. Eadie-Scatchard plot of substrate-velocity data from immobilised Gl.

The rate of fructose formation from a substrate of 0.5 M glucose in batch isomerization for a reaction time of 10 minutes was 0.9 mg/ml-minute. It was 0.41 mg/ml-minute in the continuous system for a reaction time of 12 minutes and 0.5 M glucose. The higher figure in the batch than in the continuous system was probably because of stirring during the batch isomerization. Nevertheless, it is expected that the unproductive time of the batch system would be longer than in the continuous system, and this should be considered in a commercial application.

CONCLUSION

The absorption or binding capacity of GI by DEAE-cellulose was higher than by DEAE-sephadex A-50, and its activity was also greater. The values of Km and Vmax of immobilized GI on DEAE-cellulose with continuous isomerization were 0.45 M and 4.43 micromole per minute, respectively.
REFERENCES


CARACTÉRISTIQUES DU GLUCOSE ISOMÈRE IMMOBILISÉ

Gading F. Hutasoit
Indonesian Sugar Research Institute, Pasuruan, Indonesia

EXTRAIT

La production d'un sucre liquide contenant une proportion élevée de fructose à partir de la mélasse peut être obtenue en séparant le glucose du fructose et en convertissant le glucose séparé du fructose par isomérisation du glucose (GI). La 'GI' peut être utilisée efficacement quand elle est en immobilisation. L'échangeur ionique deae-cellulose comme base a été meilleur que deae-sephadex A-50, en ce qui concerne la quantité et l'activité de l'immobilisation GI le flux plus lent et la concentration plus élevée du substrat du glucose, une plus grande quantité de fructose se forment dans une isomérisation continue. La quantité de fructose formée dans un système de battage (intermittent) a été plus grande que dans celui en continu, pendant la même durée de réaction.

CARACTERISTICAS DE LA GLUCOSA ISOMERASA INMOVILIZADA

Gading F. Hutasoit
Indonesian Sugar Research Institute, Pasuruan, Indonesia

RESUMEN

La producción de azúcar líquido con un alto contenido de fructuosa a partir de mieles puede ser lograda separando glucosa de la fructuosa y convirtiendo la glucosa separada en fructuosa mediante la glucosa isomerasa (GI). La GI podría ser usada eficientemente cuando está de forma inmovilizada. El soporte intercambiador iónico celulósico DEAE - resultó mejor que el DEAE-sephadex A-50 en términos de la cantidad y actividad de la GI inmovilizada. A un flujo más lento y mayor concentración de sustrato de glucosa, correspondió una mayor cantidad de la fructuosa formada en isomerización continua. La cantidad de fructuosa formada en un sistema discontinuo fue mayor que en el continuo a aproximadamente la misma duración de la reacción.