KINETICS OF GLUCOSE ISOMERASE ENZYME.

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ABSTRACT

The effect of substrate concentration on reaction velocity of glucose isomerase enzyme (Sweetzyme type Q) indicated that the maximum velocity (V_{max}) was 16.8 µmM/l and its Michaelis constant was (0.028 mM/l). The effect of pH on reaction activity showed that the optimum pH of this enzyme equals 8.0 while the optimum temperature was 50°C with reaction activity 5.93 µmM/l/min.

The reaction activity increased as the Mg^{++} ion concentration increased up to 0.03 M/l with immobilized enzyme concentration of 600 mg/10 ml.

The maximum conversion value to fructose isomer was obtained with initial glucose syrup concentration equals (28.70%). The needed time for isomerization achievement could be decreased to almost 50%.

INTRODUCTION

The production of high fructose syrup from hydrolyzed starch can certainly cover the shortage of sucrose sugar to a great extent in Egypt. The resulted syrup is more sweet than glucose, besides it has higher osmotic pressure as an inverted sugar to almost twice of that of sucrose. This property is important when the product used as a preservative (Mermelstein,1975).

The kinetic of this isomerase enzyme and the factors affecting its reaction velocity are to be considered in studying this enzyme.Novo (1982) stated that reasonable immobilized glucose isomerase enzyme (Sweetzyme) lies normally in the range 2000 - 3000 Kg dry substance per Kg enzyme. Mohamed (1986) found that the optimum activity of immobilized glucose isomerase enzyme (type A) was 0.6 mM/l; Km 0.029 mM with temperature 65°C, pH 7.4,reaction time one hour and enzyme concentration 250 mg/20 ml syrup.

Oestergaard and Knudsen (1976), Aschengreen et al. (1979), Mamta and Prabhu (1980) and Novo (1982) have emphasized that the optimum activity was reached at pH 7.4 to 8.4 for sweetzyme. Tsumura and Sato (1965b) found that the maximal activity for another strain of glucose isomerase enzyme obtained from Aerobacter cloacae was achieved at 50°C with incubation period of 30 min. Aschengreen et al. (1979) and Novo (1982) stated that the optimum activity for sweetzyme type Q was achieved at 60°C. Tsumura and Sato (1965a) and Novo (1982) showed that glucose isomerase enzyme requires magnesium ions to activate its reaction since Mg^{++} ions were bound with the enzyme protein.

This work is mainly concerned with studying the most suitable conditions of glucose isomerase enzyme (Sweetzyme type Q) for the production of high fructose syrup.

MATERIALS AND METHODS

1: Immobilized glucose isomerase enzyme (Sweetzyme type Q), E.C.5.5.99.1-D-glucose - ketal - isomerase, was supplied by Industrial A/S, Denmark. It was obtained from a strain of Bacillus coagulans in powdered form with an activity equals to 200 IGIC/g.

2: Pure glucose and fructose sugars were purchased from British Drug House (B.D.H.).

3: Conversion of cassava starch to glucose syrup was carried out in two distinct steps:

3-1. Liquefaction process:

Standard liquefaction process was accomplished on cassava starch according to the method described by Novo (1979) using amylase enzyme (Termamyl 120 L).

3-2. Saccharification process

The liquefied starch from the previous process was saccharified as described by Novo (1979) using glucoamylase enzyme (AMG 200L). The resulted saccharified syrup was purified by filtration after treatment with activated carbon powder. The cations were removed using ion exchange chromatography (cation resin: IR - 120 H; anion resin: Dowex 1x8 - 400).

The following experiments were carried out on the purified resulted glucose syrup.

4: Evaluation of sweetzyme type Q:

4-1. Effect of substrate concentration on reaction velocity:

The method of Mamata and Prabhu (1980) was accomplished and the produced fructose was determined by cysteine - carbazole - H_2SO_4 method, as described by Dische and Borefreund (1951). A blank experiment was carried out under the same conditions.

4-2. Effect of pH on reaction activity:

The activity of glucose isomerase enzyme (Sweetzyme) was tested at different pH values, i.e., 5.0, 5.5, 6.0, 7.0, 7.2, 7.4, 7.8, 8.0, 8.2, 8.4, 8.6, 8.8 and 9.0 in tris-buffer (0.05 M). The reaction was carried out with 4 ml. of $MgSO_4 . 7H_2O$ soln. (0.01 M) and 500 mg. of this enzyme. Temperature was adjusted at 50°C for one hour. The fructose content was estimated as mentioned before.

4-3. Effect of temperature on reaction activity:

The mixture of glucose isomerase enzyme (600 mg. enzyme + 0.8 mM glucose syrup), 4ml. of $MgSO_4 . 7H_2O$ soln. (0.01 M) and 4ml. of tris - buffer (0.05, pH 8.2) were incubated at different temps., 30, 40, 45, 60, 55, 60, 65, 70 and 75°C for one hour, then cooled and the fructose content at each temperature was estimated as mentioned before.

4-4. Effect of enzyme concentration on reaction activity:

The activity of glucose isomerase enzyme was determined by the method of Mamta and Prabhu (1980) with some modifications ;

The reaction mixture was adjusted to pH 8.2 in a solution composed of 4ml. tris - buffer (0.05 M), 4ml. of $MgSO_4 . 7H_2O$ soln. (0.01M), 4 ml. of glucose syrup soln. as substrate (concentration 0.8 mM) and mixed with 50, 100, 200, 300, 400, 500, 600, 700 and 800 mg. of the immobilized enzyme. The seperation of the enzyme was accomplished on filter paper and the formed fructose was determined as mentioned before. A blank experiment was carried out under the same conditions.

4-5. Effect of Mg^{++} ion concentration on the activity of isomerase enzyme:

Different concentrations of Mg^{++} ion, i.e., 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.05, 0.07 and 0.1 M were used, all other factors were standardized as substrate concentration (0.8 mM) pH 8.2, temp. 50°C and incubated for one hour.

RESULTS AND DISCUSSION

1. Effect of different parameters on reaction velocity and activity of glucose isomerase (Sweetzyme type Q) enzyme:

1-1. Effect of substrate concentration:

The effect of substrate concentration on reaction velocity of glucose isomerase (Sweetzyme type Q) is shown in Table (1) and Fig.(1-a). The obtained results show that the reaction velocity to convert glucose to fructose isomer was increased up to 0.8 mM/l of -D- glucose. At higher concentration of glucose, i.e. 1.0 and 1.1 mM/l the conversion process was inhibited and the reaction slow down until no further change in velocity was observed (Zero order reaction) such observation was noted by Segel (1968). On plotting (v) as fructose content in µmM against (S) which is the substrate concentration in mM, Michaelis constant (K_m) of isomerase enzyme (Sweetzyme type Q) could be obtained as shown in Fig. (1-b) and equals 0.028 mM/l.

The Michaelis constant (K_m) of glucose isomerase was also determined using Lineweaver and Burk (1954) by plotting 1/S against 1/v as shown in Fig.(1-c). The obtained (K_m) was in agreement with that previously obtained by half way point of the experimental curve as shown in Fig.(1-a).

On the other hand, the obtained K_m is very similar to that mentioned before by Mohamed (1986) for another type of glucose isomerase (Sweetzyme type A).

Table (1): Effect of substrate concentration on reaction velocity of glucose isomerase (Sweetzyme type Q).

D-glucose (S) mM	Fructose content µmM/l	Reaction velocity	1/S	1/v (x 10^1)
0.05	0.699	1.70	20	5.88
0.10	1.388	3.00	10	3.33
0.20	2.630	5.09	5	1.96
0.40	5.485	7.80	2.5	1.28
0.60	14.444	9.51	1.27	1.05
0.80	16.805	10.66	1.25	0.94
1.00	16.525	11.51	1.00	0.87
1.10	12.775	11.85	0.91	0.84

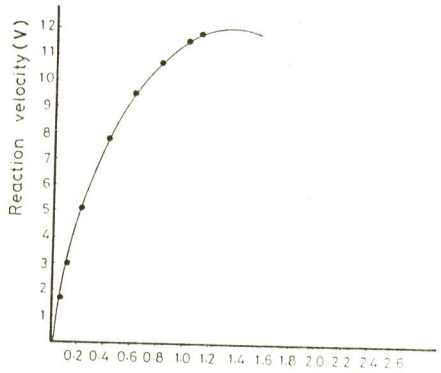

Fig.(1,a) Relationship between substrate concentration and reaction velocity of glucose isomerase enzyme.

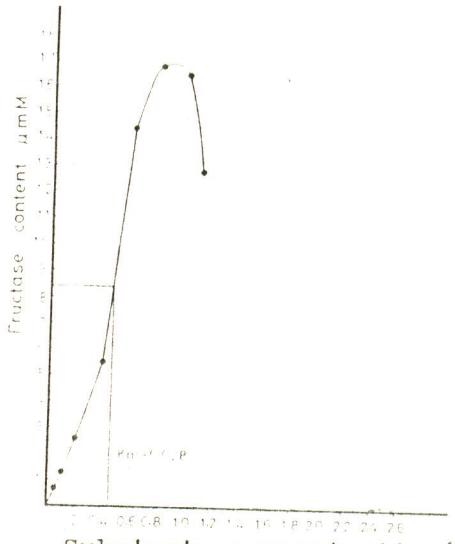

Fig.(1,b) Relationship between substrate concentration and fructose content of glucose isomerase enzyme.

1-2. Effect of pH:

The effect of pH on reaction activity of glucose isomerase enzyme (Sweetzyme type Q) is illustrated in Table (2) and shown in Fig. (2). The obtained bell - shaped curve indicates indicated that the optimum activity was achieved at pH 8.0 and reached to 12.2 µmM/l/min. The value of pH 8.0 is almost in agreement with that reported by Standberg and Smiley (1971); Oestergaard and Knudsen (1976); Aschengreen et al. (1979); Mamta and Prabhu (1980).

Novo enzyme comp. (1982), the producer of this enzyme, stated that the maximum activity is obtained in the range of pH 7.8 - 8.3 and above pH 8.5 stability decreases rapidly besides high pH increases the colour formation during isomerization.

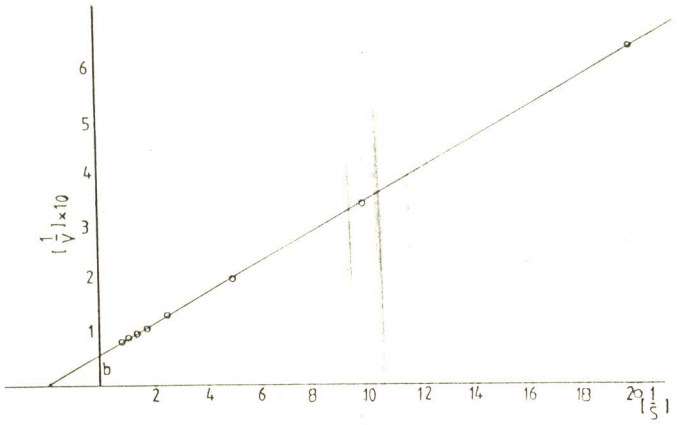

Fig.(1-c): Effect of substrate concentration on the reaction velocity of glucose isomerase enzyme (Sweetzyme type Q)

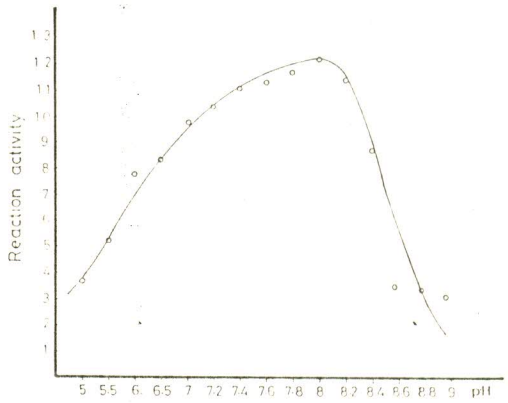

Fig. (2): Effect of pH on the reaction activity of immobilized glucose isomerase enzyme (Sweetzyme type-Q).

Because glucose isomerase enzyme has alkaline optimum pH, it was believed by Dixon and Webb (1964) that the active form of such enzyme must depend upon the ionization of certain groups at the active center which is capable to bind 2 protons, the monoprotonated form being the active form.

$$H_2\ \text{Enz.}\ \underset{\text{inactive}}{} \xleftarrow{\text{Proton}} \text{H Enz.}\ \underset{\text{active}}{} \xrightarrow{\text{Proton}} \underset{\text{inactive}}{\text{Enz.}}$$

Table (2): Effect of pH on activity of glucose isomerase enzyme (Sweetzyme type Q).

pH	Fructose content µmM/l	Reaction activity µmM/l/min
5.0	222.2	3.7
5.5	311.1	5.2
6.0	466.6	7.8
6.5	516.0	8.3
7.0	588.0	9.8
7.2	622.2	10.4
7.4	666.6	11.1
7.6	678.5	11.3
7.8	700.0	11.7
8.0	733.3	12.2
8.2	683.4	11.4
8.4	462.2	7.7
8.6	188.8	3.1
8.8	177.7	2.9
9.0	166.6	2.7

1-3. Effect of temperature:

The effect of temperature on reaction activity of glucose isomerase enzyme (Sweetzyme type Q) is illustrated in Fig.(3) and Table (3), nine different temperatures degrees started from 30°C and ended with 75°C were

employed. At first increase of temperature was accompanied with a rapid increase in the activity of the enzyme to form almost straight line. The optimum temperature was 50°C with reaction activity 5.93 μmM/min. The time reaction curve begins to fall down after 50°C at which temperature increased leading to enzyme inactivation and the enzyme inactivated at 75°C. However, the optimum temperature for this enzyme is concident with that stated before by Tsumura and Sato (1965-b); Aschengreen et al. (1979) and Novo (1982).

Table (3): Effect of temperature on the reaction activity of glucose isomerase enzyme.

Temperature °C	Fructose content μmM/l	Reaction activity μmM/l/min.
30	41.6	0.69
40	225.0	3.75
45	341.6	5.69
50	355.5	5.93
55	219.4	3.66
60	87.4	1.46
65	58.2	0.97
70	19.4	0.32
75	19.7	0.31

1-4. Effect of enzyme concentration:

Table (4) and Fig.(4) illustrate the effect of different sweetzyme type Q concentrations on the activity of glucose isomerase. The maximum reaction activity, 44.9 μmM/l/min. was reached at 600 mg/10 ml. It could

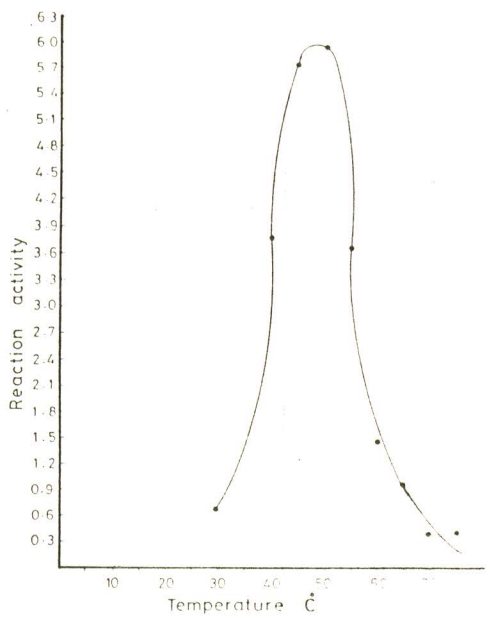

Fig.(3) Effect of temperature on the activity of glucose enzyme (Sweetzyme type-Q).

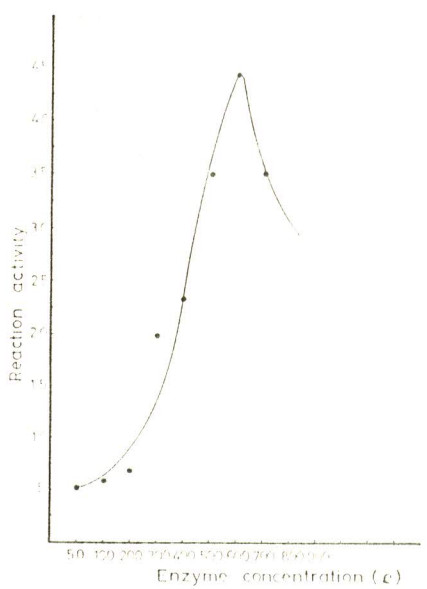

Fig.(4) Effect of enzyme concentration on the reaction activity of glucose isomerase enzyme (Sweetzyme type -Q).

be stated that increasing enzyme concentration to extra high levels lead to a noticed decrease in the overall reaction activity. This might be attributed to the rate of the reverse reaction which might proceeded in opposite direction leading to a general decrease in the final fructose product, Yudkin and Offord (1980).

Table (4): Effect of enzyme concentration on the reaction activity of glucose isomerase enzyme (Sweetzyme type Q).

Enzyme concentration mg/10 ml.	Obtained D-fructose µmM/l	Reaction activity µmM/l/min.
50	311.1	5.2
100	338.8	5.6
200	388.8	6.5
300	1208.3	20.1
400	1416.6	23.6
500	3222.2	35.7
600	2694.4	44.9
700	2111.1	35.2
800	1888.8	31.5

1-5. Effect of Mg^{++}ion concentration on the reaction activity of glucose isomerase enzyme (Sweetzyme type Q):

Different concentrations of Mg^{++} ions were used and the results were recorded in Table (5) and shown in Fig.(5). The reaction activity increased as the Mg^{++} ion concentration increased up to 0.03 (M) and the obtained D-fructose was 290 µmM/l with maximum enzyme reaction activity i.e. 4.83 µmM/ l/ min. At higher concentration of Mg^{++} ion, the reaction activity began to fall down owing to the autoinhibition effect of the high concentration of the Mg^{++} ion.

Table (5): Effect of Mg^{++} ion conc. on the activity of glucose isomerase enzyme (Sweetzyme type Q).

Mg^{++} ion[M] concentration	Fructose content µmM/l	Reaction activity µmM/l/min.
0.005	58	0.97
0.010	122	2.03
0.015	130	2.22
0.020	280	4.67
0.025	285	4.75
0.030	290	4.83
0.035	180	3.00
0.040	160	2.67
0.050	150	2.50
0.070	130	2.17
0.100	25.2	0.42

Similar results were reported by Oestergaard and Knudsen (1976), Aschengreen et al. (1979), Novo (1982) and Mohamed (1986).

2- The production of high fructose syrup from cassava starch:

The liquefaction and saccharification stages were carried out on cassava starch using enzymes under optimum conditions as mentioned before. The resulted syrup with dextrose equivalent (D.E.) and glucose 94.2% was used in the isomerization process. The resulted glucose syrup was purified to eliminate impurities, e.g., peptides, aminoacids and salts which inhibit the isomerase enzyme. The glucose syrup was concentrated to different dry substances content (D.S.) as shown in Table (6), while other parameters were mostly constant of glucose isomerase enzyme (Sweetzyme type Q) according to optimum conditions.

From Table (6) and Fig. (6), it could be concluded that the conversion value to fructose isomer was increased as the initial glucose concentration of the syrup was increased up to 28.70% glucose content. At higher conc. of glucose 35.42%, the conversion value was decreased to 59.29%. This observation was pointed out by Novo (1982).

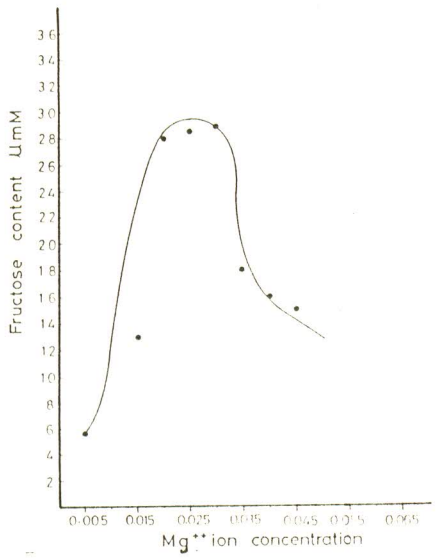

Fig.(5) Effect of Mg^{++} ion concentration on the activity of glucose isomerase enzyme (Sweetzyme type-Q).

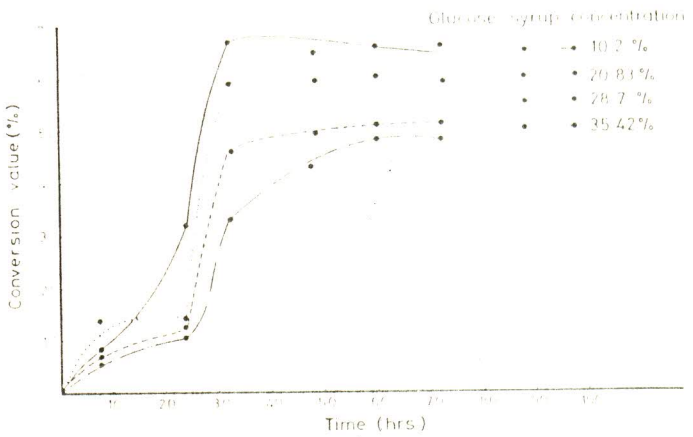

Fig. (6) Effect of glucose syrup concentration on the conversion of glucose to fructose.

Such phenomenon might be attributed to the diffusion resistance occurred in the enzyme particles owing to the high content of dry substrate.

From the result obtained, it seems that there is no need for 72 hours for process duration since 32 hours is quite enough. The different initial glucose concentration reached almost its maximum conversion value after only 32 hours. Such conclusion in very important from the economical stand point of view, since decreasing the time required for isomerization process to almost 50%, simply means great saving in the final expencies of the process. However, such point need to be more investigated under different condition to come to conclusive results.

Table (6): Effect of glucose syrup concentration on conversion of glucose to fructose

Glucose syrup		Fructose content at different periods											
D.S	Glucose	8 hrs.		24 hrs.		32 hrs.		48 hrs.		60 hrs.		70 hrs.	
%	%	g/100 ml	%	g/100 ml	%	g/100 ml	%	g/100 ml	%	g/100 ml	%	g/100 ml	%
12.0	10.20	0.63	6.18	1.80	17.09	3.42	33.53	4.40	43.14	4.90	48.04	4.90	48.04
33.5	20.83	1.63	7.83	2.63	12.60	9.53	45.75	10.40	49.92	10.63	51.03	10.75	51.61
31.5	28.70	2.33	8.10	9.00	31.28	19.30	67.09	18.75	65.17	19.25	66.91	19.00	66.04
40.0	35.42	5.00	14.12	5.00	14.12	20.90	59.01	19.38	54.71	21.25	59.99	21.00	59.29

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السلوك الحركى لانزيم جلوكلوز ايزوميريز .

عزت رزق جندى ـ صلاح مصطفى سعد ـ عبد السلام محمد حلمى ـ فرحات فوده فوده .

قسم الأراضى والكيمياء الحيوية ـ كلية الزراعة بمشتهر ـ جامعة الزقازيق .

أجريت دراسة تأثير مادة التفاعل على كينيكية أنزيم الجلوكز ايزوميريز (سويت زيم Q) وأوضحت النتائج أن السرعة القصوى لهذا الانزيم تساوى ٨ ر١٦ ميكروملليمول / لكل كما أن ثابت ميكاليس لهذا الانزيم يساوى ٢٨ ر٠ ملليمول /لتر . وقد أظهرت النتائج أن درجة الحموضه المثلى لهذا الانزيم كانت ٨ درجة بينما درجة حرارته المثلى كانت ٥٠ م مع نشاط انزيمى ٩٣ ر٥ ميكرومول/ لتر / دقيقة .

وقد وجد أنه بزيادة تركيز أيون المغنسيوم فى بيئة التفاعل أدى ذلك الى زيادة نشاط الانزيم حتى تركيز ٠٣ ر٠ مول /لتر مع تركيز انزيمى محمل يساوى ٦٠٠ ملليجرام / ١٠ ملليلتر .

وقد أوضحت الدراسة أن أقصى معدل لانتاج السكر المحول (شراب الفركتوز) حصل عليها عند استخدام تركيز ابتدائى ٧٠ ر٢٨ ٪ للجلوكوز فى شراب الجلوكوز وقد وجد أن العملية يمكن أن تتم بعد ٥٠ ٪ من الوقت الذى أنجزت فيه التجربة وهذه النقطة مهمة تكنولوجيا لتوفير الطاقة اللازمة لعملية التحضير أثناء العملية .