Kasetsart J. (Nat. Sci.) 34 : 262 - 269 (2000)

Enzymatic Production of Fructo-Oligosaccharides from Sucrose

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ABSTRACT

To increase an efficiency of fructo-oligosaccharide production from sucrose, a mixed-enzyme system of β-fructofuranosidase (*Aspergillus niger* ATCC 20611) and glucose oxidase (Sigma Chemical Co., Ltd.) was carried out in a 2-l stirred tank reactor with the following controlled conditions; temperature 40°C, pH 5.5, aeration rate 1 vvm and agitation rate 550 rpm, for 32 hours of incubation time. With an initial sucrose concentration of 400 g/l, the optimal condition was obtained by using β-fructofuranosidase and glucose oxidase 10 and 15 units per gram sucrose, respectively. As a result, the yields of kestose ($Y_{GF2/S}$) and nystose ($Y_{GF3/S}$) from sucrose and their volumetric productivities ($Q_{GF2/S}$ and $Q_{GF3/S}$) were 0.44, 0.49 g/g, and 4.97, 5.44 g/l h, respectively.

Key words : fructo-oligosaccharides, *Aspergillus niger* ATCC 20611, β-fructofuranosidase

INTRODUCTION

Enzymatic production of fructo-oligosaccharides was industrially performed with the aid of fructosyltransferase (EC 2.4.1.9) and β-fructofuranosidase (EC 3.2.1.26) prepared from *Aspergillus niger* ATCC 20611 (Hidaka *et al*., 1988) and *Aureobasidium pullulans* KFCC 10524 (Jung *et al*., 1987, 1993; Yun and Song, 1993; Yun *et al*., 1994).

For the production of high content of fructo-oligosaccharides, the use of glucose oxidase or glucose isomerase can reduce glucose inhibition in the enzyme reaction with β-fructofuranosidase. Under an optimal condition, the 98% high fructo-oligosaccharides could be produced using a mixed enzyme system of β-fructofuranosidase (*Aureobasidium pullulans* KFCC 10524) and glucose oxidase. (Yun and Song, 1993; Yun *et al*., 1994).

Aspergillus niger ATCC 20611 was previously reported as a good strain for preparing high activities of fructo-oligosaccharide producing enzymes (Hidaka *et al*., 1988). When sucrose was used as substrate, fructo-oligosaccharides were formed as a structure of $1^F(1\text{-}\beta\text{-fructofuranosyl})_n$-sucrose, i.e., n = 1-3, such as 1-kestose (GF_2), nystose (GF_3) and fructofuranosyl nystose (GF_4).

In this work, the study on fructo-oligosaccharide production was performed in a laboratory scale of 2-l fermenter. In a mixed enzyme system of β-fructofuranosidase and glucose oxidase, fructo-oligosaccharides were produced under controlled conditions by varying the concentrations of sucrose and mixed enzymes.

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MATERIALS AND METHODS

Microorganism *Aspergillus niger* ATCC 20611 was used as a source for preparation of β-fructofuranosidase (β-FFase) (Sirisansaneeyakul *et al*., 1998).

Chemicals Sucrose, glucose, and other chemicals used for producing β-fructofuranosidase and fructo-oligosaccharides and for samples analyzing were laboratory grade purchased from commercial sources in Thailand. Glucose oxidase (EC 1.1.3.4) was an enzyme product from Sigma Chemical Co.,USA, prepared by using *Aspergillus niger*, and contained total enzyme activity of 25,000 U/g.

Preparation of β-fructofuranosidase Using freeze-thaw method followed by ammonium sulfate precipitation and dialysis, semi-purified β-fructofuranosidase was produced from fungal culture broth of *Aspergillus niger* ATCC 20611. The enzyme was kept at –20°C before use (Sirisansaneeyakul *et al*., 1998).

Production of fructo-oligosaccharides using β-fructofuranosidase and glucose oxidase The 800 ml reaction mixtures containing sucrose (varied concentrations of 200, 400 and 600 g/l), semi-purified β-fructofuranosidase (10 U/g sucrose) and glucose oxidase (15 U/g sucrose) were made for the production of fructo-oligosaccharides in a 2-l stirred tank reactor. The reaction mixtures were preserved using 0.1% benzoic acid and controlled at pH 5.5, 40°C, 1-vvm aeration and 550-rpm agitation for 32 h of incubation time. Samples were taken once every 30 min, 1 and 2 h within 10, 10-25 and 25-32 h of incubation time, respectively. After withdrawing the samples, an enzyme reaction was immediately stopped using acetonitrile (1:1) and kept at –20°C before analyzing. The analyses were sugars, i.e., sucrose, glucose, 1-kestose and nystose, while the remaining activities of β–fructofuranosidase and glucose oxidase were measured. An effect of glucose oxidase on the production of fructo-oligosaccharides was studied at 15, 30 and 60 U/g sucrose using 400 g sucrose/l and 10 U β-fructofuranosidase/g sucrose.

Analytical methods

Glucose, sucrose, 1-kestose and nystose The sugars were determined using HPLC with carbohydrate column (250×46 mm, Water Corp. USA), controlled at 30°C. Acetonitrile:distilled water (75:25 v/v) was used as mobile phase at controlled flow rate of 1.4 ml/min. Refractometer (LDL Analytica) was a refractive index detector used in HPLC system. Glucose, sucrose, 1-kestose and nystose were prepared at the concentration of 10 mg/ml and used as standards by varying injection volume; 5, 10 and 20 μl.

β-fructofuranosidase (β-FFase) One unit of β-fructofuranosidase was defined as 1 μmole per minute of glucose liberated from the enzyme reaction in the specified condition of pH 5.0, 40°C and incubated for 10 min (Bernfeld, 1955). The enzyme reaction was terminated by adding DNS and boiling at 100°C for 5 min, followed by immediate cooling at 10-15°C. The glucose formed was detected as reducing sugar using 3,5-dinitrosalicylic acid (Miller, 1959).

Glucose oxidase (GO) One unit of glucose oxidase was defined as 1 mmole per minute of glucose converted to gluconic acid and hydrogen peroxide under the specified condition; pH 5.1 and 40°C for 15 min. The glucose was determined using glucose oxidase-peroxidase (Sigma diagnostics kit no. 510-A) (Yun *et al*., 1994).

RESULTS AND DISCUSSION

Effect of initial concentrations of sucrose on fructo-oligosaccharide production

The production of fructo-oligosaccharides was made at controlled pH of 5.5, 40°C and aeration

of 1 vvm. Varying sucrose concentrations of 200, 400 and 600 g/l, the concentrations of β–fructofuranosidase and glucose oxidase used in the experiments were 10 and 15 U/g sucrose, respectively. From the experimental observation, it was found that ca. 50% of sucrose content was rapidly utilized in the early 5 h, 5 h and 8 h of reaction time for initial concentrations of 200, 400 and 600 g/l sucrose, respectively. For initial concentrations of 200 and 600 g/l, the utilization of sucrose, subsequently, decreased slowly and their concentrations became constant at 12 h of reaction time. As a result, the remaining sucrose concentrations of 26.89 and 39.36% were obtained, respectively. In case of 400 g/l initial sucrose concentration, sucrose was continuously utilized after 5 h of reaction time and remained constant at 8 h (13.47%). At 32 h of reaction time, 25.74, 10.40 and 31.94% of sucrose concentration were finally obtained for initial sucrose concentrations of 200, 400 and 600 g/l, respectively (Table 1a).

Similarly, 1-kestose was rapidly produced from sucrose in the early state of sucrose consumption. For initial concentrations of 200, 400 and 600 g/l sucrose, 52.39, 61.24 and 46.31% of maximum 1-kestose concentration were obtained at 10, 8 and 12 h of reaction time. Finally, at 32 h of reaction time, 1-kestose concentrations remained at 35.26, 43.51 and 32.03%, respectively.

For the formation of glucose, glucose increased spontaneously up to 5.64, 8.52 and 4.63% in 30 minutes of incubation time, for the initial

Table 1 Effect of initial concentrations of sucrose on the production of fructo-oligosaccharides using β-fructofuranosidase and glucose oxidase.

(a) Composition of sugar mixtures

Sucrose concentrations (g/l)	Composition of sugar mixtures (%)				Yields (GF_2+GF_3)	(%)
		Fructo-oligosaccharides (FOS)		FOS		
	Sucrose (GF)	1-Kestose (GF_2)	Nystose (GF_3)	Glucose (G)		
200	25.74	31.82	35.26	6.28	67.08	99.10
400	10.40	39.75	43.51	3.79	83.26	97.45
600	31.94	30.26	32.03	4.97	62.29	99.20

(b) Yields and productivities

Sucrose concentrations (g/l)	Yields (g/g)			Productivities (g/l h)		
	1-Kestose (GF_2) $Y_{GF2/GF}$	Nystose (GF_3) $Y_{GF3/GF}$	FOS $Y_{FOS/GF}$	1-Kestose (GF_2) Q_{GF2}	Nystose (GF_3) Q_{GF3}	FOS Q_{FOS}
200	0.428	0.475	0.903	1.99	2.20	4.19
400	0.444	0.486	0.929	4.97	5.44	10.41
600	0.445	0.471	0.915	5.67	6.01	11.68

sucrose concentrations of 200, 400 and 600 g/l, respectively. As for 200 and 600 g/l sucrose, the increasing was continued slightly and their concentrations remained constant until 32 h of incubation time. However, the 400 g/l initial sucrose concentration was found to reach its maximal concentration at 5.5 h of incubation time (9.76%) and decreased continuously to 3.79% at 32 h of incubation time. This indicated that glucose oxidase in the 400 g/l sucrose was able to work quite well for converting glucose to gluconic acid, as compared to those in the 200 and 600 g/l sucrose.

It was found that the mixed soluble enzymes of β-fructofuranosidase and glucose oxidase were very similarly inactivated during the incubation, but β-fructofuranosidase showed its greater stability comparing to glucose oxidase. At 32 h of incubation time, β-fructofuranosidase remained to have its relative activity of 0.32-0.42, whereas glucose oxidase remained to have its relative activity of 0.12-0.24. As for the 400 g/l sucrose, the relative activities of β-fructofuranosidase and glucose oxidase were finally obtained more 23.47 and 50.06% than those for the 600 g/l sucrose, respectively.

Studying the initial concentrations of 200, 400 and 600 g/l sucrose on fructo-oligosaccharide production, the changes for sucrose, glucose, 1-kestose, nystose, β-fructofuranosidase and glucose oxidase were shown comparatively in Figure 1a. And the comparison of sugar composition, yields and productivities of 1-kestose (GF_2), nystose (GF_3) and fructo-oligosaccharides (FOS = GF_2+GF_3) were shown in Figure 2a. As a result, the conclusion was summarized in Table 1, as (a) composition of sugar mixtures and (b) yields and productivities. As for the 400 g/l sucrose, the sugar mixture contained maximal content of fructo-oligosaccharides (83.26%) and minimal contents of sucrose (10.40%) and glucose (3.79%) (Table 1a). Therefore, the maximal FOS yield of $Y_{FOS/GF}$ = 0.929 g/g and the relatively high FOS productivity of Q_{FOS} = 10.41 g/l h were obtained (Table 1b). This FOS productivity was 2.5 times higher than the 200 g/l sucrose's.

Effect of initial concentrations of glucose oxidase on fructo-oligosaccharide production

The production of fructo-oligosaccharides using mixed enzymes was made at the controlled pH of 5.5, 40°C and aeration of 1 vvm. With varying glucose oxidase concentrations of 15, 30 and 60 U/g sucrose, the initial concentrations of β-fructofuranosidase and sucrose used in the experiments were 10 U/g sucrose and 400 g/l, respectively. From the experimental results, sucrose was consumed very rapidly within the first 10 h of reaction time. When using 15, 30 and 60 U glucose oxidase/g sucrose, the final sucrose concentrations of 13.16, 9.08 and 7.83% were remained, respectively. As for 30 and 60 U glucose oxidase/g sucrose, glucose was completely converted to gluconic acid at 9 and 6.5 h of incubation time, respectively. This showed that an increase of 2 and 4 times glucose oxidase could rapidly remove an inhibitory glucose from the resulting reaction of β-fructofuranosidase. As a result, their both relative activities of β-fructofuranosidase (14.10 and 28.26%) and glucose oxidase (68.93 and 78.97%) were remained higher than those for 15 U glucose oxidase/g sucrose at 32 h of incubation time, respectively.

The formation of 1-kestose and nystose could slightly be increased by increasing glucose oxidase 2 and 4 times originally from 15 U/g sucrose. With glucose oxidase of 15, 30 and 60 U/g sucrose, maximal 1-kestose of 61.24, 63.51-64.78 and 65.47-66.12% were obtained at 8, 9-13 and 13-14 h of reaction time, respectively. Subsequently, at 32 h of reaction time, the remaining 1-kestose was 39.75, 41.58 and 43.95%, respectively. On the other hand, nystose of 43.51,

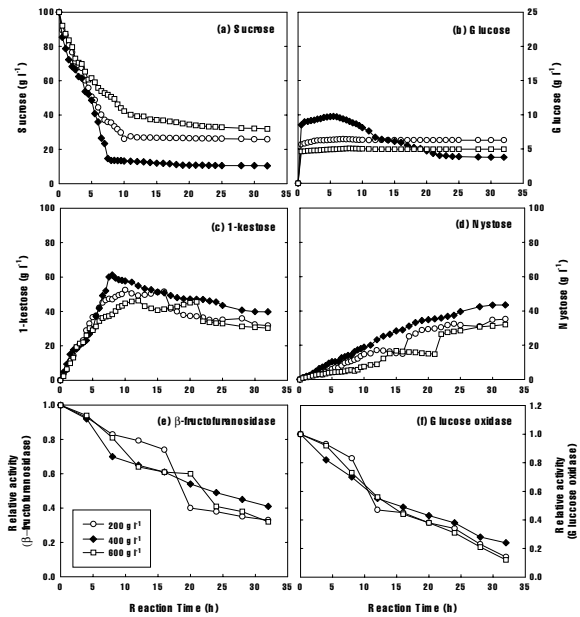

Figure 1a Effect of initial sucrose concentrations on the production of fructo-oligosaccharides; (a) sucrose, (b) glucose, (c) 1-kestose, (d) nystose, (e) β-fructofuranosidase and (f) glucose oxidase.

42.82 and 46.03%, were remained at 32 h of reaction time, respectively (Table 2a).

The study of 15, 30 and 60 U glucose oxidase/g sucrose affecting fructo-oligosaccharide production, the changes for sucrose, glucose, 1-kestose, nystose, β-fructofuranosidase and glucose oxidase were shown in Figure 1b. And the comparison of sugar composition, yields and productivities of 1-kestose (GF_2), nystose (GF_3) and fructo-oligosaccharides (FOS = GF_2+GF_3) were shown in Figure 2b. As a result, the composition of sugar mixtures, yields and productivities were also summarized in Table 2. Removing inhibitory glucose using glucose oxidase was absolutely succeeded using 2 and 4 times higher glucose oxidase in the reaction system. Therefore, fructo-oligosaccharides (FOS = GF_2+ GF_3) were increased 1.37 and 8.07% in the sugar mixtures, whereas FOS productivities (Q_{FOS}) were 1.01 and 1.08 times higher, respectively (Table 2).

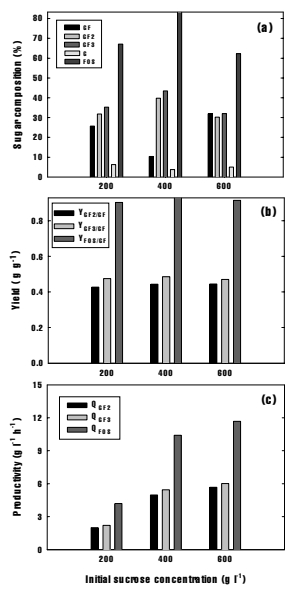

Figure 2a Effect of initial sucrose concentrations on the production of fructo-oligosaccharides; (a) sugar composition, (b) yields and (c) productivities.

Table 2 Effect of glucose oxidase on the production of fructo-oligosaccharides.

(a) Composition of sugar mixtures

Glucose oxidase (GO) (U/g sucrose)	Composition of sugar mixtures (%)					
		Fructo-oligosaccharides (FOS)		FOS	Yields	
	Sucrose(GF)	1-Kestose (GF_2)	Nystose (GF_3)	Glucose (G)	(GF_2+GF_3)	(%)
15	10.40	39.75	43.51	3.79	83.26	97.45
30	8.12	41.58	42.82	0	84.40	92.52
60	2.33	43.95	46.03	0	89.98	92.31

(b) Yields and productivities

Glucose oxidase (GO) (U/g sucrose)	Yields (g/g)			Productivities (g/l h)		
	1-Kestose (GF_2) $Y_{GF2/GF}$	Nystose (GF_3) $Y_{GF3/GF}$	FOS $Y_{FOS/GF}$	1-Kestose (GF_2) Q_{GF2}	Nystose (GF_3) Q_{GF3}	FOS Q_{FOS}
15	0.444	0.486	0.929	4.97	5.44	10.41
30	0.453	0.466	0.919	5.20	5.35	10.55
60	0.450	0.471	0.921	5.49	5.75	11.25

So, we found that the increasing addition of glucose oxidase was not totally necessary for improving FOS productivity. Moreover, the maximal FOS yield ($Y_{FOS/GF}$ = 0.929 g/g) was obtained using 15 U glucose oxidase/g sucrose. Therefore, β-fructofuranosidase (10 U/g sucrose) and glucose oxidase (15 U/g sucrose) in a reaction mixture containing 400 g/l sucrose were concluded as relatively optimal for the production of fructo-oligosaccharides.

CONCLUSION

In the mixed enzyme system with 400 g/l sucrose, maximal fructo-oligosaccharides (83.26%) and minimal sucrose (10.40%) and glucose (3.79%) were finally obtained in the sugar mixture. The FOS yield ($Y_{FOS/GF}$) and productivity (Q_{FOS}) were 0.929 g/g and 10.41 g/l h, respectively. This FOS productivity was calculated as 2.5 times higher than that obtained from 200 g/l sucrose. On the other hand, when glucose oxidase was increased from 15 U/g sucrose to 30 and 60 U/g sucrose, fructo-oligosaccharides were increased only 1.37 and 8.07% in the sugar mixture, respectively. Their FOS productivities (Q_{FOS}) obtained were increased to 1.01 and 1.08 times, respectively. Therefore, it is not recommended to increase glucose oxidase concentration in a mixed enzyme system for the production of fructo-oligosaccharides under this specific condition.

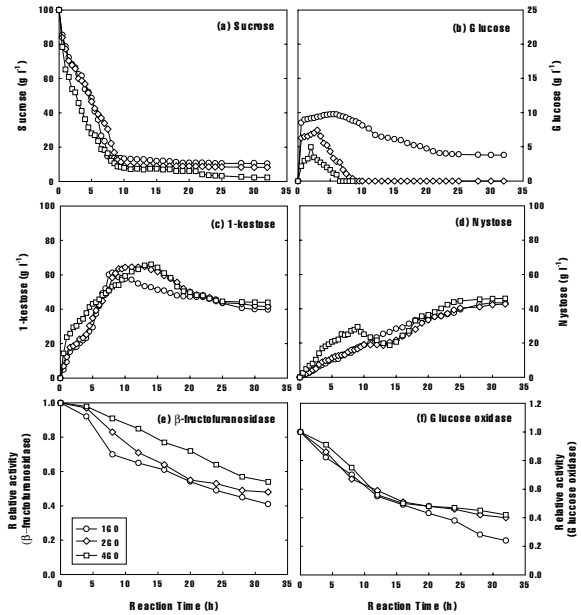

Figure 1b Effect of glucose oxidase concentrations on the production of fructo-oligosaccharides; (a) sucrose, (b) glucose, (c) 1-kestose, (d) nystose, (e) β-fructofuranosidase and (f) glucose oxidase.

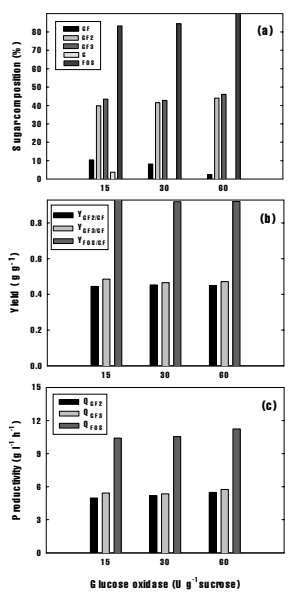

Figure 2b Effect of glucose oxidase concentrations on the production of fructo-oligosaccharides; (a) sugar composition, (b) yields and (c) productivities.

ACKNOWLEDGEMENT

This serial work was financially supported by NRCT grant for FY 1997-1998 entitled enzymatic production of fructo-oligosaccharides. The authors would like to express their sincere thanks to NRCT for giving this research opportunity.

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Received date : 19/01/00
Accepted date : 30/06/00