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Production of Fructooligosaccharides (FOS) Syrup from Sucrose using *Aspergillus Niger* ATCC 20611

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Abstract: The production of fructooligosaccharides from sucrose by the enzyme glucose oxidase was done for 72 hrs. In order to determine the relationship between enzyme activity and transformation of sucrose into fructooligosaccharides, the transfructosylating and hydrolyzing activities in the cell and culture both of microorganisms, including *Aspergillus niger* ATCC 20611, were assayed. The optimum conditions for reaction were as follows: pH, 5.5; temperature, 40°C; sucrose concentration, 70% (700g/L), agitation speed, 260 rpm. Products were analyzed by high performance liquid chromatograph (HPLC).

Key words: Fructooligosaccharides, sucrose, glucose oxidase, *aspergillus nige* 2061 ATCC 20611

Introduction

Fructooligosaccharides exist naturally in many kinds of plants. It can be prepared from high concentration of sucrose using *Aspergillus niger* ATCC 20611 and enzyme glucose oxidase. Recently, fructooligosaccharides (J. H., 1989, Yun *et al.*, 1990, Yun *et al.*, 1992a, Yun *et al.*, 1993, Hayashi *et al.*, 1991 and Hidaka *et al.*, 1987) has had a significant impact on the sugar industry because of their excellent functional advantages in foods such as anti-diabetes and low-cariogenic properties (Hidaka *et al.*, 1986, Oku *et al.*, 1984, Kohmoto *et al.*, 1991 and Wada *et al.*, 1992). In particular, microbial fructooligosaccharides (FOS) from sucrose have attracted much attention and their industrial importance is gradually increasing because mass production is relatively simple and sweet taste is very similar to that of sucrose, a conventional sweetener.

Microbial FOS are produced from sucrose by the action of glucose oxidasease (Kohmoto *et al.*, 1991, Cheetham *et al.*, 1985 and Yun *et al.*, 1992b) and consist mainly of 1-kestose, nystose and fructofuranosyl nystose, in which one to three fructosyl units are bound at the β-2,1 position of sucrose, respectively. This is in contrast to microbial levan (Ohtsuka *et al.*, 1992) and fructosan originating from plants (Edelman and Jefford, 1968). Currently, the industrial production of FOS is carried out by microbial enzyme derived from *Aspergillus niger* ATCC 20611 (Hidaka *et al.*, 1987 and Hidaka *et al.*, 1988). Most of the commerical FOS products presently available contain large amounts of sucrose and glucose; therefore, they are a mixture of a FOS, sucrose and glucose. The maximum FOS content is known to be 55-60% on a dry substance basis (Yun *et al.*, 1992a, Yun *et al.*, 1993, Hayashi *et al.*, 1991 and Hidaka *et al.*, 1987). Our aim of this paper has been focused on searching for a more economical method by using glucose oxidase with which to produce high- content FOS. This study focused on optimizing the reaction conditions for batch experiments to produce high-content FOS using the enzyme of glucose oxidase. The optimum values for pH, temperature, agitation speed, sucrose concentration and enzyme dosage were evaluated. Experiments were performed in an attempt to increase FOS conversion through the efficient removal of sucrose and glucose.

Materials and Methods

Microorganism and Cultivation Conditions (according to J. H., 1989):

Fungi ⟶ Reculture into PDA (Potato Dextrose Agar) media

Strain was subcultured in a medium (100ml in a 250 ml flask) containing 5% sucrose, 0.7% maltoextract, 1.0% polypeptone, 0.5% carboxymethylcellulose (CMC) and 0.3% NaCl with incubation at 28°C for 28 hrs with reciprocal shaking (240 rpm). After growing cell the subculture was transfer at different concentration in different flasks, 6 flasks (500ml) for each concentration. 100 ml of medium consisting of 50-70% (w/v) of sucrose solution, (sucrose and water were sterilized separately then cooled) 5% yeast extract and 0.5% CMC (adjusted pH 5.0-6.0, maintains by Na acetate buffer, 0.05ml/L ~ 68g/L). Two flasks were incubated at 30-40°C with reciprocal shaking (240-260 rpm) for 60 and 72 hrs respectively. After cultivation, the cultures were centrifuged (5°C) and the supernatant solutions and cells were separated for enzyme assays. The same experiments were done with the enzyme, glucose oxidase. Some experiments were done without buffer, some experiments were done without centrifuge and some experiments were done in shaking water bath as an alternative of shaking incubator.

Enzyme Assays: The reaction mixture for determining the enzyme activities consisted of 50-70% (w/v) sucrose (1.0 ml) as the substrate, 5.6 M Na acetate buffer (pH 4.9-5.5, 1.0 ml) and the enzyme solution (0.5 ml) containing an adequate amount of cells or the supernatant solution. The enzyme reaction was carried out at 28°C for 1 hr with moderate shaking in L-type test tubes and terminated by heating in boiling water. Quantitative analysis of tri-saccharides, glucose, fructose and sucrose in the reaction mixture was performed by high performance liquid chromatography (HPLC). The transfructosylating activity and the hydrolyzing activity were determined from the amount of tri-saccharides and fructose respectively. One unit of each of these enzyme activities was defined as the amount of enzyme required to produce (or consume) 1 μ mol of the corresponding saccharide per min under the above conditions.

Sugar Anaysis by High Performance Liquid Choromatography (HPLC): HPLC grade sucrose, glucose and fructose standards were purchased from Sigma Chemical Co., USA and were used as a reference samples. Reference såmples for furctooligosaccharides were HPLC grade and were purchased from Wako Pure Chemical Industries, Ltd. Osaka, Japan. A commercial sample containing 34% 1-kestose, 53% nystose and 10% 1-fructofuranosyl-D-nystose, a gift from Meiji Seika Kaisa, Ltd. Tokyo, Japan. Reference sample for stachyose was purchased from Sigma Chemical Co. USA.

HPLC Analysis was Carried out with Water 410 Under the following Conditions: Column, Sugar-Pak C_{18} column (4.6 mm x 15 cm); temperature, 90°C; mobile phase, calcium EDTA; flow rate, 1.2 ml min: and detector, Waters RI model 410 differential refractive index monitor. The samples were filtered through a Waters Sep-Pak cartridge and a Millipore filter Millex-HV (pore size, 0.45 μm), appropriately diluted before injection.

Effect of the Sucrose Concentration on the Formation of Fructooligosaccharides and Analysis of the Products: All reactions were performed at 30-40°C with the cells in 5.6 M *Na* acetate buffer (pH 4.9-5.5) containing 60-70% sucrose (w/v). Samples taken at appropriate times during the reaction were inactivated by boiling and then the saccharide composition was analyzed by HPLC.

Calorie from Available Sugars and pH: Total calorie was calculated after estimating total sugar in g/100ml of the samples of the available sugars (sucrose, glucose and fructose), pH of samples was measured using a model 691 Digital pH meter, Metrohm Herisau, Switzerland. In most cases, a total run time of 30 min was required to elute all of the components of interest in the samples. A typical HPLC chromatogram of a sample has a major 3 peaks, which comprised of sucrose, glucose and fructose. Trace amounts of oligosaccharides were also present.

Preparation of Fructooligosaccharides: During the transfructosylation reaction on sucrose with cells of *Aspergillus niger* ATCC 20611, the changes in the carbohydrate composition were followed. Sucrose was rapidly converted into fructooligosaccharides (GF_2 and GF_3) and the content of these saccharides reached maxium for glucose 44.4%, fructose 47% and sucrose 33% of the total carbohydrates within 72 hrs.

Results and Discussion

Effect of Sucrose Concentration on the Formation of Fructooligosaccharides: An increase in the sucrose concentration caused an increase in the formation of fructooligosaccharides and the hydrolyzed products increased with the decrease in the sucrose concentration (Kohmoto *et al.*, 1991). That is the largest amount of fructooligosaccharides was produced with 70% sucrose by a strain of *Aspergillus niger* ATCC 20611.
The difference in the bond-formation in the products was investigated by quantitative HPLC analysis of the oligosaccharides obtained.

Sugar ratio: Sugar composition was expressed as sugar ratio 1 and sugar ratio 2, where;

Sugar ratio 1 = [GF] /{[G]} + [F]} (1) and,
Sugar ratio 2 = [GF] / total [Gfn] (2)

Where $2 \leq n \leq 4$ and, GF, G, F and GFn are sucrose, glucose, fructose and fructo-oligosaccharides respectively. In almost all instance, six distinct peaks were identified as stachyose (G_3F), nystose (GF_3), 1-kestose (GF_2), sucrose (GF), glucose (G) and fructose (F). In a few cases, particularly at longer incubation times, peak for 1-fructofuranosyl-D-nystose (GF_4) was also identified. The available sugar sucrose, glucose and fructose were 33%, 44.4% and 47% respectively (Table 2 and Table 3). The percentage sugar composition of the sample including the fructooligosaccharides (FOS) is shown in Table 1.

Table 1: The result of fructooligosaccharides produced by shaking incubator, rpm 240

Temperature	Time	Buffered/pH	Enzyme	% of sucrose in solution	% of FOS	FOS in g/L
30°C	60hrs	Unbuffered pH 4.9	no	50	24	120
30°C	60hrs	Unbuffered pH 4.9	no	50	23	120
40°C	60hrs	Buffered, pH 5.5	glucose oxidase	50	32	160
40°C	60hrs	unbuffered pH 4.9	glucose oxidase	50	26	130
40°C	60hrs	Buffered, pH pH 5.5, sterilized	glucose oxidase	50	35	168
40°C	60hrs	unbuffered pH 4.9	glucose oxidase	70	25	175
40°C	60hrs	Buffered, pH 5.5	glucose oxidase	70	27	189
40°C	60hrs	Buffered, pH 5.5, sterilized	glucose oxidase	70	48	154

Table 2: The result for fructooligosaccharides composition produced from 70% sucrose solution using *Aspergillus niger* ATTC 20611 in shaking incubator at rpm 240, temperature 40°C and pH 5.5 for 72 hrs

Quantity of cells	Condition	% of FOS	FOS g/L	% of sucrose	% of glucose	% of fructose	% of EF
5ml	340 ml substrate in llitre conical flask, buffered	42	294	19	30	7.7	81
3ml	170 ml substrate in 500 ml conical flask buffered	45	315	25	23	6.6	75
4ml	170ml substrate in 500 ml conical flask unbuffered	40	280	15	31	8.5	85
2ml	170 ml substrate in 500 ml conical flask buffered	52	364	15	27	6	85
1 ml	170 ml substrate in 500 ml conical flask buffered	27	189	17.9	44.4	10	82

Ef = sucrose conversion efficiency

Production of fructooligosaccharides from sucrose was existing in various microorganisms. There were quantitative differences among the latter, the enzyme of *Aspergillus niger* ATCC 20611 being the most efficient for the preparation of fructooligosaccharides (Hidaka *et al.*, 1988, Oku *et al.*, 1984 and Cheetham *et al.*, 1985).
From Table 1 we found, at temperature 30°C, sucrose concentration 50% with enzyme glucose oxidase or without enzyme in both condition after 60 hrs obtained FOS 24% (i.e. 120g/L). At temperature 40°C, with the same condition by using Buffered (after sterilization), pH 5.5 it was 35% (i.e. 168g/L) and unbuffered pH 4.9 it was 26% (130/L). But at 40°C temperature in 70% sucrose solution, Buffered pH 5.5 (after sterilization) after 60 hrs the FOS production was 48% (154g/L) and after 72 hrs it was maximum i.e. 62%. By using shaking water

Table 3: The result for fructooligosaccharides composition produced from 70% sucrose solution using *Aspergillus niger* ATCC 20611in bioreactor/shaking incubator at rpm 260, buffered and pH 5.5 for 72 hrs

Quantity of cells	Condition	% of FOS Obtained	FOS in g/L	% of Sucrose	% of Glucose	% of Fructose	% of Ef
10ml	40°C water bath shaker at rpm 240, 340ml substrate in 1L conical flask, no enzyme	61	420	12	22	47	88
5 ml	40°C, water bath shaker at rpm 240, 170 ml substrate in 500 ml conical flask glucose oxidase (2ml)	19	133	24	31	24	75
5 ml	33°C shaking incubator rpm 260 no enzyme, 170 ml substrate in 500 ml conical flask	60	420	13	22	25	88
20 ml	33°C bioreactor rpm 260, substrate 200 ml, glucose oxidase (10 ml)	62	420	14	23	26	88
20 ml	40°C bioreactor rpm 260, 2 litre substrate, glucose oxidase (10 ml)	52	364	33	4.2	9.4	
2 ml	40°C, shaking incubator rpm 260, 170 ml substrate in 500 ml conical flask, glucose oxidase (10ml)	52	364	15	27	16	72
20 ml	33°C shaking water bath rpm 260, substrate 2 litre (250ml flask x 8) glucose oxidase (10 ml), sterilized at 120°C for 15 min.	60	420	14	20	24	78

Ef = sucrose conversion efficiency

bath and without using enzyme the maximum FOS obtained was 61%, because of using 1L flask for 340 ml substrate to get enough oxigen supply (Wada *et el.*, 1992 and Ohtsuku *et al.*, 1992). With the same condition by using 500 ml flask for 170 ml substrate with enzyme the lowest amount of FOS obtained i.e. 19% only, glucose oxidase cosume the insuffient O_2 produced by shaking (Table 2).

It is known that B-Fase commonly possesses for both transfructosylating (U_t) and hydrolyzing activity (U_h) (15). (U_t) and (U_h) were assayed by HPLC as the amounts of fructose formed from sucrose. Therefore, the U_t/U_h ratio indicated the relative strength of the transfructosylating activity of the strain. For the efficient production of fructooligosaccharides, it is preferable to have both a high U_t/U_h ratio and high enzyme productivity Yun *et al.*, 1990 and Hayashi *et al.*, 1991).

With the regard to the sucrose concentration, it was confirm that the transfer reaction proceeded smoothly even in a 60% solution and that the transferred products increased as the sucrose concentration increased until 70% solution. It is known that transfer to a substrate competes with transfer to water in the reaction catalyzed by enzyme, Glucose Oxidase (Edelman and Jefford, 1968 and Hidaka *et al.*, 1988) and fructooligosaccharides could be effectively prepared with a high sucrose concentration.

References

Cheetham, P.S.J., C. Garrett and Clark, 1985. Isomaltulose production using immobilized cells.Biotechnol. Bioeng., 27: 471-481.

Edelman, J. and T. G. Jefford, 1968. The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. New Phytol., 67:517-531.

Hayashi, S., K. Ito, M. Nonoguchi, Y. Takasaki and K. Imada, 1991. Immobilization of a fructosyl-transferring enzyme from *Aureobasidium* sp. on *Shirasu* porous glass. J. Ferment. Bioeng., 72: 68-72.

Hidaka, H., T. Eida, T. Takizawa, T. Tokunaga and Y. Tashiro, 1986. Effect of fructooligosaccharides on intestinal flora and human health. Bifidobacteria Microflora, 5:37-50.

Hidaka, H., T. Eida and Y. Saitoh, 1987. Industrial production of fructooligosaccharides and its application for human and animals.Nippon Nogeikagaku Kaishi, 61:915-923.

Hidaka, H., M. Hirayama and N. Sumi, 1988. A fructooligosaccharide producing enzyme from *Aspergillus niger* ATCC 20611. Agric. Biol. Chem., 52:1181-1187.

J. H., 1989. Mathematical model for enzymatic production of fructooligosaccharides from sucrose. Enzyme Microb. Technol., 11: 491-494.

Kohmoto, T., F. Fukui, H. Tanaka and T. Mitsuoka, 1991. Dose response test of isomalto-oligosaccharides for increasing fecal Bifidobacteria. Agric. Biol. Chem., 55: 2157-2159.

Ohtsuka, K., S. Hino, T. Fukushima, O. Ozawa, T. Kanematsu and T. Uchita, 1992. Characteristics of levansucrase from *Rahnella aquatilis* JCM-1683. Biosci. Biotech. Biochem., 56: 1373-1377.

Oku, T., T. Tokunaga and N. Hosoya, 1984. Nondigestibility of a new sweetener "Neo- sugar" in the rat.J. Nutr., 114:1574-1581.

Wada, K., J. Watanabe, J. Mizutani, M. Tomoda, H. Suzuki and Y. Saitoh, 1992. Effect of soybean oligosaccharides in a beverage on human fecal flora and metabolites.Nippon Nogeikagaku Kaishi, 66: 127-135.

Yun, J. W., K. H. Jung, J. W. Oh and J. H. Lee, 1990. Semi-batch production of fructooligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. Appl. Biochem. Biotechnol., 24/25: 299-308.

Yun, J. W., K. H. Jung, J. W. Oh and J. H. Lee, 1992a. Continuous production of fructooligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. J. Microbiol. Biotechnol., 2: 98-101.

Yun, J.W., K.K. Oh, J.H. Kim, Y.J. Jeon and J.H. Lee, 1992b. Production of palatinose by immobilized cells of *Erwinia raphontici*. Korean J. Biotechnol. Bioeng., 7: 79-83.

Yun, J. W., Y. J. Jeon, M. G. Lee and S. K. Song, 1993. Modelling the effect of mass transfer on the kinetics of fructooligosaccharides production by immobilized cells. Korean J. Biotechnol. Bioeng., 8: 266-271.