

**THE EFFECT OF SUBSTRATE AND ENZYME CONCENTRATION TO THE
FRUCTOOLIGOSACCHARIDES (FOS) PRODUCTION**

RODIANAH BINTI ALIAS

A thesis submitted in fulfillment of the
requirement of the award of the degree of
Bachelor in Chemical Engineering

Faculty of Chemical Engineering & Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2009

I declare that this thesis entitled “*Effect of Substrate and Enzyme Concentration to the Fructooligosaccharides (FOS) Production*” is the result of my own researched except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

Name of candidate : Rodianah binti Alias

Date : 28 April 2009

Special Dedication of This Grateful Feeling to My...

Beloved mother;

Mrs. Asiah binti Awang,

My brothers

And also to all my dearest friends

ACKNOWLEDGEMENT

In preparing this thesis, I was in contact with many people, researchers, lecturers, academicians and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my beloved supervisor, Dr. Mimi Sakinah binti Abdul Munaim for precious encouragement and advice, a lot of guidance and also constructive critics.

I am also indeed indebted to FKKSA technical staff especially to Mr. Razak and Mr. Zaki for her guideline and for their help in my works at the lab. Unforgotten to Ms. Kamariah at FKASA for her guidance and friendly in finishing my research. Without their continued support and interest, this thesis would not be the same as presented here.

My sincere appreciation also extends to all postgraduate students especially Madam Mazlina and Ms. Norhafizah who have provided assistace at various occasions. For the last, I am very grateful to all my friends in UMP and all my family members.

ABSTRACT

The fructooligosaccharides (FOS) are oligosaccharides of fructose that have simple molecule. They can produced by action of fructosyltransferase with sucrose. The fructooligosaccharides is significant to the diabetec and colon disease due to its function reducing the content of sugar in blood. FOS also has a function of prebiotic whereas capable help in human digestion colon. The purpose of this research to determine the effect of substrate and enzyme and the optimum both two of these factor to produce FOS The experiment is carried out used 40 to 80% (w/v) of sucrose concentration and 5% to 9% (w/v) of enzyme concentration in water bath at temperature of 60 °C at 100 rpm. The samples were analyzed by using HPLC, UV-Vis, and TOC to determine FOS concentration, sucrose residual and TOC content respectively. The Design Expert Software also involve to determine the optimum substrate and enzyme to produce FOS. From the result acquire, Increased 40 to 60% of sucrose concentration resulted in increased 96% FOS production and constant after 60% of sucrose concentration approximately at 258 mg/ml. As increasing of enzyme concentration, the production of FOS also increase up to the maximum point of 241 mg/ml FOS production at 5% to 7% enzyme concentration and decrease after 7% enzyme concentration to 134 mg/ml. By using Design Expert Software, increased of sucrose concentration, the FOS production also increase. Increased of enzyme concentration, the FOS production also increase. The optimum condition of substrate and enzyme concentration is 50% and 7% to produce 552.857 mg/ml concentration of FOS.

ABSTRAK

Fruktooligosakarida (FOS) adalah fruktosa oligosakarida yang mempunyai struktur molekul yang ringkas. Ia dihasilkan daripada tindakbalas antara *fructosyltransferase* dengan sukrosa. Fructooligosakarida sangat berguna untuk pesakit diabetes kerana ia dapat menurunkan kandungan gula dalam darah. Selain itu, FOS juga berfungsi sebagai prebiotik dimana ianya dapat membantu pesakit usus dalam sistem penghadaman. Tujuan penyelidikan ini dijalankan adalah untuk mengkaji kesan kepekatan sukrosa, kepekatan enzim dan keadaan optimum kedua-dua faktor ini dalam penghasilan FOS. Ujikaji yang dijalankan menggunakan 40% hingga 80% kepekatan sukrosa dan 5% hingga 9% kepekatan enzim ditidakbalaskan di dalam pemanas air seragam pada suhu (60 °C) dan kelajuan (100 rpm). Sampel ujikaji dianalisa menggunakan *High Performance Liquid Chromatography* (HPLC), *UV Visible Spectrophotometer* (UV-Vis), dan Penganalisa Jumlah Organik Karbon (TOC) untuk menentukan kepekatan FOS, baki sukrosa, dan kandungan organik karbon. Perisian *Design Expert* digunakan untuk menentukan keadaan optimum kepekatan sukrosa dan enzim dalam penghasilan FOS. Daripada keputusan ujikaji yang telah dijalankan, kandungan FOS meningkat dengan peningkatan sebanyak 96% pada 40% hingga 60% kepekatan sukrosa dan penghasilan FOS malar pada 258 mg/ml selepas 60% kepekatan sukrosa. Kandungan FOS meningkat kepada nilai 241 mg/ml dari 5% hingga 7% kepekatan enzim dan penghasilan FOS menurun hingga 134 mg/ml selepas 7% kepekatan enzim. Dengan menggunakan perisian *Design Expert*, penghasilan FOS meningkat dengan peningkatan kepekatan sukrosa dan enzim. Keadaan optimum bagi sukrosa dan enzim adalah 50% dan 7% untuk menghasilkan 552.857 mg/ml kepekatan FOS.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	LIST OF TABLES	xi
	LIST OF FIGURES	xiii
1	INTRODUCTION	1
	1.1 Introduction	1
	1.2 Problem Statement	2
	1.3 Significant of study	3
	1.4 Objective	4
	1.5 Scope of study	4

2	LITERATURE REVIEW	5
2.1	Sucrose	5
2.1.1	Molecular structure of sucrose	5
2.1.2	Physical and Chemical Properties	6
2.1.3	Commercial production and uses	7
2.1.4	Sugar as macronutrien	8
2.1.5	Analysis of sugar	9
2.2	Fructosyltransferase	10
2.2.1	Introduction	10
2.2.2	Production of novel disaccharides	10
2.2.3	Production of Ftase by recycling cell culture	11
2.2.4	Production of Ftase by intentive method	12
2.2.5	Assay for fructosyltransferase	12
2.2.6	Biomass production and enzyme activities	13
2.2.7	Fructofuranosidase activity	15
2.2.8	Crude enzyme properties	16
2.2.9	Enzyme charcterization	16
2.2.10	Hydrolase and Ftase activity	17
2.2.11	Kinetic properties of Ftase	18
2.2.12	Ftase activity and substrate specificity	21
2.2.13	Kinetic of transfructosylation	24

	2.2.14	Characterization of the isolated fructosyltransferase	26
	2.3	Fructooligosaccharides	27
	2.3	Introduction	27
	2.3.2	Advantages of fructooligosaccharides	28
	2.3.3	Usages of fructooligosaccharides	29
	2.3.4	FOS Production	32
3	3.0	METHODOLOGY	55
	3.1	The overall methodology	55
	3.2	List of chemical, glassware and equipment	56
	3.3	Sample preparation	57
	3.3.1	Phosphate Buffer Preparation	57
	3.3.2	Sample preparation	58
	3.3.3	Sample reaction	59
	3.4	Preparation of calibration curve for pure sucrose	61
	3.5	Sample Analysis	62
	3.5.1	By using UV-Vis	62
	3.5.2	By using HPLC	66
	3.5.3	Calibration curve for total carbon (TC) and inorganic carbon (IC)	67
	3.5.4	By using TOC Analyzer	68
	3.5.5	By using Design Expert	70

		x
4	RESULT AND DISCUSSION	71
	4.1 Introduction	71
	4.2 Calibration curve for pure sucrose	71
	4.3 Analysis using UV-Vis	73
	4.4 Analysis using HPLC	75
	4.5 Calibration curve for TC and IC	79
	4.6 Analysis using TOC Analyzer	81
	4.7 Analysis using Design Expert	85
5	5.0 CONCLUSION	88
	5.1 Introduction	88
	5.2 Recommendation	89
	REFERENCE	90 - 92
	S	
	APPENDIX A-D	93 - 100

LIST OF TABLES

TABLE NO.	TITLE	PAGE
3.1	Phosphate buffer solution	57
3.2	Reaction of five different sucrose concentration with enzyme data	58
3.3	Reaction of five different enzyme concentration with sucrose data	59
3.4	Sample of pure sucrose data	61
4.1	ABS value for calibration curve data	71
4.2	Sucrose residual at different sucrose concentration data	73
4.3	Sucrose residual at different enzyme concentration data	74
4.4	Concentration of FOS at different sucrose concentration data	76
4.5	Concentration of FOS at different enzyme concentration data	77
4.6	Area of the graph at different concentration of TC data	79
4.7	Area of the graph at different concentration of IC data	80
4.8	TOC before reaction at different sucrose concentration data	81
4.9	TOC after reaction at different sucrose concentration data	81

4.10	TOC before reaction at different enzyme concentration data	83
4.11	TOC after reaction at different enzyme concentration data	83
4.12	Anova data in FOS production	85

LIST OF FIGURES

NO. OF FIGURE	TITLE	PAGE
2.1	Sucrose molecule structure	5
2.2	Ftase molecule structure	10
2.3	Novel disaccharides production	10
2.4	Time versus pH and DO	13
2.5	Ezyme activity profile	14
2.6	The influence of initial sucrose concentration	22
2.7	Progress curve of saccharides	23
2.8	Dependence of reduced initial rate of transfructosylation reaction	25
2.9	Production of FOS using Ftase obtained recycling cell culture	33
2.10	Crossview and axial view of the design filtration module	34
2.11	Reactor dimension	38
2.12	Sugar profile in a batch process	42
2.13	Effect of incubation time on cellular growth and β -fructofuranosidase	45
2.14	Effect of pH on activity of intracellular β -fructofuranosidase	46

2.15	Temperature effect on the activity and stability of β -fructofuranosidase	47
2.16	Sucrose concentration and FOS production by IM	47
2.17	Reaction time and FOS production by IM	49
2.18	Batch production of FOS	52
2.19	Influence of immobilization cell content in gluten matrix	53
3.1	Five samples of different sucrose concentration	60
3.2	Five samples of different enzyme concentration	60
3.3	Optical system diagram	62
3.4	DNS reagent solution (1%) and potassium sodium tartrate solution (40%)	63
3.5	The color solution before and after heating	65
3.6	The samples were cold at room temperature	65
3.7	HPLC	66
3.8	TC and IC standard solution	67
3.9	Dessicator	68
3.10	TOC Analyzer	69
3.11	Multiple sample has been run	69
3.6	Axial point	70

4.1	Sucrose concentration versus ABS value	72
4.2	Sucrose concentration versus sucrose residual	74
4.3	Enzyme concentration versus sucrose residual	75
4.4	Sucrose concentration versus FOS production	77
4.5	Enzyme concentration versus FOS production	78
4.6	TC concentration versus area	79
4.7	IC concentration versus area	80
4.8	Sucrose concentration versus TOC before reaction	82
4.9	Sucrose concentration versus TOC after reaction	82
4.10	Enzyme concentration versus TOC before reaction	84
4.11	Enzyme concentration versus TOC after reaction	84
4.12	Contour plot of sucrose and enzyme concentration versus FOS production	86
4.13	3-D Graph of FOS production	87

CHAPTER 1

INTRODUCTION

1.1 Introduction

Fructooligosaccharides (FOS) are oligosaccharides of fructose containing a single glucose moiety and have a simple molecule structure rather than the complex molecule from its original sucrose. Fructooligosaccharides can be produced by the action of fructosyltransferase from many plants and microorganisms (Yun, 1996; Hidaka et al., 1988).

Fructooligosaccharides are composed by 1-kestose (GF₂), nystose (GF₃), and 1- β -fructofuranosyl nystose (GF₄), in which fructosyl units (F) are bound at the $\beta(2 \rightarrow 1)$ position of sucrose molecule (GF) (Sangeeta *et al.*, 2005; Kaplan, Hutkins and Yun, 1996 ; Kucbauch, 1972). When the polymeric grade of fructo-oligosaccharides is low, it has a better therapeutic properties compared than the high polymeric grade.

Fructooligosaccharides derived from sucrose using microbial enzymes have attracted special attention due to their sweet taste being very similar to that of sucrose, a traditional sweetener. Fructooligosaccharides are widely used in pharmaceutical industry as a functional sweetener, it is known 0.4 and 0.6 times sweet than sucrose.

The properties of these oligosaccharides such as low caloric values, non-cariogenic properties, decrease level of phospholipids, triglycerides and cholesterol.

(Biedrzycka and Bielecka, 2004). Fructo-oligosaccharides are industrially produced from sucrose by microbial enzymes with transfructosylating activity. This enzyme naturally found in such food such as onion, bananas, artichokes, tomato and in trace amounts as natural components in fruits, vegetables and honey.

1.2 Problem Statement

Diabetes is a disease in which blood sugar levels are above normal. People with diabetes have problem in converting food into energy. There are two main types of diabetes, type 1 diabetes is the person that cannot make any insulin and often occur before age thirty. Type 2 is the person that has adequate insulin, but the cells have become resistant to it and usually occurs in adults over thirty five years old. There are also have a gestational diabetes that effect about 4 % of all pregnant women.

At the normal condition, hormone insulin in the pancreas help process blood sugar into energy. However the sucrose that we take everyday in meal is more complex and is a dimer composed of two sugar monomers, β -D-fructose and α -D-glucose, and is non-reducing. Long term effects of the diabetes are usually their glucose level remain elevated for long period of time. Excess blood sugar levels have a horrible effect on the body. Some common effects from diabetes include vision problems, kidney damage, nerve damage, heart and circulation problem. About 250 million people are diabetics worldwide and it is expected to reach 380 million by 2025, representing 7.1 % of the adult population. Between 1999 and 2004, Health Ministry statistics show a 56 % increase of those below 19 years old being admitted to hospitals due to diabetes, from 837 to 1305 cases. (National Diabetes Statistics, 2007 from national Institute of Diabetes and Digestive and Kidney Disease). To help the diabetes patient to take sugar in healthy way, the sugar or sucrose can converted into fructo-oligosaccharide using fructosyltransferase that more simple molecule structure.

Colon is also known as the large intestine, is part of digestive system. The common problem for the colon disease is irritable bowel syndrome. Treatment for colonic disease involve dietary fiber, medicine and in some case surgery. But the special treatment for the pra-stage of colon disease with daily intake of prebiotics in fructooligosaccharide.

1.3 Significant of the study

Fructooligosaccharides help the absorption of calcium and magnesium, it is useful for diabetec products and are used as prebiotics to stimulate the bifidobacteria growth in the human colon. (Sangeeta *et al.*, 2005).

Fructose is also called monosaccharide compared to the sucrose that called disaccharide. The more simple molecule of fructooligosaccharide, more easy of insulin to convert the food into energy. So, its really help the diabetics to take their sugar in their daily diet. Another benefit of fructooligosaccharide is to treat the colon disease, because the fructooligosaccharide have a prebiotic.

Prebiotics have a good bacteria that destroy the bad bacteria in body. It also may reduce the production of potentially toxic or carcinogeric compounds by disturb the activity of certain enzymes. A prebiotics was defined as a non-digestible food ingredient that stimulating the activity of good bacteria in colon. Prebiotic can found in oligosaccharide also fructooligosaccharide and polysaccharide including dietary fiber but not all dietary carbohydrate are prebiotics.

1.4 Objectives

- i) To determine the effect of the substrate concentration to the production of Fructose-oligosaccharides.
- ii) To determine the effect of the enzyme concentration to the production of Fructose-oligosaccharides.
- iii) To determine the optimum substrate and enzyme concentration to Fructose-oligosaccharide production.

1.5 Scope of study

- i) Enzyme

Commercial enzyme were used from Sigma Alrich and concentration of enzyme were from 5 % (w/v) to 9 % (w/v).

- ii) Substrate

Commercial sucrose were used from Sigma Alrich and concentration of sucrose were from 40 % (w/v) to 80 % (w/v).

- iii) Reaction condition

The samples were reacted in phosphate buffer with pH 5.5 in temperature of 60 speed of °C and 100 rpm. All these conditions are constant during the experiment.

CHAPTER 2

LITERATURE REVIEW

2.1 Sucrose

2.1.1 Molecular structure of sucrose

Sucrose is a IUPAC name, sucrose also called saccharose, is a disaccharide of glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$, or common name is a table sugar. Its systematic name is α -D-glucopyranosyl- (1 \leftrightarrow 2)- β -D-fructofuranoside. (Douglas, 2002)

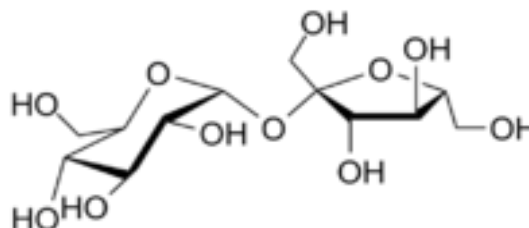


Figure 2.1 Sucrose molecular structure

2.1.2 Physical and Chemical Properties

Sucrose usually prepared as fine, white, odorless, crystalline powder with a pleasing and sweet taste. It was generally isolated from natural sources, where consist of two monosaccharides, α -glucose and fructose, joined by a glycosidic bond between carbon atom 1 of the glucose unit and carbon atom 2 of the fructose unit. The ratio of hydrogen to oxygen in sucrose is 2:1.

Unlike most saccharides, the glycosidic bond in sugar is formed between the reducing ends of both glucose and fructose, and not between the reducing end of one and the non reducing end of other. Since it contains no anomeric hydroxyl groups, it is classified as a non reducing sugar. Acidic hydrolysis can be used in laboratories to achieve the hydrolysis of sucrose into glucose and fructose.

Sucrose melts and decomposes at 186 °C to form caramel, and when combusted produces carbon, carbon dioxide, and water. If the enzyme sucrose is added however, the reaction will proceed rapidly. Reacting sucrose with sulfuric acid dehydrates the sucrose and forms the element carbon.

2.1.3 Commercial production and use

Sucrose is the most common food sweetener, although it has been replaced in American industrial food production by other sweeteners such as fructose syrups or combinations of functional ingredients and high intensity sweeteners. This is due to the subsidization of sugar by the US government, raising the price of sucrose to levels above those of the rest of the world.

Sugar is also used in the manufacturing of an amateur rocket motor propellant called rocket candy. In that propellant it is the fuel with potassium nitrate as the oxidizer (Ghazi, 1995).

Sucrose is the most important sugar in plants, and can be found in the phloem sap. It is generally extracted from sugar cane or sugar beet and then purified and crystallized. Other commercial sources are sweet sorghum and sugar maples.

Sucrose is ubiquitous in food preparations due to both its sweetness and its functional properties; it is important to the structure of many foods including biscuits and cookies, cakes and pies, candy canes, ice cream and sorbets, and also assists in the preservation of foods. As such it is common in many processed and so-called “junk foods.”

2.1.4 Sugar as macronutrien

In mammals, sucrose is very readily digested in the stomach into its component sugars, by acidic hydrolysis. This step is performed by a glycoside hydrolase, which catalyzes the hydrolysis of sucrose to the monosaccharides glucose and fructose. Glucose and fructose are rapidly absorbed into the bloodstream in the small intestine. Undigested sucrose passing into the intestine is also broken down by isomaltase glycoside hydrolases. These products are also transferred rapidly into the bloodstream. Sucrose is digested by the enzyme invertase in bacteria and some animals.

Sucrose is an easily assimilated macronutrient that provides a quick source of energy to the body, also help a rapid rise in blood glucose upon ingestion. However, pure sucrose is not normally part of a human diet balanced for good nutrition, although it may be included sparingly to make certain foods more palatable. Large crystals are sometimes grown from aqueous solutions of sucrose on a string to form rock candy, a confection.

Exceed consumption of sucrose can cause some adverse health effects. The most common is dental caries or tooth decay, in which oral bacteria convert sugars from food into acids that attack tooth enamel. Sucrose, as a pure carbohydrate, has an energy content of 3.94 kilocalories per gram or 17 kilojoules per gram (Chang, 1999).

When a large amount of foods that contain a high percentage of sucrose is consumed, beneficial nutrients can be displaced from the diet, which can contribute to an increased risk for chronic disease. It has been suggested that sucrose-containing drinks may be linked to the development of obesity and insulin resistance. Although most soft drinks in the USA are now made with high fructose corn syrup, not sucrose, this makes little functional difference, since high fructose corn syrup contains fructose and glucose in a similar ratio to that produced metabolically from sucrose.

The rapidity with which sucrose raises blood glucose can cause problems for people suffering from defects in glucose metabolism, such as persons with hypoglycemia or

diabetes mellitus. Sucrose can contribute to development of the metabolic syndrome. In an experiment with rats that were fed a diet one-third of which was sucrose, the sucrose first elevated blood levels of triglycerides.

2.1.5 Analysis of sugars

The analysis of sugars was performed by high-performance liquid chromatography (HPLC). The HPLC equipment consisted of a pump Waters 515 with an on line degasser, a refractive index (RI) detector Waters 410 and an injection valve with a 20 μL loop.

A Sugar-PakTM (Waters) column was used for sucrose, glucose and fructose identification and quantification. Chromatographic conditions were column temperature, 84 °C; mobile phase, water at a flow rate of 0.4 $\text{cm}^3 \text{min}^{-1}$ and RI detector temperature, 40 °C (Sánchez, 2006).

A Shodex[®] column was used for 1-kestose, nystose and 1- β -fructofuranosyl nystose identification and quantification. Chromatographic conditions were column temperature, 65 °C; mobile phase, water–acetonitrile (72:28) at a flow rate of 1.0 $\text{cm}^3 \text{min}^{-1}$ and RI detector temperature, 45 °C (Sánchez, 2006).