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

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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 canditure of any other degree.

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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

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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 peghasilan 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

LIST OF TABLES

LIST OF FIGURES

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 produced by the action of fructosyltransferase from many plants and microorganisms (Yun, 1996; Hidaka et al., 1988).

Fructooligosaccharides are composed by 1-kestose (GF_2), nystose (GF_3), and 1-β-fructofuranosyl nystose (GF_4), 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, types 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↔2)-β-D-fructofuranoside. (Douglas, 2002)

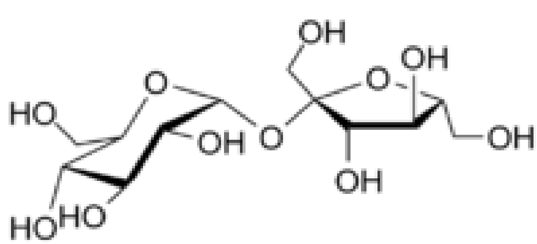

Figure 2.1 Sucrose molecular structure

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-Pak™ (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 cm^3 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 cm^3 min^{-1} and RI detector temperature, 45 °C (Sánchez, 2006).