INFLUENCE OF REACTION TEMPERATURE AND MIXING INTENSITY ON FRUCTO-OLIGOSACCHARIDES PRODUCTION

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

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2009

I declare that this thesis entitled "Influence of Temperature and Mixing Intensity on Fructo-Oligosaccharides Production" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature **:……**
Name **: MOHD ARIFF BIN MOHD NOOR**
Date **: 20th APRIL 2009**

Special Dedication of This Grateful Feeling to My...

Beloved parent;

Mr. Mohd Noor bin Ab Rahman & Mrs. Nik Nah binti Nik Wan

Loving brothers and sisters;

Understanding and helpful friends;

For Their Love, Support and Best Wishes.

ACKNOWLEDGEMENT

First and foremost, I wish to express my sincere appreciation to my project supervisor, Dr. Mimi Sakinah binti Abdul Munaim, for constantly guiding and encourage me throughout this study. Thanks a lot for giving me a professional training, advice and suggestion to its final form.

I am grateful to the staff of Faculty of Chemical Engineering of Universiti Malaysia Pahang for their cheerfulness and professionalism in handling their work. In preparing this thesis, I was in contact with many people, researches, academicians and practitioners. They have contributed towards my understanding and thoughts.

In particular, my sincere thankful is also extends to all my colleagues and others who have provided assistance at various occasions. Their opinions and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. And last, but not least I thank my family for their continuous support while completing the thesis.

ABSTRACT

This study investigates the influence of temperature and mixing intensity on the reaction of Ftase with sucrose. It is important in order to increase the efficiency of the fructo-oligosaccharides (FOS) production. FOS may highly contribute to the food industries, which give beneficial effect to diabetes and colon disease. The objective of this research is to determine the influence of temperature and mixing intensity on the FOS production. The conical flask and shaking water bath is used for the reaction of sucrose with Ftase. The process will be done by varying the temperature and mixing intensity respectively. For the first parameter, different temperature is used in this study which is 30°C, 40°C, 50°C, 60°C, 70°C, 80°C and constant mixing intensity of 100 rpm. For the second parameter, different mixing intensity is used which is 40 rpm, 60 rpm, 80 rpm, 100 rpm, 120 rpm,140 rpm and constant temperature of 65°C. All the samples are then heated up to 100°C to denature the enzyme activity. The samples are analyzed using UV-Vis spectrometer to measure the sucrose residual of the sample. The result of the experiment shows that the optimum condition of FOS production at 60°C and 60 rpm which is 40 g/L and 55 g/L of sucrose residual. From the experiment, it can be concluded the FOS production is increased from 30°C to 60°C, then decreased from 60°C to 80°C. Meanwhile the effect mixing intensity shows the FOS production is increased from 40 rpm to 60 rpm, then decreased from 60 rpm to 100 rpm before increased again from 100 rpm to 140 rpm.

ABSTRAK

Penyelidikan ini mengkaji kesan suhu dan kadar campuran kepada tindakbalas Fructosyltransferase dan sukrosa. Ini penting untuk meningkatkan kecekapan penghasilan frukto-oligosakarida (FOS). FOS memberikan sumbangan yang besar kepada industri makanan, yang mana ia merupakan makanan tambahan kepada penyakit diabetes dan kanser usus. Objektif penyelidikan ini ialah untuk menentukan kesan suhu dan kadar campuran ke atas penghasilan FOS. Kelalang kon dan pemanas air bergetar digunakan untuk tindakbalas sukrosa dan Ftase. Bagi parameter yang pertama, suhu yang berbeza digunakan dalam penyelidikan ini iaitu 30°C, 40°C, 50°C, 60°C, 70°C, 80°C dan kadar campuran yang tetap iaitu 100 rpm. Bagi parameter yang kedua pula, kadar campuran yang berbeza digunakan iaitu 40 rpm, 60 rpm, 80 rpm, 100 rpm, 120 rpm,140 rpm dan suhu tetap 65°C. Semua sampel kemudiannya akan dipanaskan untuk menyahfungsikan aktiviti enzim. Sampel kemudiannya akan dianalisis menggunakan Spektrometer Ultraviolet-Cahaya Tampak untuk menyukat baki sukrosa yang tidak bertindakbalas. Hasil eksperimen ini menunjukkan keadaan optimum penghasilan FOS adalah pada suhu 60°C dan 60 rpm iaitu 40 g/L dan 55 g/L baki sukrosa. Daripada eksperimen yang dijalankan, secara kesimpulannya penghasilan FOS bertambah dari 30°C hingga 60°C, kemudian berkurang dari 60°C hingga 80°C. Sementara itu kesan kadar campuran menunjukkan penghasilan FOS bertambah dari 40 rpm hingga 60 rpm, kemudian berkurang dari 60 rpm hingga 100 rpm sebelum bertambah semula dari 100 rpm hingga 140 rpm.

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FOS	-	Fructo-Oligosaccharides
Ftase		Fructosyltransferase
UV-Vis	-	Ultraviolet – Visible
TOC		Total Organic Carbon
HPLC		High Performance Liquid Chromatography

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CHAPTER 1

INTRODUCTON

1.1 Introduction

Fructo-oligosaccharides (FOS) are included in the class of oligosaccharides, usually used as artificial or alternative sweetener. The word oligosaccharide refers to short chain of sugar molecule. In this case, the sugar molecule is fructose molecule. According to Oxford Dictionary, oligo means few, containing a relatively small number of units, and saccharide means sugar. FOS are constructed from glucose-terminated fructose chain of 3 to 5 units in length. The main sources of FOS usually come from vegetable and fruits such as bananas, onions, garlic, asparagus, barley, wheat, jícama, and tomatoes.

FOS consumption stimulates the growth of bifidobacteria in the gastrointestinal tract of humans and animals with important associated benefits for health. They are calorie-free, since they are hardly hydrolyzed by the digestive enzymes, comprising a safefood for diabetics. They are related to the decrease in total cholesterol, triglyceride and phospholipid levels in the serum and to the reduction in diastolic blood pressure (Katapodis *et al.*, 2004)

Recent studies about FOS indicate the possibility to produce in large scale of FOS using enzymatic synthesis. These studies show the enzyme with high transfructosylation activity is the best enzyme for the synthesis of fructooligosaccharides. The enzymes that usually used in the research such as *Aspergillus niger, Aspergillus japonicus, Aureobasidium pullulans*, and *Fusarium oxysporum*. (Andrelina *et al*., 2007)

1.2 Problem Statement

FOS is used widely in food industry as sweetener. It also used in pharmaceutical industry to produce medicine due to the beneficial effect to diabetes and colon disease. Since there was highly concern on its contribution to these industries, there are a lot of researches done to optimize its production. FOS or fructose shows better advantages to the diabetes and colon disease. In addition, sucrose is one of the main food source that caused diseases related to corpulence,cariogenecity and artherosclerosis. Corpulence or obesity can cause the people get the diabetes. FOS also acts as prebiotic. Prebiotics are described as non-digestible food ingredients that beneficially affect the colon by selectively stimulating the growth and/or activity of the bacteria in the colon that improve health. When human eat something contain prebiotic thing, it will go through stomach first, then go to the colon. Basically, the ingestion of prebiotic has been shown to stimulate the growth of bifidobacteria in the host colon. Bifidobacteria is a friendly bacteria which operate in the lower part of the digestive system. Thus, the colonic environmental is improved by increasing the bifidobacteria and reducing the number of harmful bacteria such as *E-coli* and *Salmonellas*. Since the colon is a critical area which may become the source for several chronic diseases and besides, the correlation between this colon and FOS, it is important to produce and optimize the FOS production.

1.3 Statement of the Objective

The main objective of this study is:

1. To determine the effect of the temperature on fructo-oligosaccharides production
2. To determine the effect of mixing intensity on fructo-oligosaccharides production.
3. To determine the optimum production of fructo-oligosaccharides.

At the end of this study, this objective will help in understanding of the influence of temperature and mixing intensity to optimize the production of the fructo-oligosaccharides.

1.4 Scope of the study

1. The parameters that going to control in this research are:
    i. Temperature
    ii. Mixing intensity
2. The range for temperature is between 30-80°C.
3. The range for mixing intensity is between 150-250 rpm.
4. The parameters that should be constant are enzyme concentrations, volume of enzyme, concentrations and volume of sucrose, time reaction and pH of buffer solution
5. The enzyme used in this research is commercial enzyme.
6. The volume of enzyme is 5ml
7. The volume of sucrose should be 100 ml for each sample of production.
8. The reaction time is 1 hours.
9. Equipment used- jacketed stirred reactor where the rotation and temperature can be control.

1.5 Rationale and Significance

The production of fructo-oligosaccharides has a large significance to industry, especially food and pharmaceutical industry. If we compared to the sucrose, the FOS should be more valuable than sucrose. As the FOS value increase, the price to sell it should be expensive. By doing this research, the price of the FOS can be reduced because of the large production of FOS using the least raw material. This can save the cost of production. Since the price of FOS is cheap, it can be used widely by diabetics and colon disease sufferers. As a result, the percentage of both sufferers can be reduced at least about 20 to 40 percent.

CHAPTER 2

LITERATURE REVIEW AND THEORY

This chapter will review the research that has already been done to gain additional information for the project. It will provide background and information about sucrose, fructo-oligosaccharides (FOS) and enzyme that involved in the reaction as guidance to determine the effect of temperature and mixing intensity on the FOS.

2.1 Fructooligosaccharides (FOS)

2.1.1 Introduction

Since 1980s, the fructooligosaccharides (FOS) always been used in health food. In earlier production of FOS, the enzymatic production of FOS was reported as using *Aureobasidium pullulans* type of enzyme which produced such as 1-kestose and nystose from sucrose. This FOS included in oligosaccharides group, which is an important group of polymeric carbohydrates that are found either free or in combined forms in all living organism. The term of "oligosaccharides" usually used for saccharides that have the degree of polymerization of 2-10. Oligosaccharides usually hydrolyzed to their constituent monosaccharides either by acid or by specific enzymes. Figure 2.1 shows the chemical structure oligosaccharides.

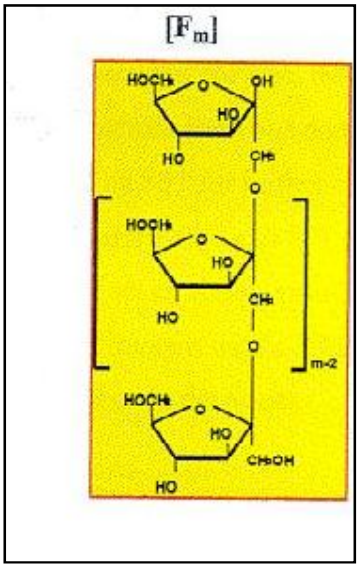

Figure 2.1: Chemical structure of oligofructose.

Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, milk, honey, and Japanese traditional foods such as sake and sweet sake used as seasonings. Also, oligosaccharides are functional food ingredients that have a great potential to improve the quality of many foods. In addition to providing useful modifications to physicochemical properties of foods, it has been reported that these oligosaccharides have various physiological functions such as the improvement of intestinal microflora based on the selective proliferation of bifidobacteria, stimulation of mineral absorption, non-or anticariogenicity, and the improvement of both plasma cholesterol and blood glucose level. (Nakakuki, 2002)

2.1.2 Advantages

The presents of biofunction in this enzyme will increase the number of Bifidobacteria, normally occur in man large intestine. The mixture of FOSs is used as a nondigestible sweetener for humans and exhibits a physiological benefit for improving the growth of the intestinal population of *Bifidusbacteria*. It contributes

many healthful benefits such as immune system activation, resistance to some infections, synthesis of B-complex vitamins, and calcium absorption.

These oligosaccharides are calorie free and noncarcinogenic. FOS has low intensity of sweetness since there are only about one-third as sweet as sucrose. It is scarcely hydrolyzed by the digestive enzymes and not utilized as an energy source in the body. They are non-cariogenic, encourage the growth of beneficial bifidobacteria, anddecrease the levels of serum cholesterol, phospholipids, and triglycerides.

FOS with low polymeric grade has better therapeutic properties than those with a high polymeric degree. FOS are about 0.4 and 0.6 times as sweet as sucrose and have been used in the pharmaceutical industry as a functional sweetener. Until recently, industrial application of enzymes has been restricted to only their hydrolytic action. So, the greatest number of biocatalysts available whom correspondence should be addressed market was represented by lipases, proteases and carbohydrases, aimed exclusively at the degradation of their respective substrates. (Sangeetha *et al*., 2005)

In recent years, some carbohydrases have also started to acquire importance because of their synthesis ability, especially in the food industry. Among these, perhaps the most important is Gfructofuranosidase with fructosyltransferase activity that catalyzes fructooligosaccharides (FOS) synthesis from sucrose; (Yun et al., 1993).

The FOS are composed of sucrose attached by a p(2-+l)linkage to one to three fructose units and are called, respectively, 1-kestose, nystose and fructosyl nystose. Like lactosucrose, galactooligosaccharides, isomaltooligosaccharides and glucosylsucrose, FOS have attracted attention because of their special physiological effect in promoting the growth of Bifidobacteria in the intestinal tract and in decreasing the content of putrefactive substances. (Toshiaki, 1995).

Besides this, FOS are non-cariogenic and non-caloric but have a sweet taste, with sweetening power from 40 to 60% that of sugar. Some microorganisms of the *Aspergillus, Fusarium* and *Aureobasidium genera* have been described as good producers of that enzyme, with potential for industrial purposes. After extensive screening a strain identified as *Aspergillus japonicus* showed the highest ability to produce the enzyme intracellularly. To study the possibility of employing this enzyme in industrial production of FOS, the mycelia were immobilized in calcium alginate, inoculated into highly concentrated sucrose solution and some reaction parameters were established (Andrelina *et al*., 2007)

2.1.3 Usage

Fructooligosaccharides are added in pig and chicken food to improve growth. In humans, they could be used to treat breast cancer, diarrhea, and constipation. In addition, incidence of otitis media could be reduced by daily intake of fructooligosaccharides. FOS present properties such as low caloric values, non-cariogenic properties, decrease levels of phospholipids, triglycerides and cholesterol, help gut absorption of calcium and magnesium, are useful for diabetic products and are used as prebiotics to stimulate the bifidobacteria growth in the human colon (Sangeetha *et al*., 2005).

FOS are industrially produced from sucrose by microbial enzymes with transfructosylating activity, mainly found in fungi such *as Aspergillus, Aureobasidum, Arthrobacter* and *Fusarium*. The theoretical yield of FOS from sucrose is 75% if 1-kestose is the only FOS produced (Yoshikawa *et al*., 2008). Commercial FOS may contain glucose, fructose and sucrose in more than 500 g/kg of total FOS dry weight. Thus, the search of new potent transfructosylating-enzyme producers with their best reaction conditions is desirable in order to scale-up the process. (Yun *et al*., 1997).

Fructooligosaccharides (FOSs) have attracted attention and commercially produced in response to an increasing demand from the consumer for so-called health foods. These fructose oligomers are mainly composed of 1-kestose (GF2), nystose (GF3), and 1F-b-fructofuranosyl nystose (GF4), and can be produced from sucrose through the transfructosylating action of enzymes obtained from various microorganism and plants. The mixture of FOSs is used as a nondigestible sweetener for humans and regarded to be physiologically useful because it improves the intestinal population of Bifidusbacteria.

The FOS-producing enzyme is usually classified as a b-D-fructofuranosidase, despite the fact that many researchers designate it as fructosyltransferase. The former denomination is probably due to the fact that transfructosylating activity was originally found from invertase when acting on high concentration of sucrose. The enzymes that are potential for industrial application come from several fungi including *Aureobasidium sp*., *Aureobasidium pullulans* and *Aspergillus niger*.

In the past few years, several *Aspergillus japonicus* strains have been reported as potentially adequate for industrial production of FOSs. Previous studies described that *A. japonicas* can produce a b-D-fructofuranosidase with high transfructosylating activity. The production of FOSs catalyzed by this enzyme is quite similar to that *from A. pullulans* and *A. niger*.

A recent study suggests that in the crude extract of *Aspergillus niger* an invertase (b-D-fructofuranosidase) exhibits only hydrolytic activity producing exclusively fructose and glucose from sucrose, while a separate enzyme fructosyltransferase catalyzes fructosyltransfer reaction producing glucose and FOSs. The use of whole cell as the biocatalyst avoids the purification of FOS-producing enzyme from the cell extract.

The continuous production of FOSs can be achieved by using immobilized enzyme or cell containing FOS producing, enzyme. However, two food companies in Japan and Korea use different commercial processes involving the immobilized cells

of *Aspergillus niger* and *Aureobasidium pullulans*, respectively, both entrapped in calcium alginate gel. Calcium alginate has also been employed for immobilization of *A. japonicus* mycelia in order to establish FOS-producing processes.

Previously there were researches used a synthetic polymer polymethacrylamide as the matrix for entrapment of *A. japonicus* mycelia. The present paper describes the application of gluten for the entrapment of *A. japonicus* cells. Gluten is a natural polymer and the major by-product from the production of wheat flour. Gluten is advantageous for use as the matrix for immobilization of cells or cellular components because it is biodegradable, inexpensive, nontoxic and readily available.

As described in a previous paper, this protein-based polymer is effective for microencapsulating a fungal antibiotic, entrapping spores of a bio-control microorganism and cell-associated enzymes. Immobilization of cell-associated enzyme was achieved by entrapping bacterial cells containing enzyme of interest within gluten matrices for carrying out a specific biotransformation. The immobilized preparations can be in the form of a thin membrane, a sheet or small pieces. In this work, gluten was used for entrapping fungal mycelia containing FOS-producing enzyme. Batch and continuous productions of FOSs used the immobilized mycelium-associated enzyme were studied.

They are manufactured either from sucrose by transfructosylation or from inulin by controlled enzymatic hydrolysis. Although FOS is present, plant sources like garlic, honey, barley, onion, banana, rye, asparagus, Jerusalem artichoke, etc, the concentration of FOS is low and mass production is limited by seasonal conditions. Hence, microbial production by the action of fungal fructosyl transferase (FTase) on sucrose is more feasible at industrial level. Microbial production of oligosaccharides provides a cost effective and convenient alternative to chemical synthesis (Prapulla *et al.*, 2000).

Fructooligosaccharides (FOSs), including 1-kestose (GF2), nystose (GF3), and 1f-b-fructofuranosyl nystose (GF4), currently have received particular attention and have been commercially produced in response to an increasing demand from the consumer for the so-called health foods (Hidaka *et al*., 1988).

2.2 Fructosyltransferase

2.2.1 Introduction

Fructosyltransferase (FTase) is the enzyme used to catalyze the formation of fructooligosaccharides from sucrose. This catalyst does not change itself, but only catalyze the formation of fructooligosaccharides. There are several microorganisms that have been observed to possess FTase activity and produce FOS from sucrose. One of the microorganism is *Aureobasidium pullulans*, which from previous research reported that it have a potential source of FTase activity. This is the major source for the FTase to produce the FOS from sucrose. The purification and characterization of FTase have been reported from various sources. However, the information about FTase differs from one source to another, from one microorganism to another, even from one strain to another, thereby making it imperative to purify the enzyme from each source.

The present study deals with the purification and characterization of FTase obtained from *Aureobasidium pullulans*. This strain showed very high enzyme productivity and FTase activity, producing 59% of FOS within 9 h of reaction. The purification and partial characterization of the enzyme were undertaken as necessary steps towards understanding some of its properties.