OPTIMUM CONDITIONS FOR SUCROSE PRODUCTION USING ULTRASONIC TREATMENT

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OPTIMUM CONDITIONS FOR SUCROSE PRODUCTION USING ULTRASONIC TREATMENT

TEA LEI KWAN

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

Faculty of Chemical and Natural Resources Engineering
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APRIL 2009

I declare that this thesis entitled “Optimum Conditions for Sucrose Production Using Ultrasonic Treatment” is the result of my own research 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	:	…….
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To my beloved father and mother

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ABSTRACT

The purpose of this study is to determine the optimum conditions for sucrose production using ultrasonic treatment. The raw material used in this study is coconut sugar. With the increasing number of diabetics in the world, the demands for natural, organic, non-calorific and cheap sugar are on the rise. Thus, the sucrose obtained from this coconut sugar can be used to produce fructooligosaccharides (FOS) from the reaction with fructosyl transferase (FTase) enzyme. FOS is non-digestible carbohydrates, exhibits sweetness levels between 30 to 50 per cent of sugar, low caloric value and prebiotic to stimulate the bifidobacteria growth in human colon. As such, FOS can replace the common table sugar and also artificial sweeteners that are supposedly harmful to human. Ultrasonic technology can also be promoted for the usage in the food processing industry as an alternative method to conventional heat treatment that is degradable to food quality and nutrients. The coconut sugar concentrations that were used in this study were 40, 60 and 80 % w/v, diluted in pH 5.5 acetate buffer solution. Ultrasonic frequencies of 25, 68 and 132 kHz were used to treat these coconut sugar concentrations for 5 hours with the samples being analyzed every 30 minutes. The determination of sucrose composition in the treated coconut sugar was by dinitrosalicylic acid (DNS) methods, with measurement of absorbance at 580 nm using UV-Visible Spectrophotometer. It was observed that the sucrose compositions in the coconut sugar increased with time, with occasional drops during treatment. The highest amount of sucrose was found to be in 80 % w/v coconut sugar concentration at 68 kHz ultrasonic treatment. However, the highest percentage of sucrose increased was found in 60 % w/v coconut sugar concentration. Hence, it can be concluded that the highest sucrose production was 68.63% of the original amount at conditions of 60 % w/v coconut sugar concentration and treated at 68 kHz ultrasonic treatment.

ABSTRAK

Kajian ini bertujuan untuk mengenalpasti keadaan optima untuk produksi sukrosa dengan mengunakan kaedah ultrasonik. Bahan yang digunakan dalam kajian ini ialah gula kelapa. Dengan pertambahan bilangan penghidap diabetes dalam dunia, permintaan untuk gula asli, organik, tiada kalori and murah semakin bertambah. Oleh itu, sukrosa yang didapati daripada gula kelapa boleh digunakan untuk menghasilkan fruktooligosakarida (FOS) dengan tindakbalas enzim *fructosyl transferase* (FTase). FOS ialah sejenis karbohidrat yang tidak boleh dihadam, tahap kemanisan di antara 30 hingga 50 peratus dari gula biasa, nilai kalori rendah dan prebiotik untuk pertumbuhan bifidobakteria dalam usus manusia. Dengan itu, FOS dapat menggantikan gula biasa dan juga gula sintetik yang dikatakan mendatangkan bahaya kepada manusia. Teknologi ultrasonik boleh digunakan dalam industri pembuatan makanan sebagai kaedah alternatif kepada penggunaan konvensional haba yang boleh memusnahkan kualiti dan nutrient makanan. Kepekatan gula kelapa yang digunakan dalam kajian ini ialah 40, 60 and 80 % b/i, dilarutkan dalam larutan penampan asetik pH 5.5. Frekuensi ultrasonik 25, 68 and 132 kHz digunakan untuk merawat gula kelapa selama 5 jam, dengan analisis sampel setiap 30 minit. Kandungan sukrosa dalam gula kelapa yang dirawat, diuji dengan menggunakan kaedah asid dinitrosalisilik (DNS), seterusnya menggunakan *UV-Visible Spectrophotometer* untuk menentukan bacaan penyerapan pada 580nm. Data yang diperoleh menunjukkan kandungan sukrosa meningkat, walaupun terdapat beberapa kekurangan semasa eksperimen dijalankan. Kandungan sukrosa yang tinggi terdapat dalam kepekatan gula kelapa 80 % b/i pada frekuensi ultrasonik 68 kHz. Walau bagaimanapun, peratusan peningkatan sukrosa yang tertinggi didapati dalam kepekatan gula kelapa 60 % b/i. Peratus peningkatan penghasilan sukrosa adalah pada kepekatan gula kelapa 60 % b/i dan frekuensi ultrasonik 68 kHz, iaitu sebanyak 68.63%.

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LIST OF ABBREVIATIONS/SYMBOLS

b/i	-	berat per isipadu
DNS	-	dinitrosalicylic acid
USD	-	United States Dollar\
g	-	gram
g/L	-	gram per liter
kcal/g	-	kilocalorie per gram
kHz	-	kilohertz
L	-	liter
M	-	Molar
ml	-	mililiter
mmol/l	-	milimol per liter
N	-	normality
nm	-	nanometer
°C	-	degree Celsius
%	-	percent
% w/v	-	percent weight over volume
µL	-	microliter

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

The word sugar originates from the Arabian word *assukkar* (Ponce-Lee *et al*., 2004). As stated by Mohammad *et al*. (2005), sugar act as sweetening agents, gel or paste-forming and thickening agents, stabilizers and also precursors for aroma and colouring substances generated within the food by a series of reactions and during handling and processing. Sugar, or also called as sucrose, occurs naturally in every fruit and vegetable (Mundt and Wedzicha, 2003). The common, white table sugar crystal that is in everyday diet is made from sugar cane and sugar beet.

Other types of sugars that are gaining reputation in the food and beverages industries are alternative sweeteners such as high fructose corn syrup (HFCS), corn syrup, maple syrup, honey, malt sugar, brown sugar, powdered sugar and so on. However, few studies have shown that these sweeteners could be harmful to human health, for example the sweeteners are sweeter and higher calorie than common table sugar. Synthetic sweeteners are also being introduced into these industries as these sweeteners have absolutely no calories. Dietitians and health practitioners alleged that the sweeteners can cause cancer to human as these types of sweeteners are

chemically made. These claims and allegations could not be proven true or false as research and studies are not done extensively and on human (National Cancer Institute, 2008).

Nevertheless, to be on a safer side, health-conscious consumers are looking for another type of sweetener that is organic, natural and most important of all, safe. One particular sugar that is in this study is the coconut sugar which is crude, dark brown color and in solid form (De Leon and Dolores, 1996). Previously, coconut sugar is not well-known in the world as it is typically produced traditionally and in small scale, mostly in rural areas. Nowadays, Philippines, Indonesia and Thailand have emerged to be the biggest producer of coconut sugar in the world. This success is due to the few studies which showed that coconut sugar has many benefits. As such, other products made from the coconut sugar also cropped up; include beauty products such as skin care, soap, lotions and others.

Coconut sugar contains 80% sugar, essential vitamins and amino acids, and macro and micronutrients (Punchihewa and Arancon, 1996). The main sugars are glucose, fructose and sucrose. It is less sweet than table sugar, thus has lower calorie. It is also classified as a low gylcemic index (GI) food, with GI of 35, which means that the blood glucose level does not rise rapidly and suddenly after consumption (Philippines Coconut Authority). These great properties are beneficial especially for diabetics who need to control their blood sugar level. What is more is that this sugar is natural and does not contain any additive chemicals.

Coconut sugar is made from the sap of the coconut tree. The sap is sweet and watery, derived from the bud or flower of the coconut tree. The sap is boiled at high temperature and long duration time, with constant stirring. The juice will turn into viscous liquid. When the liquid become viscous, it is poured into moulds and the liquid will solidified to form the hard-rock coconut sugar (Apriyantono *et al*., 2002).

Conventional treatment, which is the heat treatment, is popular for so many years in the processing and preservation industry. Heat treatment uses very high temperature to inactivate and kill microorganisms in food products. This inadvertently degrades and spoils the quality and nutrients in the food products. At the present time, new technologies, such as microwave, ultraviolet irradiation, high pressure, ultrasonic and so on, emerged to counteract this shortcoming (Ashokkumar *et al*., 2008). These technologies, which do not use heat, can be used alone or together with heat treatment at lower temperature to give more effective treatment.

Ultrasonic treatment is used in this study to treat the coconut sugar. Ultrasonic waves generate vibrations through liquid medium and at high frequency, cavitations will occur. Cavitations is the making and breaking of the microscopic bubbles. These bubbles form and collapse inwardly and violently, generating mechanical forces (Cameron *et al*., 2008). These forces replace the heat used in heat treatment.

Ultrasonic treatment is still a new technology in the food industry. It is a promising technology as it has many advantages. Ultrasonic treatment has advantage compare to the conventional treatment, where the treatment can be done at much lower temperature and absolutely no heating is required. This can also kill pathogens and microorganisms. Thus, it does not degrade the quality and nutrients of the food products, maintaining its original and fresh-like quality (Cameron *et al*., 2008).

The aim of this research is to study the effects of varieties of ultrasonic frequencies on the treatment of three different concentrations of coconut sugar. The sucrose compositions are determined over time in order to determine the effect of the ultrasonic treatment. The conditions where the highest sucrose composition presents are identified.

1.2 Problem Statement

Over the years, the number of people suffering from diabetes is ever increasing. According to American Diabetes Association, diabetes is a disease where the body does not produce or properly use insulin, causing the blood sugar level to increase drastically. Insulin is a hormone that is needed to move sugar, starches and other food, which is converted into energy needed for daily life, into the cells. Diabetics are not allowed to consume high calories foods, such as fatty foods, high carbohydrates and sugar, and have a proper consumption of the three main nutrients, such as proteins, carbohydrates and fats (Hagura, 2000).

Common table sugar is mostly made of sugar cane and sugar beet. It is thought to absorb quickly, thus rapidly increasing the level of blood sugars (Khazrai, 2006). Based on Food and Nutrition Research Institute (FNRI) and Department Of Science Technology (DOST) in Philippines (2007), the glycemic index (GI) of cane sugar is 50, which is quite high. Glycemic index, according to a scientist from Food and Nutrition Research Institute, is the glucose response of an individual from food relative to a standard glucose solution. Cane sugar is also sweeter than any other sugar and has high calorie. Thus, common table sugar is not suitable for consumption of a diabetic.

Sucrose is a raw material for a compound name fructooligosaccharides (FOS). FOS is beneficial for diabetics, colon disease patients and consumers who want a healthy diet. FOS has properties such as low in sweetness level, low in calories, rich in fibers and as a prebiotic to promote bifidobacteria growth in human colon. Nevertheless, FOS is sold at higher price. This is because the production of FOS requires a lot of work, from the making of sugar, extracting sucrose and to reaction with fructosyl transferase (FTase) enzyme to form FOS. Moreover, production is little as not much study is done to promote and commercialized the use of FOS as an alternative sweetener.

Besides, alternative sweeteners such as high fructose corn syrup, corn syrup and malt sugar, are replacing sucrose as sweetener products in the food and beverages industry. These sweeteners contain one or more of sucrose and the other saccharides such as glucose, fructose, galactose and lactose. Few studies had been done to determine the effect of these other saccharides, alone and combine. The results were not as encouraging as using sucrose as the sweetener. Moreover, some saccharides are little and not obtained naturally.

Synthetic sugars or artificial sweeteners are not natural as they are made from chemical processes and have no calories, which also mean no nutritive values (Sardesai and Waldshan, 1991). Synthetic sugars contain active ingredients that have strong biological effects and the safety is not always assured in all users (Food and Drug Administration, 2008). Although there are no prove to determine the safety of their usage, some people are skeptic about the usage as there are many claims, one leading to cancer and also death. Some synthetic sugars could cause diarrhea while others contain more fat and calories in food products, while the most serious effect is cancer (American Diabetes Association).

1.3 Objectives

The objectives of this study are:

i) To determine the effect and optimal value of ultrasonic frequencies to the sucrose composition during ultrasonic treatment of coconut sugar.
ii) To determine the effect and optimal value of coconut sugar concentration during ultrasonic treatment of coconut sugar.
iii) To determine the effect of exposure time during ultrasonic treatment to the sucrose compositions.

1.4 Scope of Study

The scopes of this study are:

i) The material that is used for the treatment of coconut sugar is the food grade coconut sugar.
ii) The treatment of coconut sugar using ultrasonic treatment with ultrasonic frequencies of 25, 68 and 132 kHz.
iii) The temperature of treatment is set at 25 °C.
iv) Coconut sugar at concentrations of 40, 60 and 80 % w/v are used.
v) The pH set for this treatment is pH 5.5 acetate buffer.
vi) The exposure time for the treatment of coconut sugar is 300 minutes with sampling at time interval of 30 minutes.
vii) The concentration of sucrose in coconut sugar is analyzed by using dinitrosalicylic acid (DNS) method with measurement using UV-Visible Spectrometer with absorbance at 580nm.

1.5 Significant of Study

Coconut sugar has many potential benefits that are yet to be discovered. Coconut sugar is totally natural, contain nutrient, free from additives and artificial flavouring. As such, coconut sugar can be used to replace the common sweeteners and artificial sweeteners, reduce the calories intake and can also be used by diabetes patients. The natural property of coconut sugar will also make the sugar the first choice to consume rather than consuming chemically-made sugars which are surrounded by myths of causing danger to health.

Coconut sugar contains sucrose which is the raw material for fructooligosaccharides (FOS). This compound is important for diabetics and colon disease patients. The importance for diabetics is that FOS does not increase the body weight of the person, thus proving that FOS is low in calorie, low in sweetness and suitable for diabetics (Gudiel and Goni, 2002). FOS increase potentially beneficial fecal bacteria, including bifidobacteria, which is vital to fight colon disease (Euler *et al*., 2005). With these properties, coconut sugar could provide a lot of benefits to diabetics and consumers who want to have a healthy and controlled diet.

Coconut sugar is usually produced traditionally. This contributes to the coconut sugar being sold at a low price. Sucrose can be optimized from the coconut sugar. Sucrose can be obtained and used to produce FOS. Therefore, FOS can be sold commercially and at a much cheaper price than usually sold.

Coconut sugar contains nutrients and it is much better than common table sugar and synthetic sugars in many ways. The glycemic index of coconut sugar is 35 (Philippines Coconut Authority, 2004/5), which is much lower then cane sugar, enables coconut sugar to be a substitute sweetener for diabetics. It is also less sweet than cane sugar. Coconut sugar is purely organic, prepared directly from coconut sap and contains no artificial colouring and flavouring. It is also much cheaper than the synthetic sugars sold over the counter. The benefits should be studied more extensively to recognize its potential in replacing unhealthy common table sugar, high fructose corn syrup and other sugars in the food processing industry.

This would in a way, promote the benefit of planting coconut trees and add values to the coconut plantations. More plantations will be opened, thus creating more jobs for the locals. Coconut farmers can also expand their businesses and venture into the coconut sugar market, which will bring extra income.

More lands will be opened for the plantation of coconut trees if the benefits of coconut sugar are proven. This would encourage growth in the country's economic as the sugar can be exported as demands increase. So far, the three biggest producers of coconut sugar products are Philippines, Indonesia and Thailand (Punchihewa and Arancon, 1996). Coconut sugar is not produced in large quantities to meet demands from overseas. Thus, the study of coconut sugar would give Malaysia a head start as being the main producer of coconut sugar.

On the other hand, the usage of ultrasonic in this study will benefit this technology in the food and beverages industry. Ultrasonic treatment could replace the conventional heat treatment which uses high temperature that tends to degrade the food qualities and nutrients. Ultrasonic treatment does not use any heat and can be operated at ambient temperature. It only uses ultrasonic waves to form bubbles in the medium and creating a mechanical force (Cameron *et al*., 2008). This force could kill pathogens and microorganisms while maintaining the qualities and nutrients of the products. As such, more benefits could be gained from the use of non-thermal technologies to treat food and beverages products.

CHAPTER 2

LITERATURE REVIEW

2.1 Coconut Tree

Coconut tree or its scientific name, *Cocos nucifera*, is the most extensively grown tree in the world and the most important palm. *Cocos* means monkey in Portuguese, because the three indentations on the hairy nut resembles the head and face of a monkey, and *nucifera* means nut bearing (Ombrello, 2008). Coconut tree can reach up to 80 to 100 feet high upon maturity. The tree is seldom straight due to the wind, fruit load and instability of the soil. After four or five years old, the tree begins producing flowers and thereafter the fruits (nuts). The nuts reach full size in about six months to a year for maturity. The nut has a hard outside and white meat beneath with a hollow center in which there is coconut water or juice (Jessica, 2001). Coconut tree can be expected to produce 25 to 50 nuts a year. The tree's lifespan is approximately 80 to 90 years.

The origin of coconut tree is unknown, but it is believed to be from the Pacific Ocean or the Indian Ocean regions. Coconut trees can be found growing mostly throughout the tropical regions in the early years. This is because coconut tree has specific requirements in order for it to survive and prosper, namely: sandy

soils, high amounts of sunlight, high amounts of rain, and also high humidity (Carlson, 2007). Today, distribution of coconut trees around the world is due to humans, having been carried from place to place by immigrants and explorers.

Total world coconut production in 2007 was estimated at 60 million tonnes and around 93 percent is found in the Asian and Pacific region. Two biggest producers are the Philippines and Indonesia; produce about 17 million tonnes and 15.58 million tonnes respectively. India is the third largest producer with 9.4 million tonnes (Food and Agricultural Organization).

Figure 2.1 Coconut tree

2.1.1 Products of Coconut Tree

Practically every part of the coconut tree is important. Coconut tree provides people with basic needs such as food, drink, shelter, fuel, furniture, medicine, decorative materials and much more. More than 50 percent of the coconut production is processed into copra. Another main product is the coconut oil, obtained from copra, which gives almost 7 percent of the total vegetable oils in

the world (Sumner-Fromeyer, 2008). While a small portion is converted into desiccated coconut and other edible kernel products, the rest is consumed as fresh nuts. The coconut palm also provides a series of by-products such as fibre, charcoal, handicrafts, vinegar, alcohol, sugar, furniture, roofing, fuel among others, which provide an additional source of income (Ombrello, 2008).

Coconut is highly nutritious and rich in fiber, vitamins, and minerals. It is classified as a functional food because it provides many health benefits beyond its nutritional content. Coconut oil possesses healing properties far beyond that of any other dietary oil and is extensively used in traditional medicine among Asian and Pacific populations (Ombrello, 2008). The coconut tree is as highly valued as both a source of food and medicine that it is called “The Tree of Life” (Sumner-Fromeyer, 2008).

Table 2.1: Products from coconut tree

Products	Description
Copra (dried kernel)	Produced from dried coconut. Make coconut oil, copra cake and desiccated coconut.
Coconut oil	Obtained from copra. Used as a vegetable oil, soaps, food products, candles, ointments, lubricants, plastics, insecticides.
Desiccated coconut	Dried, white, shredded from freshly peeled coconut kernels. Used as food products in household and bakery and confectionary industries.
Coconut fibre	Coir and coir products, mats, mattings, brushes, brooms, rubberized coir mattresses.
Coconut shell products	Charcoal, activated carbon, filler, insect repellent.
Coconut-based food products	Coconut milk, cream, coconut water, nata de coco, coconut vinegar, coconut jam, nutmeat, young tender coconut, coconut toddy, coconut sugar.

2.1.2 Coconut Sugar

The coconut sugar is crude, dark brown color in solid form. White sugar can also be produced from coconut sap if small amounts of water are added during the crystallization stage. It has the characteristic flavour of coconut and smells like burnt coconut meat (De Leon and Dolores, 1996). Coconut sugar contains 80% sugar, essential vitamins and amino acids, and macro and micronutrients (Punchihewa and Arancon, 1996). Coconut sugar is less sweet compare to cane sugar.

Many perceived that coconut sugar is made from coconut water or juice but it is really made from the coconut sap, as stated in an article by the Philippines Coconut Authority. The Philippines, Indonesia and Thailand are the biggest producer of coconut sugar in the world. Coconut sugars are produced from dwarf and hybrid species of coconut trees as these trees produced more sap. The tall coconut varieties can still produced sugars but the work is laborious.

Figure 2.2 Coconut sugar

Coconut sap contains 12 to 18% sugar and is obtained from cut flower buds. The coconut sap is then boiled at high temperature with constant stirring and long duration time. The sap will turn into viscous liquid. When the liquid become

viscous, it is poured into moulds and the liquid will solidified to form coconut sugar (Apriyantono *et al*., 2002).

In an experiment done by Apriyantono *et al*. (2002), coconut and palm sap were heated to produced coconut and palm sugar. The rate of browning reaction and the sugar composition at the end of heating were analyzed and determined. The browning intensity increased while the glucose, fructose and sucrose compositions decreased with time. The main sugar contain in coconut sugar is sucrose where the amount sucrose left after heat treatment was 83.7% of the initial amount.

2.2 Sugar

The word sugar originates from the Arabian word *assukkar* (Ponce-Lee *et al*., 2004). As stated by Mohammad *et al*. (2005), sugar act as sweetening agents, gel or paste-forming and thickening agents, stabilizers and also precursors for aroma and colouring substances generated within the food by a series of reactions and during handling and processing.

Sugar can be classified in the carbohydrates group, together with oligosaccharides and polysaccharides. Sugar is simple carbohydrates that are monosaccharide and disaccharides (Mohammad *et al*., 2005). Examples of monosaccharide are glucose, fructose and galactose. Disaccharides are formed by two molecules of monosaccharide, which are sucrose, lactose and maltose.

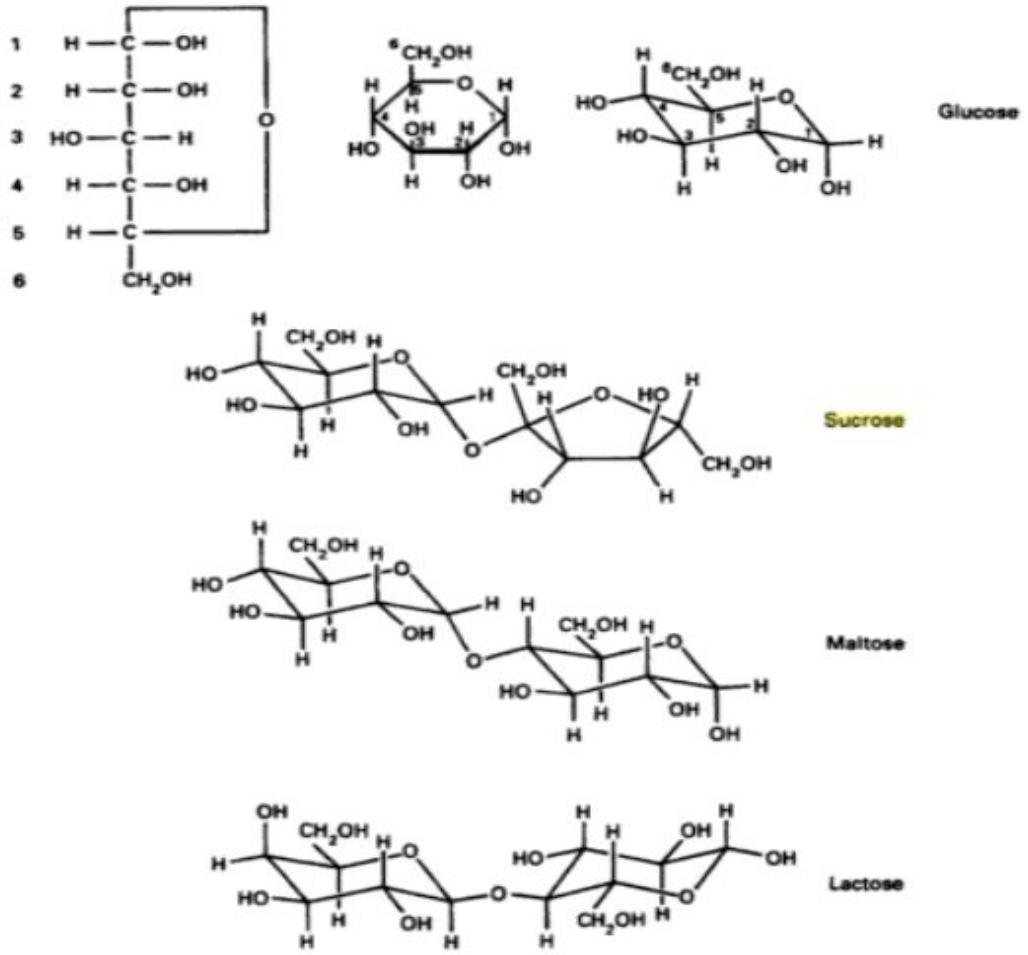

Figure 2.3 Chemical structures of sugars (Brody, 1999)

Sugar or, more commonly known as sucrose, occurs naturally in every fruit and vegetable and it occurs in greatest quantities in sugar cane and sugar beet which are extracted for commercial use (Mundt and Wedzicha, 2003). About 60% of sugar produced around the world comes from sugar cane while 40% from sugar beet. The largest producer of sugar in the world is Brazil, followed by the European Union, India, China and United States (Boughner, 2001). According to Food and Agriculture Organization (FAO) in a market analysis in June 2008, global sugar production was estimated to exceed consumption because of more high technologies inventories and increase in stocks-to-use ratio. This shows that the future markets for sugar depend on nutritional trends: sugar in many foods may replace fats, as over consumption of fats is now considered a more serious health hazard than over consumption of carbohydrates (Mathlouthi and Reiser, 1995).

2.2.1 Types of Sugar

Glucose is the primary form of sugar that is stored in the body for energy and is always linked to diabetics. Fructose, the primary sugar found in fruits, is also found in honey and high fructose corn syrup (HFCS). Galactose is less likely found in nature. It often combines with glucose to form lactose, a milk sugar. Fructose and galactose are metabolized to glucose for the use by body. Maltose is malt sugar, produced from the chemical decomposition of starch, which occurs during the germination of seeds and the production of alcohol.

Sugars or sucrose are mainly found in sugar cane and sugar beets. These sugars are refined to make granulated table sugar. Different degree of purification and processes changes the final products from white, brown to powdered sugars but all these forms of sugars are still sucrose. The types of sugars are shown in Table 2.2.

Table 2.2: Types of sugars (Rasberry, 2008)

Sugar	**Carbohydrate**	**Monosaccharide or disaccharide**	**Additional information**
Beet sugar or cane sugar	Sucrose	Disaccharide (fructose and glucose)	Similar to white and powdered sugar, but varied degree of purification
Brown sugar	Sucrose	Disaccharide (fructose and glucose)	Similar to white and powdered sugar, but varied degree of purification
Corn syrup	Glucose	Monosaccharide	
Fruit sugar	Fructose	Monosaccharide	Very sweet
High-fructose corn syrup	Fructose	Monosaccharide	Very sweet and inexpensive Added to soft drinks and canned or frozen fruits
Honey	Fructose and glucose	Monosaccharides	
Malt sugar	Maltose	Disaccharide (glucose and glucose)	Formed by the hydrolysis of starch, but sweeter than starch
Maple syrup	Sucrose	Disaccharide (fructose and glucose)	
Milk sugar	Lactose	Disaccharide (glucose and galactose)	Made in mammary glands of most lactating animals
Powdered sugar	Sucrose	Disaccharide (fructose and glucose)	Similar to white and brown sugar, but varied degree of purification
White sugar	Sucrose	Disaccharide (fructose and glucose)	Similar to brown and powdered sugar, but varied degree of purification
SOURCE: Mahan and Escott-Stump, 2000; Northwestern University; Sizer and Whitney, 1997; and Wardlaw and Kessel, 2002.			

2.2.2 Sucrose

Sucrose is the most abundant disaccharide occurring in nature, produced about 150 million tons per year (Jarosz and Lewandowski, 2008). It is the common table sugar that is used in daily life. Sucrose occurs naturally in many green plants as a product of photosynthesis. Sucrose is present in limited quantities in many plants, including various palms and the sugar maple, but the sugar beet and the sugar cane are the only commercially important sources. Other natural sources of sucrose are found in many fruits, seeds, roots, and honey.

Sucrose is made up of two monosaccharide molecules, glucose and fructose, with molecular formula of $C_{12}H_{22}O_{11}$. It is a non-reducing sugar, has sweet taste and stable in air. The melting point of sucrose is 186 °C and produced caramel smell when burned (Ponce-Lee *et al*., 2004). Enzymes, sucrase or invertase, are used to hydrolyze sucrose into glucose and fructose.

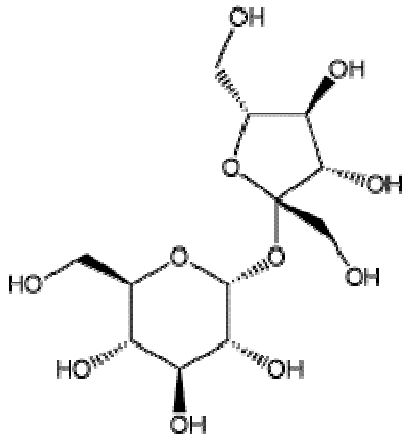

Figure 2.4 Chemical structure of sucrose

Sucrose is the basic ingredient for traditional sugar confectionery, and the whole industry has been built around its physical and chemical properties (Dodson and Pepper, 1985). Nevertheless, it has been displaced in industrial food production

by some other sweeteners such as glucose syrups or combinations of functional ingredients and high intensity sweeteners. Sucrose, normally used in the food industry, is finding ways into other fields as a cheap organic raw material. Considerable effort has been applied to promote sucrose as a scaffold for surfactants, pharmaceuticals or biodegradable polymers (Jarosz and Lewandowski, 2008).

2.2.3 Fructooligosaccharides (FOS)

Fructooligosaccharide (FOS) is non-digestible carbohydrates that possess interesting functional and physiological attributes like low sweetness, non-cariogenicity, low caloric value, prebiotic to stimulate the bifidobacteria growth in human colon, hypolipidemic, hypocholestrolemic properties, facilitate mineral absorption and inhibit growth of pathogenic bacteria in colon (Mabel *et al*., 2008). FOS exhibits sweetness levels between 30 to 50 per cent of sugar in commercially prepared syrups. Its use emerged in the 1980s in response to consumer demand for healthier and calorie-reduced foods. FOS with low polymeric grade has better curative properties than those with high polymeric grade (Sanchez *et al*., 2008). It is present in trace amounts in natural foods like onions, asparagus, wheat, banana, tomato and honey.

FOS is produced from sucrose by microbial transferase like fructosyl transferase (FTase). It is mainly composed of 1-kestose, nystose and 1-β-fructofuranosyl nystose, in which fructosyl units are bound at the β position of sucrose molecule (Sanchez *et al*., 2008). As stated in Mabel *et al*. (2008) papers, many fungal and bacterial strains were reported to produce FTase. The structure and linkage of FOS differ depending on the microbial source of FTase used for its production.

The food diet in a diabetic must be rich of fibres, low in calories and also low in sweetness. Thus, FOS, containing all those, will provide an excellent diet treatment for diabetics (Kaufhold *et al.*, 2000). Some studies demonstrated that FOS can help bring down high blood sugar levels in diabetics, cut down elevated cholesterol levels and normalizes blood fat content. FOS is also said to maintain the body weight of a person upon high consumption (Gudiel and Goni, 2002). It is these properties that FOS may be considered as a non-nutritive, natural sweetener (Mabel *et al.*, 2008).

2.2.4 Comparison of Sucrose and Other Saccharides

Nowadays, producers of carbonated soft drinks are replacing sucrose with HFCS as it is less expensive, easier to handle and has longer shelf life. The producers also stipulated that consuming HFCS is more beneficial than consuming sucrose. HFCS is made from corn starch which is broken down and enzymatically-treated to produce fructose. Fructose is then blended with glucose to achieve the desired ratio of the two sugars (Asian Food Information Centre, 2008).

Melanson *et al.* (2007) had done a study on the effect of substituting sucrose with HFCS as sweetener in carbonated soft drinks on a group of lean women's body weight and appetite. The study on pure fructose showed connection with obesity. This was because fructose does not stimulate the production of insulin that moves glucose into the cells, thus storing up energy in the adipose tissues and become fats. However, pure fructose is not consumed directly but through HFCS and sucrose. Accordingly, the results showed that the effects of sucrose and HFCS on the women's metabolism were the same. More studies are needed as understandings on the effects of HFCS are scarce.

While sucrose and HFCS differ in their bonding, the metabolisms to produce energy for the body are similar. In fact, HFCS has the same amount of calorie as sucrose (4 calories per gram), and contains more fructose, which has been linked with obesity. Sucrose contains 50 percent fructose while HFCS has 55 percent fructose. HFCS also has the same sweetness level as sucrose.

In a study by Yukie and Kazuo (1999), a comparison was done between cube sugar solution, sucrose solution, glucose solution and traubenzucker, grape sugar candy, tested on laboratory rats. It was shown that cube sugar solution and sucrose solution have lower value of blood sugar and longer time to reach the maximum value compared to glucose and traubenzucker solution. Thus, glucose is more easily absorbed into blood compare to sucrose.

Table 2.3: Comparison between sucrose and other saccharides

Saccharides	**Explanations**
Sucrose	- Found in abundance occurring in nature. - Raw material for FOS production. - Must first be hydrolyzed into glucose for energy to be used in body. - Lower blood sugar level. - Longer time to increase blood sugar level. - Benefit for diabetics.
Glucose	- Directly enters blood stream for energy. - Increased blood sugar level drastically. - Not suitable for diabetics consumption.
Fructose	- Linked with obesity. - Not consumed directly but through HFCS. - Consumed in large amount as HFCS is being used in carbonated soft drinks.
Galactose	- Less likely found in nature. - Often combines with glucose to form lactose, milk sugar.

2.3 Diabetes

Diabetes is a chronic condition where the blood glucose level or the amount of glucose in blood is abnormal, affecting millions of people around the world. The body's ability to convert food into energy is impaired. After a meal, a healthy body breaks down most food into glucose and enters into the cells. Nevertheless, diabetic's body does not produce or use insulin, a hormone that helps move glucose, energy needed for daily life, into the cells of the body. Without insulin, glucose builds up in the blood. Over time, high blood glucose levels caused by diabetes can damage many parts of the body.

The World Health Organization (WHO) had predicted that diabetes will increase drastically every year if nothing is done to prevent it. In 1985, there were estimated 30 million people with diabetes. Today, more than 230 million people are affected around the world. The number of people with diabetes is expected to grow to 350 million in less than 20 years if action is not taken (World Diabetes Foundation, 2008). In 2007, the five countries with the largest numbers of people with diabetes were India (40.9 million), China (39.8 million), the United States (23.6 million), Russia (9.6 million) and Germany (7.4 million) (International Diabetes Federation, 2008).

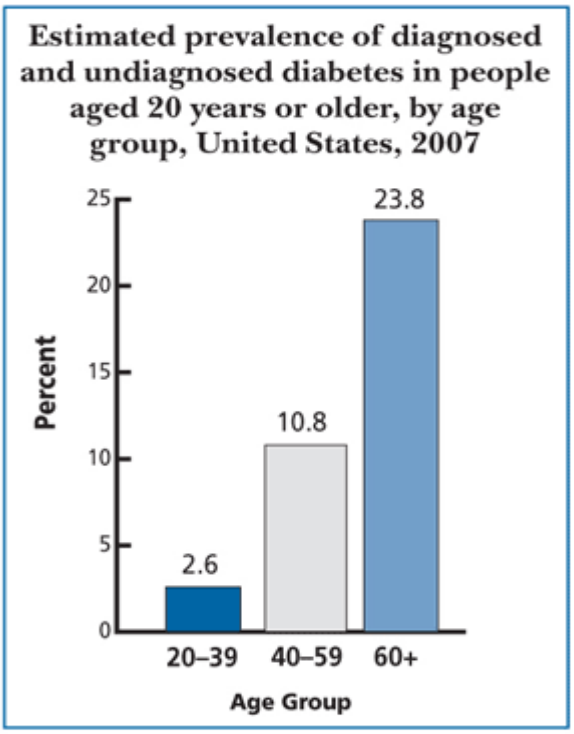

Figure 2.5 Graph of diabetics aged 20 years and above in United States 2007 (*Source*: 2004–2006 National Health Interview Survey estimates projected to year 2007)

Most diabetics were inherited from past generations. However, diabetes is beginning to occur in all stages of life around the world with increasing numbers seen in children and adolescents. This is due to the modern lifestyle most people are living where obesity and unhealthy diets are on the rise (Diabetes Control Program, 2008). At least, 50 percent of all people with diabetes are unaware of their condition while in some countries this figure may reach 80 percent.

Diabetes and diabetes-related disease is widely recognized as the fourth leading causes of death and disability around the world although it is likely to be underreported as the cause of death. Each year about 3.8 million deaths related to diabetes. Diabetes can cause heart disease, stroke, blindness, kidneys damage, nerves damage and foot problems that can lead to amputations (Medline Plus, 2008). Kidney failure is the largest cause of diabetes in developed countries and up to 10 to 20 percent of people die due to this failure (International Diabetes Federation, 2008).

2.3.1 Types of Diabetes

There are three types of diabetes; Type 1, Type 2 and gestational diabetes. Type 1 diabetes, which accounts for 5 to 10 percent of cases, usually strikes children and young adults. Type 2 diabetes occurs more frequently in older people, which accounts for 90 to 95 percent of all cases (Carlton, 2000). Gestational diabetes, the third form, develops in some women during pregnancy.

Type 2 diabetes is mostly given priority in any studies as this type of diabetes is controllable and can be prevented. Type 2 diabetes is often associated with older age, obesity, a family history of diabetes, women who have had gestational diabetes, impaired glucose tolerance, physical inactivity and race/ethnicity (CAM and Diabetes, 2008). Over the recent years, this type of diabetes is beginning to occur in younger generations due to changes in lifestyle such as inactivity and diets rich in saturated fats (Khazrai, 2006). More description of the types of diabetes is shown in Table 2.3.

Table 2.4: Types of diabetes

Types	Target People	Descriptions	Symptoms	Treatment
1	Often in children and young adults but can appear at any age.	Failure to produce insulin. Due to body's immune system attacking and destroy the insulin-producing beta cells in pancreas.	-Very thirsty -Urinating often -Feeling very hungry or tired -Weight loss -Sores that heal slowly -Dry, itchy skin -Lose feeling in feet or tingling in feet -Blurry eyesight	-Insulin medication -Healthy eating -Physical activity
2	Older people, children, people with obesity, family history of diabetes, physical inactivity, history of gestational diabetes.	Most common. Begins as insulin resistance, cells do not use insulin properly, combine with insulin deficiency.	-Develop gradually -Fatigue -Frequent urination -Excessive thirst and hunger -Weight loss -Blurred vision -Slow-healing wounds/sores -Possible to have no symptoms	-Insulin -Healthy eating -Physical activity
Gestational	Women during pregnancy.	Too much glucose is not good for the baby. Diabetes that happens for the first time during pregnancy. It goes away when baby is born.	-Often no symptoms -Increase risk of high blood pressure during pregnancy -Increase risk of large baby	-Follow meal plan -Exercise -Test blood sugar and keep under control -Insulin medication (if needed)

2.3.2 Diabetes Treatment

Diabetes is a costly disease due to its chronic nature, severity of its complications and the methods to control them. In 2007, the world spent an estimated 375 billion USD to care for diabetes and its complications. Those who cannot afford for a proper health care are likely to be diagnosed late and suffered from diabetes related complications because of delay and improper treatment.

In an article by Khazrai (2006), the Diabetes Prevention Program (DPP) investigated that diet and physical activities were more effective than pharmaceutical methods in preventing or delaying the onset of Type 2 diabetes in patients with impaired glucose tolerance and a family history of diabetes. The results showed that those who underwent weight loss program and physical activities lost seven percent of their body weight, with a 58 percent reduction in the incidence of diabetes versus 31 percent in the pharmaceutical group.

Type 2 diabetes can be prevented by a healthy balance of food diet. Diabetics should have fewer intakes on high-energy food such as carbohydrate, protein and saturated fats as these would trigger a high blood glucose level (Hagura, 2000). A healthy diet should provide 55 percent of calories from carbohydrate, 10 to 20 percent from protein and 30 percent or less from fat. Carbohydrate is the body's main energy source and is divided into three groups: sugars, oligosaccharides and polysaccharides.

Simple sugars are thought to be absorbed quickly, thus rapidly increasing the level of blood sugars. Hence, sugars should be restricted in the diet as these are high-calorie foods. Restricting intake of sugars may prevent triggering the triglyceride levels, dental caries and maintain a healthy body weight. Polysaccharides are preferred because the more fibre food contains, the more slowly it is digested, slowly raising the blood sugar level (Khazrai, 2006). However, some

diabetics, who cannot live without sugar in their food, are already replacing their sugar with synthetic sweeteners which promote low glucose release.

Conventional medical treatments, such as insulin injections, are available to control diabetes. However, some people opt for alternative medicine including dietary supplements, such as alpha-lipoic acid (ALA), chromium, omega-3 fatty acids, polyphenols, magnesium, garlic and so on. They may also try acupuncture or biofeedback to help with painful symptoms (CAM and Diabetes, 2008). At times, organ transplant is another resort to treat diabetes. Diabetics with kidney failure may choose to have kidney transplant and there are also pancreas transplant (American Diabetes Association).

2.3.3 Glucose Test Kits

Diabetics should keep their blood glucose level in a healthy range. Normal blood glucose level stays at 4 to 8 mmol/l. They are higher after meals and usually lowest in the morning (Henriksen and Bech-Nielsen, 2008). Monitoring the blood glucose level will help learn how foods, activity levels, stress, medicine and insulin change the blood sugar level. This information will help prevent or delay diabetic complications as well as maintain a healthy body (Family Doctor, 2006).

Diabetics can monitor their own blood glucose with a glucose meter and with the help of professionals using other diabetes management tests. By using the glucose meter, a small sample of blood is placed on a disposable test strip and place in the meter. The test strips are coated with chemicals (glucose oxidase, dehydrogenase, or hexokinase). The meter measures how much glucose is present and display as number (Holt, 2008).

Figure 2.6 Glucose test kit (Holt, 2008)

Most glucose meters are able to read glucose levels over a broad range of values from as low as 0 to as high as 600 mg/dL. Since the range is different among meters, very high or low values are interpreted carefully. Glucose readings are not linear over their entire range. Most glucose meters need blood to be taken from the fingertips. However, consumers were beginning to complain over the lost of sensation at the fingertips due to frequent pricking (U.S. Food and Drug Administration, 2005).

Therefore, new technologies are emerging, allowing testing of blood from alternative sites, such as the upper arm, forearm, base of thumbs and thigh. Sampling blood from alternative sites may be desirable, but it may have some limitations. Blood in the fingertips show changes in glucose levels more quickly than blood in other parts of the body. This means that alternative site test results may be different from fingertip test results not because of the meter's ability to test accurately, but because the actual glucose concentration can be different (U.S. Food and Drug Administration, 2005).

2.3.4 Synthetic Sugars

Artificial sweeteners or synthetic sugars are becoming popular nowadays, especially for diabetics and health-conscious consumers. These are used to replace sucrose or sugars to sweeten foods and beverages. This is related to the fact that sugar is believed to be bad for health and associated with obesity and diabetes. These sweeteners are low in calorie and thus, assisting in weight maintenance and preventing dental cavities. As these sweeteners are many times sweeter than normal sugar, small amounts are only needed to create the same level of sweetness (National Cancer Institute, 2008).

There are two categories of artificial sweeteners; bulk sweeteners and intense sweeteners. Bulk sweeteners are mannitol, sorbitol, xylitol and hydrogenated glucose syrup. Bulk sweeteners have half the calorific value of sugar and used in many processed foods but they are not so readily absorbed. This type of sweeteners helps prevent dental caries but can cause diarrhea if consumed in excess of 25g daily. Intense sweeteners are aspartame, saccharin, acesulfame potassium, sucralose and neotame which have no calories and mainly used in diet drinks, desserts and tabletop sweeteners (Reader's Digest Asia, 2008).

In a study done by Cardoso and Bolini (2007), sucrose in peach nectar was substituted by five artificial sweeteners and the concentrations and magnitudes of these artificial sweeteners sweetness were studied. In order to substitute sucrose successfully, it was necessary to know artificial sweetener concentrations that would be used and their sweetness equivalency related to sucrose. It was also shown that each sweetener has different sweetness intensity and characteristics in different kinds of foods and beverages, and they can promote undesirable effects, such as bitterness or residual tastes.

Artificial sweeteners, at the present, are widely available in different varieties with competitive prices and flavour compare to normal sugars. Even though these sweeteners are popular, there are claims that they are dangerous and can be harmful human health and yet it is still not entirely clear as how these would effect human. Artificial sweeteners are chemically made, and they have no food value and have by-products of harmful toxic side effects (Mayo Clinic, 2008). Aspartame, for example, was discovered as an ulcer drug and thus, taking in aspartame is the same as taking in any dose of medication (Hull, 2002). Although many researches and studies have been done, these testing were only done on laboratory animals. The effects of these sweeteners on animals have proven to be different than on human (National Cancer Institute, 2008).

2.3.4.1 Aspartame

Aspartame is commercialized with names as Nutrasweet and Equal. It is a dipeptide of aspartic acid and a methyl ester of phenylalanine. It is approved for use in pharmaceutical products and used increasingly in chewable tablet and sugar-free formulations. 200 times sweeter than sugar, it has caloric value similar to sugar (4 kcal/g) but the amounts used are small enough to consider aspartame free of calories.

Aspartame was first approved by the FDA in 1981 as a tabletop sweetener, and for use in gum, breakfast cereal, and other dry products. The use of aspartame was expanded to sodas in 1983, and then to use as a general-purpose sweetener in all foods and drinks in 1996 (U.S. Food and Drug Administration, 2006).

In mid-1990s, a researcher raised concerns that a rise in brain cancer was linked to aspartame use. However, there was no scientific evidence supporting this claim (National Cancer Institute, 2008). Due to the phenylalanine component, patients with autosomal recessive phenylketonuria should avoid aspartame. The side effects claims are headache, panic attacks, mood changes, hallucinations, dizziness, nausea, depression and such (Hull, 2002).

2.3.4.2 Saccharin

Saccharin derives from O-toluene sulfonamide. It may be present in drugs in substantial amounts. It is 200 to 700 times sweeter than sugar and has no calories. Its brand names include Sweet'N Low, Sweet Twin and Necta Sweet. Saccharin is used in tabletop sweeteners, baked goods, soft drinks, jams and chewing gum.

Saccharin was discovered in 1879 and had been considered generally as safe until 1972. In 1977, Food and Drug Administration proposed a ban because studies on laboratory rats linked saccharin to the development of bladder cancer after receiving high doses of saccharin. Further studies were performed and no evidence of cancer appeared in human (National Cancer Institute, 2008). In 2000, National Toxicology Program determined saccharin was not a cancer-causing agent and the saccharin warning label removed (U.S. Food and Drug Administration, 2006).

Saccharin can cause dermatologic reactions, such as pruritus, urticaria, eczema, photosensitivity and prurigo. Other reactions include wheezing, nausea, diarrhea, tongue blisters, tachycardia, headache, diuresis and such. Children with 'sulfa' allergy should avoid saccharin (Hull, 2002).

2.3.4.3 Acesulfame Potassium

Acesulfame potassium, or acesulfame-K, is distributed as Sweet One and Sunett. A derivative of acetoacetic acid, it is 200 times sweeter than sugar, also with no calories. Acesulfame-K can be found in baked goods, frozen desserts, candies, beverages, cough drops, and breath mints. It is often combined with other sweeteners and has excellent shelf life and does not break down when cooked or baked (American Heart Association, 2008).

Acesulfame-K was first approved by the Food and Drug Administration in 1988 for specific uses, including tabletop sweetener. The use in beverages was approved in 1998, followed by approved for general use in foods, but not in meat or poultry in December 2003 (U.S. Food and Drug Administration, 2006).

Studies on animals showed acesulfame-K stimulate insulin secretion and could possibly generate reactive hypoglycemia (Hull, 2002). Nevertheless, the Food and Drug Administration and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives had evaluated the sweetener's safety and supported it.

2.3.4.4 Sucralose

Known commercially as Splenda, it is from chlorinated sucrose derivative. It is 600 times sweeter than sugar on average and has no calories. Although sucralose is made from sugar, it adds no calories because it is not digested in the body. Chlorine added is similar to chlorine atom in salt molecule. Taking in sucralose may be more like ingesting tiny amounts of chlorinated pesticides (Hull, 2002).

After reviewing more than 110 animal and human studies, the Food and Drug Administration approved sucralose in 1998 for use in 15 food categories, including as a tabletop sweetener and for use in products such as beverages, chewing gum, frozen desserts, fruit juices, and gelatins. In 1999, sucralose was allowed as a general-purpose sweetener in all foods (U.S. Food and Drug Administration, 2006).

2.3.4.5 Neotame

Neotame is 7000 to 13000 times sweeter than sugar, depending on how it is used in food and has no calories. It is similar in structure to aspartame. The Food and Drug Administration approved neotame in 2002 as a general-purpose sweetener in a wide variety of food products other than meat or poultry. It has been approved for use in baked goods, soft drinks, chewing gum, frosting, frozen desserts, jams, jellies, gelatins, puddings, processed fruit and fruit juices, toppings, and syrups (U.S. Food and Drug Administration, 2006).

2.4 Benefits of Coconut Sugar

Coconut sugar has not gain wide reputation as being beneficial to health-conscious consumers. A few studies had shown that coconut sugar is especially good for diabetics, who prefer natural sugar. Although cane sugar and beet sugar come from nature too, they have high caloric value and sweetness as compared to coconut sugar.

According to the Philippines Coconut Authority, tests on coconut sugar revealed a glycemic index (GI) of 35 which is classified as Low GI food. Cane sugar and beet sugar has GI of 50 which is higher than that of coconut sugar. The GI is a ranking system for carbohydrates based on the immediate effect on blood glucose levels. The higher the number, the greater the blood sugar response. Example a low GI food will cause a small rise in blood glucose level, which is needed by diabetics to control their blood sugar level.

Table 2.5: Classification of food glycemic index

Level	**Index**
High	> 70
Medium	56 – 69
Low	< 55

Coconut sugar can replace many naturally and artificial sweeteners in the food and beverages industries. Artificial sweeteners are chemically-made and have no calories and nutrients. These sweeteners are said to posed dangerous health effects to consumers although not much evident arise to support these claims. High fructose corn syrup (HFCS) that is replacing sucrose in the food and beverages industries lately is also said to cause many health risks. Hence, coconut sugar, with its natural properties, is mostly beneficial in replacing all these ill-health claims of sugars.

2.5 Treatments in Food Processing Applications

Thermal treatment for processing and preservation gained popular attention for a long time in the food industry. While this maybe so, the quality and nutritional value in food are sometimes questioned. As there are growing numbers of consumers demanding for high quality food products, new alternatives must exist to compliment this. Therefore, more and more non-thermal technologies emerge for the application of food processing and preservation (Ashokkumar *et al*., 2008). Examples of non-thermal processing technologies are microwave, ultraviolet irradiation, high pressure, high intensity pulsed electric field, ultrasonic and such.

Non-thermal treatments are generally carried out at a lower temperature than conventional methods, so that heat effect on food quality is minimized. These technologies have the advantage of preserving the qualities with acceptable inactivation of spoilage and pathogenic microorganisms (Walkling-Ribeiro *et al*., 2008). These treatments can be used together with a milder heat treatment to ensure enough microbial inactivation while reducing the impact on quality.

Apriyantono *et al*. (2002) used heat treatment to heat the coconut and palm sap to produce coconut and palm sugar. This heat treatment had led to the formation of brown colour and the decreasing of sucrose composition. Hence, the effects of heat treatment are very large, degrading the quality of coconut sugar. This study uses ultrasonic treatment to treat the coconut sugar. The brown colour formation and sucrose concentration are measured to determine the effects of ultrasonic treatment. With this study, it is hope that ultrasonic treatment could provide a new and better chapter in the processing and preservation of food and beverages products.

2.5.1 Thermal Treatment

The most general treatment for food processing and preservation applications is thermal or heat treatment. Pasteurization and microbial inactivation, especially, used conventional thermal treatment for the treatment of their food products as it is widely known that microorganisms can not survive high temperature. However, thermal treatment has disadvantage in these food industries. Heat that is used can promote many chemical reactions within the food and degrade the quality of the food. Sugar undergoes thermal degradation with the formation of furan derivatives (Piva *et al*., 2008). Degradation of sucrose during heating also may affect the colour and antioxidant capacity of a food product (Tsai *et al*., 2005).

Changes done by heat treatment can be shown in a study by Atasoy and Turkoglu (2008), where milk, which was used for the production of cheese, was pasteurized by high temperature to eliminate pathogens which may be present. However, this pasteurization changed the biochemistry, microbiology and also the flavour of the cheese. Inactivation of indigenous milk pro-enzymes and enzymes, elimination of milk microorganisms and modification in activity of starter bacteria are characteristics of milk, which were changed by heat treatment. Milk without heat treatment produced cheese which matured faster and had more intense and unique flavour compared to pasteurized milk. Although so, using milk without pasteurization could create health risk as the milk still contain pathogens.

Nevertheless, in a study done by Muratore *et al*. (2008), thermal treatment on tomatoes, dipped in an aqueous solution of citric acid, sodium and calcium chloride, lowers the risk for cancer of the digestive tract and prostate. This was because lycopene, an antioxidant that reduced the risk of cancer, was stable during heating and the treatment was able to improve lycopene bioavailability.

Nowadays, heat treatment is combined with other non-thermal treatment to give a better effect while preventing the degradation of the food qualities. In a study by Fang *et al.* (2008) on kiwifruit peroxidase, heat was combined with high pressure treatments to inactivate the enzyme peroxidase that could cause negative changes in the colour and flavour of vegetables during storage. Conventional heat treatment that was used to inactivate the enzyme had raised concerns with the lost of nutrients and degrade the food quality. Thus, high pressure treatment was combined so that the heat temperature used was lower than usual and will not degrade the food quality.

2.5.2 Ultrasonic Treatment

Many areas have already been using ultrasonic technology to make or modify materials (Cui *et al*., 2007). Its application has been introduced in many new areas, such as food, agricultural, environmental, chemical engineering and so on, as many great benefits arrived from this technology. Ultrasonic treatment, as will be done in this study, is still new in the food industry and few studies have addressed the use of ultrasound in processing and preservation (Fernandes *et al*., 2008).

Ultrasonic waves, generated by mechanical vibrations with frequencies between 20 to 800 kHz, are transmitted through water, gas and vapour. Alternating compressions and rarefactions are produced. If the amplitude of the wave is high enough, cavitations, the making and breaking of microscopic bubbles, will occur. When the bubbles reach a critical size, they collapse violently, generating mechanical forces (Cameron *et al*., 2008). The phenomenon is called acoustic cavitations. Water undergoes thermolysis in the bubbles to release radical species (Li *et al*., 2008).

Ultrasound has been used to accelerate the rates of numerous chemical reactions. The rate of enzyme-catalysed reaction was known to increase when treated with ultrasonic but it is still unclear and less study has been done on this (Shah and Gupta, 2008). Nevertheless, studies have been done for the application of ultrasonication as a laboratory based technique for assisting extraction process from plant, foods and herbs (Vilkhu *et al*., 2007). The usage of ultrasound have shown increased yield of extraction, increased rate of extraction and reduction in extraction time.

Ultrasonic treatment has also found its use in pasteurization and microbial inactivation in food products without generating negative side effects associated with thermal treatment. Cameron *et al*. (2008) studied the effect of ultrasonication on milk microbes. It was shown that ultrasound structurally damaged the microbial cell wall and within the cell extensively. However, further studies are needed as some microbial are destroyed internally only and some are resistant to treatment.

Ultrasonic technology is a new promising non-thermal technology that will provide a great breakthrough for the food industry. The advantage of ultrasonic treatment is that the process requires no heating and causes no common side effects associated with conventional heat treatments, such as nutrient and flavour loss (Cameron *et al*., 2008). This treatment can improve product quality, lower production costs, environmental friendly and other benefits (Cui *et al*., 2007). In this study, ultrasonic treatment is used to investigate the effect upon coconut sugar treatment and to determine the conditions suitable to achieve high sucrose composition in coconut sugar.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This study was done in order to determine the composition of sucrose in coconut sugar after treatment with different ultrasonic frequencies. The method used to analyze the composition is by using dinitrosalicylic acid (DNS) method with measurement of absorbance. The experiments were done in duplicate and average results were obtained as the end results. The research methodology was done in the order shown in Figure 3.1. The detailed methods are shown below.

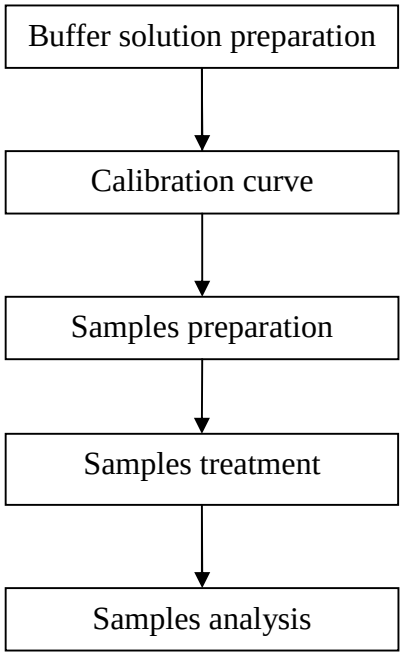

Figure 3.1 Flow chart of the research methodology

3.2 Research Apparatus/Chemical Reagents

3.2.1 Apparatus

The apparatus used in this study were:

i) Ultrasonic equipments with 25, 68 and 132 kHz.
ii) UV-Visible Spectrophotometer
iii) Water bath equipment
iv) Micropipette
v) Test tubes
vi) Beakers

3.2.2 Chemical Reagents

The chemical reagents used in this study were:

i) 1 Liter 0.1M acetate buffer pH 5.5
   a) 5.675 ml acetic acid
   b) 13.608 g sodium acetate

ii) 1 Liter dinitrosalicylic acid (DNS) reagent solution 1%
   a) 10 g dinitrosalicylic acid
   b) 0.5 g sodium sulfite
   c) 10 g sodium hydroxide

iii) 40% potassium sodium tartrate solution

iv) Hydrochloric acid, concentrate (37.3%, 11.9N) solution

v) Potassium hydroxide 5N solution

3.2.3 Research Samples

The research samples used in this study was food grade coconut sugar. About 1080 g of coconut sugar was used in this study. Coconut sugar with concentrations of 40, 60 and 80 % w/v were prepared for every ultrasonic frequency. Thus, about 90 samples in 90 test tubes were prepared for this study.

Figure 3.2 Coconut sugar

3.3 Research Methodology

3.3.1 Acetate Buffer

Acetate buffer solution was used in this study for the reaction medium for coconut sugar. Buffers are composed of weak acids and their salts, or weak bases and their salts. They are used to minimize changes that can occur during reactions, that is the acidity and alkalinity of a solution can be prevented from changing drastically. The acetate buffer used in this study was at concentration of 0.1M at pH 5.5.

About 1 liter of 0.1M acetic acid solution and 1 liter of 0.1M sodium acetate solution were prepared prior to the buffer solution. Thus, 5.675 ml of acetic acid and 13.608 g of sodium acetate was mixed with distilled water in 1L of volumetric flask each. The detailed calculations are shown in Appendix A-1.

A certain amount of acetic acid solution and sodium acetate solution are needed to obtain the desired pH of the buffer solution. Table 3.1 shows the proportions of acetic acid and sodium acetate to get the desired pH values from previous experiments.

Table 3.1: Proportions used to get the desired pH values (Source: AnalChem Resources)

ph	volume of 0.1M acetic acid	volume of 0.1M sodium acetate
3	982.3 ml	17.7 ml
4	847.0 ml	153.0 ml
5	357.0 ml	643.0 ml
6	52.2 ml	947.8 ml

Acetic acid solution and sodium acetate solution were added slowly to achieve the desired pH 5.5. Thus, the amount used in this study was 153.1 ml of acetic acid solution and 846.9 ml of sodium acetate solution. These amounts total up to 1 liter of acetate buffer solution with concentration of 0.1M and pH 5.5.

3.3.2 Calibration Curve

Calibration curve was prepared to find the concentration of sucrose in samples through absorbance of the samples. Calibration curve was prepared from pure sucrose solution. 100 ml of 20 g/L stock solution of sucrose solution was prepared. Thus, 2 g of sucrose was needed and mixed with 100 ml of buffer solution. The stock solution was then diluted to 8, 10, 12, 14, 16 and 18 g/L of sucrose solutions. To prepare 10 ml of sucrose solution for each concentration, the following calculations were done.

$M_1V_1 = M_2V_2$

$20\ V_1 = 8\ (10)$

$V_1 = 4$ ml

Thus, 4 ml of stock solution was mixed with 6 ml of water to obtained 10 ml of 8 g/L sucrose solution. Table 3.2 lists the amount of stock solution and water required for other sucrose concentrations.

Table 3.2: Volume of stock solution and water required for sucrose concentration

Concentration of Sucrose (g/L)	**Volume of Stock Solution (ml)**	**Volume of Water (ml)**
8	4	6
10	5	5
12	6	4
14	7	3
16	8	2
18	9	1
20	10	0

The analysis of sucrose concentration was done by using DNS method, which will be explained in the later subtopic. The absorbance of sucrose solution was measured at 580 nm using UV-Visible Spectrophotometer. The graph of absorbance versus sucrose concentration was drawn.

3.3.3 Samples Preparation

The concentrations used for this study were 40, 60 and 80 % w/v of coconut sugar. The coconut sugar was first mashed into smaller pieces and 300 ml of every concentration were prepared by diluting with buffer solution. Then, 10 test tubes were prepared for each concentration and frequency and 10 ml of the coconut sugar was placed in each test tubes. These test tubes were taken for ultrasonic treatment.

40 % w/v coconut sugar concentration = 40 g of coconut sugar / 100 ml of buffer solution

60 % w/v coconut sugar concentration = 60 g of coconut sugar / 100 ml of buffer solution

80 % w/v coconut sugar concentration = 80 g of coconut sugar / 100 ml of buffer solution

Figure 3.3 40 % w/v coconut sugar concentration

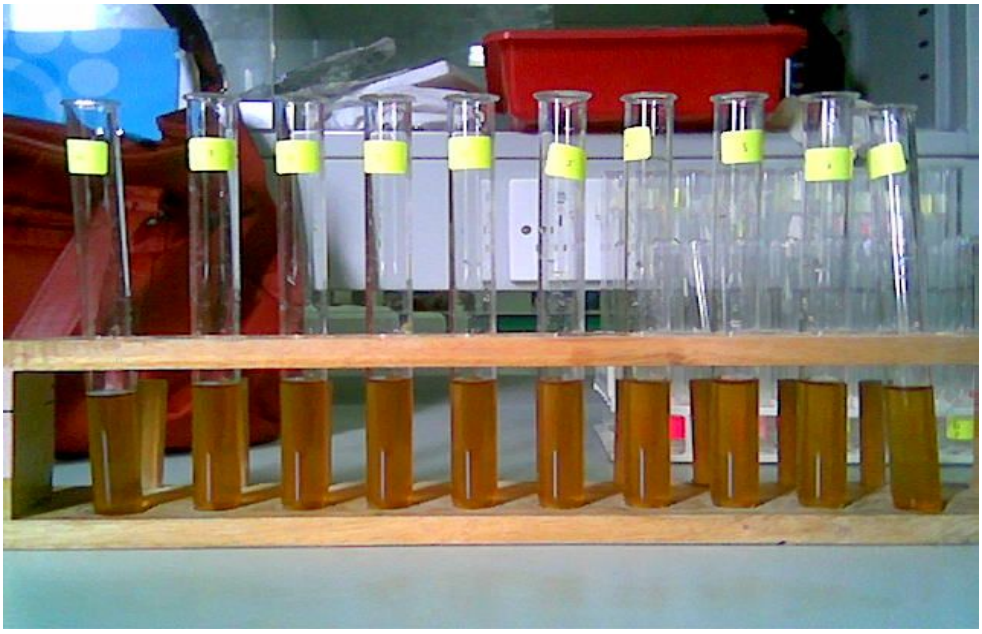

Figure 3.4 60 % w/v coconut sugar concentration

Figure 3.5 80 % w/v coconut sugar concentration

3.3.4 Coconut Sugar Characterization

The moisture, sucrose and glucose content of the coconut sugar were determined so that the same coconut sugars were used throughout the whole experiment. This is also to act as a control for the coconut sugars treatment.

The moisture content was determined by means of drying. The initial weight of coconut sugar was first measured. Drying last for 6 hours at 110 °C in an oven. After 6 hours, the coconut sugar was weighted again to determine the weight after drying. Moisture was lost from the coconut sugar during this drying process. Thus, the percentage of moisture content was determined by subtracting the initial weight with the final weight.

The sucrose and glucose contents in coconut sugar were determined by DNS methods. The coconut sugar was prepared with concentrations of 40, 60 and 80 % w/v and the sucrose and glucose contents were determined in each coconut sugar concentrations.

3.3.5 Samples Treatment

Ultrasonic treatment was used in this study to treat the coconut sugar. The frequencies used were 25, 68 and 132 kHz. The temperature was set at room condition which was at 25 °C. Around 10 samples of 10 ml of coconut sugar at one concentration were treated in an ultrasonic frequency. The samples were treated for 5 hours with one sample taken out at every 30 minutes. These samples were then analyzed for sucrose concentration using dinitrosalicylic acid (DNS) method.

Figure 3.6 25 kHz ultrasonic equipment

Figure 3.7 68 kHz ultrasonic equipment

Figure 3.8 Ultrasonic compartment for 25 and 68 kHz

Figure 3.9 132 kHz ultrasonic equipment

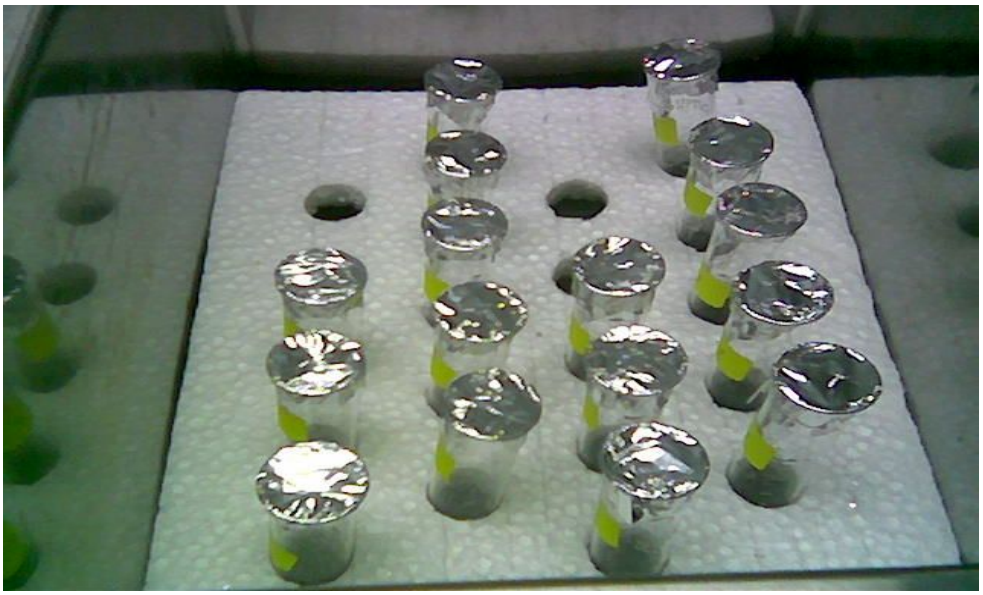

Figure 3.10 Samples in ultrasonic equipment

3.3.6 Samples Analysis

The treated coconut sugar samples were analyzed using dinitrosalicylic acid (DNS) method. DNS method is used to determine the concentration of glucose. Thus, in order to find the concentration of sucrose, the samples were first hydrolyzed so that sucrose was breakdown into its components; glucose and fructose. The absorbances were measured twice, that is before hydrolysis and after hydrolysis. Sucrose concentration could be determined by subtracting the concentrations after hydrolysis with concentrations before hydrolysis.

About 3 ml was taken out from every coconut sugar samples in test tubes after treatment with ultrasonic frequencies. Then, 60 µL of hydrochloric acid was added to each of the samples and allowed to hydrolyze at 90 °C for 5 minutes in a water bath. Potassium hydroxide was added with the amount of 150 µL after the hydrolysis to neutralize the acid. This also gave the samples in alkaline condition where a red brown colour can be developed from DNS methods.

After hydrolysis was done, 3 ml of DNS reagent was added to each sample and the test tubes were close lightly. These samples were taken to water bath and heated at 90 °C to develop the red brown colour. After 5 minutes, the test tubes were taken out and 1 ml of potassium sodium tartrate solution was added to stabilize the colour. The samples were then allowed to cool to room temperature in cold water bath.

Figure 3.11 Samples taken for heating in water bath

Before hydrolysis:
3 ml sample + 3 ml DNS reagent + 1 ml potassium sodium tartrate solution

After hydrolysis:
3 ml sample + 60 µL hydrochloric acid + 150 µL potassium hydroxide + 3 ml DNS reagent + 1 ml potassium sodium tartrate solution

The absorbance of every samples were measured at 580 nm using UV-Visible Spectrophotometer. The concentrations of samples were determined from the calibration curve. The concentrations of sucrose in samples were obtained by

subtracting the concentrations at absorbance after hydrolysis with concentrations at absorbance before hydrolysis.

Figure 3.12 UV-Visible Spectrophotometer

Figure 3.13 Analysis using UV-Visible Spectrophotometer

3.4 Dilution Factor

Dinitrosalicylic acid (DNS) method is a very sensitive method. At higher concentration, the brown colour formed in the samples was too dark and UV-Visible Spectrophotometer was unable to give an accurate reading. Thus, the sucrose solutions and samples needed to be diluted before measuring the absorbance, using the dilution factor to give more accurate readings. The dilution was done based on trial and error. The best dilution gave out the lightest colour and that can be detected by the UV-Visible Spectrophotometer.

The condition for dilution factor is as follows:

2x = 1 part sample + 1 part water
5x = 1 part sample + 4 part water
10x = 1 part sample + 9 part water
100x = 1 part sample + 99 part water

Table 3.3: Dilution factor for sucrose concentrations for calibration curve

Concentration of Sucrose (g/L)	**Dilution Factor**	**Dilution Volume**
8	20x	200 µL sample + 3800 µL H_2O
10	20x	200 µL sample + 3800 µL H_2O
12	20x	200 µL sample + 3800 µL H_2O
14	20x	200 µL sample + 3800 µL H_2O
16	20x	200 µL sample + 3800 µL H_2O
18	20x	200 µL sample + 3800 µL H_2O
20	20x	200 µL sample + 3800 µL H_2O

Table 3.4: Dilution factor for coconut sugar samples

Concentration of Coconut Sugar (%w/v)	**Dilution Factor**	**Dilution Volume**
40	30x	100 µL sample + 2900 µL H_2O
	100x	100 µL sample + 9900 µL H_2O
	200x	100 µL sample + 19900 µL H_2O
	300x	100 µL sample + 29900 µL H_2O
60	50x	200 µL sample + 9800 µL H_2O
	200x	100 µL sample + 19900 µL H_2O
	300x	100 µL sample + 29900 µL H_2O
80	70x	100 µL sample + 6900 µL H_2O
	400x	100 µL sample + 39900 µL H_2O
	500x	100 µL sample + 49900 µL H_2O

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

Results were obtained after all the experimental methods have finished. All the results were from the analysis using UV-Visible Spectrophotometer to find the sucrose concentrations. The experiments were done in duplicate and the average results were taken. The results were tabulated and drawn in graphs to show the comparisons between concentrations and frequencies.

The samples were divided into 2 parts for analysis. One part was analysis before hydrolysis, where only the original glucose concentration was measured first. The second part was analysis after hydrolysis, where sucrose was broken down into glucose and fructose. The absorbance was then measured to determine the amount of glucose after sucrose break down. The amount of sucrose concentration was obtained by subtracting the concentration after hydrolysis with concentration before hydrolysis.

4.2 Calibration Curve

Calibration curve was done to determine the concentration of sucrose in coconut sugar samples. Calibration curve was obtained from pure sucrose concentrations, where these concentrations were tested by dinitrosalicylic acid (DNS) method and analyzed using UV-Visible Spectrophotometer to measure the absorbances.

The pure sucrose concentrations used were 8, 10, 12, 14, 16, 18 and 20 g/L. These concentrations were hydrolyzed to break down the sucrose into glucose first and diluted in order to get accurate results. Dilutions were done according to dilution factor. The results were tabulated and graph of calibration was drawn. This graph was used to find the concentration of sucrose from absorbance. A linear graph was obtained which showed that the absorbance is proportional to the concentration.

Table 4.1: Absorbance readings for calibration curve

Concentration of Sucrose (g/L)	**Dilution Factor**	**Dilution Concentration**	**Absorbance**		**Average**
8	20	0.4	0.135	0.134	0.135
10	20	0.5	0.172	0.172	0.172
12	20	0.6	0.282	0.285	0.284
14	20	0.7	0.284	0.284	0.284
16	20	0.8	0.342	0.341	0.342
18	20	0.9	0.419	0.415	0.417
20	20	1.0	0.472	0.472	0.472

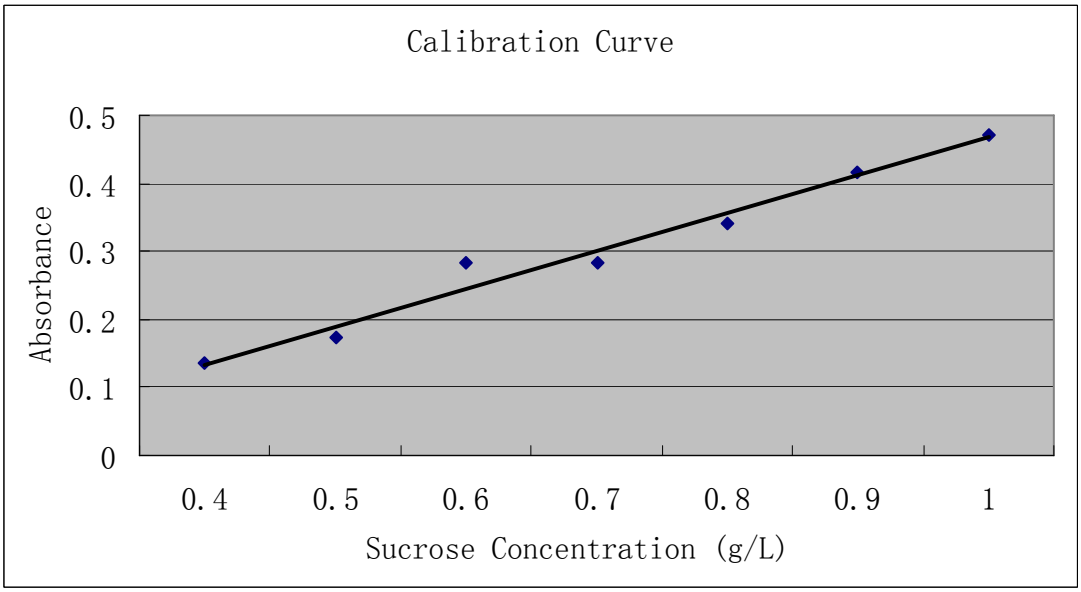

Figure 4.1 Graph of calibration curve

4.3 Coconut Sugar Characterization

The moisture, sucrose and glucose content of the coconut sugar were determined as these will be the control samples for the treated samples. The moisture content was determined by means of drying and the sucrose and glucose contents were by dinitrosalicylic acid (DNS) methods, absorbance and calibration curve. The moisture content was determined to be 8.54 % in 6.3 g of coconut sugar. Thus, the initial amount of sucrose and glucose present in each coconut sugar concentration were shown in Table 4.2.

Table 4.2: Sucrose and glucose contents in coconut sugar samples

	40 % w/v Coconut Sugar	**60 % w/v Coconut Sugar**	**80 % w/v Coconut Sugar**
Sucrose (g/L)	172.436	222.133	297.129
Glucose (g/L)	27.920	31.022	38.516

4.4 Effect of Coconut Sugar Concentrations and Exposure Time during 25 kHz Ultrasonic Treatment

Coconut sugar with concentrations of 40, 60 and 80 % w/v were treated in 25 kHz ultrasonic equipment for 5 hours. About 10 samples for each concentration were prepared and for every 30 minutes, one sample was taken out. The samples were taken for analysis by dinitrosalicylic acid (DNS) method and the measurement of absorbances were done from UV-Visible Spectrophotometer.

The absorbances of before and after hydrolysis for each samples were recorded and tabulated in Appendix B-3. Table 4.3 showed the sucrose concentration present in each coconut sugar concentration during ultrasonic treatment at every 30 minutes. Graph of sucrose concentrations in coconut sugar concentrations versus time was drawn as in Figure 4.2.

Table 4.3: Sucrose concentrations in coconut sugar concentrations during 25 kHz ultrasonic treatment

Time (min)	40 % w/v Coconut Sugar	60 % w/v Coconut Sugar	80 % w/v Coconut Sugar
30	212.951	264.356	403.573
60	214.071	299.556	395.893
90	207.947	312.267	410.596
120	199.698	283.378	449.600
150	169.689	259.289	388.462
180	210.613	348.267	440.764
210	213.467	316.800	417.760
240	225.964	319.822	418.204
270	212.302	364.978	435.609
300	246.000	335.022	422.818

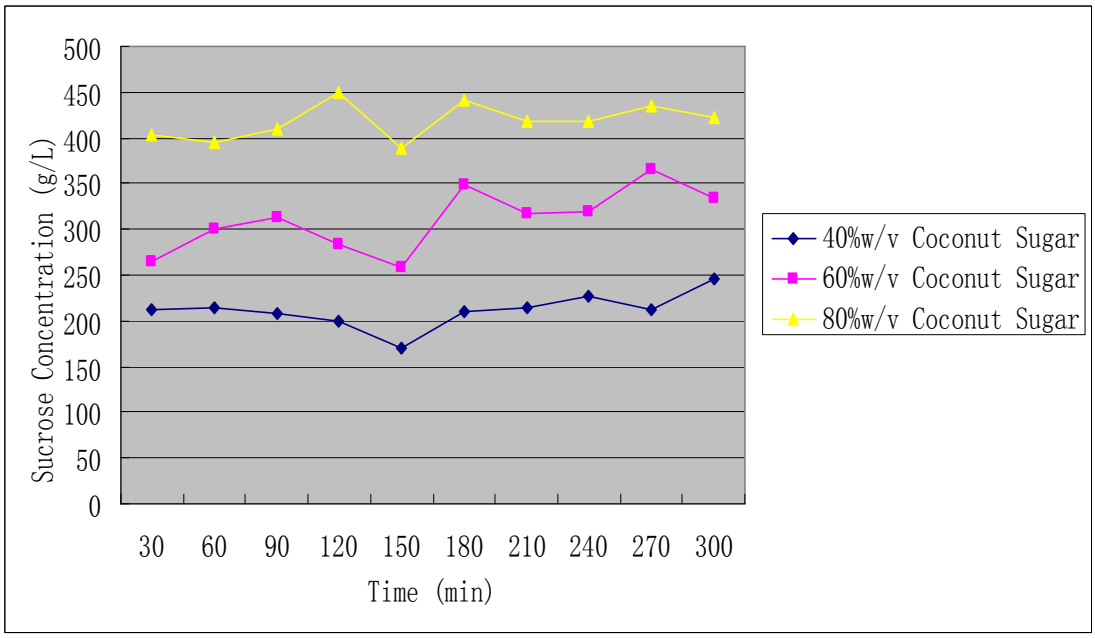

Figure 4.2 Graph of sucrose concentrations in three coconut sugar concentrations versus time during 25 kHz ultrasonic treatment

After 5 hours of treatment, the sucrose concentration in 40 % w/v coconut sugar concentration was 246 g/L, while for 60 % w/v coconut sugar concentration was 335.02 g/L and 80 % w/v coconut sugar concentration was 422.82 g/L. These meant the increases for sucrose concentration from the original amount were 42.66%, 50.82% and 42.3%, respectively. Coconut sugar concentration of 80 % w/v has the highest amount of sucrose, followed by 60 % w/v and 40 % w/v coconut sugar concentrations. These increases could be due to the forces created by the ultrasonic wave to breakdown the long-chain molecules present in coconut sugar into sucrose.

As shown in the graph in Figure 4.2, the sucrose concentration increased with time, although there were occasional drops during ultrasonic treatment. The most significant drops occurred at 150 minutes for all three coconut sugar concentrations. The reason for this was unclear as more studies on the physical and chemical properties of sucrose in the coconut sugar during that time needed to be done. This could probably due to the instability of the sucrose structure after some time of treatment and thus, breakdown of sucrose occurred.

Coconut sugar concentration of 40 % w/v showed the highest amount of sucrose concentration present was at 300 minutes, with 246 g/L. Coconut sugar concentration of 60 % w/v has the highest sucrose concentration at 270 minutes, with 364.98 g/L. On the other hand, the highest sucrose amount for 80 % w/v coconut sugar concentration was at 120 minutes, with 449.6 g/L. The difference in time was probably due to how the sucrose reacted in different concentrations of coconut sugar.

Figure 4.3 40 % w/v coconut sugar concentration after 25 kHz ultrasonic treatment

Figure 4.4 60 % w/v coconut sugar concentration after 25 kHz ultrasonic treatment

Figure 4.5 80 % w/v coconut sugar concentration after 25 kHz ultrasonic treatment

4.5 Effect of Coconut Sugar Concentrations and Exposure Time during 68 kHz Ultrasonic Treatment

Coconut sugar with concentrations of 40, 60 and 80 % w/v were also treated in 68 kHz ultrasonic equipment for 5 hours. About 10 samples for each concentration were prepared and for every 30 minutes, one sample was taken out. The samples were taken for analysis by dinitrosalicylic acid (DNS) method and the measurement of absorbances were done from UV-Visible Spectrophotometer.

The absorbances of before and after hydrolysis for each samples were recorded and tabulated in Appendix B-4. The sucrose concentration present in each coconut sugar concentration during ultrasonic treatment were obtained for every 30 minutes and tabulated in Table 4.4. Graph of each sucrose concentrations in coconut sugar concentrations versus time was drawn as in Figure 4.6.

Table 4.4: Sucrose concentrations in coconut sugar concentrations during 68 kHz ultrasonic treatment

Time (min)	40 % w/v Coconut Sugar	60 % w/v Coconut Sugar	80 % w/v Coconut Sugar
30	257.751	427.511	384.427
60	274.018	422.711	434.702
90	180.720	417.244	467.742
120	262.764	365.244	464.276
150	284.613	426.800	405.298
180	274.587	458.756	435.520
210	255.316	451.422	395.502
240	263.040	367.156	444.764
270	271.413	354.222	452.924
300	268.880	374.578	450.293

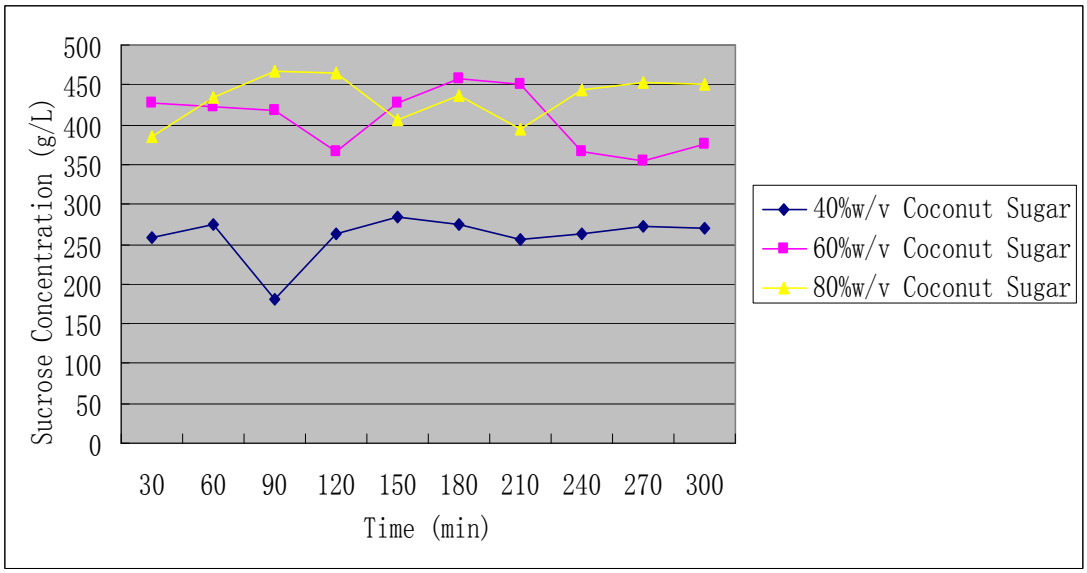

Figure 4.6 Graph of sucrose concentrations in three coconut sugar concentrations versus time during 68 kHz ultrasonic treatment

The sucrose concentration after 5 hours of treatment was 268.88 g/L for 40 % w/v coconut sugar concentration, while for 60 % w/v coconut sugar concentration was 374.58 g/L and 80 % w/v coconut sugar concentration was 450.29 g/L. This showed that the 80 % w/v coconut sugar concentration has the highest amount of sucrose, followed by 60 % w/v and 40 % w/v coconut sugar concentrations. Thus, the increases for sucrose concentration from the original amount were 55.93%, 68.63% and 51.55%, respectively. These increases could also be due to the breakdown of long-chain molecules into sucrose during ultrasonic treatment.

As shown in the graph in Figure 4.6, the sucrose concentration increased with time. There were also occasional drops during ultrasonic treatment. However, for 60 % w/v coconut sugar concentration, the sucrose concentration decreased from the beginning of the treatment through the end, with some increases in the middle of treatment. Significant drop were seen in 90 minutes for 40 % w/v coconut sugar concentration. Coconut sugar with concentration of 60 % w/v showed drops in 120, 240 and 270 minutes while 80 % w/v coconut sugar concentration showed in 150 and 210 minutes. More studies were needed to be done in order to know the causes in the drops.

Coconut sugar with concentration of 40 % w/v showed the highest amount of sucrose concentration present was at 150 minutes, with 284.61 g/L. Coconut sugar concentration of 60 % w/v has the highest sucrose concentration at 180 minutes, with 458.76 g/L. On the other hand, the highest sucrose amount for 80 % w/v coconut sugar was at 90 minutes, with 467.74 g/L. The variation in time was probably due to how the sucrose reacted in different concentrations of coconut sugar.

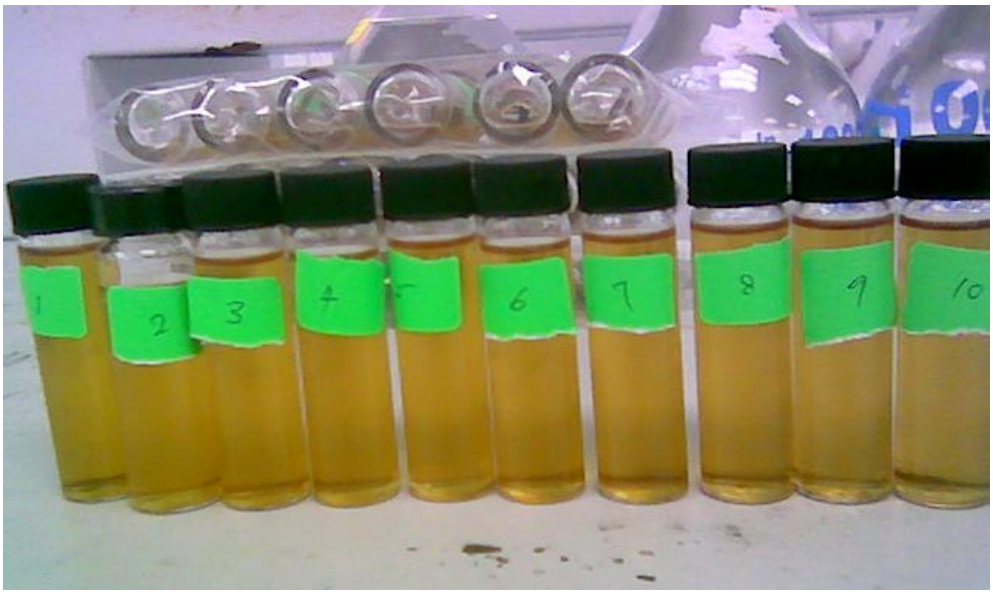

Figure 4.7 40 % w/v coconut sugar concentration after 68 kHz ultrasonic treatment

Figure 4.8 60 % w/v coconut sugar concentration after 68 kHz ultrasonic treatment

Figure 4.9 80 % w/v coconut sugar concentration after 68 kHz ultrasonic treatment

4.6 Effect of Coconut Sugar Concentrations and Exposure Time during 132 kHz Ultrasonic Treatment

The same methods were also done for 132 kHz ultrasonic treatment. Coconut sugar with concentrations of 40, 60 and 80 % w/v were treated in 132 kHz ultrasonic equipment for 5 hours. About 10 samples for each concentration were prepared and for every 30 minutes, one sample was taken out. The samples were taken for analysis by dinitrosalicylic acid (DNS) method and the measurement of absorbances were taken from UV-Visible Spectrophotometer.

The absorbances of before and after hydrolysis for each samples were recorded and tabulated Appendix B-5. Sucrose concentrations for every coconut sugar concentrations during ultrasonic treatment were obtained from the absorbances and tabulated in Table 4.5. Graph of each sucrose concentrations versus time was drawn as in Figure 4.10.

Table 4.5: Sucrose concentrations in coconut sugar concentrations during 132 kHz ultrasonic treatment

Time (min)	40 % w/v Coconut Sugar	60 % w/v Coconut Sugar	80 % w/v Coconut Sugar
30	222.693	309.022	365.991
60	223.440	322.578	366.889
90	252.400	327.422	380.258
120	253.840	306.000	392.213
150	239.973	301.956	383.858
180	244.187	318.089	390.471
210	238.240	317.378	397.458
240	239.973	327.333	366.018
270	226.933	308.044	422.178
300	239.547	312.444	396.098

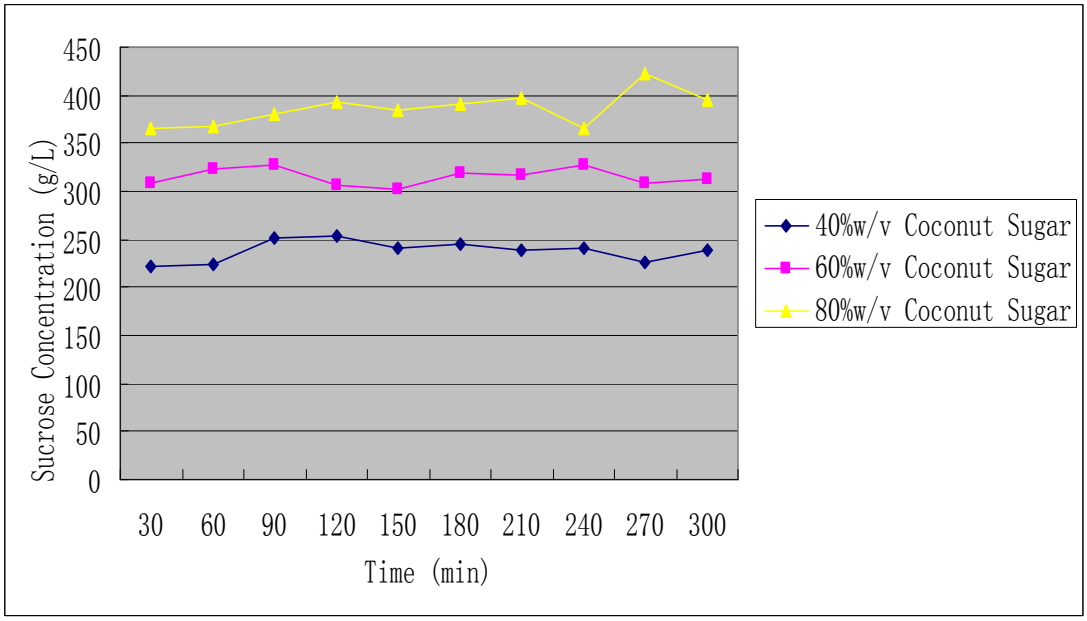

Figure 4.10 Graph of sucrose concentrations in three coconut sugar concentrations versus time during 132 kHz ultrasonic treatment

After 5 hours of treatment, the sucrose concentration for 40 % w/v coconut sugar concentration was 239.55 g/L, for coconut sugar with concentration of 60 % w/v was 312.44 g/L and for 80 % w/v coconut sugar concentration was 396.1 g/L. This showed that 80 % w/v coconut sugar concentration has the highest amount of sucrose, followed by 60 % w/v and 40 % w/v coconut sugar concentration. Accordingly, the increases for sucrose concentration from the original amount were 38.92%, 40.65% and 33.31%, respectively. These increases could be due to the breakdown of long-chain molecules present in the coconut sugar to form sucrose during ultrasonic treatment.

As shown in the graph in Figure 4.10, the sucrose concentration increased with time, as there were also slight drops as the treatment progress. Sucrose concentrations in 40 % w/v and 60 % w/v coconut sugar concentrations varied as the treatment time progress. Significant drop in 80 % w/v coconut sugar concentration can also be seen at 240 minutes. The reason was unclear and more studies are needed to be done.

Coconut sugar of concentration 40 % w/v showed the highest amount of sucrose concentration present was at 120 minutes, with 253.84 g/L. Coconut sugar with concentration of 60 % w/v has the highest sucrose concentration at 90 minutes, with 327.42 g/L. On the other hand, the highest sucrose amount for 80 % w/v coconut sugar concentration was at 270 minutes, with 422.18 g/L. The differences in time were probably due to how the sucrose reacted in different concentrations of coconut sugar.

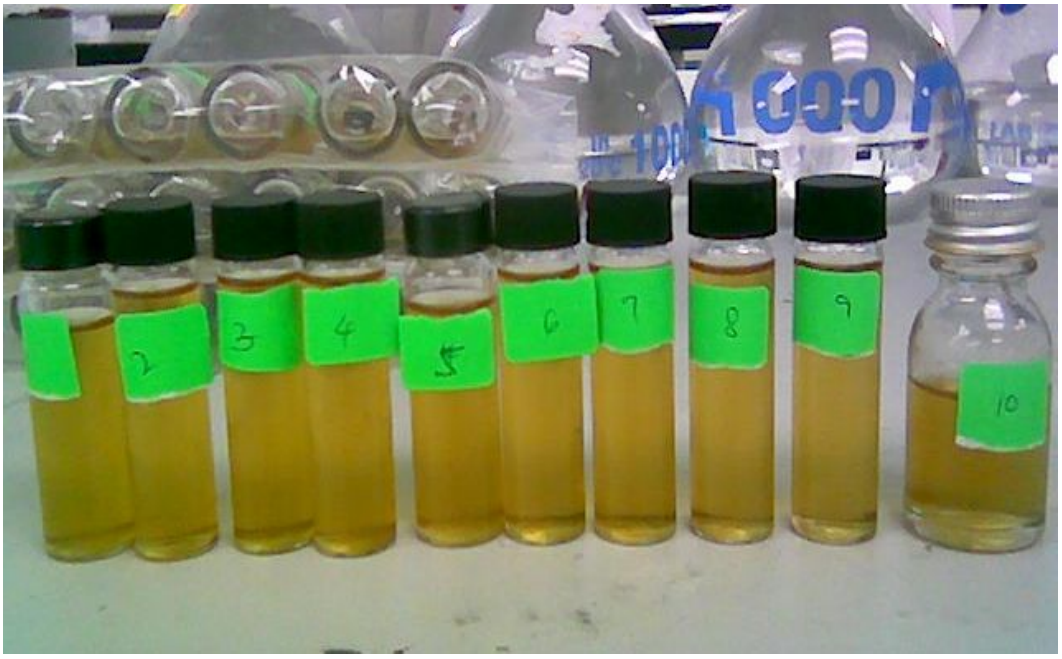

Figure 4.11 40 % w/v coconut sugar concentration after 132 kHz ultrasonic treatment

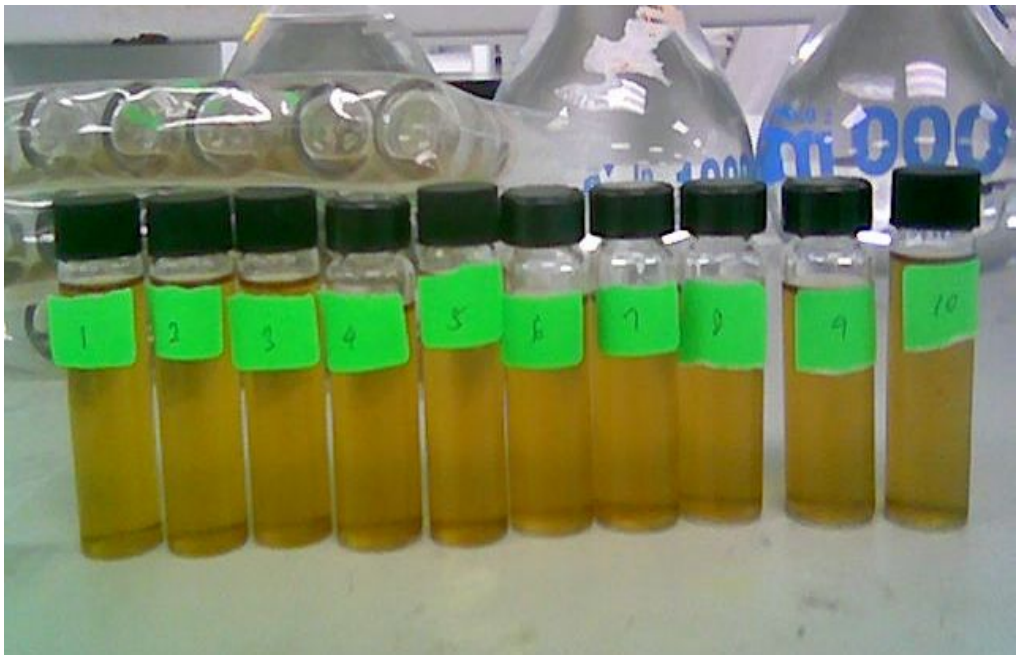

Figure 4.12 60 % w/v coconut sugar concentration after 132 kHz ultrasonic treatment

Figure 4.13 80 % w/v coconut sugar concentration after 132 kHz ultrasonic treatment

4.7 Comparison of Ultrasonic Frequencies and Coconut Sugar Concentrations

Comparisons were made between the percentages of sucrose increased in coconut sugar concentrations with ultrasonic frequencies. This was done in order to determine the optimum conditions for a higher sucrose production.

The final amount of sucrose concentrations were used to calculate the percentages of increased, that is the sucrose concentrations after 5 hours of ultrasonic treatment. The final amount of sucrose was subtracted with the initial amount of sucrose to obtain the percentages of sucrose increased after treatment. The detailed calculations were shown in Appendix B-6. The values were then tabulated in Table 4.6 and graph of percentages of sucrose increased versus coconut sugar concentrations was drawn for the three ultrasonic frequencies.

Table 4.6: Percentages of sucrose concentration increased in coconut sugar concentrations and ultrasonic frequencies

Frequency (kHz)	40 % w/v Coconut Sugar	60 % w/v Coconut Sugar	80 % w/v Coconut Sugar
25	42.66%	50.82%	42.30%
68	55.93%	68.63%	51.55%
132	38.92%	40.65%	33.31%

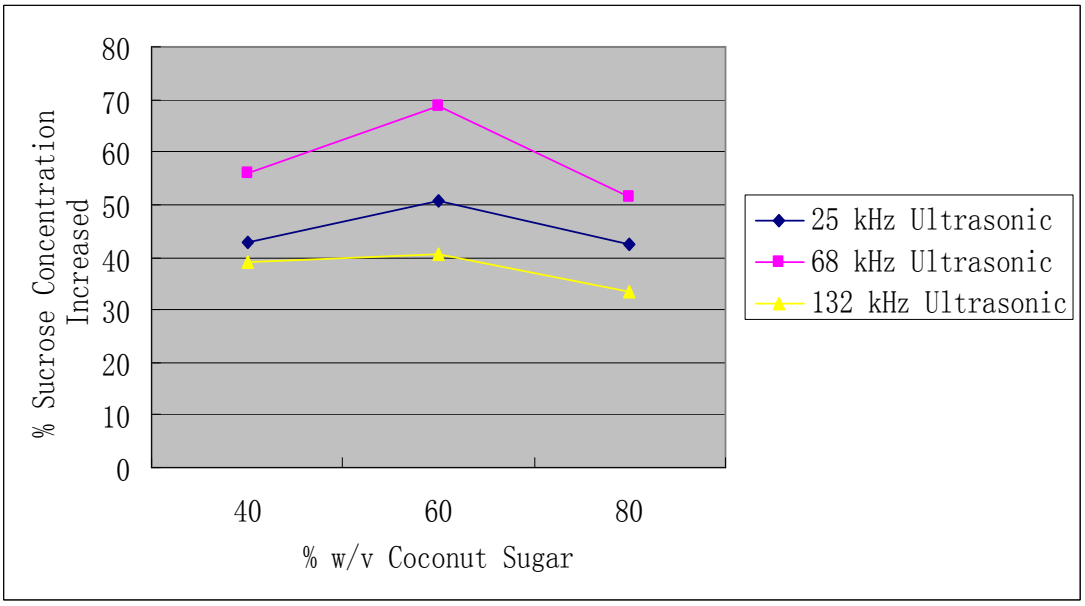

Figure 4.14 Comparison between percentages of sucrose concentration increased in coconut sugar concentrations and ultrasonic frequencies

From the graph in Figure 4.14, 60 % w/v coconut sugar concentration showed the highest percentage of sucrose concentration increased after ultrasonic treatment in every frequency. This was followed by 40 % w/v coconut sugar concentration and 80 % w/v coconut sugar concentration has the least percentage of sucrose increased. The increased of sucrose in every coconut sugar concentration after ultrasonic treatment was probably due to the breakdown of long-chain molecules in the coconut sugar. These long-chain molecules were broken down into sucrose, thus increasing the amount in coconut sugar. The differences in sucrose increased could be probably due to the different initial amount of sucrose before treatment and how they reacted in these differences.

The percentages of sucrose increased also showed variations in ultrasonic frequencies. Ultrasonic treatment with frequency of 68 kHz gave the highest sucrose composition in all coconut sugar concentrations. Ultrasonic frequency at 25 kHz generated the second highest of sucrose concentration followed by 132 kHz ultrasonic frequency. This could be due to the mechanical forces created by the frequencies on the effect of sucrose compositions.

The higher the frequency, the smaller the cavitations occurred. These small cavitations probably created large mechanical forces on the coconut sugar and thus, the mechanism should probably occurred faster. As a result, more sucrose was most likely to form in the coconut sugar, giving a higher percentage of sucrose increased. Ultrasonic frequency of 132 kHz was expected to give a higher sucrose composition, though the experiment showed that it was the least in the percentage of sucrose increased. This could probably be where the long-chain molecules reach its peak point in breaking down into sucrose. The ultrasonic frequency after 60 kHz probably promoted the breakdown of sucrose into other smaller molecules, thus, causing less sucrose increased in coconut sugar with 132 kHz ultrasonic frequency.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The ultrasonic frequency that produced the highest percentage of sucrose increased is 68 kHz, followed by 25 and 132 kHz. The coconut sugar concentrations with highest percentage of sucrose increased is 60 % w/v, followed by 40 and 80 % w/v. On the other hand, the sucrose concentrations in the three coconut sugar concentrations increased with time, with the occasional drops during the five hours ultrasonic treatment.

Thus, it can be concluded that the highest sucrose production was 68.63% of the original amount at conditions of 60 % w/v coconut sugar and treated at 68 kHz ultrasonic treatment. This sucrose can be obtained to produce fructooligosaccharides (FOS) in abundance which is beneficial for diabetics and everyone, and also it is cheap.

Ultrasonic technology can be recommended for use in food processing industry. It was shown in this study that ultrasonic treatment can increase the sucrose composition in coconut sugar. Thus, with the usage of this ultrasonic, sucrose can be increased in the coconut sugar. Further studies are needed to determine why the sucrose composition in coconut sugar reacted in that manner. Therefore, this could lead to more sucrose production from coconut sugar.

5.2 Recommendations

To improve the experiment and to get better results, it is recommended that the determination of sucrose composition is done by using High Performance Liquid Chromatography (HPLC). This method is recommended to get accurate reading of sucrose composition present in the coconut sugar. The DNS method used in this experiment to determine the sucrose composition is a very sensitive method. The readings obtained might not be as accurate as possible and the readings maybe contaminated with other compositions contained in the coconut sugar.

The molecules' structures present in coconut sugar must also be studied in order to gain more understanding on how more sucrose is present after the ultrasonic treatment. Scanning electron microscope (SEM), x-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy can be used to determine the molecules present in the coconut sugar. The type of molecules that breakdown into sucrose and how sucrose breaks down into smaller molecules at higher frequency must be determined to find the effectiveness and the relevancy of the ultrasonic treatment used.

REFERENCES

All About Diabetes (2008). *American Diabetes Association*. Diabetes Organisation, United States.

Apriyantono, A., Aristyani, A., Nurhayati, Lidya, Y., Budiyanto, S. and Soekarto, S. T. (2002). Rate of Browning Reaction during Preparation of Coconut and Palm Sugar. *International Congress Series*, 1245, 275-278.

American Heart Association (2008). *Artificial Sweeteners*. American Heart Association, United States.

Artificial Sweeteners (2008). *Reader's Digest Asia*. Retrieved December 19th, 2008, from http://www.readersdigest.com.my/

Artificial Sweeteners: A Safe Alternative to Sugar. (September 13th, 2008). *MayoClinic.com*. Mayo Clinic Online, United States.

Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilku, K. and Versteeg, C. (2008). Modification of Food Ingredients by Ultrasound to Improve Functionality: A Preliminary Study on a Model System. *Innovative Food Science and Emerging Technologies*, 9, pp.155-160.

Atasoy, A. F. and Turkoglu, H. (2008). Changes of Composition and Free Fatty Acid Contentts of Urfa Cheeses (A White-Brined Turkish Cheese) During Ripening: Effects of Heat Treatments and Starter Cultures. *Food Chemistry*, 110, pp. 598-604.

Boughner, D. S. (2001). Industry and Trade Summary: Sugar, pp. 1-3. Washington, DC: Diane Publishing Co.

Brody, T. (1999). Chapter 2: Digestion and Absorption. *Nutritional Biochemistry* (2nd edition), pp. 104. Academic Press.

CAM and Diabetes: A Focus on Dietary Supplements (July 31st, 2008). *National Center for Complementary and Alternative Medicine (NCCAM)*, publication no. D416. Retrieved August 6th, 2008, from http://nccam.nih.gov/

Cameron, M., McMaster, L. D. and Britz, T. J. (2008). Electron Microscopic Analysis of Dairy Microbes Inactivated by Ultrasound. *Ultrasonics Sonochemistry*, 15, pp. 960-964.

Cardoso, J. M. P. and Bolini, H. M. A. (2007). Different Sweeteners in Peach Nectar: Ideal and Equivalent Sweetness. *Food Research International*, 40, pp. 1249-1253.

Carlson, P. (2007). The Coconut: Traditional Crop to Threatened Lifestyle. *Culture, Food and Agriculture*.

Carlton, F. B. (2000). Recent Advances in the Pharmacologic Management of Diabetes Mellitus. *Emergency Medicine Clinics of North America*, vol. 18 issue 4, pp. 745-753.

Philippines Coconut Authority (PCA) (November 2004/Octoboer2005). *Coconuts Today.* [Brochure]. Philippines.

Coconut – Tree of Life (2008). *Food and Agricultural Organization: Corporate Document Repository*. Retrieved November 20th, 2008, from http://www.fao.org/

Cui, G. W., Zhu, S. Q., Zou, L. Z. and Xie, W. W. (2007). Effects of High-Power Ultrasonic Treatment on the Slurryability of Coals. *Journal of China University of Mining & Technology*, vol. 17, no. 4, pp. 562-565.

Diabetes (September 25th, 2008). *Medline Plus: U.S. National Library of Medicine and National Institutes of Health*. Retrieved November 15th, 2008, from http://www.nlm.nih.gov/

Diabetes Facts (2008). *World Diabetes Foundation*. Retrieved December 5th, 2008, from http://www.worlddiabetesfoundation.org/

Diabetes Information: Food and Meal Planning (2008). *U.S. Food and Drug Administration (FDA)*. United States.

Dodson, A. G. and Pepper, T. (1985). Confectionary Technology and The Pros and Cons of using Non-sucrose Sweeteners. *Food Chemistry*, vol. 16, issues 3-4, pp. 271-280.

Euler, A. R., Mitchell, D. K., Kline, R. and Pickering, L. K. (2005). Prebiotic Effect of Fructo-Oligosaccharide Supplemented Term Infant Formula at Two Concentrations Compared with Unsupplemented Formula and Human Milk. *Journal of Pediatric Gastroenterology and Nutrition*, vol. 40, pp.157-164.

Facts and Figures (2008). *International Diabetes Federation*. Retrieved December 5th, 2008, from http://www.idf.org/

Fernandes, F. A. N., Linhares Jr., F. E. and Rodrigues, S. (2008). Ultrasound As Pre-Treatment for Drying of Pineapple. *Ultrasonics Sonochemistry*, 15, pp. 1049-1054.

Food Outlook: Global Market Analysis (June 2008). *Food and Agriculture Organization (FAO)*. United States.

Gudiel, U. M. and Goni, I. (2002). Effect of Fructooligosaccharide on Nutritional Parameters and Mineral Bioavailability in Rats. *Journal of the Science of Food and Agriculture*, vol. 82, num. 8, pp. 913-917.

Hagura, R. (2000). Diabetes Mellitus and Lifestyle – for the Primary Prevention of Diabetes Mellitus: The Role of Diet. *British Journal of Nutrition*, 84, Suppl. 2, S191-S194.

Henriksen, Dr. J. E. and Bech-Nielsen, Dr. H. (2008). Blood Glucose Levels. *Diabetes*. [Brochure]. Retrieved December 20th, 2008, from http://www.netdoctor.co.uk/

Holt, E. H. (June 17th, 2008). Medical Encyclopedia: Monitor Blood Glucose-Series: Part 1. *Medline Plus: Trusted Health Information for You*. Retrieved December 20th, 2008, from http://www.nlm.nih.gov/

Hull, J. S. (2002). *Aspartame – Other Sweeteners*. [Brochure]. Aspartame Detox Program: United States.

Jarosz, S. and Lewandowski, B. (2008). Synthesis and Complexation Properties Towards the Ammonium Cation of Aza-Coronand Analogues Containing Sucrose. *Carbohydrate Research*, 343, pp. 965-969.

Jessica, F. (2001). *Coconut Tree*. [Brochure]. United States.

Kaufhold, J., Hammon, H. M. and Blum, J. W. (2000). Fructo-Oligosaccharides Supplementation: Effects on Metabolic, Endocrine and Hematological Traits in Veal Calves. *Journal of Veterinary Medicine*, vol. 47, pp. 17-29.

Khazrai, M. (2006). Developing Food Strategies for the Prevention of Diabetes. *Functional Ingredients: Focus on the World of Ingredients*.

Li, H., Jin, Y., Mahar, R. B., Wang, Z. and Nie, Y. (2008). Effects of Ultrasonic Disintegration on Sludge Microbial Activity and Dewaterability. *Journal of Hazardous Materials*.

Mabel, M. J., Sangeetha, P. T., Platel, K., Srinivasan, K. and Prapulla, S. G. (2008). Physicochemical Characterization of Fructooligosaccharides and Evaluation of Their Suitability as a Potential Sweetener for Diabetics. *Carbohydrate Research*, 343, pp. 56-66.

Mathlouthi, M. and Reiser, P. (Eds.) (1995). Sucrose: Properties and Applications, pp. 293. UK: Chapman Hall.

Melanson, K. J., Zukley, L., Lowndes, J., Nguyen, V., Angelopoulos, T. J. and Rippe, J. M. (2007). Effects of High-Fructose Corn Syrup and Sucrose Consumption on Circulating Glucose, Insulin, Leptin, and Ghrelin and on Appetite in Normal-Weight Women. *Nutrition*, 23, pp. 103-112.

Mohammad, F. A., Bassam, M. E. A. and Speight, J. G. (2005). Chapter 6: Sugar. *Handbook of Industrial Chemistry: Organic Chemicals* (pp. 163-199). New York: McGraw-Hill.

Muratore, G., Rizzo, V., Licciardello, F. and Maccarone, E. (2008). Partial Dehydration of Cherry Tomato at Different Temperature, and Nutritional Quality of the Products. *Food Chemistry*, 111, pp. 887-891.

National Cancer Institute (2008). Artificial Sweeteners and Cancer: Questions and Answers. *National Cancer Institute Fact Sheet*. National Cancer Institute: United States.

Ombrello, Dr. T. (2008). Coconut Palm. *Plant of the Week*, UCC Biology Department. Faculty of Biology Department, University College Cork, United States.

Piva, A., Di Mattia, C., Neri, L., Dimitri, G., Chiarini, M. and Sacchetti, G. (2008). Heat-Induced Chemical, Physical and Functional Changes During Grape Must Cooking. *Food Chemistry*, vol. 106, issue 3, pp. 1057-1065.

Ponce-Lee, E. L., Olivares-Perez, A. and Fuentes-Tapai, I. (2004). Sugar (Sucrose) Holograms. *Optical Materials*, 26, pp. 5-10.

Punchihewa, P. G. and Arancon, R. N. (1996). CHAPTER XV COCONUT: Post-harvest Operations. *Asian and Pacific Coconut Community*. Food and Agriculture Organization (FAO). United States.

Rasberry, C. N. (2008). Carbohydrate. *Nutrition and Well-Being*. Retrieved December 15th, 2008, from http://www.faqs.org/

Sanchez, O., Guio, F., Garcia, D., Silva, E. and Caicedo, L. (2008). Fructooligosaccharides Production by *Aspergillus* sp. N74 in a Mechanically Agitated Airlift Reactor. *Food and Bioproducts Processing*, 86, pp. 109-115.

Sardesai, V. M. and Waldshan, T. H. (1991). Natural and Synthetic Intense Sweeteners. *The Journal of Nutritional Biochemistry*, vol. 2, pp. 236-244.

Shah, S. and Gupta, M. N. (2008). The Effect of Ultrasonic Pre-Treatment on the Catalytic Activity of Lipases in Aqueous and Non-Aqueous Media. *Chemistry Central Journal*, 2:1.

Sonia Y. De Leon and Milagros I. Dolores (1996). Chapter 14: Coconuts. Laszlo P. Somogyi, Diane M. Barrett, Yiu H. Hui. *Processing Fruits: Science and Technology* (pp. 440). CRC Press.

Sumner-Fromeyer, Dr. J. (2008). *"The Tree of Life" – Coconuts and Copra*. Retrieved November 20th, 2008, from http://www.ms-starship.com/

Tsai, P. J., Delva, L., Yu, T. Y., Huang, Y. T. and Dufosse, L. (2005). Effect of Sucrose on the Anthocyanin and Antioxidant Capacity of Mulberry Extract during High Temperature Heating. *Food Research International*, 38, pp. 1059-1065.

U. S. Food and Drug Administration (June 14th, 2005). Glucose Meters and Diabetes Management. *Diabetes Information*. Retrieved December 18th, 2008, from http://www.fda.gov/

U. S. Food and Drug Administration (July-August, 2006). Artificial Sweeteners: No Calories…Sweet! *FDA Consumer Magazine*. Retrieved December 17th, 2008, from http://www.fda.gov/

Vilkhu, K., Mawson, R., Simons, L. and Bates, D. (2007). Applications and Opportunities for Ultrasound Assisted Extraction in the Food Industry - A Review. *Innovative Food Science and Emerging Technologies*, 9, pp. 161-169.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Riener, J., Lyng, J. G. and Morgan, D. J. (2008). Reduction of *Straphylococcus aureus* and Quality Changes in Apple Juice Processed by Ultraviolet Irradiation, Pre-Heating and Pulsed Electric Fields. *Journal of Food Engineering*, 89, pp. 267-273.

Yukie, F. and Kazuo, I. (1999). Comparison of Glucose (Grape Sugar) and Sucrose (Cube Sugar) in Hyperglycemic Action. *Journal of Practical Pharmacy*, vol. 50, no. 2, pp. 905-909.

APPENDIX A

METHODOLOGY

Appendix A-1: Calculation for Acetate Buffer Preparation

To prepare 1 liter of 0.1M acetic acid solution:

Purity = 99.8%

SG = 1.06

Molecular Weight (MW) = 60.05 g/mol

$$M_1 = \frac{\text{purity x SG x 1000}}{\text{MW}}$$

$$= \frac{0.998 \times 1.06 \times 1000}{60.05}$$

$$= 17.62M$$

$M_1V_1 = M_2V_2$

$17.62\ V_1 = 0.1\ (1)$

$V_1 = 5.675$ ml

Thus, 5.675 ml of acetic acid was mixed with distilled water in 1L of volumetric flask.

To prepare 1 liter of 0.1M sodium acetate solution:

Molecular Weight (MW) = 136.08 g/mol

0.1 mol/L x 136.08 g/mol = 13.608 g/L

Thus, 13.608 g of sodium acetate was mixed with distilled water in 1L of volumetric flask.

APPENDIX B

RESULTS AND DISCUSSIONS

Appendix B-1: Calibration Curve

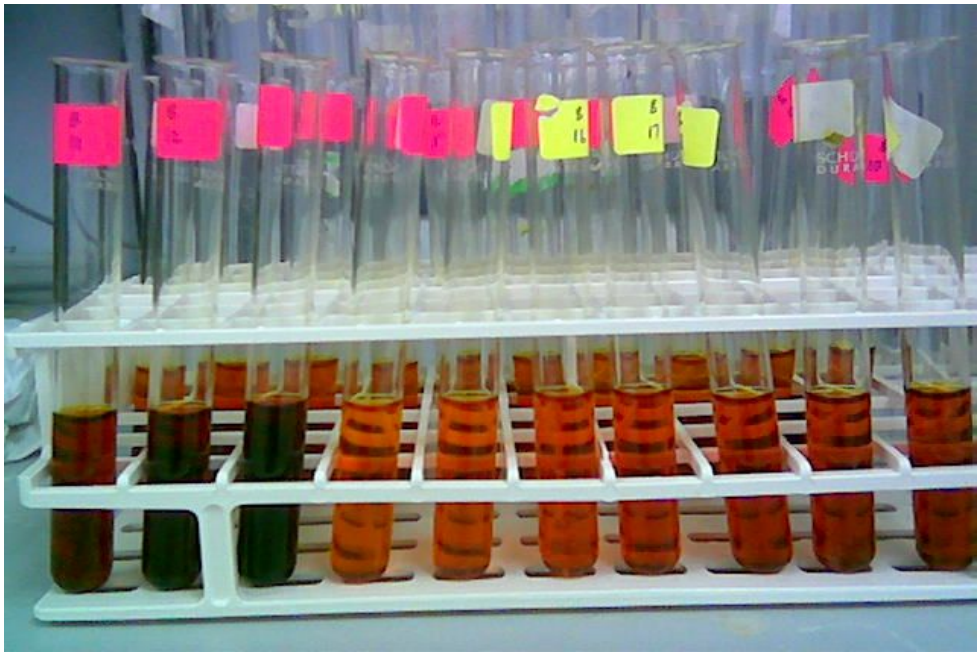

Figure B-1.1 Calibration samples after DNS methods

Appendix B-2: Coconut Sugar Characterization

Table B-2.1: Control samples of 40 % w/v coconut sugar concentration

Hydrolysis	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	0.433	0.433	0.433	0.931	27.920
After	100	1.037	1.036	1.037	2.004	200.356

Table B-2.2: Control samples of 60 % w/v coconut sugar concentration

Hydrolysis	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	50	0.258	0.259	0.259	0.620	31.022
After	200	0.622	0.621	0.622	1.266	253.156

Table B-2.3: Control samples of 80 % w/v coconut sugar concentration

Hydrolysis	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	70	0.219	0.219	0.219	0.550	38.516
After	400	0.381	0.382	0.382	0.839	335.644

Sucrose Concentrations

40 % w/v coconut sugar concentration:

Sucrose concentration = Concentration after hydrolysis – concentration before hydrolysis

= 200.35556 - 27.92

= 172.436 g/L

60 % w/v coconut sugar concentration:

Sucrose concentration = Concentration after hydrolysis – concentration before hydrolysis

= 253.15556 - 31.022222

= 222.133 g/L

80 % w/v coconut sugar concentration:

Sucrose concentration = Concentration after hydrolysis – concentration before hydrolysis

= 335.64444 - 38.515556

= 297.129 g/L

Moisture Content

(1) Weight of container = (1.048 + 1.0249)/2 = 1.03645 g

(2) Weight of initial coconut sugar + container = (7.3208 + 7.3527)/2 = 7.33675 g

(3) Weight of final coconut sugar + container = (6.792 + 6.8059)/2 = 6.79895 g

$\therefore$ % Moisture Content = $\frac{(2) - (3)}{(2) - (1)} \times 100\%$

= 8.54 %

Appendix B-3: Effect of Coconut Sugar Concentrations and Exposure Time during 25 kHz Ultrasonic Treatment

B-3.1: 40 % w/v Coconut Sugar Concentration

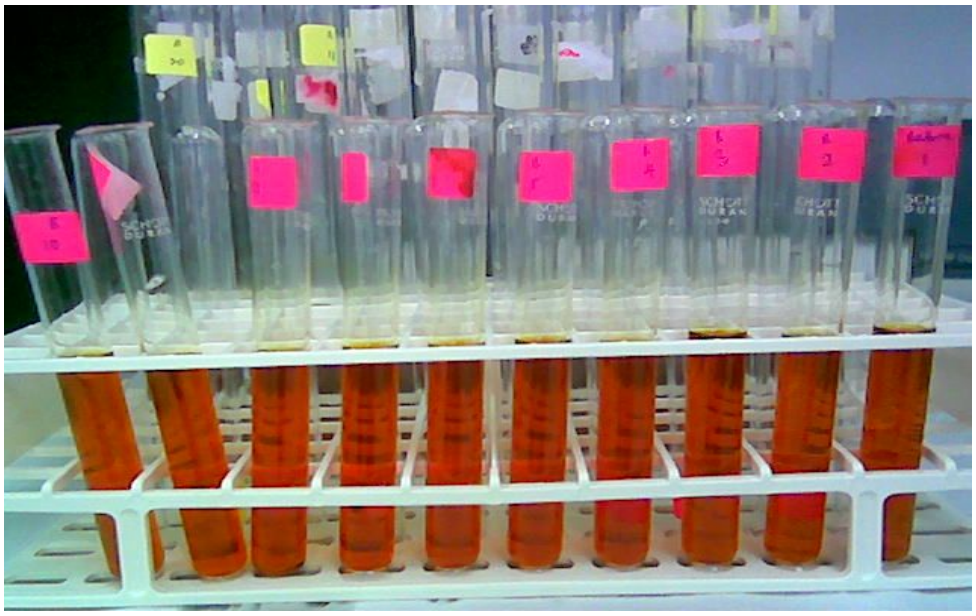

Figure B-3.1.1 DNS treatment before hydrolysis

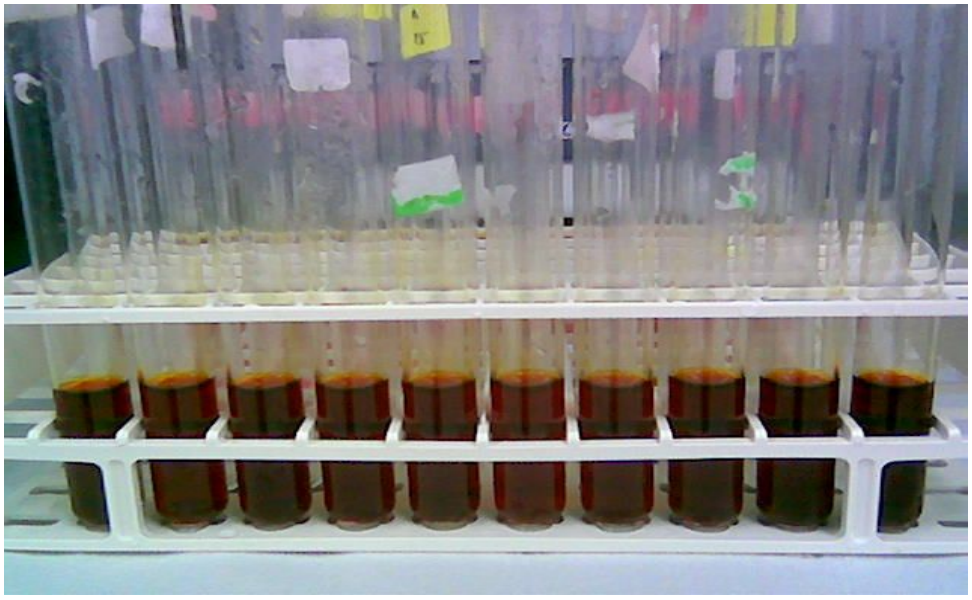

Figure B-3.1.2 DNS treatment after hydrolysis

Table B-3.1.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	30	0.291	0.289	0.290	0.676	20.293
	60	30	0.285	0.283	0.284	0.666	19.973
	90	30	0.296	0.295	0.296	0.686	20.587
	120	30	0.309	0.308	0.309	0.709	21.280
	150	30	0.325	0.324	0.325	0.738	22.133
	180	30	0.321	0.320	0.321	0.731	21.920
	210	30	0.307	0.307	0.307	0.707	21.200
	240	30	0.307	0.305	0.306	0.705	21.147
	270	30	0.286	0.285	0.286	0.668	20.053
	300	30	0.292	0.292	0.292	0.680	20.400
After	30	100	1.221	1.222	1.222	2.332	233.244
	60	100	1.226	1.226	1.226	2.340	234.044
	90	100	1.194	1.196	1.195	2.285	228.533
	120	100	1.152	1.153	1.153	2.210	220.978
	150	100	0.989	0.988	0.989	1.918	191.822
	180	100	1.217	1.218	1.218	2.325	232.533
	210	100	1.229	1.230	1.230	2.347	234.667
	240	100	1.299	1.300	1.230	2.471	247.111
	270	100	1.216	1.217	1.217	2.324	232.356
	300	100	1.407	1.409	1.408	2.664	266.400

B-3.2: 60 % w/v Coconut Sugar Concentration

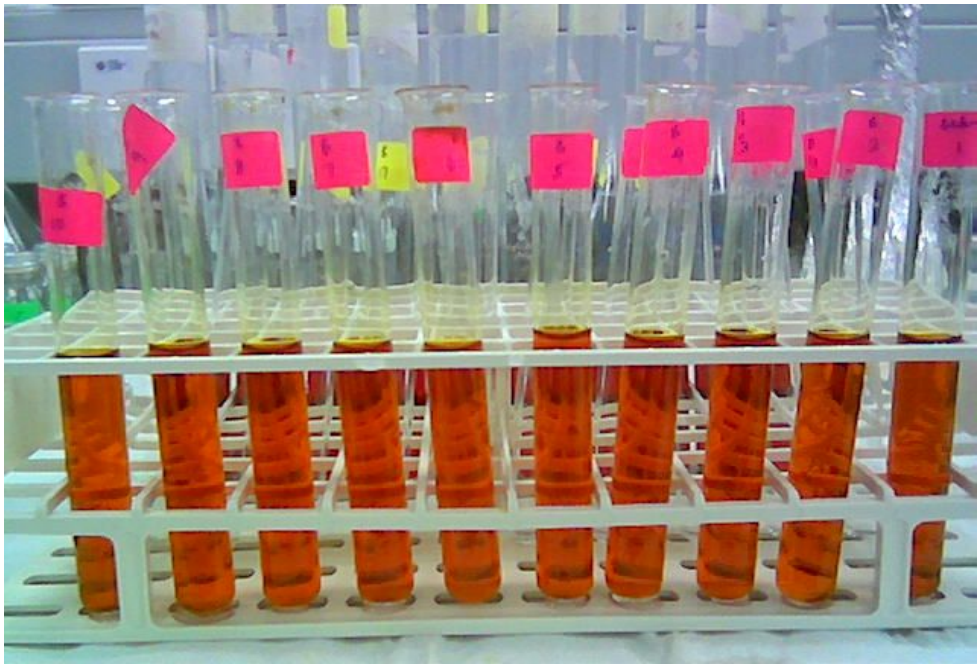

Figure B-3.2.1 DNS treatment before hydrolysis

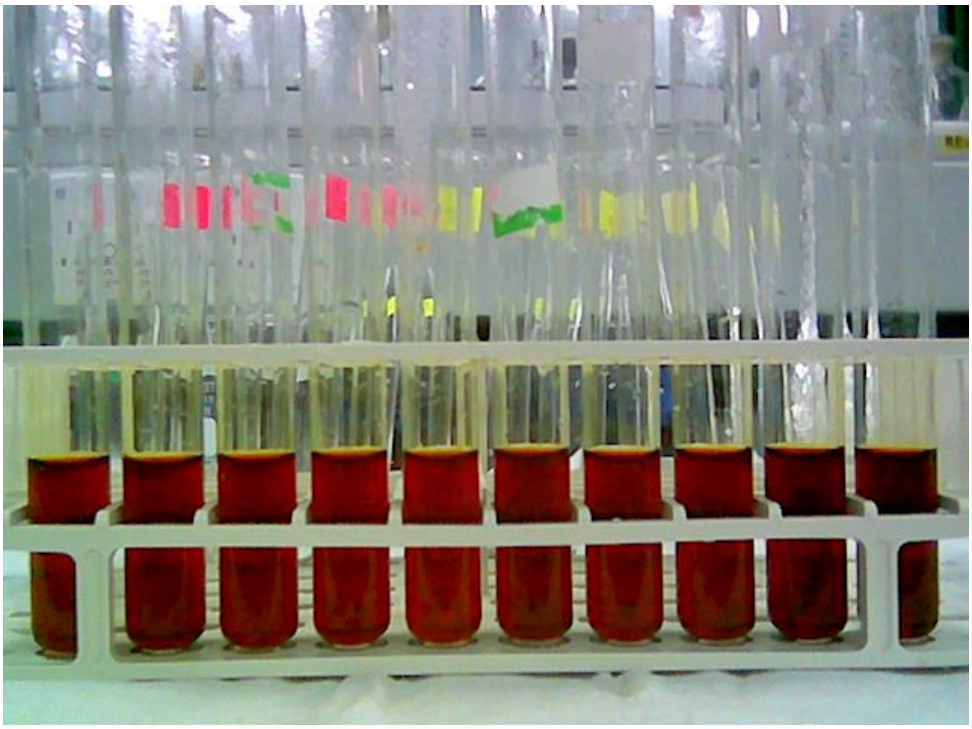

Figure B-3.2.2 DNS treatment after hydrolysis

Table B-3.2.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	50	0.275	0.276	0.276	0.651	32.533
	60	50	0.243	0.244	0.244	0.594	29.689
	90	50	0.276	0.277	0.277	0.652	32.622
	120	50	0.283	0.284	0.284	0.665	33.244
	150	50	0.256	0.257	0.257	0.617	30.844
	180	50	0.243	0.244	0.244	0.594	29.689
	210	50	0.279	0.280	0.280	0.658	32.889
	240	50	0.287	0.288	0.288	0.672	33.600
	270	50	0.287	0.288	0.288	0.672	33.600
	300	50	0.264	0.265	0.265	0.631	31.556
After	30	200	0.743	0.746	0.745	1.484	296.889
	60	200	0.835	0.836	0.836	1.646	329.244
	90	200	0.878	0.881	0.880	1.724	344.889
	120	200	0.799	0.801	0.800	1.583	316.622
	150	200	0.723	0.728	0.726	1.451	290.133
	180	200	0.970	0.975	0.973	1.890	377.956
	210	200	0.891	0.895	0.893	1.748	349.689
	240	200	0.901	0.906	0.904	1.767	353.422
	270	200	1.030	1.031	1.031	1.993	398.578
	300	200	0.939	0.942	0.941	1.833	366.578

B-3.3: 80 % w/v Coconut Sugar Concentration

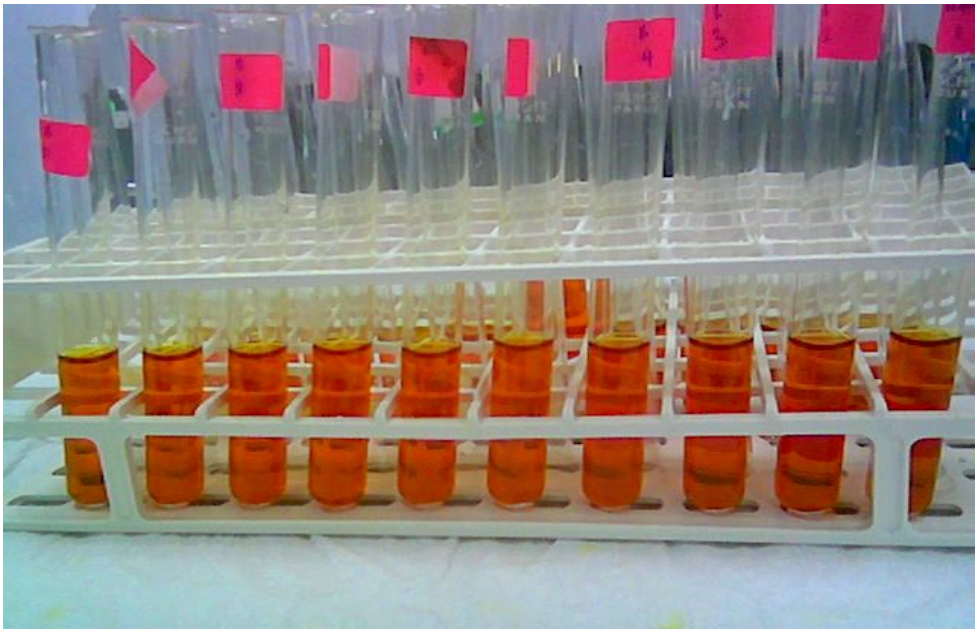

Figure B-3.3.1 DNS treatment before hydrolysis

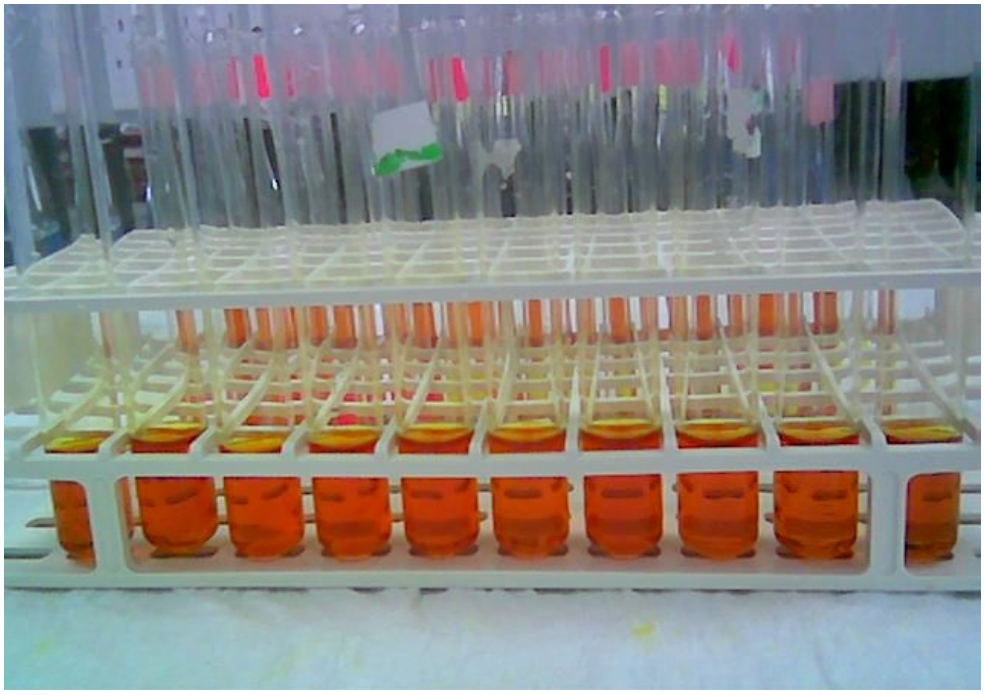

Figure B-3.3.2 DNS treatment after hydrolysis

Table B-3.3.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	70	0.192	0.191	0.192	0.501	35.093
	60	70	0.193	0.192	0.193	0.503	35.218
	90	70	0.207	0.206	0.207	0.528	36.960
	120	70	0.210	0.219	0.215	0.542	37.956
	150	70	0.192	0.191	0.192	0.501	35.093
	180	70	0.211	0.210	0.211	0.535	37.458
	210	70	0.228	0.227	0.228	0.565	39.573
	240	70	0.203	0.202	0.203	0.521	36.462
	270	70	0.231	0.230	0.231	0.571	39.947
	300	70	0.259	0.229	0.244	0.595	41.627
After	30	500	0.403	0.403	0.403	0.877	438.667
	60	500	0.395	0.394	0.395	0.862	431.111
	90	500	0.413	0.413	0.413	0.895	447.556
	120	500	0.458	0.458	0.458	0.975	487.556
	150	500	0.386	0.386	0.386	0.847	423.556
	180	500	0.447	0.448	0.448	0.956	478.222
	210	500	0.424	0.424	0.424	0.915	457.333
	240	500	0.421	0.421	0.421	0.909	454.667
	270	500	0.444	0.445	0.445	0.951	475.556
	300	500	0.432	0.432	0.432	0.929	464.444

Appendix B-4: Effect of Coconut Sugar Concentrations and Exposure Time during 68 kHz Ultrasonic Treatment

B-4.1: 40 % w/v Coconut Sugar Concentration

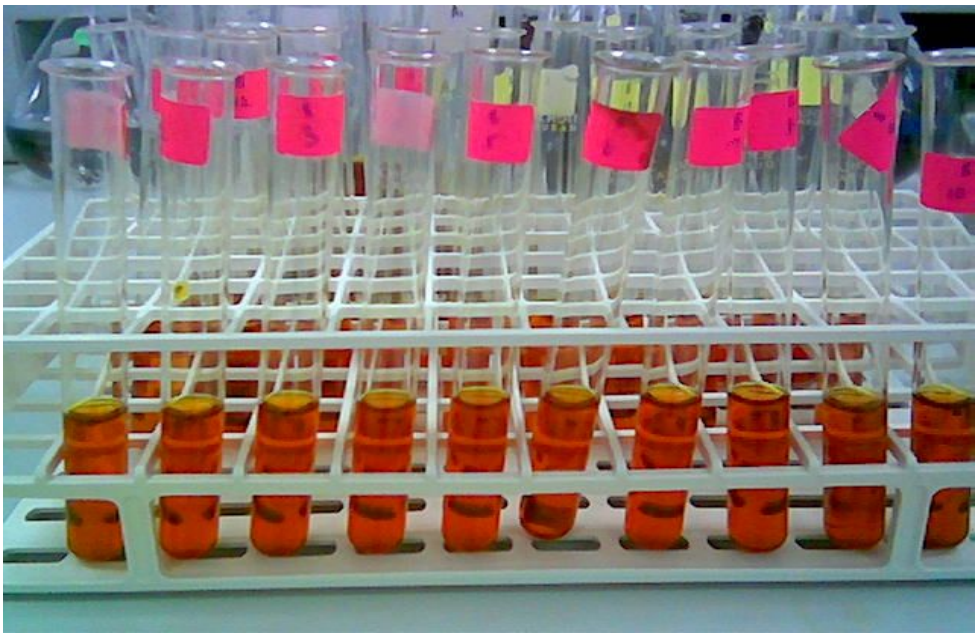

Figure B-4.1.1 DNS treatment before hydrolysis

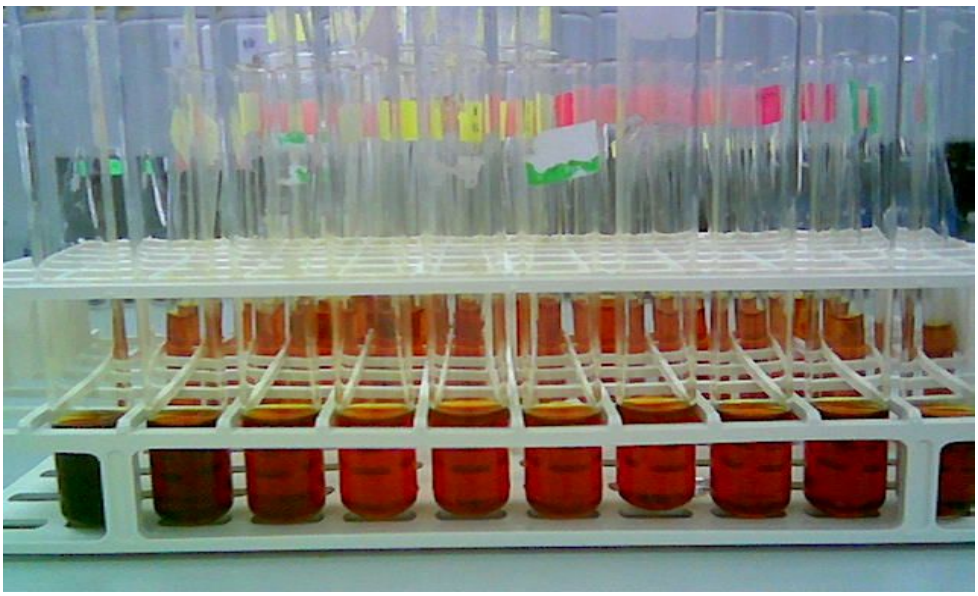

Figure B-4.1.2 DNS treatment after hydrolysis

Table B-4.1.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	30	0.305	0.305	0.305	0.703	21.093
	60	30	0.350	0.350	0.350	0.783	23.493
	90	30	0.351	0.351	0.351	0.7849	23.547
	120	30	0.356	0.356	0.356	0.794	23.813
	150	30	0.373	0.373	0.373	0.824	24.720
	180	30	0.321	0.321	0.321	0.732	21.947
	210	30	0.369	0.369	0.369	0.817	24.507
	240	30	0.377	0.378	0.378	0.832	24.960
	270	30	0.361	0.360	0.361	0.802	24.053
	300	30	0.368	0.368	0.368	0.815	24.453
After	30	100	1.477	1.479	1.478	2.788	278.844
	60	100	1.582	1.584	1.583	2.975	297.511
	90	100	1.058	1.059	1.059	2.043	204.267
	120	200	0.715	0.716	0.716	1.433	286.578
	150	200	0.779	0.780	0.780	1.547	309.333
	180	200	0.743	0.744	0.744	1.483	296.533
	210	200	0.696	0.697	0.697	1.399	279.822
	240	200	0.719	0.720	0.720	1.440	288.000
	270	200	0.740	0.741	0.741	1.477	295.467
	300	200	0.734	0.735	0.735	1.467	293.333

B-4.2: 60 % w/v Coconut Sugar Concentration

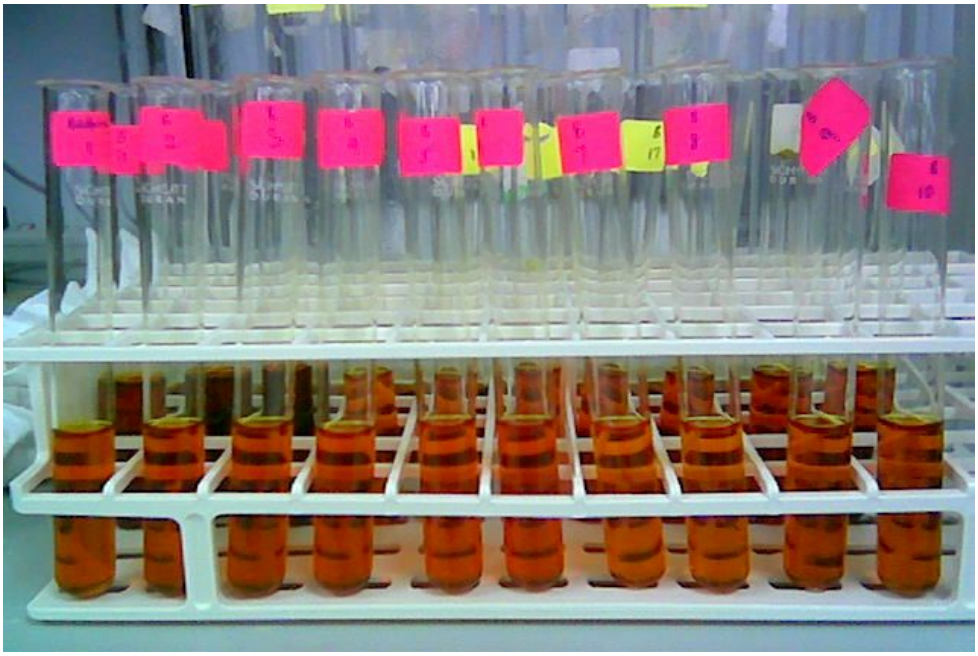

Figure B-4.2.1 DNS treatment before hydrolysis

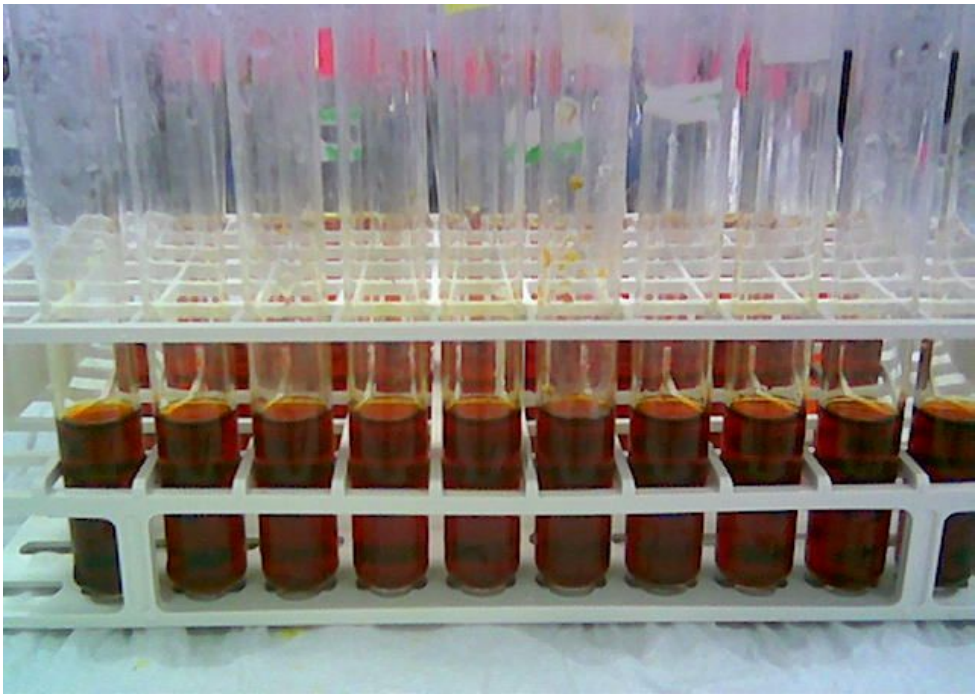

Figure B-4.2.2 DNS treatment after hydrolysis

Table B-4.2.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	50	0.234	0.234	0.234	0.577	28.844
	60	50	0.266	0.266	0.266	0.634	31.689
	90	50	0.290	0.289	0.290	0.676	33.778
	120	50	0.235	0.234	0.235	0.578	28.889
	150	50	0.206	0.206	0.206	0.527	26.356
	180	50	0.286	0.287	0.287	0.670	33.511
	210	50	0.124	0.126	0.125	0.383	19.156
	240	50	0.131	0.131	0.131	0.394	19.689
	270	50	0.284	0.285	0.285	0.667	33.333
	300	50	0.259	0.260	0.260	0.622	31.111
After	30	200	1.194	1.192	1.193	2.282	456.356
	60	200	1.190	1.185	1.188	2.272	454.400
	90	200	1.179	1.177	1.178	2.255	451.022
	120	200	1.016	1.020	1.018	1.971	394.133
	150	200	1.183	1.185	1.184	2.266	453.156
	180	200	1.294	1.294	1.294	2.461	492.267
	210	200	1.230	1.236	1.233	2.353	470.578
	240	200	0.997	0.998	0.998	1.934	386.844
	270	200	0.999	1.000	1.000	1.938	387.556
	300	200	1.050	1.051	1.051	2.028	405.689

B-4.3: 80 % w/v Coconut Sugar Concentration

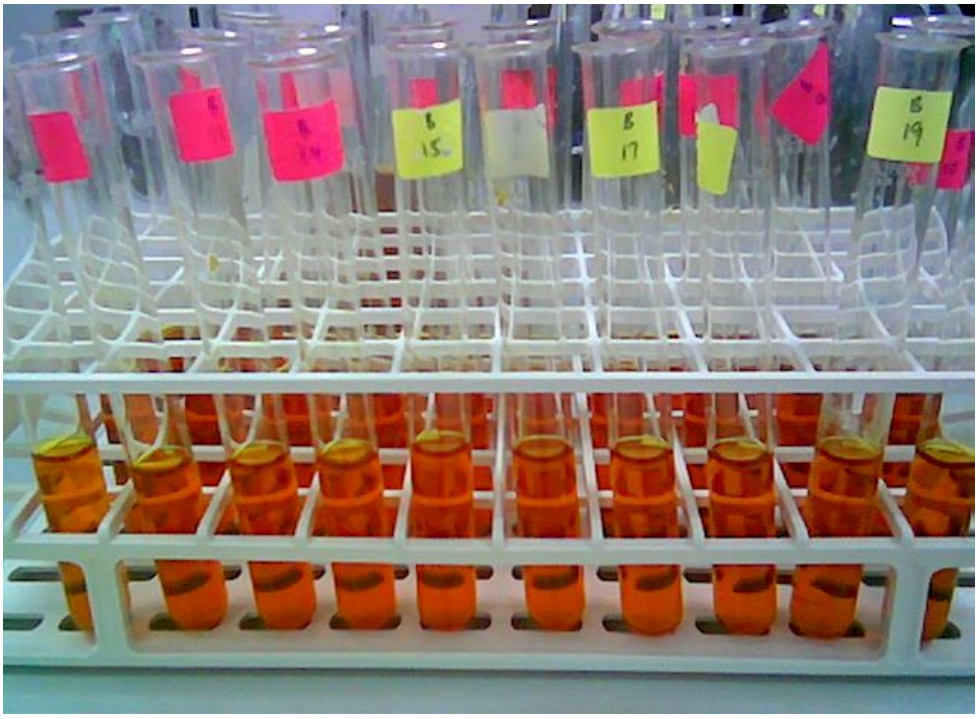

Figure B-4.3.1 DNS treatment before hydrolysis

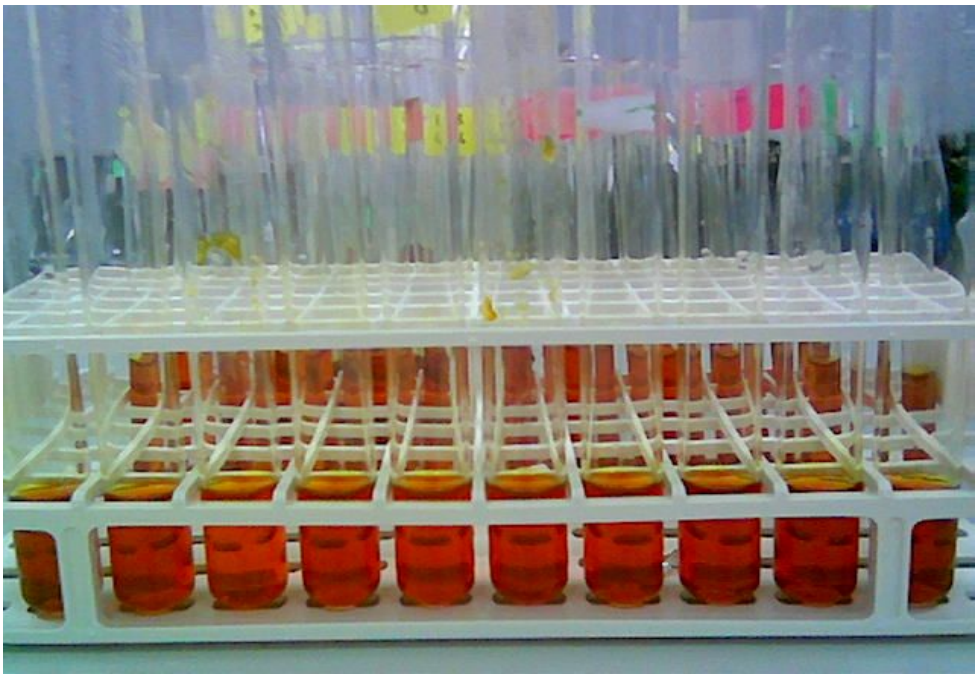

Figure B-4.3.2 DNS treatment after hydrolysis

Table B-4.3.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	70	0.157	0.158	0.158	0.441	30.862
	60	70	0.223	0.224	0.224	0.558	39.076
	90	70	0.208	0.208	0.208	0.531	37.147
	120	70	0.218	0.218	0.218	0.548	38.391
	150	70	0.220	0.221	0.221	0.553	38.702
	180	70	0.195	0.196	0.196	0.508	35.591
	210	70	0.188	0.189	0.189	0.496	34.720
	240	70	0.185	0.186	0.186	0.491	34.347
	270	70	0.198	0.199	0.199	0.514	35.964
	300	70	0.237	0.238	0.238	0.583	40.818
After	30	400	0.493	0.494	0.494	1.038	415.289
	60	500	0.442	0.443	0.443	0.948	473.778
	90	500	0.477	0.478	0.478	1.010	504.889
	120	500	0.475	0.475	0.475	1.005	502.667
	150	500	0.408	0.410	0.409	0.888	444.000
	180	500	0.439	0.440	0.440	0.942	471.111
	210	500	0.393	0.394	0.394	0.860	430.222
	240	500	0.448	0.449	0.449	0.958	479.111
	270	500	0.460	0.459	0.460	0.978	488.889
	300	500	0.462	0.462	0.462	0.982	491.111

Appendix B-5: Effect of Coconut Sugar Concentrations and Exposure Time during 132 kHz Ultrasonic Treatment

B-5.1: 40 % w/v Coconut Sugar Concentration

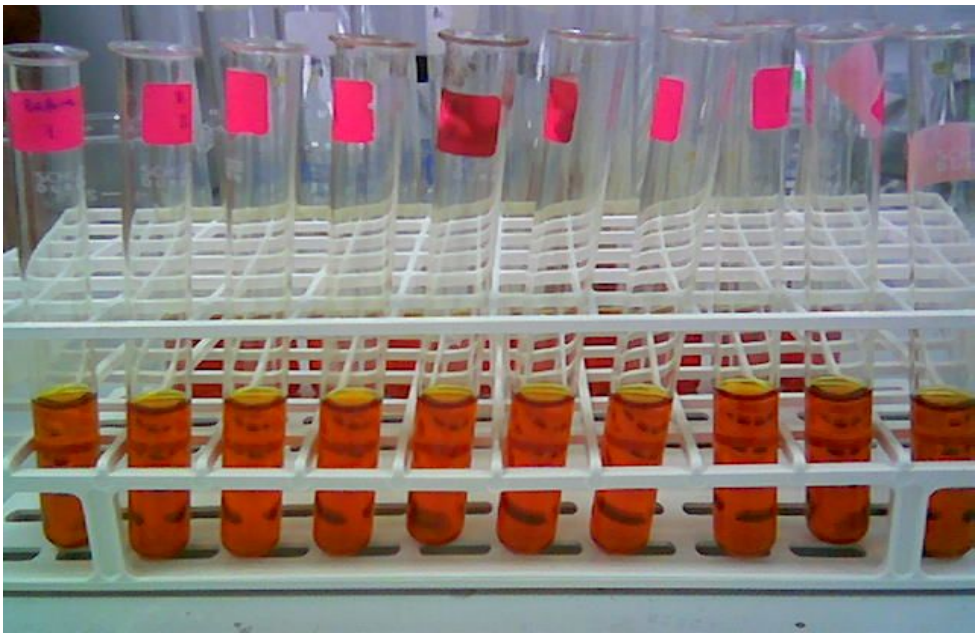

Figure B-5.1.1 DNS treatment before hydrolysis

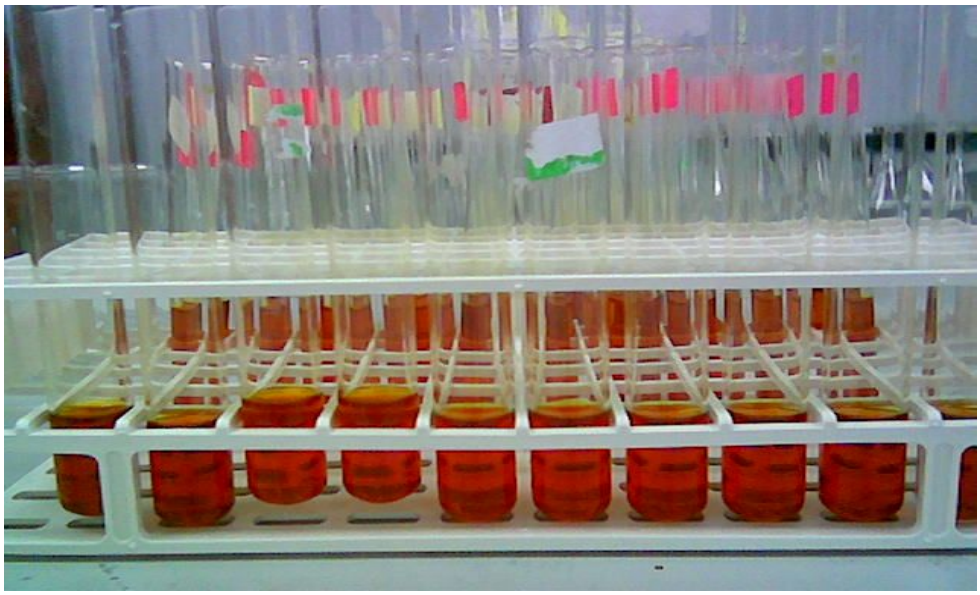

Figure B-5.1.2 DNS treatment after hydrolysis

Table B-5.1.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	30	0.294	0.294	0.294	0.684	20.507
	60	30	0.340	0.340	0.340	0.765	22.960
	90	30	0.307	0.307	0.307	0.707	21.200
	120	30	0.330	0.330	0.330	0.748	22.427
	150	30	0.290	0.290	0.290	0.676	20.293
	180	30	0.306	0.306	0.306	0.705	21.147
	210	30	0.292	0.293	0.293	0.681	20.427
	240	30	0.320	0.320	0.320	0.730	21.893
	270	30	0.329	0.330	0.330	0.747	22.400
	300	30	0.308	0.308	0.308	0.708	21.253
After	30	200	0.593	0.594	0.594	1.216	243.200
	60	200	0.602	0.603	0.603	1.232	246.400
	90	300	0.422	0.423	0.423	0.912	273.600
	120	300	0.428	0.427	0.428	0.921	276.267
	150	300	0.397	0.398	0.398	0.868	260.267
	180	300	0.406	0.408	0.407	0.884	265.333
	210	300	0.394	0.395	0.395	0.862	258.667
	240	300	0.400	0.401	0.401	0.873	261.867
	270	300	0.377	0.377	0.377	0.831	249.333
	300	300	0.398	0.399	0.399	0.869	260.800

B-5.2: 60 % w/v Coconut Sugar Concentration

Figure B-5.2.1 DNS treatment before hydrolysis

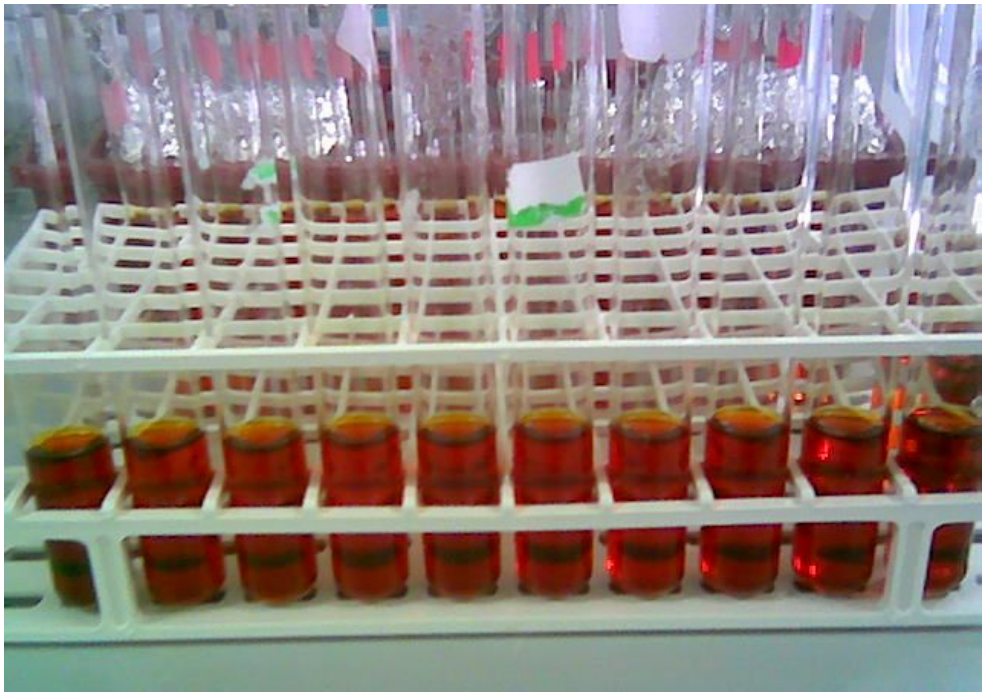

Figure B-5.2.2 DNS treatment after hydrolysis

Table B-5.2.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	50	0.243	0.243	0.243	0.593	29.644
	60	50	0.228	0.229	0.229	0.567	28.356
	90	50	0.231	0.231	0.231	0.572	28.578
	120	50	0.244	0.244	0.244	0.595	29.733
	150	50	0.268	0.269	0.269	0.638	31.911
	180	50	0.228	0.228	0.228	0.566	28.311
	210	50	0.227	0.227	0.227	0.564	28.222
	240	50	0.250	0.250	0.250	0.605	30.267
	270	50	0.254	0.254	0.254	0.612	30.622
	300	50	0.234	0.235	0.235	0.578	28.889
After	30	200	0.862	0.862	0.862	1.693	338.667
	60	300	0.567	0.568	0.568	1.170	350.933
	90	300	0.577	0.577	0.577	1.187	356.000
	120	300	0.539	0.539	0.539	1.119	335.733
	150	300	0.535	0.536	0.536	1.113	333.867
	180	300	0.559	0.559	0.559	1.155	346.400
	210	300	0.556	0.559	0.558	1.152	345.600
	240	300	0.580	0.580	0.580	1.192	357.600
	270	300	0.544	0.545	0.545	1.129	338.667
	300	300	0.549	0.550	0.550	1.138	341.333

B-5.3: 80 % w/v Coconut Sugar Concentration

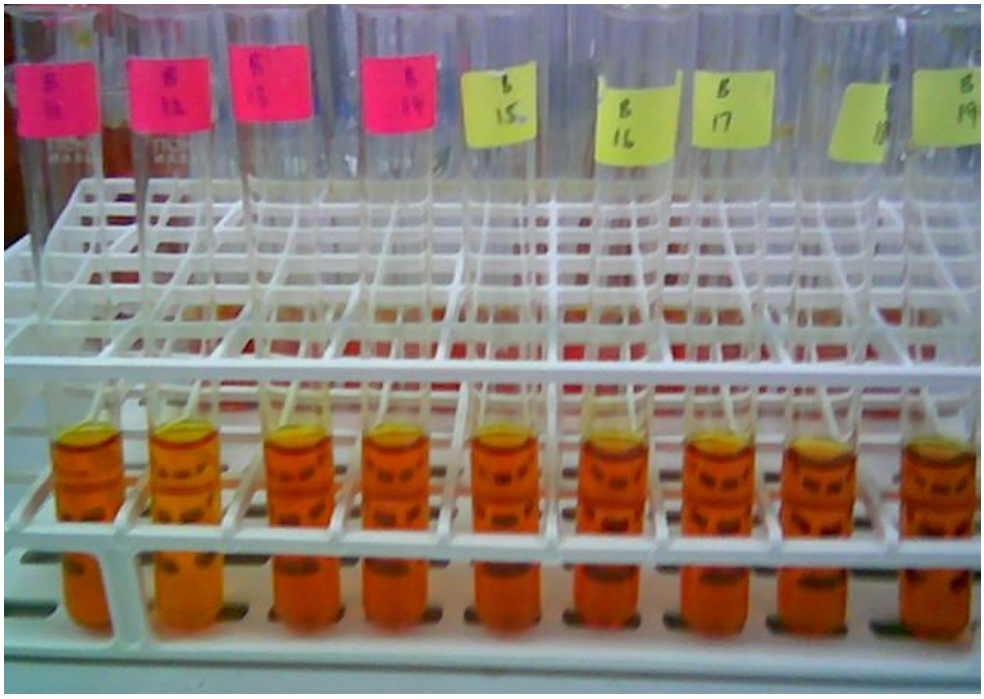

Figure B-5.3.1 DNS treatment before hydrolysis

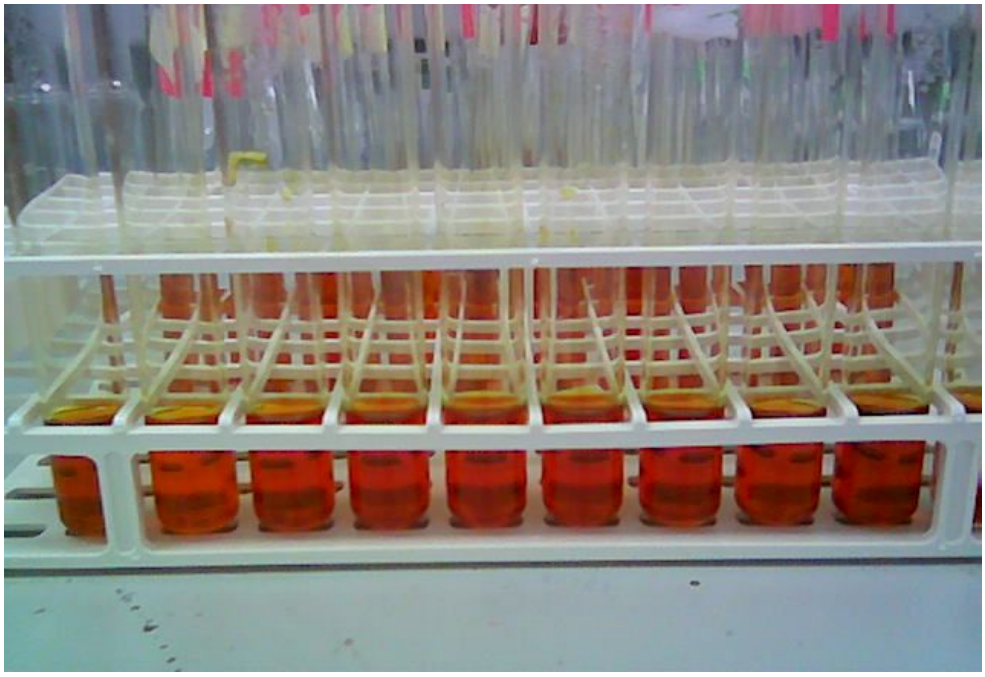

Figure B-5.3.2 DNS treatment after hydrolysis

Table B-5.3.1: Absorbances and concentrations of before and after hydrolysis

Hydrolysis	Time (min)	Dilution Factor	Absorbance		Average	Concentration (g/L)	Actual Concentration (g/L)
Before	30	70	0.193	0.194	0.194	0.505	35.342
	60	70	0.097	0.097	0.097	0.333	23.333
	90	70	0.211	0.211	0.211	0.536	37.520
	120	70	0.193	0.194	0.194	0.505	35.342
	150	70	0.203	0.204	0.204	0.523	36.587
	180	70	0.207	0.208	0.208	0.530	37.084
	210	70	0.208	0.209	0.209	0.532	37.209
	240	70	0.204	0.204	0.204	0.524	36.649
	270	70	0.217	0.217	0.217	0.547	38.267
	300	70	0.248	0.248	0.248	0.602	42.124
After	30	500	0.360	0.362	0.361	0.803	401.333
	60	500	0.348	0.349	0.349	0.780	390.222
	90	500	0.379	0.380	0.380	0.836	417.778
	120	500	0.390	0.391	0.391	0.855	427.556
	150	500	0.382	0.383	0.383	0.841	420.444
	180	500	0.390	0.391	0.391	0.855	427.556
	210	500	0.398	0.399	0.399	0.869	434.667
	240	500	0.362	0.363	0.363	0.805	402.667
	270	500	0.427	0.428	0.428	0.921	460.444
	300	500	0.402	0.403	0.403	0.876	438.222

Appendix B-6: Calculations for Percentages of Sucrose Increased in Treated Coconut Sugar

$$\% \text{ sucrose increased} = \frac{\text{Final amount} - \text{Initial amount}}{\text{Initial amount}} \times 100\%$$

Table B-6.1: Amount of sucrose composition in coconut sugar concentrations and ultrasonic frequencies

Ultrasonic Frequency		**25 kHz**		**68 kHz**		**132 kHz**	
Coconut Sugar Concentration	**Initial (g/L)**	**Final (g/L)**	**%**	**Final (g/L)**	**%**	**Final (g/L)**	**%**
40 % w/v	172.436	246.000	42.66	268.880	55.93	239.547	38.92
60 % w/v	222.133	335.022	50.82	374.578	68.63	312.444	40.65
80 % w/v	297.129	422.818	42.30	450.293	51.55	396.098	33.31