

APPLICATION OF THERMAL TREATMENT FOR VARIOUS *COCOS*  
*NUCIFERA* SAMPLES TO THE SUCROSE CONTENT ANALYSIS

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UNIVERSITI MALAYSIA PAHANG

# UNIVERSITI MALAYSIA PAHANG

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**JUDUL: APPLICATION OF THERMAL TREATMENT FOR VARIOUS  
COCOS NUCIFERA SAMPLES TO THE SUCROSE  
CONTENT ANALYSIS**

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SITI NOREDYANI BINTI ABDUL RAHMAN

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

Faculty of Chemical and Natural Resources Engineering  
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APRIL 2009

## DECLARATION

I declare that this thesis entitled “ Application of Thermal Treatment for Various *Cocos Nucifera* Samples to the Sucrose Content Analysis ” is the result of my own research except as cited in the references. The thesis has not been accepted for and degree and is not concurrently submitted in candidature of any other degree.

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**To Beloved Dad and Mom, Haji Abdul Rahman Bin Jusoh and Hajjah  
Shamsiah Bt Mamat, My Lovely Fiance, Abdul Halim Bin Abdul Razik and  
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## ABSTRACT

The percentage of diabetics has shown unwelcomed increment recently and one of the methods to mitigate this situation is by increasing the production of Fructo-Oligosaccharides (FOS) in the market. FOS is very useful product for diabetics as it can reduce the amount of sugar in their blood. Sucrose which can be found in coconut samples is the raw material to produce FOS. Through thermal treatment of the coconut samples, the percentages of sucrose production can be determined. In this research, three types of coconut samples being used which are coconut water (22.41% sucrose content), coconut sugar A (17.88% sucrose content) and coconut sugar B (14.95% sucrose content). The thermal treatment involves specific procedures of heating and cooling which can give effects to the microstructures of each sample. Two parameters has been analyzed in this study which are temperature and exposure time. The changes of sucrose concentration in each sample have been analyzed using UV-Vis Spectrometer. The experimental results show that increased in temperature and exposure time resulted in increased of sucrose concentration. Optimal conditions for coconut water are 181.64 minutes exposure time and 70°C with the concentration of sucrose produced is 343.53 g/L. Without the thermal treatment, the concentration of sucrose is only 224.05 g/L or 34.78% lower than with thermal treatment. Meanwhile, optimal conditions for coconut sugar A and coconut sugar B are 199.24 minutes and 202.08 minutes respectively, both at temperature of 70°C. At these optimal conditions, coconut sugar A produced 398.06 g/L of sucrose (55.08% higher than without thermal treatment) and coconut sugar B produced 305.37 g/L of sucrose (51.04% higher than without thermal treatment). In conclusion, after performing the thermal treatment for the three types of coconut samples, coconut sugar A is the best sample where the production percentage of sucrose has been increased up to 55.08% after 199.24 minutes exposure time at 70°C.



## ABSTRAK

Pada masa kini, peratusan pesakit diabetes semakin meningkat disebabkan oleh peningkatan kandungan gula di dalam darah. Salah satu kaedah bagi mengurangkan risiko menghidap penyakit ini adalah dengan menggunakan frukto-Oligosakarida (FOS) sebagai penggantian kepada gula tebu kerana FOS dapat mengawal kandungan gula di dalam darah. Sukrosa merupakan bahan mentah dalam penghasilan FOS. Dalam kajian ini, kaedah rawatan haba dijalankan ke atas beberapa jenis sampel kelapa bagi mengenal pasti peningkatan peratusan penghasilan sukrosa. Tiga jenis sampel kelapa digunakan iaitu air kelapa (22.41% sukrosa), gula kelapa A (17.88% sukrosa) dan gula kelapa B (14.95% sukrosa). Kaedah pemanasan dan penyejukan yang dijalankan memberikan kesan terhadap mikrostruktur dan seterusnya memberikan kesan terhadap sifat-sifat sampel tersebut. Dua parameter dikaji iaitu suhu (40°C, 50°C, 60°C, 70°C dan masa (setiap 30 minit, 0 minit hingga 300 minit) terhadap sampel-sampel kelapa dan seterusnya perubahan kepekatan sukrosa dianalisa menggunakan Spektrometer UV-Vis. Hasil daripada eksperimen yang dijalankan, didapati semakin meningkat suhu dan masa yang dikenakan, semakin banyak penghasilan sukrosa di dalam sampel-sampel. Tempoh optima untuk sampel air kelapa ialah pada 181.64 minit pada suhu 70°C dengan kepekatan sukrosa 343.53 g/L. Berbanding dengan kepekatan sukrosa sebelum rawatan, 224.05 g/L menunjukkan peningkatan peratusan sebanyak 34.78%. Manakala untuk gula kelapa A dan gula kelapa B, tempoh optima pada 199.24 minit dan 202.08 minit, pada suhu 70°C dengan kepekatan sukrosa, 398.06 g/L dan 305.37 g/L. Berbanding sebelum rawatan, kandungan sukrosa, 178.794 g/L dan 149.507 g/L, peratusan meningkat kepada 55.08% dan 51.04%. Kesimpulannya, gula kelapa A adalah sampel terbaik kerana menghasilkan kandungan sukrosa yang tertinggi (55.08%) pada suhu 70°C dan masa 199.25 minit berbanding dengan sampel air kelapa dan gula kelapa B selepas menggunakan kaedah rawatan haba.

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**LIST OF SYMBOLS**

°C	Degree Celcius
Min	Minute
g/L	Gram per Litre
yr	Year
C=O	Free Carbonyl Group
COOH	Carboxylate Anion
OH	Oxidation radical



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## CHAPTER 1

### INTRODUCTION

#### 1.0 Introduction

#### 1.1 Background of Study

The scientific name for the coconut is *Cocos nucifera*. It is perhaps one of the most widely spread fruit through natural means in the world, and because of this and the many utilitarian uses of this plant it has become an extremely important plant for indigenous populations. But the coconut now is facing the effects of globalization. From indigenous crop, to disappearing resource, the coconut is a threatened crop. The ever increasing use of monocrops being forced upon the developing world is destroying the traditional methods of agriculture in indigenous populations all in the name of profit for the Western world.

The sucrose, fructose and glucose containing in natural sources such as barley malt syrup, date sugar, white grape juice, honey, maple syrup, banana and coconut sugar is also one of them. Sucrose is usually made from sugar beets or sugar cane that gives you quick energy. However too much consumption of sucrose can cause the excess carbohydrates to be stored in fat cells just like the consumption of others high-glycemic carbohydrates. Another disadvantage of sucrose is that it encourages acid production in the mouth and in turn this promotes dental caries. In this study, the sucrose produced will be raw material in order to convert it become Fructo-Oligosaccharide (FOS). Fructo-oligosaccharide (FOS) can be used as a curing agent for colon diseases and diabetic. Fructo-oligosaccharide (FOS) as a prebiotic and also as a sweetener.

## 1.2 Problem Statement

The incidence of diabetes has risen from almost nothing a century ago to a level of major concern today. It is now the sixth biggest killer in America. Diabetes not only can cause death but can lead to kidney disease, heart disease, high blood pressure, stroke, cataracts, nerve damage, hearing loss, and blindness. It is estimated that 65% percent of the population is at risk of developing diabetes. With diabetes, heart attacks occur earlier in life and often result in death. By managing diabetes, high blood pressure and cholesterol, people with diabetes can reduce their risk. One of the ways reducing the sugar in blood is by using synthetic sugar. Synthetic sugar having no sucrose, low of calories and do not effecting blood sugar level but synthetic sugar are still chemicals whereas food additives that could cause side effects.

There are so many foods and beverages on the market today that that contain aspartame. Some on the list are diet soft drinks, yogurt, instant breakfasts, candy, breath mints, sugar free gum, cocoa mixes, coffee beverages, frozen desserts, juice, laxatives, milk drinks, shakes, tea beverages, topping mixes, wine coolers, and the list goes on. Many people make the mistake of not checking labels, and continue to poison themselves and their children, unknowingly. So, by using nutritious natural sugar, it will add benefiting nutrients to consumer.

The coconut industry is also facing the problem of decreasing prices in all coconut-based products. A main cause of this situation is oversupply of global output especially in Thailand which is one of the major suppliers of coconut based. The uses of this product at the moment are limited to food industry. Hence, too much production with lower demand can cause the value of coconut to decrease.

### 1.3 Objectives

Objective can be defined as the main target that needs to be achieved in this proposal. It is also as guidance to make sure that the problem is solved with an appropriate solution. The objectives for this proposal are:

- i. To determine the effect of temperature during production of sucrose by using thermal treatment.
- ii. To determine the effect of exposure time during production of sucrose by using thermal treatment
- iii. To identify the optimal condition of glucose production, during thermal treatment method.

### 1.4 Scopes of Study

To make this project a success, some boundary need to be specified so that the objective of this project can be achieved without questionable problem. The boundary is called scope of study. This scope will be used to get a clear view on which element that need to be studied or which component that will be used.

First, this study will be focusing on determination of the maximum amount of sucrose produced by using thermal treatment method. There are three types of sample which are coconut water (22.41% sucrose), coconut sugar A (17.88% sucrose) and coconut sugar B (14.95% sucrose).

The parameters that will be studied are the optimal temperature and exposure time. The temperatures used for this treatment are 40, 50°C, 60°C and 70°C and for exposure time at range 300 minutes with 30 minutes interval time. The pH set for this pre-treatment is pH 5.5 by using acetate buffer. The Dinitrosalicylic Calorimetric Method (DNS Method) was used to determine the amount of sucrose produced by using UV-Visible Spectrophotometer.

## 1.5 Significant of Study

Diabetes is a chronic condition in which the body cannot properly convert food into energy. Coconut sugar is the best product for diabetic patient comparing with the synthetic sugar. Nowadays, diabetic patients use synthetic sugar to replace sugar in their foods and drinks in controlling their insulin surges. The synthetic sugar and natural sugar contains the sucrose, fructose and glucose which are helping the patient to control their blood sugar. However the natural sugar is better since there is no presence of chemicals compared to the synthesis ones.

For the health conscious consumers and diabetics, coconut sugar can be considered to be the best sweetener substitute once it is available in the market because of the many benefits that it has to offer. Tests done by the Food and Nutrition Research Institute ( FNRI) and Department of Science Technology (DOST) of the Philippines has revealed a low glycemic index (GI) of 35 compared to that of cane sugar's glycemic index of 50. That's mean coconut sugar is good for proper control of diabetes mellitus. The coconut sugar consist 0.64% bonded glucose, 1.43% bonded fructose and 89.33% bonded sucrose. With amount of 89.33% of sucrose in coconut sugar, the amount FOS being converted can be produced more.

Fructo-oligosaccharide (FOS) also known as oligofructose, a dietary sugar, which the human body does not metabolize, hence its potential use for diabetics and in body weight control. Coconut was one of the best raw material with high sucrose content to produce Fructo-oligosaccharide (FOS). Fructo-oligosaccharide (FOS) can actually help bring down high blood sugar levels in diabetics. In addition, it cuts down elevated cholesterol levels and normalizes blood fat content. Moreover, increased intake of oligofructose has been associated with improved gut health because of the stimulation of (beneficial) bifidus bacteria in the colon.

From this study, at optimal temperature and time of exposure, maximum amount of could be collected and converted to Fructo-oligosaccharide (FOS). Fructo-oligosaccharide (FOS) is known from natural resource that is free from chemical which is can be used as a dietary sugar and prebiotic also as a synthetic sugar. With these advantages, demand for coconut sugar will rapidly increase as the price also.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.0 Introduction

#### 2.1 Coconut Fruit (*Cocos Nucifera*)

Coconut (*Cocos nucifera* L.), one of the key plantation crops of the tropics, is a member of the monocotyledonous family Aracaceae (Palmaceae) (Daniel *et al.*, 2005). It is the only species of the genus *Cocos* belonging to the subfamily Cocoideae which includes 27 genera and 600 species (Perera *et al.*, 2003). Now, coconut is divided into two main types which are tall coconuts, fast growing and predominantly allogamous (cross-fertilizing), and dwarf ones, slow growing and mainly autogamous (self-fertilizing) (Teulat *et al.*, 2000).

Recently, the coconut palm also occupies an important position in the international vegetable oil market (Baudouin *et al.*, 2006). In addition, coconut can also be used as food diet and other purposes. For example, lauric acid, the major fatty acid from the fat of the coconut, has long been recognized for its unique properties that it lends to nonfood uses in the soaps and cosmetics industry (Ashburner *et al.*, 1997). At the same time, in some regions, the coconut is, with few exceptions, exclusively a tropical ornamental plant, much in demand as a signature tropical landscape element (Meerow *et al.*, 2003).

Base on Figure 2.1, Dignan *et al.* (2004) have studied that the coconut products differ in their nutrient content. Coconut oil is almost 100% fat with no carbohydrate, whereas boiled coconut toddy is almost half carbohydrate with almost no fat. As it matures, the flesh of the coconut becomes higher in fat and energy. Coconut oil, mature coconut meat and coconut cream are all high in energy (calories). A small amount of coconut cream added to local root crops and starchy fruits makes a good energy food for young infants after six months of age. The soft flesh of a young drinking coconut is also a suitable food for infants and children.



**Figure 2.1** : Species Coconut fruits for Pacific Island Agroforestry (Bourdeix, 2005)

Coconut toddy is an excellent source of vitamin C, which is important for fighting infection and also helps the body absorb some forms of iron. One cup of fresh toddy provides more than the estimated daily requirement of vitamin C for most adults (45 milligrams). Coconut juice, sprouted coconut, and the flesh of immature and mature nuts are also good sources of vitamin C.



From Murai *et al.* (1958) previous studies, some coconut products are also a good source of iron, which is needed for building strong blood. Many coconut products contain niacin, riboflavin, and thiamine (essential B vitamins), which are important for body metabolism. The drinking coconut contains a refreshing nutrient rich liquid. The juice can also be given to people with diarrhoea to replace lost fluids and minerals. Soft drinks contain few nutrients (Table 2.1) and may be harmful to health because they often contain a large amount of refined sugar.

**Table 2.1** : Comparison of 100gm edible of coconut products, processed baby food and soft drink.

Comparison of 100 gram (g) edible portions of coconut products, processed baby food and soft drink.									
Food item	Kcal*	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Calcium (mg)	Iron (mg)	Niacin (mg)	Vitamin C (mg)
Sprouting coconut <sup>1</sup>	74	1.3	3.6	8.5	1.8	19	0.7	0.9	6
Coconut flesh, mature <sup>1</sup>	283	3.0	27.4	3.6	7.6	10	1.0	0.6	7
Coconut flesh, immature <sup>1</sup>	81	1.8	5.9	3.8	3.2	2	1.3	3.6	3.8
Coconut cream, fresh, no water <sup>1</sup>	325	4.4	32.3	4.7	1.7	15	1.8	0.5	1.0
Coconut water/ juice, immature nut <sup>1</sup>	16	0.1	0	3.9	0	12	trace	2.8	1.4
Coconut water, mature nut <sup>1</sup>	22	0.3	0.2	4.9	0	29	0.1	0.1	2
Coconut toddy, fresh	42	0.2	0.4	9.6	0	trace	trace	0.2	20
Coconut toddy, boiled <sup>2</sup>	217	0.9	2.1	49.4	0	trace	trace	trace	**
Coconut oil	883	trace	99.9	0	0	2	trace	trace	0
Baby food, apple and apricot <sup>1</sup>	45	0.2	0.2	9.8	1.8	6	0.3	0.1	18.0
Soft drink, cola <sup>1</sup>	43	0	0	10.9	0	trace	0	0	0

### 2.1.1 Coconut Sugar

Recently, PCA Administrator Oscar G.Garinbared in a press conference the result of Glycemic Index (GI) test done by Dr. T. P. Trinidad of the Food and Nutrition Research Institute-DOST on coconut sugar produced at Linabu, Balingasag, Misamis, Oriental. According to this test, the glycemic index of

coconut sap sugar is only 35, hence classified as Low GI food which is good for proper control & management of deadly diabetes.

The Glycemic Index (GI) is a ranking system for carbohydrates based on the immediate effect on blood glucose level, the higher the number, the greater the blood sugar response. A low GI food will cause a small rise in blood glucose level while a high GI food will trigger a dramatic rise in blood glucose level. Low GI food is good for proper control and management of the deadly diabetes mellitus, a local and global major human disease. Coconut sugar is also capable of lowering the total cholesterol and low density lipoprotein (LDL)

A disease in which the blood glucose levels are above normal or the body does not produce or properly use insulin. Insulin are hormone needed to convert sugar, starches and other food into energy needed for daily life. Currently, more than 240 million people worldwide living with diabetes. The rate of diabetes among adults in countries as Asian giants such as China and India, others like South Korea, Indonesia and Thailand are contributing to this increase. In the Philippines, there are 3.5million who are diabetics, roughly 4.6% of our population, another 4.6% unaware that they are diabetic; 8% will become diabetic.. These figures are expected to double in the next 20 years, by then the Philippines were expected to be among top 10 countries with highest incidence of diabetes.

Asian countries rich in coconut resources such as Philippines, Thailand, Indonesia, Vietnam and others should encourage coconut farmers and processors to produce sugar because a healthy natural sweetener also for people of all walks of life, particularly, the diabetics. This can be one measure to prevent or minimize the incidence of this disease especially in children. Young people are now prone to Type 2 diabetes due to current lifestyle because of the fast foods and junk foods and lesser physical activities which leads to obesity. In Thailand, public health officials are encouraging government to consider increase in taxes of food high in sugar and consequently promote healthy diet.

### **2.1.2 Coconut Water**

Coconut water is a refreshing beverage that comes from coconuts. It is a powerhouse of nutrition containing a complex blend of vitamins, minerals, amino acids, carbohydrates, antioxidants, enzymes, health enhancing growth hormones, and other phytonutrients. Because its electrolyte or known as ionic mineral content is similar to human plasma, it has gained international acclaim as natural sports drink for oral rehydration. As such, it has proven superior to commercial sports drinks. Unlike other beverages, it is completely compatible with the human body, in so much that it can be infused directly into the bloodstream. In fact, doctors have used coconut water successfully as an intravenous fluid for over 60 years ( Fife., 2008).

Coconut water's unique nutritional profile gives it the power to balance body chemistry, ward off disease, fight cancer, and retard aging. History and folklore credit coconut water with remarkable healing powers, which medical science is now confirming. Published medical research and clinical observation have shown that coconut water makes an excellent oral rehydration sports beverage, aids in exercise performance, kidney function and dissolves kidney stones. Coconut water also can reduce swelling in hands, feet and risk of heart disease, protects against cancer, provides a source of ionic trace minerals, improves blood circulation, blood cholesterol levels and digestion which are contains nutrients that feed friendly gut bacteria. Besides that, this fruit can help balance blood sugar and relieve constipation, lowers high blood pressure, prevent atherosclerosis and abnormal blood clotting, possesses anti-aging properties and enhances immune function ( Fife., 2008).

Young tender coconut water contains ascorbic acid. The concentration of ascorbic acid ranges between 2.2 to 3.7 mg per ml. This ascorbic acid content gradually diminishes as the kernel surrounding the water begins to harden up. The coconut water also contains vitamins of the B group.

The natural sugars in the forms of fructose and glucose form an important element of the young tender coconut water. The concentration of natural sugars in the water of the coconut steadily increases from about 1.5 % percent to about 5.0 to 5.5 % percent in the early months of maturation. This process slowly begins to fall back to around 2 % percent at the stage of full maturity of the coconut. It is in the early stages of maturity when the sugars that are in the form of fructose and glucose (reducing sugar) and sucrose (non reducing sugar) appear. Sucrose appears only in the later stages and increases with the maturity of the coconut, while the reducing sugars fall of. In the fully mature coconut approximately (90%) percent of the total sugars is in sucrose form.

Young tender coconut water contains many valuable minerals for our bodies such as calcium, sodium, potassium, copper, iron, phosphorous, sulphur, and chlorides. Among the minerals that accounts for more than half the concentration of the coconut water is potassium. The environment in which the coconut trees are grown markedly influences the concentration of which. Young tender coconut water with its high concentration of potassium is the perfect electrolytic balance for our body. This help in the elimination of toxic waste from the body by increasing the urinary output (Nature's Fitness Water). Coconut water contains small amounts of protein. The percentage of alanine, arginine, cystine and serene in the protein of young coconut water is higher than cow's milk. Soymilk is another great source of good protein. Since young coconut water does not contain any complex proteins the danger of producing shock to the patients is minimized.

Almost every part of coconut palm is used. It is a primary source of food, water, drink, purifier, fluid re-hydration, isotonic, energy, tonic, fuel, soil rejuvenator from the fiber, animal feed, and shelter. Nature provided us with a tree that produces the world's best water.

This varies according to the different stages of development. The mature coconut is a good source of iron and potassium. Approximately 86% of the calories in coconuts is from the white meat inside the shell and are from fat calories, most of which is saturated fat. But the water of the coconut contains less than 1 %. So the pure coconut water is Cholesterol Free and 99% fat free and young coconut hearts are high in calcium and phosphorus and low in fat (Coconut Development Board of India).

### **2.1.3 Dietary fiber**

The term dietary fiber encompasses a large number of substances with very different chemical structures. There are botanical, physiological and chemical definitions (Biesalski., 1995). According to a very broad definition, the term of dietary fiber refers to all those substances which are either not directly absorbed or directly absorbed to only an insignificant extent or which cannot be converted into absorbable forms by human enzymes in the intestinal tractö (Biesalski., 1995). However, most authors refer to the following narrower definition.

Dietary fiber consists of carbohydrates and lignin which are not digested by the human enzymes in the intestinal tract (Biesalski.,1995). The effects of not well digestion in our body can cause critical diseases such as diabetes, heart attack, kidney disease and more. Dietary fiber are good for human in order to prevent this effect and live well and healthy. This brochure deals exclusively with dietary fiber belonging to the group of carbohydrates.

The following Table 2.2 provides a complete overview of the different types of dietary fiber. The information in the top half of the table (above the double line) refers to the narrower definition such as dietary fiber equal to carbohydrates. All other nutrients which are capable of passing through the stomach and small intestine without being digested are listed below the double line.

**Table 2.2 :** Overview of all types of dietary fiber and their chemical characteristics. The narrower definition of dietary fiber applies to the substances above the dividing line (Biesalski., 1995)

Type of dietary fiber	Chemical characteristics
Non-absorbable sugars	Monosaccharides and disaccharides
Non-digestible oligosaccharides	Oligosaccharides built up from glucose molecules linked in different ways than in starches or from chemical building blocks other than glucose
Non-starch polysaccharides (NSP)	Polysaccharides built up from glucose molecules linked in different ways than in starches or from chemical building blocks other than glucose such as inulin (e.g. in Jerusalem artichokes) or methylcellulose (semi-synthetic)
Resistant starches (RS)	Starches with a dense crystalline structure which precludes attack by intestinal amylase
Lignin	Branched polycondensate made out of cross-linked phenylpropane groups
Polyols (> C4)	Sugar alcohols with a carbon chain consisting of more than four carbon atoms, e.g. sorbitol
Non-digestible proteins	Non-soluble polypeptides and/or polypeptides which cannot be hydrolyzed by intestinal enzymes
High-melting-point fats	Fats with a melting point above 400°C
Fat substitutes	Glycerol ether, fatty acid esters made from alcohols other than glycerol, esters of sugar and fatty acids

### 2.1.3.1 Sucrose

Since the beginning of the century sugar consumption has been on the rise. Sugar may be defined as any of the simple carbohydrates such as monosaccharides and disaccharides. In the Thailand, sucrose, lactose and high fructose coconut sugar represent the majority of the sugars consumed, yet there are many other forms of sugar (Linder., 1998)..The most popular monosaccharides are D-glucose, D-galactose, and D-fructose. Glucose is the main sugar composing polysaccharides and is a component in every disaccharide. Fructose is the sweetest of the monosaccharides and is sometimes referred to as fruit sugar.

Sucrose is known as table sugar and is the most common sugar whereas the fructose is what makes it taste sweet (Whitney., 1991). Sucrose is mainly produced from sugar beets and sugar cane. About 37% of the total world production of sucrose is from sugar beets. The sugar is extracted from the plants and then refined to varying degrees depending upon the type of sugar desired such as brown sugar, refined sugar, or powdered sugar (Bennion., 2007). Sucrose is important in cooking. One application of sucrose is caramelization. Dry sucrose is heated, after a time water is added. This is used in puddings, ice cream, frostings, sauces, peanut brittle, and caramel candy. Sucrose is also used in yeast fermentation yielding carbon dioxide gas and alcohol. This is important in breads and baked goods. The alcohol is volatilized during the baking process and the carbon dioxide acts as a leavening agent. Yeast fermentation is also used in wines and breweries. Other good qualities of sucrose include improving the texture and body of ice creams, retards

The growth of microorganisms in jams and jellies, essential to texture formation in baked goods, absorbs moisture from the environment so foods stay moist and lastly stabilizes egg foams, such as in angel food cake. Sucrose acts as a tenderizer which is its mode of action is to compete for water thereby inhibiting gluten formation (Bennion., 2007).

Sucrose gives people a quick boost of energy and a large number of kilocalories (kcal). This becomes a disadvantage because many people in the U.S have become sedentary and do not need extra kcals. The amount of sugar consumed in the U.S has increased within this century. Another disadvantage of sucrose is that it encourages acid production in the mouth and in turn this promotes dental caries (Geise., 2002). The American Dietetic Association says, based on research, sugar is not an independent risk factor of any particular disease and does not cause behavioral changes such as hyperactivity (Bennion., 2007). It is known that excess kcal are converted to fat within the body and stored. Obesity is a factor in various diseases such as type II diabetes and coronary heart disease.

With these disadvantages in mind, an article in the Journal of Cereal Chemistry states that the health conscious public demands high quality and low-calorie products which are low in fat and sugar. However, altering amounts of ingredients to reduce calorie content may compromise texture, mouth feel, flavor and appearance (Pong., 2007 ). In agreement with this, another article says that an increase in health and nutrition concerns and public demand have created a market for low-calorie, low-fat and reduced sugar products which are being marketed by the food industry in increasing numbers (Bulluck., 2006).

### **2.1.3.2 Fructo-Oligosaccharides: Oligofructose and Inulin**

Fructooligosaccharides (FOSs) are oligosaccharides of fructose containing a single glucose moiety, they are produced by the action of fructosyltransferase from plants and microorganisms (Yun.,*et al.*, 1988). FOS are mainly composed of 1 kestose , nystose, and 1 fructofuranosyl nystose , in which fructosyl units are bound at the position of sucrose molecule (Sangeetha *et al.*, 2005; Kaplan and Hutkins, 2000; Yun, 1996; Hidaka *et al.*, 1988). FOS with low polymeric grade has better therapeutic properties than those with a high polymeric degree. FOS are about 0.4 and 0.6 times as sweet as sucrose and have been used in the pharmaceutical industry as a functional sweetener (Sangeetha *et al.*). FOS present properties such as low caloric values, non-cariogenic properties, decrease levels of phospholipids. Triglycerides and cholesterol, help gut absorption of calcium and magnesium, are useful for diabetic products and are used as prebiotics to stimulate the bifidobacteria )growth in the human colon (Sangeetha *et al.*, 2005)..

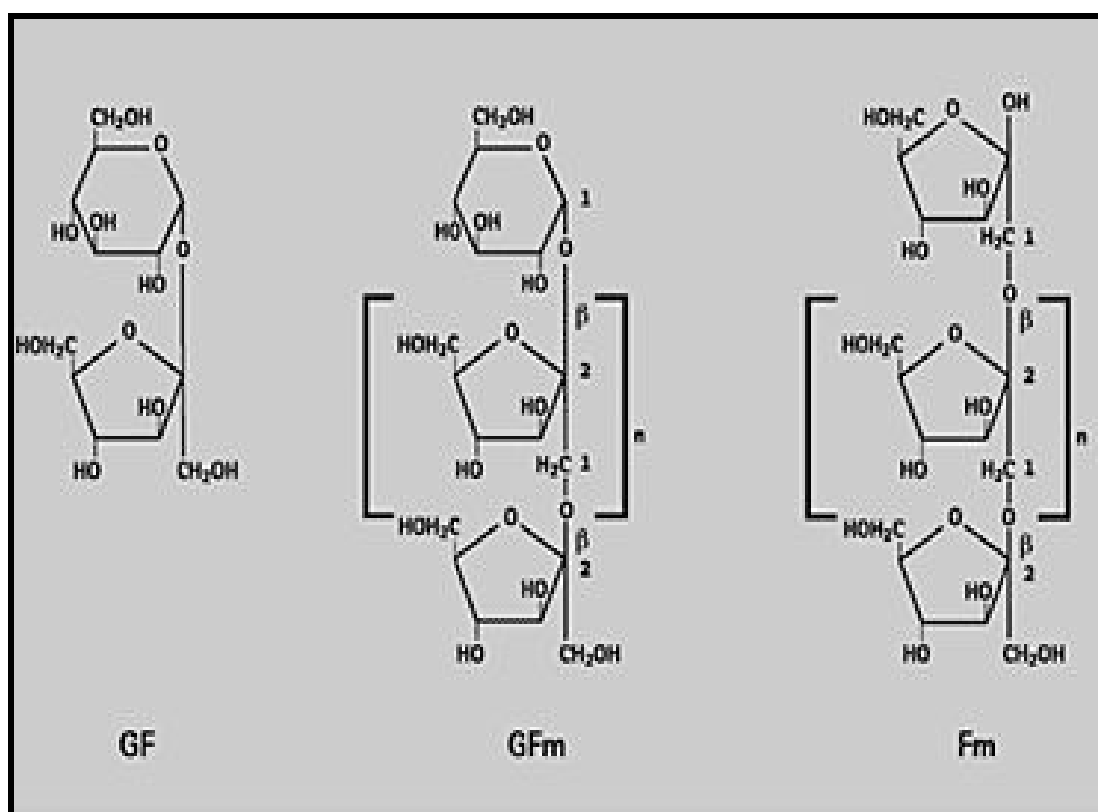
Oligofructose and inulin belong to the group of non-digestible oligosaccharides.. Oligofructose and inulin consist of chains of fructose molecules and are thus given names such as fructo-oligosaccharide (FOS), fructane or owing to their  $\beta$ -2-1 links, also as  $\beta$ -fructane. The figures given in the scientific literature for the grade of polymerization of different oligosaccharides range between 2 and 20 for oligofructose and between 2 and 65 for inulin (Gibson., 1999). Nevertheless, they



are frequently included in the group of "non-digestible oligosaccharides" in the IUPAC-IUB nomenclature.

### 2.1.3.2 1 Occurrence and intake

Inulin and oligofructose are constituents of foods derived from plants. The simplest sugar compound is sucrose which is the sucrose molecule consists of one fructose molecule and one glucose molecule. When more fructose molecules are added, the result is oligofructose or inulin. The variations in the number of inserted fructose molecules result in different grades of polymerization (Figure 2.2).



**Figure 2.2** : Chemical structures of sucrose (GF) and fructo-oligosaccharide (GF<sub>n</sub> and F<sub>m</sub>) G = glucosyl; F=frucosyl (Gibson & Roberfroid., 1995).

Inulin is present as a storage polysaccharide in more than 36,000 plant species. These include bananas (0.3 to 0.7%), Belgian endive or chicory (15 to 20%), barley, honey, garlic (9 to 16%), rye (0.5 to 1%), asparagus (1 to 30%),

tomatoes, Jerusalem artichoke (16 to 20%), triticale, wheat (1 to 4%), coconut sugar and onions (2 to 6%). The estimated amount of inulin and oligofructose eaten per person per day is between 4 and 12 g in Europe and between 2 and 4 g in the USA (Vrese., 1997). Exact figures are hard to obtain since the applicable analytical methods are fairly new and are usually not listed in food tables. In some cases substantial, the variations in the figures quoted are attributable to the different degree of sophistication of the various analytical methods used as well the different cultivation conditions and times of harvest.

Inulin is available commercially as a food additive; it is obtained for this purpose from the chicory root by hot water extraction and, in some cases, hydrolyzed by enzymes to produce short chain polymers. Another manufacturing method is enzymatic synthesis; this method involves the addition of a fructose molecule to a sugar monomer (glucose or fructose). The fructo-oligosaccharides supplied for food production are generally not pure products: they usually consist of mixtures of FOS with different grades of polymerization. To a certain extent, they also contain the original polysaccharide or monosaccharide and disaccharide (Crittenden., 1996).

Several studies performed on rats and mice showed that the addition of inulin-like fructanes (inulin, oligofructose) to the animal's feed decreased their risk of developing cancer of the large intestine. The previous project stated that the results of these animal studies were substantiated. Human studies will have to be performed to obtain definitive proof (Van *et al*, 1999).

The German Nutrition Report (2000) describes the release of toxic, genotoxic and carcinogenic substances by bacterial enzymes. The activity of these enzymes has been implicated in the development of cancer of the large intestine. Distinct differences have been noted in the nature of the enzyme activity displayed by the beneficial and detrimental bacterial groups, respectively. In comparison with other anaerobic bacteria, bifidobacteria and lactobacilli exhibit little enzyme activity of the xenobiotic-metabolizing type. In human beings the intake of probiotics and

prebiotics results in a modulation of the enzymes which activate carcinogenesis and excretion of mutagenic and genotoxic substances in urine and feces (Pool., 2000).

carcinogenic disease, the fructo-oligosaccharide (FOS) also can help diabetes patients to control their sugar blood. Mabel *et al.* (2007) has done a meaningful work in evaluation of the suitability of FOS as a sweetener in an animal study. While all the non-diabetic animals survived the duration of the experiment, considerable mortality was observed among the diabetic rats. Whereas 80% mortality was observed in the diabetic control group during the first fortnight of the experiment, the mortality was only 10% during this period in the FOS-fed group. Thus the survival rate was higher due to FOS feeding, especially at the 10% levels, while considering the mortality in subsequent weeks. There have been a few studies on the effect of FOS on diabetes.

The study by Yamashita *et al.* (1984) demonstrated that the daily intake of fructo-oligosaccharides for fourteen days by type-2 diabetic patients caused an 8% decrease in fasting blood glucose concentrations, a 6% decrease in total cholesterol and a 10% decrease in LDL cholesterol. The literature claims that these observations of decrease in fasting blood glucose and serum total cholesterol levels were statistically significant. Several studies have been conducted in rats since, all showing that fructooligosaccharides lower serum triglycerides and total cholesterol as well as LDL and VLDL cholesterol. Fructooligosaccharides may possibly bind to nutrients such as carbohydrates and lipids and to the absorptive mucosal surface, and the absorption of the nutrients from the intestinal mucosa may be prevented. Since fructo-oligosaccharides alter the composition of bacterial flora in the intestine, (Mitsuoka *et al.*, 1982) the digestion of carbohydrates and the metabolism of bile acids may be changed. Hence, absorption of carbohydrates and lipids will be reduced, resulting in lowering of blood glucose along with serum lipids (Yamashita *et al.*, 1984).

A study by Luo *et al.* (1996) showed that chronic consumption of 20 g of fructooligosaccharides by healthy humans increased basal hepatic glucose production

but had no effect on insulin-stimulated glucose metabolism or serum lipids. Pedersen *et al.* (1997) studied the effect of inulin on blood lipids and did not find any changes in total cholesterol, HDL cholesterol, LDL cholesterol or triglyceride. Thus the effects of fructooligosaccharides on blood glucose and serum lipids are not clear. Alles *et al.* (1999) have evidenced that consumption of fructooligosaccharides for three weeks by patients with type-2 diabetes does not favourably affect blood glucose and serum lipid concentrations.

Even in our present study, we did not observe any major influence of FOS on blood glucose. The FOS preparation used in the present study was around 50% in terms of FOS, and the remainder consisted of free sugars. Despite the high free-sugar content associated with it, FOS did not further aggravate the hyperglycemia and glucosuria in diabetic animals even at 10% levels. This may be attributed to the fact that, FOS as soluble fibre can adsorb nutrients such as glucose, thus retarding their absorption. As a result, the glucose flux in the blood following a carbohydrate meal is retarded, and the amount of insulin needed by the body is lowered. By virtue of its soluble fibre effect, FOS has even alleviated diabetic-related metabolic complications to a certain degree. Thus the present study suggests that the use of FOS as an alternative sweetener is without any adverse effects.

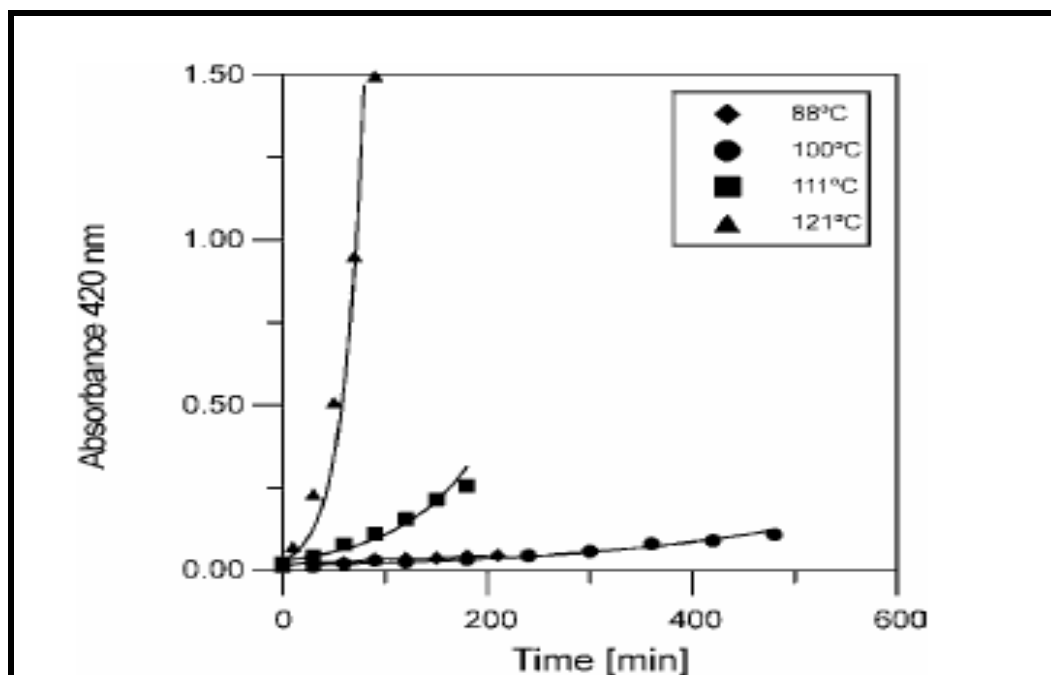
## **2.2 Thermal Treatment**

Thermal treatment is used to preserve fruit derivatives in the manufacturing process. Negative effects of thermal treatments include non enzymatic browning, loss of nutrient and formation of undesirable products. Browning due to thermal treatment is the result of several reactions known as Maillard reactions, which include condensation between reducing sugars and amino acids, caramellization, ascorbic acid browning and pigment destruction (Beveridge *et al.*, 1986). Non enzymatic browning reactions mainly cause color change, sugar and vitamin C loss food (Ibarz *et al.*, 1999). To control browning and preserve the quality of the food,

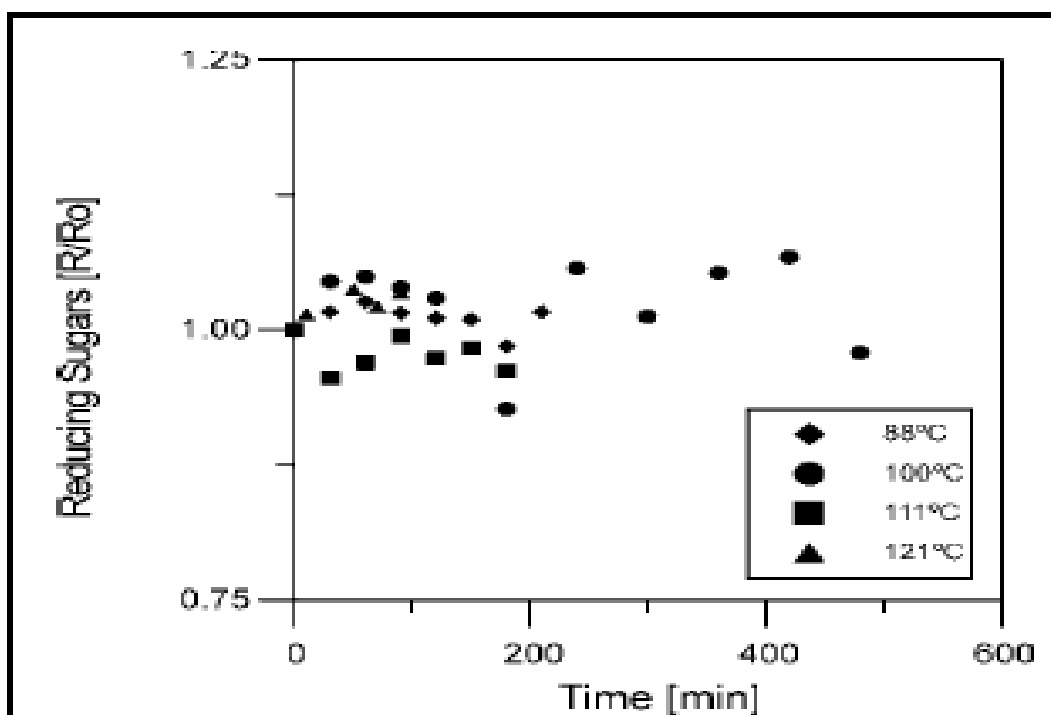
optimization of the temperature and exposure time of the coconut sugar has to be defined.

The benefits of thermal processing as a preservation technique have been well recognized, although adverse affects on sensory quality and nutritional value are sometimes observed. As a consequence, interest in emerging technologies for food processing has increased consistently in recent years because of growing consumer demands for high quality food products. Besides thermal treatment, there also have other pretreatment technologies such as pulsed electric fields (PEF), high hydrostatic pressure, ultraviolet irradiation (UV) and ultrasound (Ross *et al.*, 2003) for industrial-scale food processing.

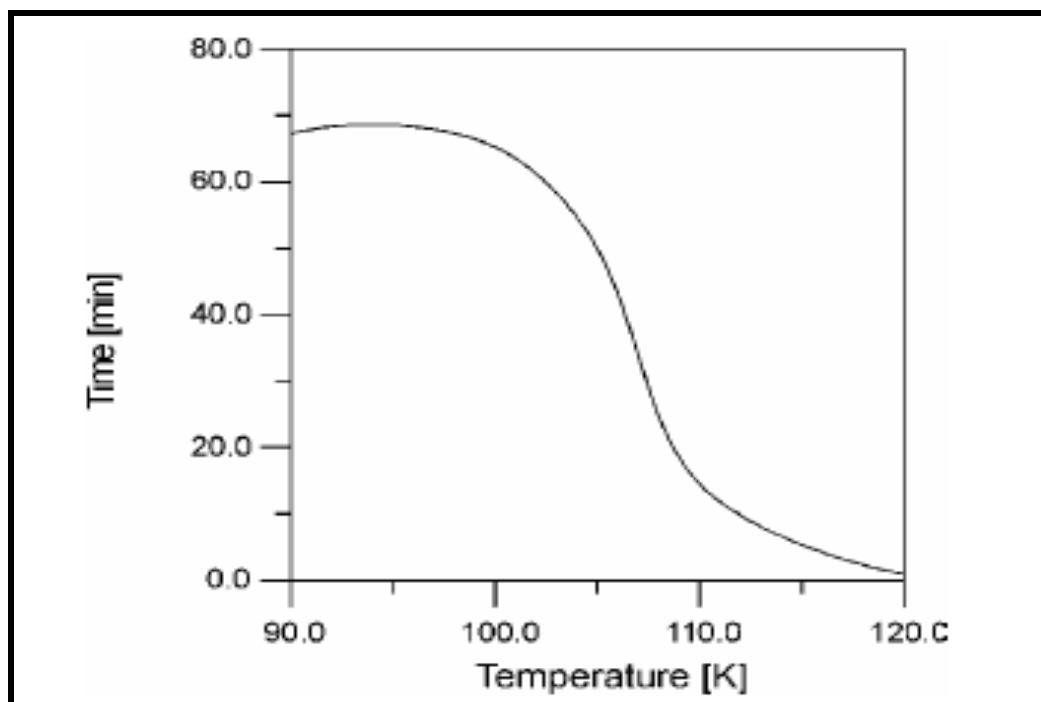
There are many approaches in chemical engineering literature that have been proposed for thermal treatment process. One of the researcher, Damasceno, L.F *et al.* (2008) was carried out the thermal treatment process on clarified cashew juice samples at four different temperatures, 88, 100, 111 and 121°C (Figure 2.3). To preserve “cajuina” from chancing, the clarified cashew apple juice is submitted to thermal treatment where a desired final color should be obtained. The experiments at 88 and 100°C were carried out in thermal water bath equipment (Fanem model 147) and the experiments at 111 and 121°C were carried out in an autoclave (Quimis model Q-190-24). Aliquots were extracted at different time intervals for each temperature and immediately brought to room temperature in an icewater bath. The kinetic models were used to optimize the thermal treatment to produce “cajuina” with an absorbance at 420 nm of 0.023. Chemical and colorimetric determinations were performed for each aliquot. Experiments and analysis were carried out in triplicate. The results shows evolution of the reducing sugars of clarified cashew apple juice during thermal treatment at different temperatures (Figure 2.4) and possible optimal time-temperature processing conditions to achieve an absorbance at 420 nm 10% higher than the initial absorbance of the juice at 420 nm (Figure 2.5).



**Figure 2.3** : Evolution of the absorbance at 420 nm of clarified cashew apple juice during thermal treatment at different temperatures.



**Figure 2.4** : Evolution of the reducing sugars of clarified cashew apple juice during thermal treatment at different temperatures.



**Figure 2.5 :** Possible optimal time-temperature processing conditions to achieve an absorbance at 420 nm 10% higher than the initial absorbance of the juice at 420 nm.

Variation in absorbance at 420 nm ( $A_{420}$ ) was measured using a Cary50conc UV-VIS Spectrophotometer . Soluble solids content was determined with an Atago PR-101 refractometer. Glucose, fructose, sucrose and 5-HMF were determined by HPLC using a Varian ProStar. For sugar analysis, water was used as mobile phase and a refractive index detector with a Varian Metacarb 87P (300 mm  $\times$  7.8 mm) column was used. For 5-HMF, a mixture of acetonitrile:water (20:80) and a UVvisible detector fixed at a wavelength of 285 nm with a Varian Microsorb (C-18) column were used

Another test in thermal treatment was carried out on food samples at four different temperatures. The experiments were carried out in thermal water bath equipment. Aliquots were extracted at different time intervals for each temperature and immediately brought to room temperature in an ice water bath. Experiments and analysis were carried out in triplicate (Damasceno *et al.*, 2008). After the pre treatment process, continue the processes by dividing the work into 3 other separate experiments. The first experiment was performed to analyze the composition of coconut sugar by using Gas Chroma The mixture was then centrifuged to remove

any fine debris present in the sample. The supernatant was filtered through a 0.45 mm filter paper and the filtrate was injected to Gas Chromatography. The amount of solution was determined using an external calibration curve (Turhana *et al.*, 2007). The second experiment was aimed at the rate of brown colour formation during heating of solution, with the constant pH in a closed system at absorbance 420 by using UV-Vis Spectrometer (Apriyantono *et al.*, 2002). The third experiment was done in order to obtain the optimal temperature and exposure time by preparing the calibration curve that used the standard solution as a comparison.

As the coconut sugar and coconut water is subjected to the pre-treatment using thermal treatment, the rate of browning reaction is increasing while the sucrose concentration is decreasing (Apriyantono *et al.*, 2002). This effects show that reactions occur within the coconut sugar as treatment is subjected to the solution. This can be because of Maillard reaction and also caramelization, where brown compound is formed and sucrose is used in the latter reaction (Apriyantono *et al.*, 2002).

Previous studies of the effect of temperature on sweetness have used only a limited number of sweeteners, and the results have been equivocal. Calvino. (1986) reported that the sweetness of sucrose solutions was greater at 37°C or 50°C than at 7°C for low concentrations (3% weight per volume), but this effect tended to disappear with increasing concentrations. Paulu. (1980) found that thresholds for sucrose were lowest in the temperature range from 20°C to 40°C, there was a slight increase in threshold at 10°C with larger increases in thresholds (up to 35%) at 60°C. However, at higher concentrations (18% weight per volume), sweetness intensity was largely independent of concentration. McBurney et al. (1973) found that taste thresholds for dulcin were lowest between 22°C and 32°C, and rose above and below this temperature range. Bartoshuk *et al.*,(1982) concluded that lower concentrations of sucrose were judged to gain in sweetness as temperature increased; this effect finally became negligible at about 0.5 Green. (1988) found that low concentrations of aspartame were perceived as less intense at 20°C than 36° C. In other studies,



however, solution temperature was not found to affect the sweetness intensity of glucose or fructose (Frankmann., 1988).

### **2.3 Dinitrosalicylic Calorimetric Method (DNS Method)**

Rochelle salt, normally present in the dinitrosalicylic acid reagent for reducing sugar, interferes with the protective action of the sulfite, but is essential to color stability. The difficulty may be resolved either by eliminating Rochelle salt from the reagent and adding it to the mixture of reducing sugar and reagent after the color is developed, or by adding known amounts of glucose to the samples of reducing sugar to compensate for the losses sustained in the presence of the Rochelle salt.

The optimal composition of a modified dinitrosalicylic acid reagent is given. The Dinitrosalicylic Acid Reagent, developed by Sumner, J.B *et al.* (1921) for the determination of reducing sugar, is composed of dinitrosalicylic acid, Rochelle salt, phenol, sodium bisulfite, and sodium hydroxide. According to the authors of the test, Rochelle salt is introduced to prevent the reagent from dissolving oxygen (Ibid, 1921 to 1925), phenol, to increase the amount of color produced and to balance the effect of phenol encountered in urine and bisulfite, to stabilize the color obtained in the presence of the phenol. The alkali is required for the reducing action of glucose on dinitrosalicylic acid.

The major defect in the test is in the loss of part of the reducing sugar being analyzed. This was pointed out by Sumner *et al.* (1921) has been observed repeatedly in this laboratory (Mandals, *et al.*, 1952). Evidence of loss of sugar is also given by the data of Hostettler, *et al.* (1951) and of Bell, *et al.* (1952). As this defect appears never to have been fully remedied, the present study was carried out to investigate the different factors which might cause it. In the course of the investigation, the effects of varying the concentrations of the different components of the reagent also

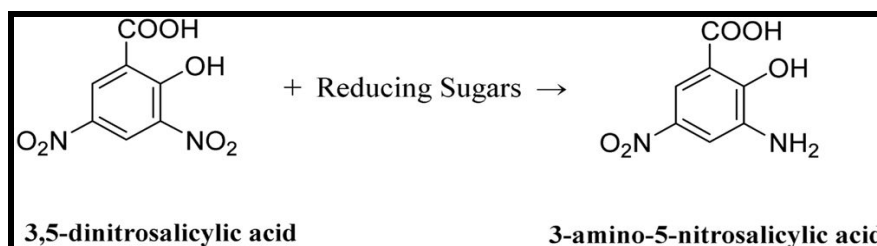
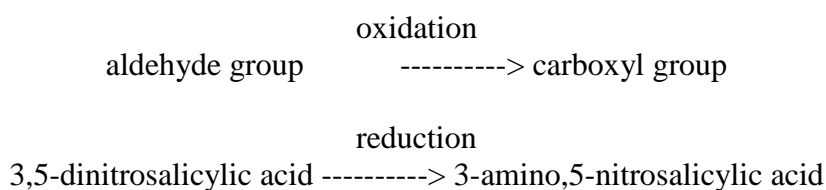
were determined. The findings which resulted have led to the development of a modified reagent and procedure.

Cellulase decomposes the cellulose fiber to small fragments of monosaccharide (glucose), disaccharide (cellobiose), trisaccharide (cellotriose), and other oligosaccharides. Since these small fragments have reducing property, they can be analyzed by reacting with a suitable reagent which can produce an intense chromophore. Therefore, the degree of cellulase treatment can be estimated by measuring the absorbance of the produced chromophore with a spectrophotometer (Lee M *et al.*, 2007). In DNS method, 3,5-dinitrosalicylic acid (DNS) is reduced by reducing sugars to 3-amino-5-nitrosalicylic acid of which absorption at 575 nm is measured spectrophotometrically. DNS method is well established and convenient. However, its signal is proportional to the number of reducing ends (Lee., *et al.*, 2007).

The objective of this experiment is to measure the concentration of sucrose. Unlike other carbohydrates, sucrose is the only non-reducing common disaccharide. Consequently, most tests for sugar detection utilizing such reagents as Benedict's solution, Fehling's solution, and DNS (3,5-dinitrosalicylic acid) solution result in negative readings for sucrose. However, these methods can still be applied if sucrose is first hydrolyzed in an acid solution to yield glucose and fructose. This method is a straightforward modification of the original DNS method for glucose analysis (Miller., 1959).

The DNS method can be applied twice to measure the individual concentrations of a mixture of glucose and sucrose. First, a small part of the original sample is consumed in measuring the glucose concentration by following the original DNS procedure. Another part of the sample is hydrolyzed and subsequently subjected to the same DNS procedure. The difference in the absorbance between the acid treated sample and the untreated sample is due to the presence of sucrose. The sucrose concentration can then be calculated from a calibration curve based on that difference in the absorbance (Miller.,1959).

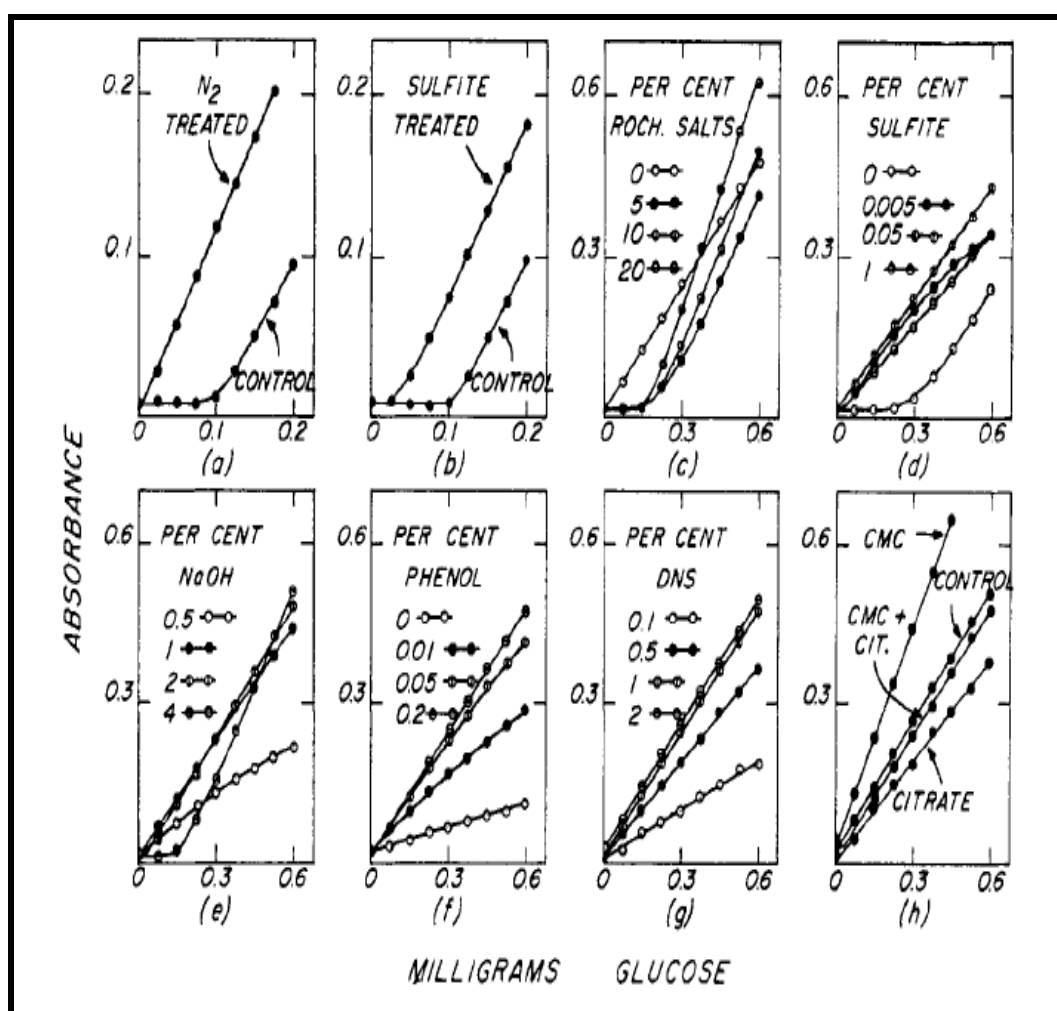
For measuring the glucose concentration, the method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, (Figure 2.6) for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions. Because dissolved oxygen can interfere with glucose oxidation, sulfite, which itself is not necessary for the color reaction, is added in the reagent to absorb the dissolved oxygen.



**Figure 2.6** : Reaction from 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid

The above reaction scheme shows that one mole of sugar will react with one mole of 3,5-dinitrosalicylic acid. However, it is suspected that there are many side reactions, and the actual reaction stoichiometry is more complicated than that previously described. The type of side reaction depends on the exact nature of the reducing sugar. Different reducing sugars generally yield different color intensities, thus, it is necessary to calibrate for each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reactions such as the decomposition of sugar also competes for the availability of 3,5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color (Miller., 1959).

Although this is a convenient and relatively inexpensive method, due to the relatively low specificity, one must run blanks diligently if the colorimetric results are to be interpreted correctly and accurately. One can determine the background absorption on the original cellulose substrate solution by adding cellulase, immediately stopping the reaction, and measuring the absorbance. When the effects of extraneous compounds are not known (Figure 2.7), one can effectively include a so-called internal standard by first fully developing the color for the unknown sample. Then, a known amount of sugar is added to this sample. The increase in the absorbance upon the second color development is equivalent to the incremental amount of sugar added (Miller., 1959).



**Figure 2.7** : Effect of variables on color produced with glucose and dinitrosalicylic acid reagent

## **CHAPTER 3**

### **METHODOLOGY**

#### **3.0 Introduction**

As was generally highlighted in the previous chapters, this work embarks with the analysis of the sucrose production in various coconut samples by using thermal treatment. This chapter will be about what are the steps and experiment that need to be worked on to complete this project and to achieve all the objectives. Several experiments had been set to make sure this project can be done correctly. The flow of the project will be start with the analyze the characteristics of the coconut sample by using DNS method, for sucrose concentration and glucose concentration and drying method to identify the water content since it is the most important part in this project in order to compare the concentration of sucrose before and after the treatment. Next after analyze the characteristic of samples, this project will continue with the major objective of this project, which is thermal treatment.

After the pre-treatment had been done, the next process involves the analysis of the samples in order to identify the sucrose production after treatment using the Dinitrosalicylic Acid Method (DNS Method). The DNS method can be applied twice to measure the individual concentrations of a mixture of glucose and sucrose. First, a small part of the original sample is consumed in measuring the glucose concentration by following the original DNS procedure. Another part of the sample is hydrolyzed and subsequently subjected to the same DNS procedure.

The difference in the absorbance between the acid treated sample and the untreated sample is due to the presence of sucrose. The sucrose concentration can then be calculated from a calibration curve based on that difference in the absorbance.

### **3.1 List of Apparatus / Equipments**

- i. Water bath
- ii. UV-Visible Spectrometer
- iii. Oven
- iv. 5 beakers
- v. 2 measuring cylinders
- vi. 30 test tubes
- vii. pH meter
- viii. 10mL micropipette
- ix. 100 L micropipette

### **3.2 List of Chemicals**

- i. Glucose Standard
- ii. Sucrose Standard
- iii. 0.1M Sodium Acetate (tri-hydrate)
- iv. 0.1M Acetic Acid
- v. DNS Reagent Acid
- vi. Sodium Sulfide
- vii. Water
- viii. KOH Solution, 5N
- ix. Potassium Sodium Tartrate Solution, 40%
- x. HCl, Concentrate (37.3%, 11.9 N) Solution

### 3.3 List of Samples

- i. Coconut Water
- ii. Coconut Sugar A
- iii. Coconut Sugar B

### 3.4 Preparation of Solution

All the solution prepared must be kept in a refrigerator at 4°C.

#### 3.4.1 Acetate Buffer Solution

- i. 0.1M of Sodium Acetate (tri-hydrate)

$$\frac{0.1\text{mol}}{1000\text{ mL}} + \frac{136.08\text{g}}{\text{mol}} = \frac{13.608\text{g}}{\text{L}}$$

$$\frac{13.608\text{g}}{\text{L}} + 1\text{L of Distilled water} = 0.1\text{M of Sodium Acetate}$$

- ii. 0.1M of Acetic Acid

$$108.8\text{ mL Acetic Acid} + 1\text{L of Distilled Water} = 0.1\text{M of Acetic Acid Solution}$$

- iii Mix in following proportions to get the required pH 5.5

**Table 3.1** : The mixture of Acetic Acid and Sodium Acetate to get required pH

pH	Volume of 0.1M acetic Acid (mL)	Volume of 0.1M Sodium Acetate
3	982.3	17.7
4	847.0	153.0
5	357.0	643.0
5.5	108.8	891.7
6.0	52.2	947.8

### 3.4.2 Preparing of Solution of Samples

Preparing solution of coconut water, coconut sugar A and coconut sugar B :-

$$60 \% (w/w) = \frac{60g \text{ of sample}}{60g + 40mL \text{ of Acetic Buffer}}$$

The sample solution must be used and analyzed in 2 days to avoid contamination.

### 3.4.3 Preparing of Dinitrosalicylic Acid Reagent Solution, 1 %

- i) Dinitrosalicylic acid: 10 g
- ii) Sodium sulfite: 0.5 g
- iii) Sodium hydroxide: 10 g
- iv) Add water to: 1 liter

The DNS reagent must be wrapped to avoid the light.

## 3.5 Identifying the Characteristics of Various Coconut Samples

### 3.5.1 Water Content (%)

#### 3.5.1.1 Samples

- i. Coconut Water Solution
- ii. Coconut Sugar A Solution
- iii. Coconut Sugar B Solution



### **3.5.1.2 Procedures**

- i. The oven started the heating at temperature 110°C
- ii. The mass of samples container was determined and recorded.
- iii. The samples were placed in the sample container.
- iv. The samples were placed in the sample container.
- v. The samples were taken out every 1 hour and determined the mass of samples until the sample mass constant.

## **3.5.2 Glucose Concentration**

### **3.5.2.1 Reagent**

- i. Dinitrosalicylic Acid Reagent Solution, 1%
- ii. Potassium sodium tartrate solution, 40%

### **3.5.2.2 Procedures**

- i. The DNS reagent was added to 3 ml of glucose sample in a lightly capped test tube. (To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used.)
- ii. Heat the mixture at 90° C for 5-15 minutes to develop the red-brown color.
- iii. 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color.
- iv. After cooling to room temperature in a cold water bath, the absorbance with a spectrophotometer at 575 nm were recorded.

### 3.5.3 Sucrose Concentration

- i. 1 drop, or 20  $\mu$ l, of concentrate HCl solution was added to 1 ml of the sucrose solution. The hydrolysis was allowed to proceed at 90°C for 5 minutes.
- ii. 3 drops, or 0.05 ml, of the 5 N KOH solution was added to neutralize the acid, because the DNS method must be applied in an alkaline condition to develop the red brown color which represents the presence of reducing sugars.
- iii. The DNS reagent was added and the DNS method henceforth was followed.
- iv. A calibration curve was generated to correlate the absorbance to the sucrose concentration.

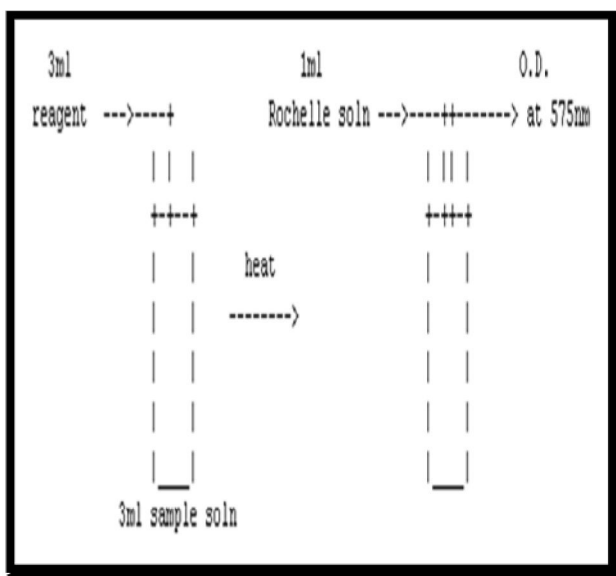
### 3.6 Thermal Treatment Method

- i. A coconut samples solution is prepared by diluting the coconut samples with acetate buffer pH 5.5.
- ii. 10 ml of coconut samples solution was filled into every 20 different test tubes and placed them in water bath with 40°C water temperature. The solution temperature was waited until reaches the water bath temperature for 300 minutes.
- iii. Every 30 minutes, the two of the samples were taken out and immediately placed them into the ice water bath before analysis.
- iv. 2 test tubes contain untreated sugar solution used as a control is also placed in the same ice water bath before the analysis.
- v. The experiment were repeated for temperature 50°C, 60° and 70°C

### 3.7 Analysis of Sucrose Production in Various Samples

#### 3.7.1 Analysis Glucose Content in Coconut Samples

This analysis about identifying the glucose content in coconut samples and Figure 3.1 shows the samples stabilizing the color in order to measuring the glucose concentration.



**Figure 3.1** : Stabilizing the samples color

- i. 3 ml of DNS reagent was added to 3 ml of glucose sample in lightly.
- ii. The mixture were heated at 90° C for 5-15 minutes to develop the red-brown color.
- iii. 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color.
- iv. After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm to stabilize the color (1).

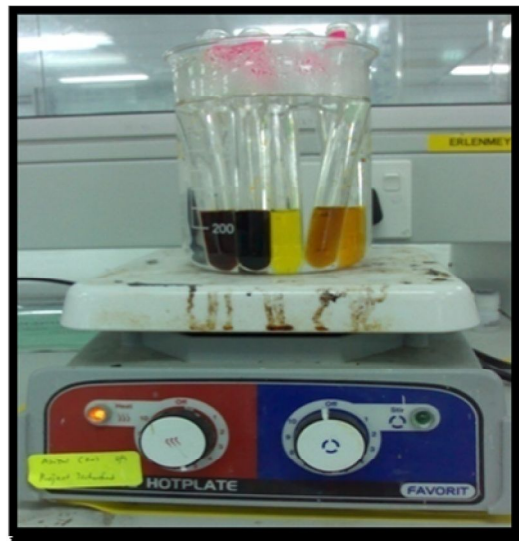
\***(1)** = Amount of sucrose sample hydrolysed into glucose

### 3.7.2 Hydrolysis of Glucose Concentration

Hydrolysis process is the process to convert the sucrose concentration to glucose concentration. Figure 3.2 and Figure 3.3 shows the procedure to mix the samples and heating process to develop red brown colour.



**Figure 3.2** : Mixture of sample and DNS solution



**Figure 3.3** : Heating in 90°C to develop red brown colour

- i. 60  $\mu$ L of concentrate HCl solution was added to 3 ml of the other sample solution from the pre-treatment. Allow the hydrolysis to proceed at 90°C for 5 minutes capped test tube.
- ii. 0.15 ml of the 5 N KOH solution was added to neutralize the acid.
- iii. Method 3.7.1 above was repeated by using DNS reagent in order to convert all sucrose concentration into glucose concentration (2).
- iv. A calibration curve was generated to correlate the absorbance to the absorbance to the sucrose concentration.

\* (2)= Amount of glucose contain in sample

### 3.7.2.1 Calibration Curve

- i. A sucrose solution is prepared at concentration of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mol/L and is measured using UV-Vis Spectrophotometer (DNS Method).
- ii. The results are recorded and the calibration curve is prepared.

### 3.7.3 Determination of Sucrose Concentration

- i. The values from (2) were minus with the values from (1) in order to get the absorbance of sucrose concentration (3).
- ii. The values of sucrose concentration (3) were reading from the calibration curve of sucrose concentration versus absorbance at 575 nm.
- iii. Method 3.7. 2 above was repeated by using DNS method in order to convert all the sucrose content into glucose content for 50°C, 60°and 70°C.

### 3.8 Analysis of Optimal Temperature during Thermal Treatment

- i. At constant temperature, the graph concentration of sucrose versus exposure time was plotted for four different temperatures.
- ii. The optimal exposure time with high concentration of sucrose based on four graphs was collected.

\*(1) = Amount of sucrose sample hydrolysed into glucose ,  
\*(2)= Amount of glucose contain in sample,  
\*(3) = Amount of sucrose produce after treatment

### **3.9. Analysis of Optimal Exposure Time during Thermal Treatment**

- i. At constant exposure time from previous analysis, the graph concentration of sucrose versus exposure temperature was plotted.
- ii. The optimal temperature with high concentration of sucrose from graph was collected

## **CHAPTER 4**

### **RESULT AND DISCUSSION**

#### **4.0 Introduction**

The previous chapters have highlighted the three main objectives and the methodology to achieve them systematically. Achieving the first and second objectives is critical as it forms the basis to accomplish the third objective, which is the main concern of this work. In other words, if the thermal treatment is failed, then analysis of sucrose production is impossible to be accomplished. In this chapter, the obtained results are discussed consecutively in achieving the desired objectives.

#### **4.1 Result for Characterization of Samples**

From the Table 4.1 shows that the characterizations of the samples coconut water, coconut sugar A and coconut sugar B that used for experimental and analysis worked. The samples were taken directly from oven and measured and recorded their mass at every 1 hour and glucose and sucrose concentration were taken from sample control before treatment and analyze by DNS Method. The detailed measurements for water content were attached on Appendix A

## 4.2 Control Samples

Referring to Table 4.1 below, the sucrose concentration and glucose concentration used as a control in this research. Comparing the coconut water, coconut sugar A and coconut sugar B before treatment (Table 4.2), coconut water is the better coconut sample which is content higher sucrose concentration, which is good and safe for diabetic patient use it directly from the coconut fruit.

**Table 4.1** : The values of sucrose and glucose concentration before treatment

Types	Without Treatment (g/L)	
	Sucrose Conc.	Glucose Conc.
Coconut Water	224.05	61.62
Coconut Sugar A	178.79	50.09
Coconut Sugar B	149.51	82.78

**Table 4.2** : Characterization of various coconut samples

Sample	Water Content (%)	Sucrose Content (%)	Glucose Content (%)
Coconut Water	41.32	22.41	6.16
Coconut Sugar A	7.34	17.88	5.01
Coconut Sugar B	16.55	14.95	8.28

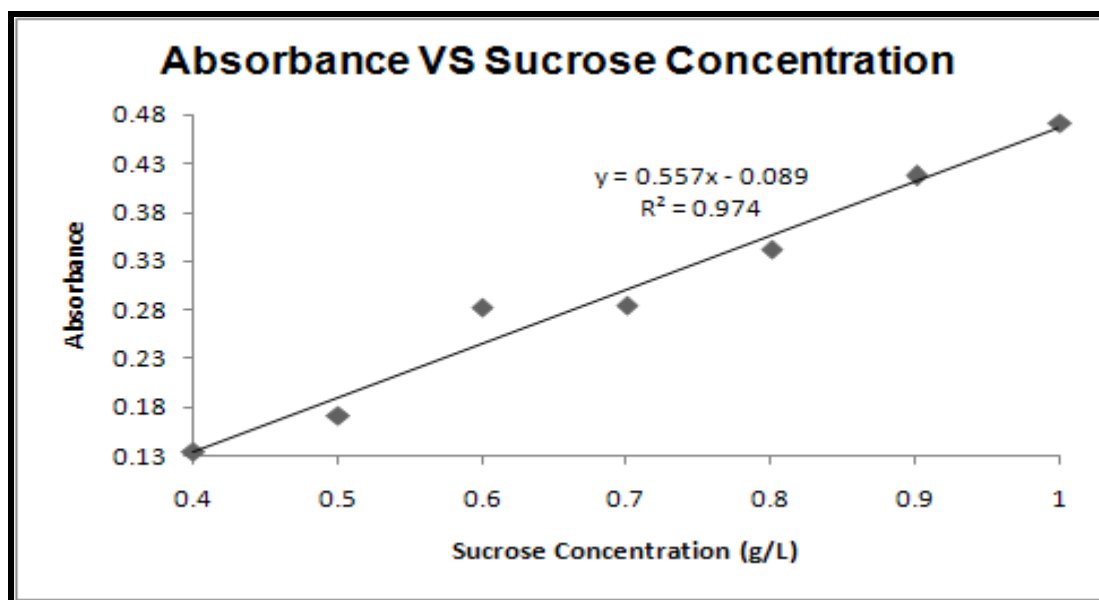
## 4.3 Result for Calibration Curve

The calibration curve is plotted base on concentration of sucrose standard versus absorbance at 575 nm (Table 4.3 and Figure 4.1).



**Table 4.3** : Calibration Curve

Absorbance (nm)	Sucrose Conc. (g/L)
0.899	2
1.883	4
2.44	6
0.135	8
0.172	10
0.281	12
0.284	14
0.342	16
0.419	18
0.472	20

**Figure 4.1** : Calibration Curve for Sucrose Concentration

#### 4.4 Effect of Exposure Time to Various Coconut Sample by using Thermal Treatment

By varying the temperature of water bath, the results were taken every 30 minutes until 300 minutes and construct the graph of sucrose concentration of various coconut samples versus exposure time. The detailed data was attached at Appendix A.

##### 4.4.1 Results and Discussion for Thermal Treatment Method at 40°C

Base on Figure 4.2 shows the sucrose concentration for three samples at 40°C by using thermal treatment in water bath. The sucrose concentration was recorded for every 30 minutes. From the Figure 4.2, it shows that the concentration of sucrose for coconut sugar A and coconut sugar B were increasing in proportional with time, while decreasing in volume of coconut water. This is because at this temperature the percentage of water content in the samples still higher. The water contains in sample help the hydration reaction occurred. In the hydrolysis of disaccharide (sucrose), a water molecule helps to break the acetal bond as shown in Figure 4.3 (red). When the acetal bond is broken, the H from the water is added to the oxygen in the glucose. While the -OH is will combined to the carbon on the fructose.

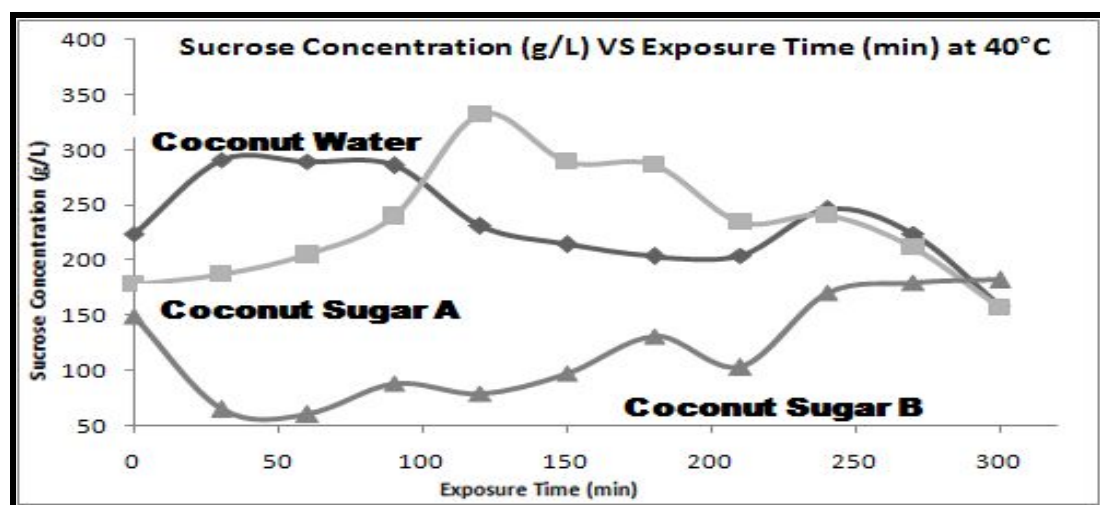
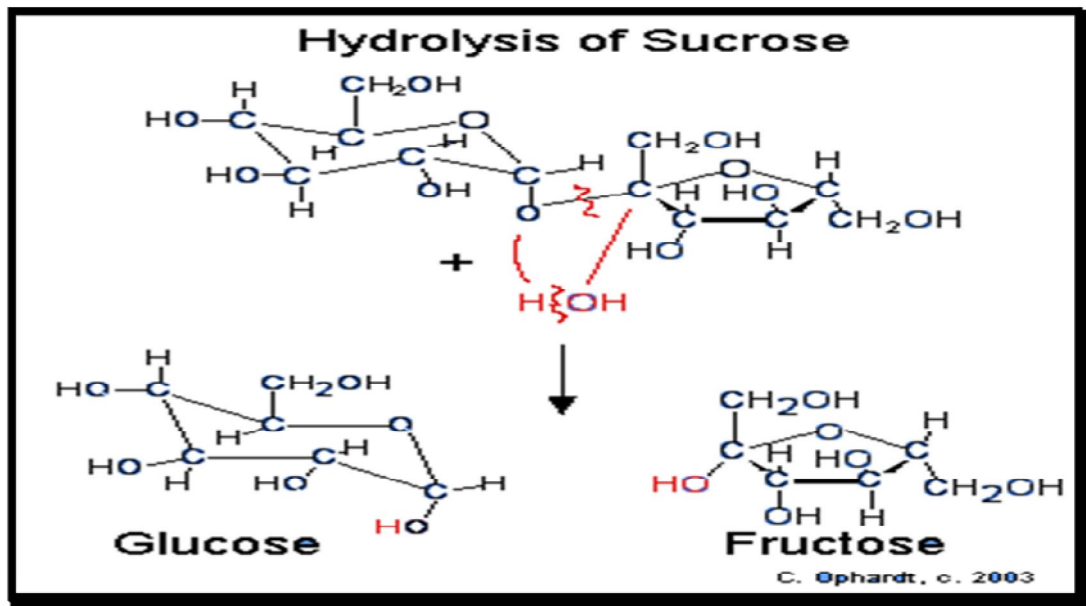


Figure 4.2 : Sucrose concentration versus exposure time at 40°C



**Figure 4.3** : Hydrolysis of sucrose in coconut sample (Charles,.,2003)

At lower temperature as  $40^\circ\text{C}$ , the water does not evaporate much, additional the coconut water contain 93% of water content, so the hydration reaction occurred more on coconut water compared to coconut sugar A, 7.34 % and coconut sugar B, 16.55 %. More reaction occurred, lowering the sucrose concentration in the sample.

#### 4.4.2 Results and Discussion for Thermal Treatment Method at $50^\circ\text{C}$

From the graph below shows the sucrose concentration for three samples at  $50^\circ\text{C}$  by using thermal treatment in water bath. The sucrose concentration was recorded for every 30 minutes. From the Figure 4.4, it shows that the concentration of sucrose for three samples of coconut product at stable condition at this temperature until to 300 minutes. The reason is at this temperature, the percentage of water evaporated higher than at  $40^\circ\text{C}$ , so the hydration reaction on the sample not much occurred.

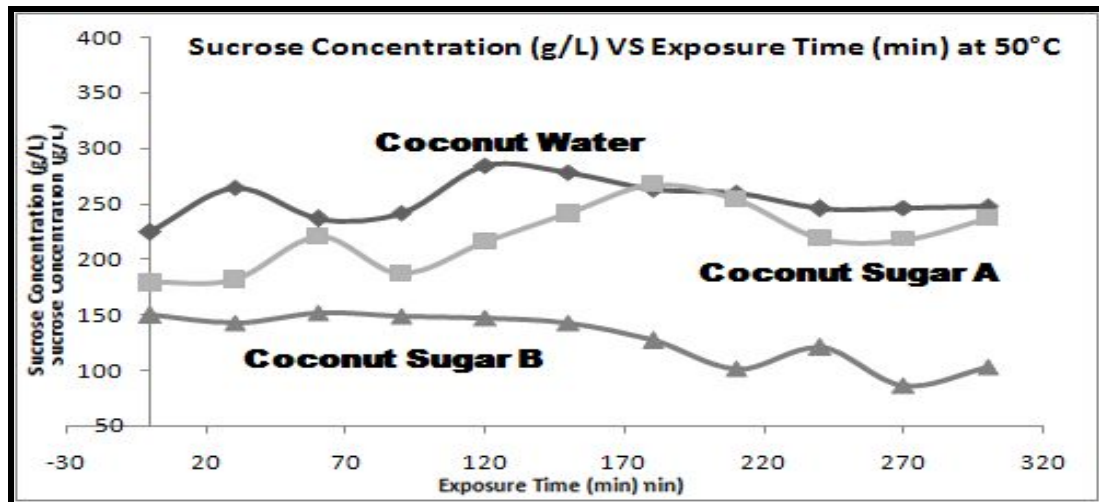


Figure 4.4 : Sucrose Concentration versus exposure time at 50°C

#### 4.4.3 Results and Discussion for Thermal Treatment Method at 60°C

Referring Figure 4.5, the result of sucrose concentration for coconut water, coconut sugar A and coconut sugar B was rather similar with result at 50°C, which is the sucrose concentration was at the stable state. However, at minute 200 until 300 minutes, the concentration of coconut water increasing at range 200g/L to 300 g/L because at this condition, the water content start to deplete, then the dehydration reaction occurred to the coconut water, which is the reversible process for dehydration reaction.

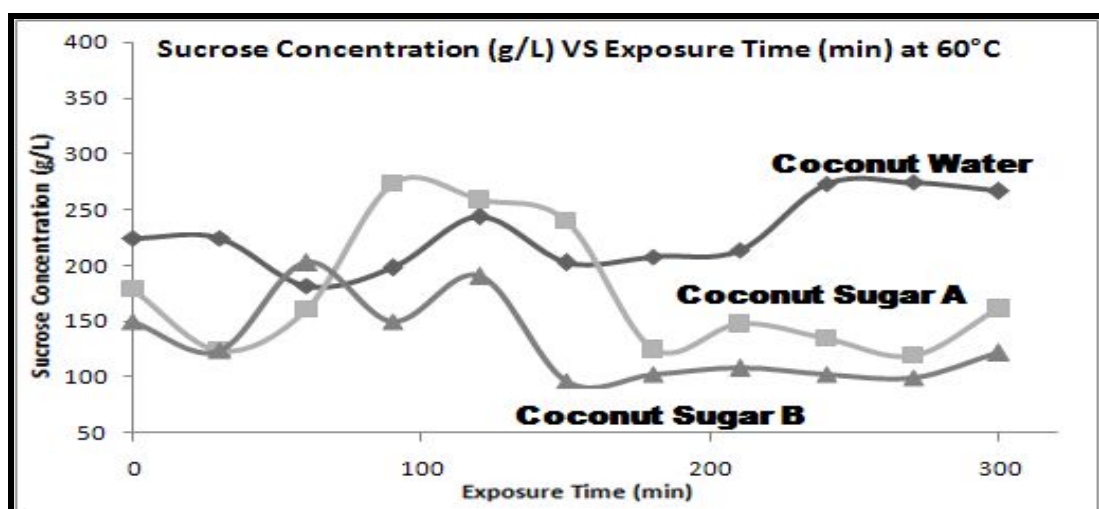


Figure 4.5 : Sucrose concentration versus exposure time at 60°C

#### 4.4.4 Results and Discussion for Thermal Treatment Method at 70°C

According to the Figure 4.6, starting from minutes 100 to 200, the sucrose concentration are increased rapidly proportional with time. These result shows that at this range with temperature 70°C, the coconut water, coconut sugar A and coconut sugar B achieve their optimal condition. This situation occurred because of the combinations of the two monosaccharides, glucose and fructose to become the disaccharides, sucrose. At the exact temperature and exposure time, the production of sucrose keep increasing until it reach maximum production, then starting to decreasing because of the disaccharide get potential to convert become polysachharides by combination of monosaccharide and disaccharide. From the results in the graph show that the effect of temperature at 70 °C can increased the production of sucrose.

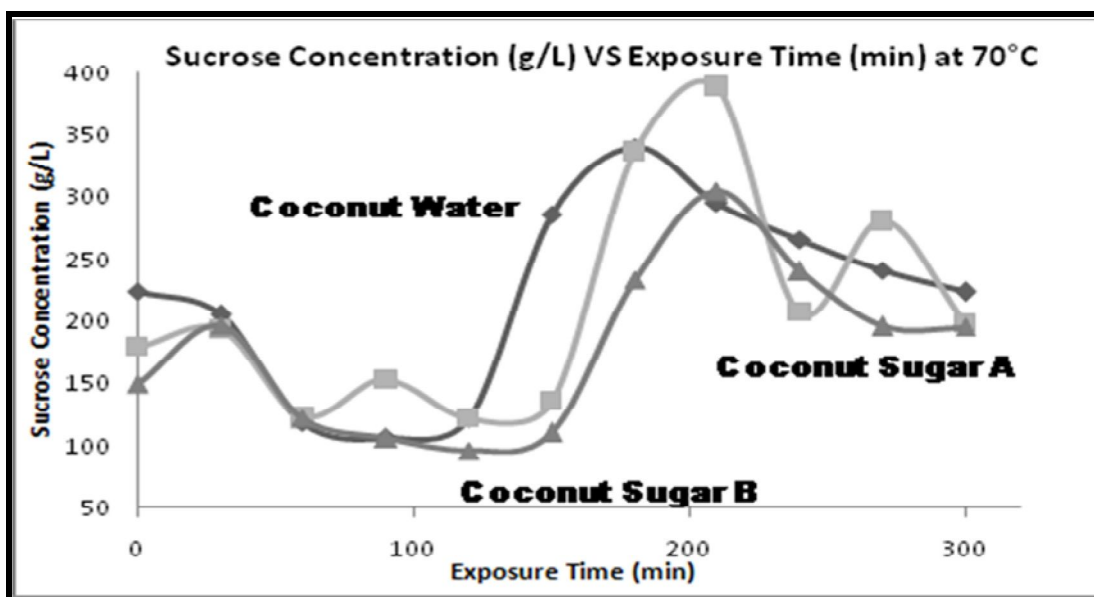


Figure 4.6 : Sucrose concentration versus exposure time at 70°C

#### **4.4.3 The Effect of Temperature to Various Coconut Samples by using Thermal Treatment**

By varying the exposure time from the previous results, the new values were obtained and the graphs of sucrose concentration for the three samples versus temperature at varying exposure times were constructed. Details about the data are attached in the Appendix A.

#### **4.5.1 Results and Discussion for Thermal Treatment Method**

According to the Figure 4.7, the higher concentration for coconut water is at 181.64 minute at temperature 70°C, coconut sugar A at 199,25 minute also at 70°C, and lastly for coconut sugar B with the same temperature at 202.8 minute. This results show at initial exposure time, the sucrose production at stable rate, then rapidly increase until in range 180 to 210 minutes. After exceeding the duration, the sucrose concentration in the samples started to become constant. Base on the results show that the optimal condition for various coconut samples at exposure time occurred at range 180 to 210 minutes. So the effect of increasing the exposure time at temperature 70 can in.

From Figure 4.7 (a), the percentage of increasing sucrose concentration for coconut water was 28.81 %, while 5% and 70 % for coconut sugar A and coconut sugar B, respectively.

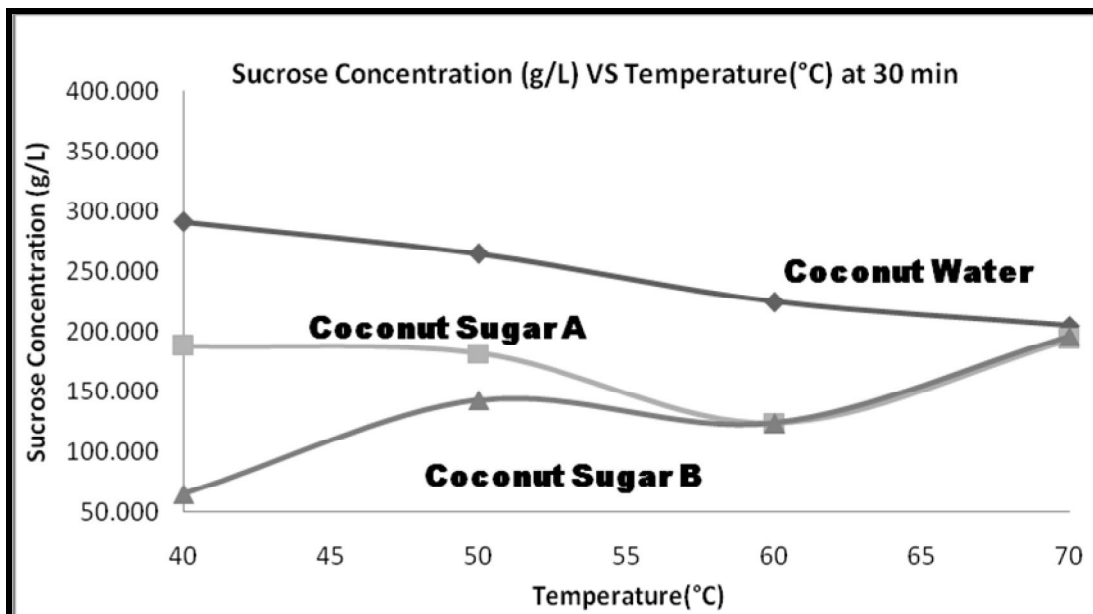


Figure 4.7 : Sucrose Concentration versus temperature at 30 minute (a)

From Figure 4.7(b), the percentage of increasing sucrose concentration for coconut sugar B was 59.32 %, while decreased for coconut sugar A, 45 % and coconut water, 55.93 %.

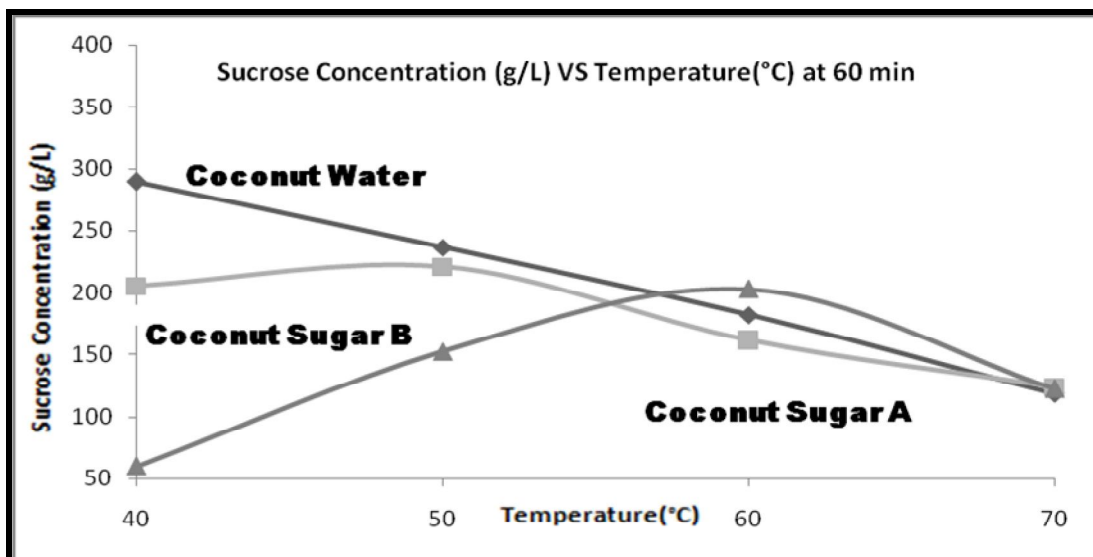
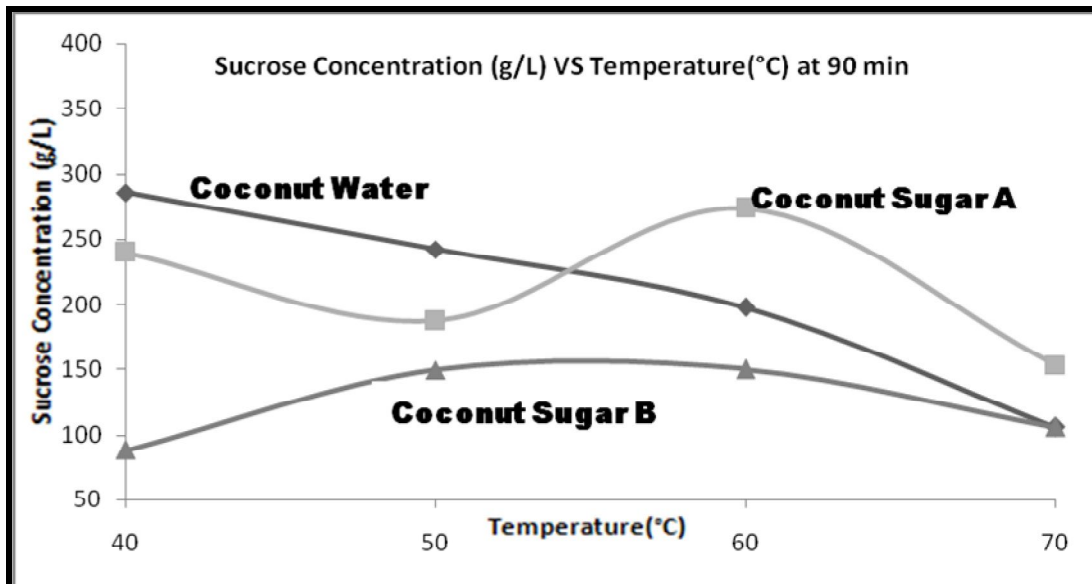


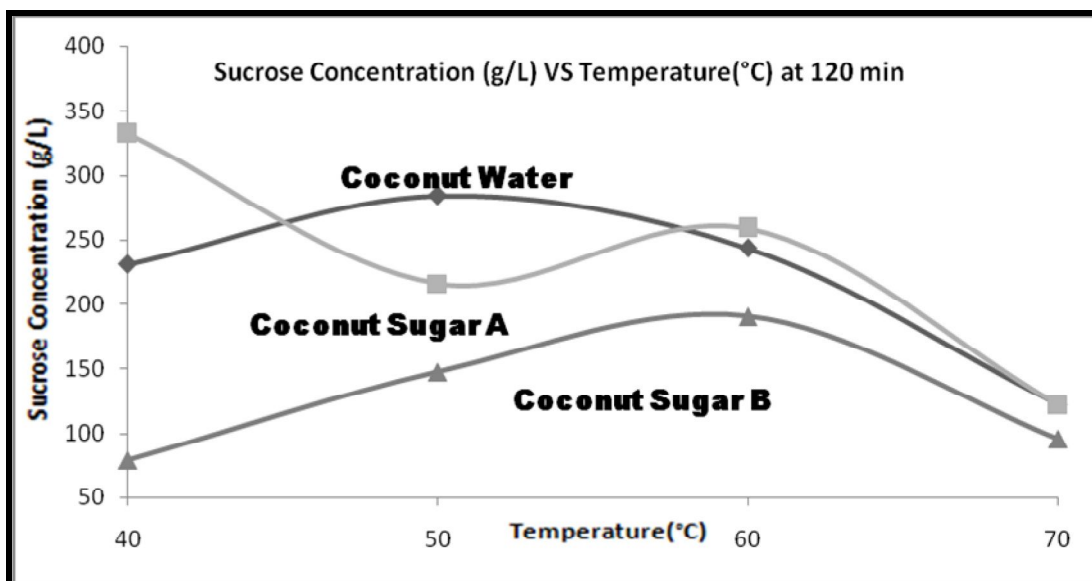
Figure 4.7 : Sucrose Concentration versus temperature at 60 minute (b)

From Figure 4.7(c), the percentage of increasing sucrose concentration for coconut sugar B was 23.81 %, while decreased for coconut sugar A, 38.78 % and coconut water, 62.71 %.



**Figure 4.7 :** Sucrose Concentration versus temperature at 90 minute (c)

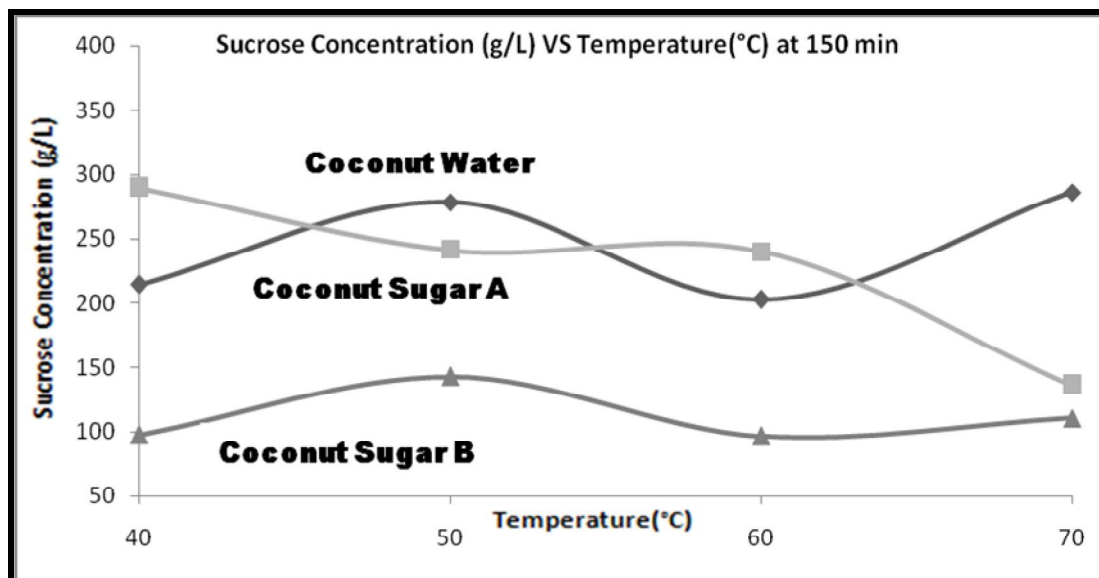
From Figure 4.7(d), the percentage of increasing sucrose concentration for coconut sugar B was 26.32 %, while decreased for coconut sugar A, 62.32 % and coconut water, 45.83 %.



**Figure 4.7 :** Sucrose Concentration versus temperature at 120 minute(d)

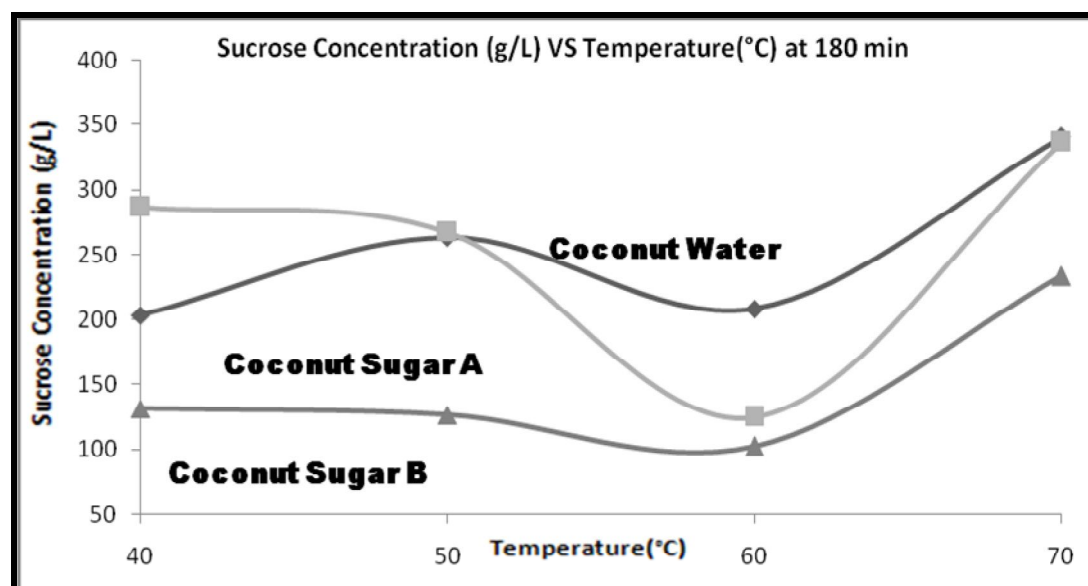


Base on Figure 4.7 (e), the percentage of sucrose concentration for coconut A was decreasing at 49.15 %, but decreased for coconut water, 30 % and coconut sugar B, 9.52 %.



**Figure 4.7 :** Sucrose Concentration versus temperature at 150 minute(e)

From Figure 4.7 (f), the percentage of increasing sucrose concentration for coconut water was 42.03 %, while 14.49 % and 48 % for coconut sugar A and coconut sugar B, respectively.



**Figure 4.7 :** Sucrose Concentration versus temperature at 180 minute(f)

From Figure 4.7 (g), the percentage of increasing sucrose concentration for coconut water was 31.67 %, while 38.75 % and 66.67 % for coconut sugar A and coconut sugar B, respectively.

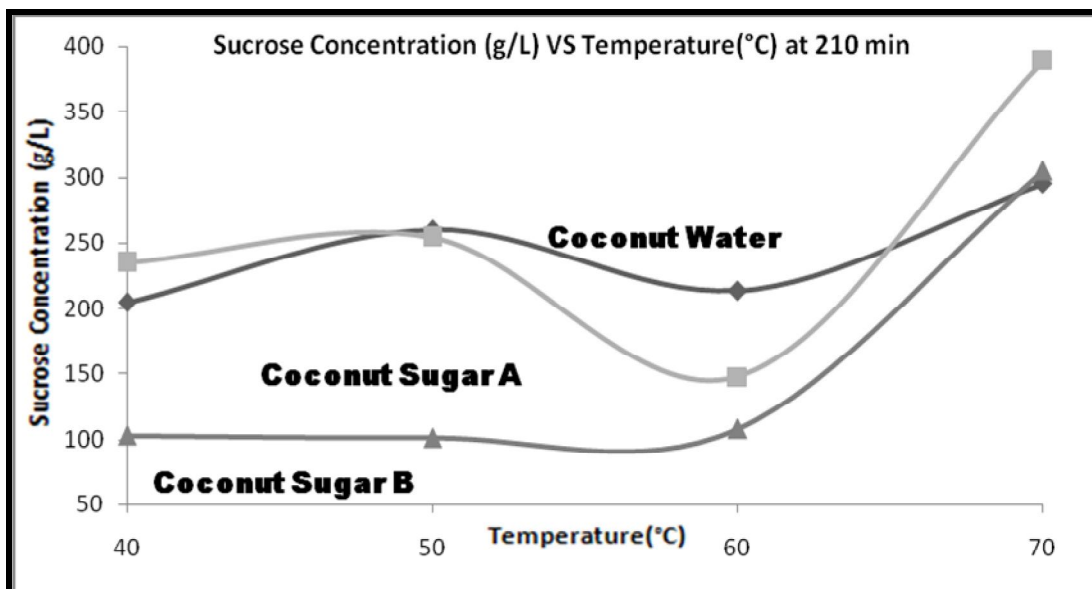


Figure 4.7 : Sucrose Concentration versus temperature at 210 minute(g)

Base on Figure 4.7 (h), the percentage of sucrose concentration for coconut A was decreasing at 19.35 %, but decreased for coconut water, 5.67 % and coconut sugar B, 30 %.

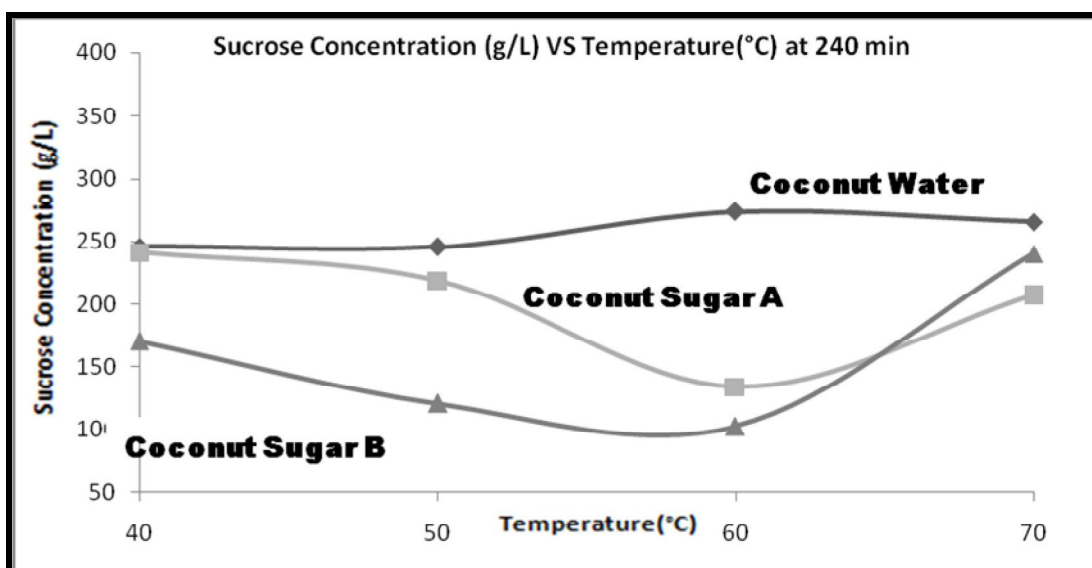


Figure 4.7 : Sucrose Concentration versus temperature at 240 minute(h)

From Figure 4.7 (i), the percentage of increasing sucrose concentration for coconut water was 10.20 %, while 30 % and 15 % for coconut sugar A and coconut sugar B, respectively.

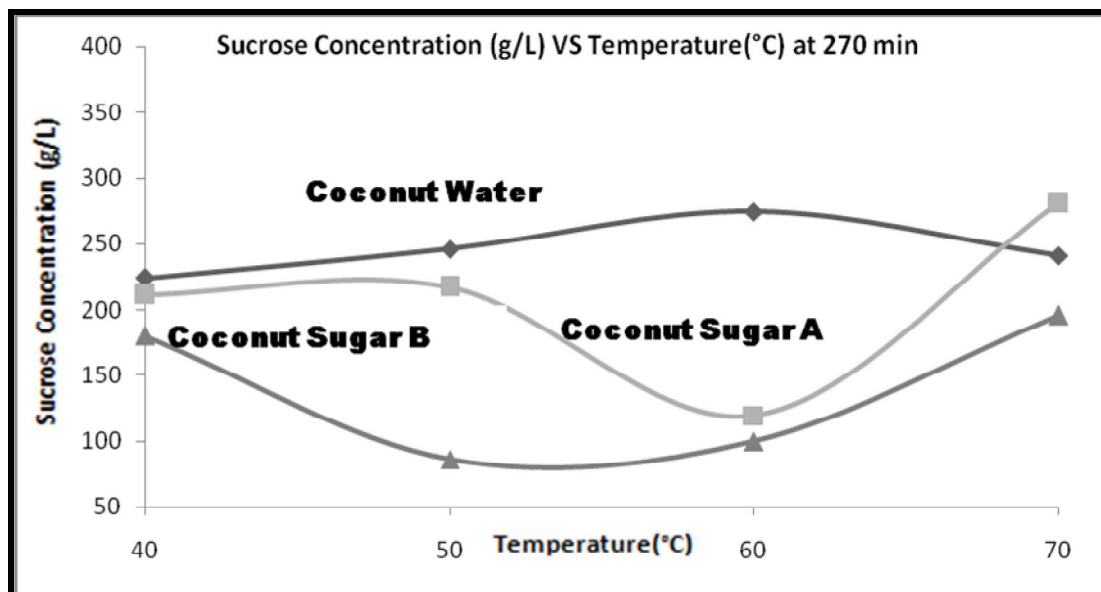


Figure 4.7 : Sucrose Concentration versus temperature at 270 minute(i)

From Figure 4.7 (j), the percentage of increasing sucrose concentration for coconut water was 25.58 %, while 20 % and 66.67 % for coconut sugar A and coconut sugar B, respectively.

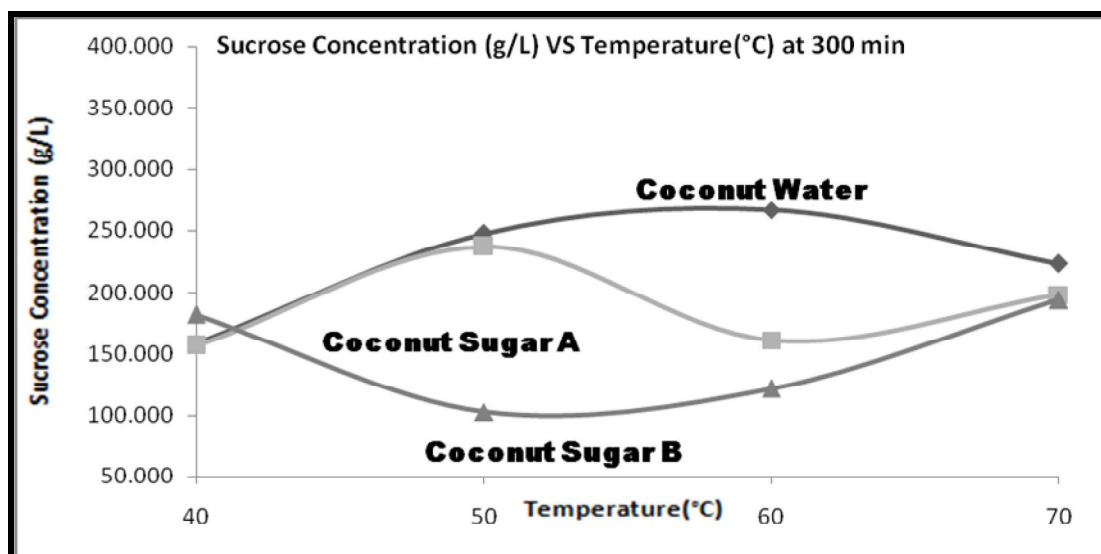


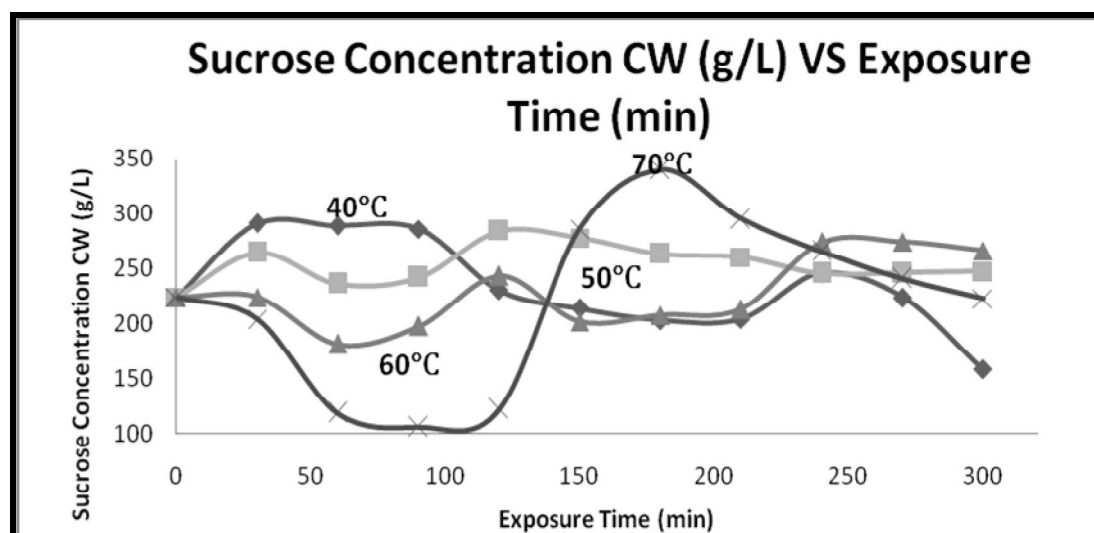
Figure 4.7 : Sucrose Concentration versus temperature at 300 minute(j)

#### 4.6 The Optimal Condition of Sucrose Production on Temperature and Exposure Time for Various Coconut Samples.

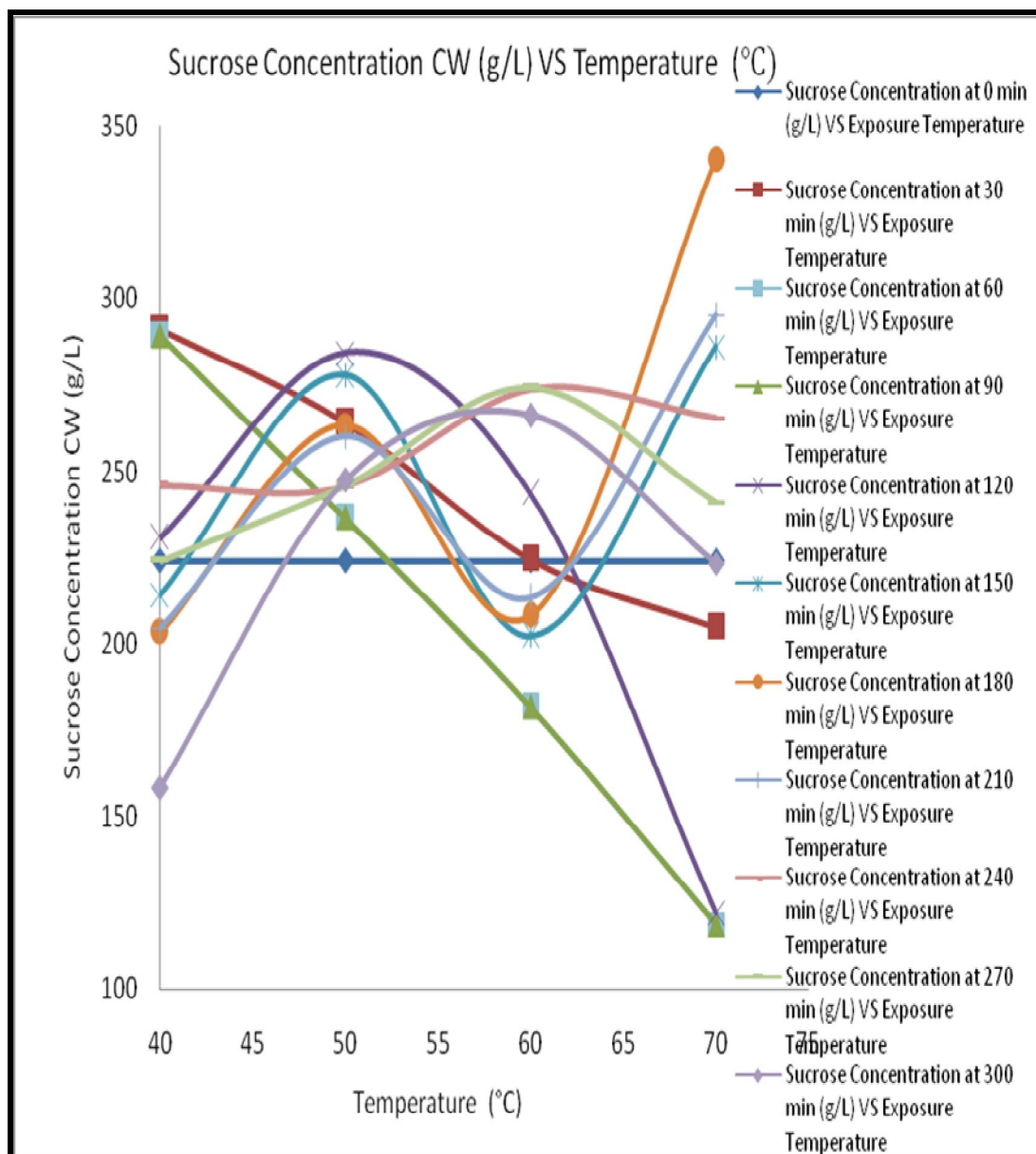
Thermal treatment plays an important role in sucrose production due to the effect of temperature and exposure time on the difference coconut samples. By increasing the temperature and exposure time until the optimum condition, thermal treatment was the best pre-treatment in order to get the highest sucrose production in various coconut samples. Referring to the Figure 4.8 to Figure 4.13, the local optimum for the temperature and exposure time for three samples is at 70°C with time 210 minutes. All the specific data can refer to Appendix A.

##### 4.6.1 Optimal Condition for Coconut Water

Base on the Figure 4.8 and Figure 4.9, the global optimum temperature and exposure time for the coconut water was at 70 °C with time 181.64 minutes with sucrose concentration 343.53 g/L. Compared the sucrose concentration before treatment, 224.05 g/L which is the percentage increasing was 34.78 %.



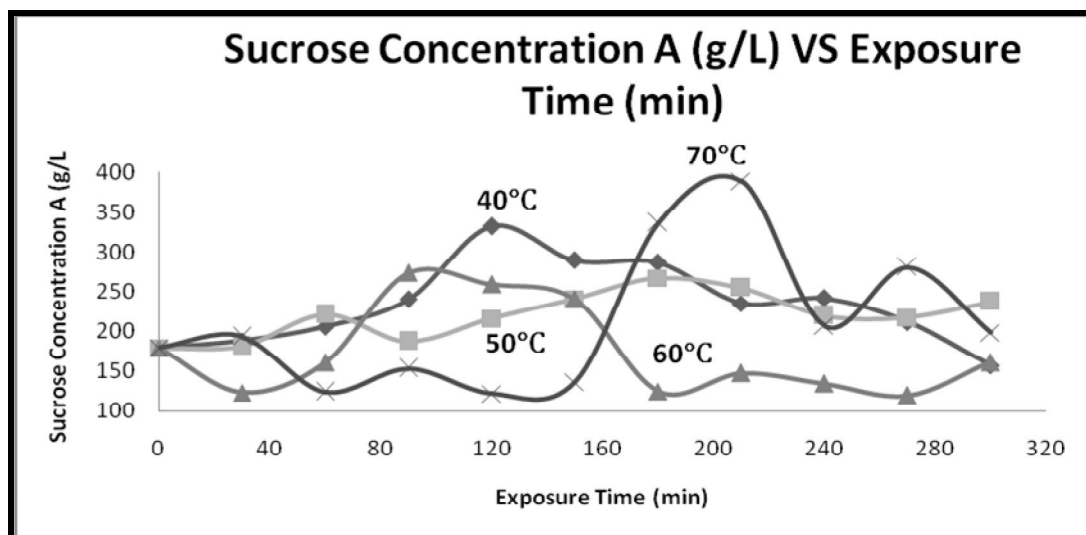
**Figure 4.8** : Sucrose concentration of coconut water versus exposure time



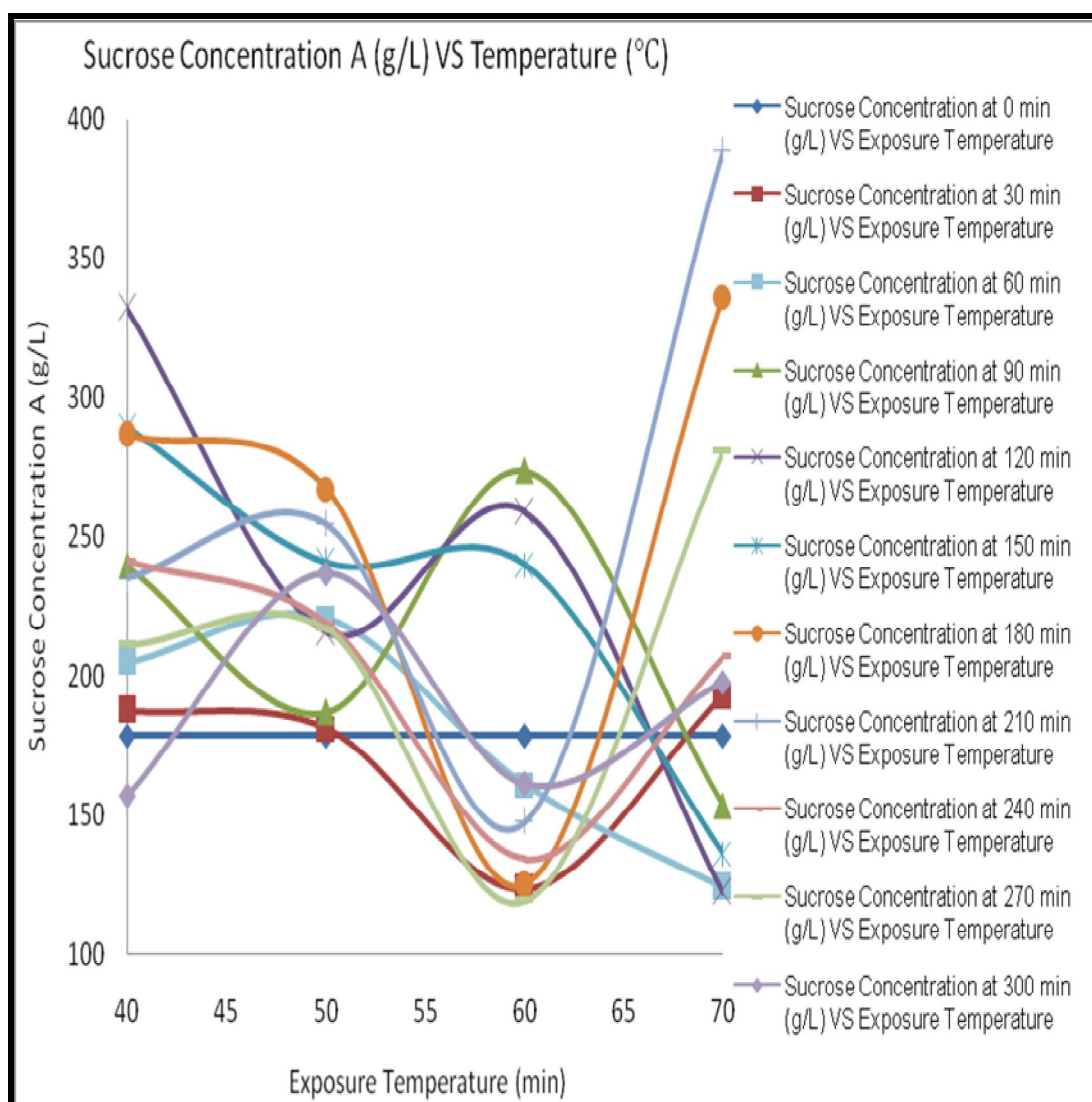
**Figure 4.9** : The sucrose concentration of coconut water versus temperature

#### 4.6.2 Optimum Condition for Coconut Sugar A

Based on the two graphs below, the global optimum temperature and exposure time was identified for the coconut sugar A at 70 °C with time 199.25 minutes with sucrose concentration 398.06 g/L. Compared to the sucrose concentration before treatment, 178.792 g/L, which is a percentage increase of 55.08 %.



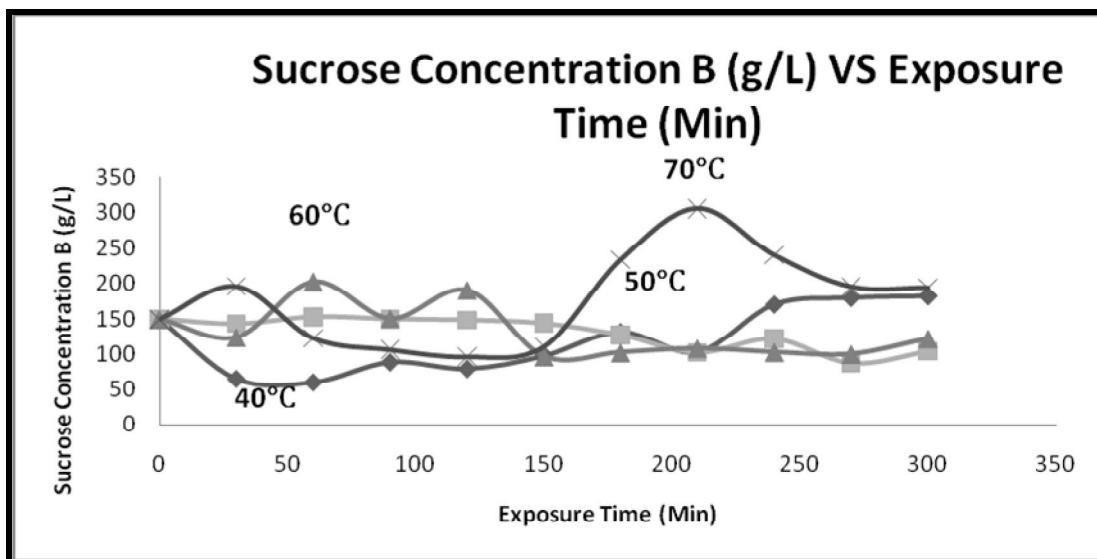
**Figure 4.10 :** Sucrose concentration of coconut sugar A versus exposure time



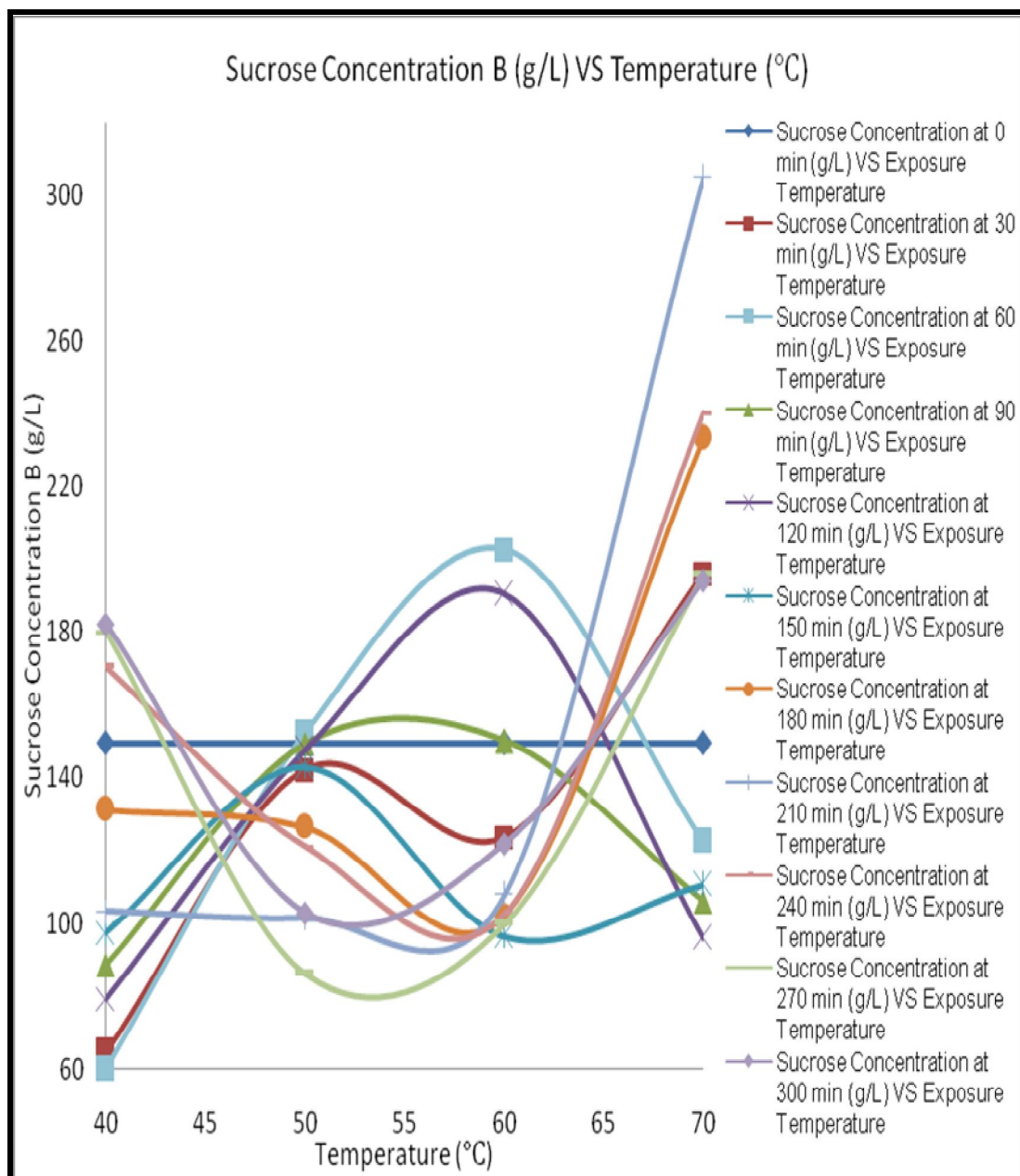
**Figure 4.11 :** Sucrose concentration of coconut sugar A versus temperature

#### 4.6.3 Optimal Condition for Coconut Sugar B

Base on the graph above, we can see that clearly of the global optimum temperature and exposure time for the coconut sugar A is at 70 °C with time 202.8 minutes with sucrose concentration 305.37 g/L Compared the sucrose concentration before treatment, 149.507 g/L which is the percentage increasing was 51.04 %.



**Figure 4.12 :** The sucrose concentration of coconut sugar B versus exposure time



**Figure 4.13 :** Sucrose concentration of coconut sugar B versus temperature



## **CHAPTER 5**

### **CONCLUSION AND RECOMMENDATION**

#### **5.0 Introduction**

This chapter will discuss about the conclusion that could be made about the experiment and recommendation for better results in the future.

#### **5.1 Conclusion**

Thermal treatment has been applied and successfully achieved in those three samples. The more higher the temperature and the longer the exposure time to the samples, the sucrose concentration obtained will be more. Optimal conditions for coconut water are at 181.64 minutes and 70°C with the concentration of sucrose produced is 343.53 g/L. Without the thermal treatment, the concentration of sucrose is only 224.05 g/L or 34.78% lower than with thermal treatment. Meanwhile, optimal conditions for coconut sugar A and coconut sugar B are 199.24 minutes and 202.08 minutes respectively, both at temperature of 70°C. At these optimal conditions, coconut sugar A produced 398.06 g/L of sucrose (55.08% higher than without thermal treatment) and coconut sugar B produced 305.37 g/L of sucrose (51.04% higher than without thermal treatment). In conclusion, after performing the thermal treatment for the three types of coconut samples, coconut sugar A is the best sample where the production percentage of sucrose has been increased until 55.08% after 199.24 minutes and at temperature of 70°C

## 5.2 Recommendation

Here is some recommendation that could be made for a better result in the future and in order to expand the research in the future.

- i. This study should be continue in using various parameters such as pH, concentration and other pre-treatment method such as pulsed electric field, high hydrostatic pressure, ultraviolet irradiation (UV) and ultrasound.
- ii. Comparing with DNS method, High Performance Liquid Chromatography (HPLC) is the best equipment to measure the results precisely.
- iii. Beside High Performance Liquid Chromatography (HPLC) equipment, microscopic analysis also one of the alternative to measuring sucrose content.
- iv. Other than using the experimental work, design expert can analyzed detail component, reducing the error occur.

## REFERENCES

- Apriyantono, A., Aristyani, A., Nurhayati, Lidya, Y., Budiyanto, S., Soekarto, S.T. (2002). Rate of Browning During Preparation of Coconut Sugar and Palm Sugar. *International Congress Series*. 1245, 275-278.
- Bennett, R. N., Mellon, F. A., and Kroon, P. A. (2004). Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair highperformance liquid chromatography negative ion electrospray mass spectrometry. *Journal of Agricultural and Food Chemistry*, 52(3).428-438.
- Chowdhury, M. G., Rahman, M. M., Tariqulislam, A.F., Islam, M.S., (2008) Processing and Preservation of Coconut Water. *j. innov.dev.strategy*. 3(1).1-5
- Damasceno, L. F., Fernandes, F. A. N., Magalhães, M. M. A., and Brito, E. S. (2008). Evaluation and Optimization of Non Enzymatic Browning of “Cajuina” during Thermal Treatment. *Brazilian Journal of Chemical Engineering*. 25(02), 313-320.
- David Van Eylen, D.V., Oey, I., Hendrickx, M., Loey, A.V. (2008). Effects of pressure / temperature treatments on stability and activity of endogenous broccoli (*Brassica oleracea* L. cv. *Italica*) myrosinase and on cell permeability, *Journal of Food Engineering*. 89, 178-186.
- Edward, C and Craigh, R.E. (2006). *Cocos Nucifera. Species Profiles for Pacific Island Agroforestry*, Version 2.
- Elevitch, C.R., and K.M. Wilkinson. (2000). *Agroforestry Guides for Pacific Islands. Permanent Agriculture. Holualoa, Hawaii*
- Finney, K.N., Ryu, C., Sharifi, V.N., Swithenbank, J. (2008). The reuse of spent mushroom compost and coal tailings for energy recovery: Comparison of thermal treatment technologies. *Bioresource Technology*.
- Gibson, G. R. and Roberfroid, M. B., (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125, 1401–1412.

- Lee, M. T., Chen, W. C., & Chou, C. C. (1999). Optimization and kinetic analysis of cholesterol oxidase production by *Rhodococcus* in submerged cultures. *Enzyme Microbial Technology*, 25, 598.
- Mabel, M. J., Sangeetha, P. T., Kalplana, P., Srinivasan, K., Prapulla, S. G. (2008). Physicochemical characterization of fructooligosaccharides and evaluation of their suitability as a potential sweetener for diabetics. *Carbohydrate Research* 343, 56-66.
- Mathlouthi, M. and Reiser, P. (Eds.). (1995). *Sucrose: Properties and Applications, UK: Chapman Hall*. 293.
- Magat, S.S. and Agustin, Y.T.V. (1997). The Philippine coconut industry. In *Proceedings of the International Cashew & Coconut Conference*, Dar es Salaam, Tanzania. 21-27
- Mohammad, F. A., Bassam, M. E. A. and Speight, J. G. (2005). Chapter 6: Sugar. *Handbook of Industrial Chemistry: Organic Chemicals New York: McGraw-Hill*, 63-199.
- Marshall, R. D.; Neuberger., (1972) A. Hen's Egg Albumin. Glycoproteins; Gottshalk, A., Ed.; Elsevier: Amsterdam,
- Maillard, L. C. (1912). Action des acides aminés sur les sucres: formation des mélanoidines par voie méthodique. *Compte rendu de l'Académie des Sciences*, 154, 66-68
- Morales, F. J.; van Boekel, M. A. J. S., (1997). A study on advanced Maillard reaction heated casain/sugar solutions: fluorescence accumulation. *Int. Dairy J.* 675-683.
- Ponce-Lee, E. L., Olivares-Perez, A. and Fuentes-Tapai, I. (2004). Sugar (Sucrose), Holograms. *Optical Materials*. 26, 5-1
- Punchihewa, P. G. and Arancon, R. (1996). CHAPTER XV COCONUT: Post-harvest Operations. *Asian and Pacific Coconut Community*. Food and Agriculture Organization (FAO).
- Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2002). Influence of media components and reaction parameters on the production of fructosyl transferase and fructooligosaccharides. *Sciences des Aliments*, 22, 277-287.
- Sardesai, V. M. and Waldshan, T. H. (1991). Natural and Synthetic Intense Sweeteners. *The Journal of Nutritional Biochemistry*. 2, 236-244.

- Turhana, I., Tetika, N., Karhana, M., Gurelb, F., Tavukcuoglu, H.R. (2008). Quality of honeys influenced by thermal treatment, *LWT* . 41, 1396-1399.
- Walkling-Ribeiro, M., Noci, F., Cronin, D.A., Riener, J., Lyng, J.G., Morgan, D.J. (2008). Reduction of *Staphylococcus aureus* and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields, *Journal of Food Engineering*. 89, 267-273.
- Zhang, W., Jia, C., Wang, Y., Kim, J. M., Jiang, P. and Zhang, X. (2008). Identification and Maillard Reaction Activities of Dilauryl Mannose Isomers Formed During Lipase-Catalysed Condensation. *Food Chemistry*.112, 421.

## APPENDIX A

**Table A.1** : The measurements of water content in various coconut sample

Sample	0 (hr)	1 (hr)	2 (hr)	3 (hr)	4 (hr)	5 (hr)	6 (hr)	Water Content (%)
Coconut Water (1) (g)	24.7001	10.7392	6.1750	2.0921	1.7561	1.7291	1.729	93.7382
Coconut Water (2) (g)	24.6948	10.7369	6.1737	1.3417	1.1817	1.1817	1.1817	
Average CW (g)	37.0475	16.1076	9.2619	2.7630	2.3470	2.3200	2.3199	
Coconut Sugar A (1) (g)	5.4718	5.1456	5.0649	5.0285	5.0146	4.9819	4.9768	8.960589829
Coconut Sugar A (2) (g)	5.4873	5.1940	5.0970	5.0353	5.0131	5.0054	5.0003	
Average CS A (g)	5.4796	5.1698	5.0810	5.0319	5.0139	4.9937	4.9886	
Coconut Sugar B (1) (g)	5.4812	5.2099	5.1191	5.1007	5.0584	4.8562	4.6087	15.98374102
Coconut Sugar B (2) (g)	5.4912	5.2291	5.1381	5.1041	5.0843	4.8846	4.6099	
Average CS B (g)	5.4862	5.2195	5.1286	5.1024	5.07135	4.8704	4.6093	

**Table A.2 : Sucrose Concentration of Samples Versus Exposure time at 40°C**

Type	Control	Exposure Time (min) for 40°C									
	0	30	60	90	120	150	180	210	240	270	300
Coconut Water	224.057	291.059	289.156	285.853	230.628	214.201	203.411	204.578	246.122	223.932	158.384
Coconut Sugar A	178.792	187.648	205.189	239.731	332.370	289.300	286.732	234.955	241.113	211.400	156.786
Coconut Sugar B	149.507	64.973	60.126	88.671	79.354	97.469	131.329	103.160	170.449	179.984	182.226

**Table A.3 : Sucrose Concentration of Samples Versus Exposure time at 50°C**

Type	Control	Exposure Time (min) for 50°C									
	0	30	60	90	120	150	180	210	240	270	300
Coconut Water	224.057	264.255	236.355	242.083	284.255	277.944	263.537	260.269	245.781	246.086	247.415
Coconut Sugar A	178.792	181.364	220.772	187.253	215.853	240.826	267.163	254.417	218.959	217.038	236.804
Coconut Sugar B	149.507	142.352	152.352	149.316	147.738	142.908	126.553	101.490	120.952	86.266	102.783

**Table A.4 : Sucrose Concentration of Samples Versus Exposure time at 60°C**

Type	Control	Exposure Time (min) for 60°C									
	0	30	60	90	120	150	180	210	240	270	300
Coconut Water	224.057	224.488	181.706	197.935	243.698	202.334	208.169	213.501	273.680	274.363	266.302
Coconut Sugar A	178.792	123.591	160.987	273.549	258.8868941	240.000	124.955	147.612	134.237	119.300	161.131
Coconut Sugar B	149.507	123.698	202.478	150.144	190.395	96.373	102.298	107.864	102.621	99.856	121.849

**Table A.5 : Sucrose Concentration of Samples Versus Exposure time at 70°C**

Type	Control	Exposure Time (min) for 70°C									
	0	30	60	90	120	150	180	210	240	270	300
Coconut Water	224.057	204.650	118.677	106.272	122.023	286.050	340.180	295.099	265.476	240.917	223.196
Coconut Sugar A	178.792	193.040	124.069	153.375	122.202	136.284	336.409	388.546	206.876	280.539	198.061
Coconut Sugar B	149.507	195.853	122.448	105.989	95.943	110.539	233.411	304.709	240.305	195.601	194.093



**Table A.6 :** Sucrose Concentration of Samples Versus Temperature at 30 min

Type	Control	Exposure Temperature for 30 (min)			
	0	40	50	60	70
Coconut Water	224.057	291.059	264.255	224.488	204.650
Coconut Sugar A	178.792	187.648	181.364	123.591	193.040
Coconut Sugar B	149.507	64.973	142.352	123.698	195.853

**Table A.7 :** Sucrose Concentration of Samples Versus Temperature at 60 min

Type	Control	Exposure Temperature for 60(min)			
	0	40	50	60	70
Coconut Water	224.057	289.156	236.355	181.706	118.677
Coconut Sugar A	178.792	205.189	220.772	160.987	124.069
Coconut Sugar B	149.507	60.126	152.352	202.478	122.448

**Table A.8 :** Sucrose Concentration of Samples Versus Temperature at 90 min

Type	Control	Exposure Temperature for 90 (min)			
	0	40	50	60	70
Coconut Water	224.057	285.853	242.083	197.935	106.272
Coconut Sugar A	178.792	239.731	187.253	273.549	153.375
Coconut Sugar B	149.507	88.671	149.316	150.144	105.989

**Table A.9 :** Sucrose Concentration of Samples Versus Temperature at 120 min

Type	Control	Exposure Temperature for 120 (min)			
	0	40	50	60	70
Coconut Water	224.057	230.628	284.255	243.698	122.023
Coconut Sugar A	178.792	332.370	215.853	258.887	122.202
Coconut Sugar B	149.507	79.354	147.738	190.395	95.943

**Table A.10 :** Sucrose Concentration of Samples Versus Temperature at 150 min

Type	Control	Exposure Temperature for 150 (min)			
	0	40	50	60	70
Coconut Water	224.057	214.201	277.944	202.334	286.050
Coconut Sugar A	178.792	289.300	240.826	240.000	136.284
Coconut Sugar B	149.507	97.469	142.908	96.373	110.539

**Table A.11 : Sucrose Concentration of Samples Versus Temperature at 180 min**

Type	Control	Exposure Temperature for 180 (min)			
	0	40	50	60	70
Coconut Water	224.057	203.411	263.537	208.169	340.180
Coconut Sugar A	178.792	286.732	267.163	124.955	336.409
Coconut Sugar B	149.507	131.329	126.553	102.298	233.411

**Table A.12 : Sucrose Concentration of Samples Versus Temperature at 210 min**

Type	Control	Exposure Temperature for 210 (min)			
	0	40	50	60	70
Coconut Water	224.057	204.578	260.269	213.501	295.099
Coconut Sugar A	178.792	234.955	254.417	147.612	388.546
Coconut Sugar B	149.507	103.160	101.490	107.864	304.709

**Table A.13 : Sucrose Concentration of Samples Versus Temperature at 240 min**

Type	Control	Exposure Temperature for 240 (min)			
	0	40	50	60	70
Coconut Water	224.057	246.122	245.781	273.680	265.476
Coconut Sugar A	178.792	241.113	218.959	134.237	206.876
Coconut Sugar B	149.507	170.449	120.952	102.621	240.305

**Table A.14 :** Sucrose Concentration of Samples Versus Temperature at 270 min

Type	Control	Exposure Temperature for 270 (min)			
	0	40	50	60	70
Coconut Water	224.057	223.932	246.086	274.363	240.917
Coconut Sugar A	178.792	211.400	217.038	119.300	280.539
Coconut Sugar B	149.507	179.984	86.266	99.856	195.601

**Table A.15 :** Sucrose Concentration of Samples Versus Temperature at 300 min

Type	Control	Exposure Temperature for 300 (min)			
	0	40	50	60	70
Coconut Water	224.057	158.384	247.415	266.302	223.196
Coconut Sugar A	178.792	156.786	236.804	161.131	198.061
Coconut Sugar B	149.507	182.226	102.783	121.849	194.093

**Table A.16 : Sucrose Concentration of Coconut Water Versus Exposure Time**

Temperature ('C)	Control	Exposure Time (min) for Coconut Water									
	0	30	60	90	120	150	180	210	240	270	300
40	224.057	291.059	289.156	285.853	230.628	214.201	203.411	204.578	246.122	223.932	158.384
50	224.057	264.255	236.355	242.083	284.255	277.944	263.537	260.269	245.781	246.086	247.415
60	224.057	224.488	181.706	197.935	243.698	202.334	208.169	213.501	273.680	274.363	266.302
70	224.057	204.650	118.677	106.272	122.023	286.050	340.180	295.099	265.476	240.917	223.196

**Table A.17 : Sucrose Concentration of Coconut Water Versus Temperature**

Time	Exposure Temperature for Coconut Water			
	40	50	60	70
0	224.057	224.057	224.057	224.057
30	291.059	264.255	224.488	204.650
60	289.156	236.355	181.706	118.677
90	285.853	242.083	197.935	106.272
120	230.628	284.255	243.698	122.023
150	214.201	277.944	202.334	286.050
180	203.411	263.537	208.169	340.180
210	204.578	260.269	213.501	295.099
240	246.122	245.781	273.680	265.476
270	223.932	246.086	274.363	240.917
300	158.384	247.415	266.302	223.196

**Table A.18 : Sucrose Concentration of Coconut Sugar A Versus Exposure Time**

Temperature ('C)	Control	Exposure Time (min) for Coconut Sugar A									
	0	30	60	90	120	150	180	210	240	270	300
40	178.792	187.648	205.189	239.731	332.370	289.300	286.732	234.955	241.113	211.400	156.786
50	178.792	181.364	220.772	187.253	215.853	240.826	267.163	254.417	218.959	217.038	236.804
60	178.792	123.591	160.987	273.549	258.887	240.000	124.955	147.612	134.237	119.300	161.131
70	178.792	193.040	124.069	153.375	122.202	136.284	336.409	388.546	206.876	280.539	198.061

**Table A.19 : Sucrose Concentration of Coconut Sugar A Versus Temperature**

Time	Exposure Temperature for Coconut Sugar A			
	40	50	60	70
0	178.792	178.792	178.792	178.792
30	187.648	181.364	123.591	193.040
60	205.189	220.772	160.987	124.069
90	239.731	187.253	273.549	153.375
120	332.370	215.853	258.887	122.202
150	289.300	240.826	240.000	136.284
180	286.732	267.163	124.955	336.409
210	234.955	254.417	147.612	388.546
240	241.113	218.959	134.237	206.876
270	211.400	217.038	119.300	280.539
300	156.786	236.804	161.131	198.061

**Table A.20 : Sucrose Concentration of Coconut Sugar B Versus Exposure Time**

Temperature ('C)	Control	Exposure Time (min) for Coconut Sugar B									
	0	30	60	90	120	150	180	210	240	270	300
40	149.507	64.973	60.126	88.671	79.354	97.469	131.329	103.160	170.449	179.984	182.226
50	149.507	142.352	152.352	149.316	147.738	142.908	126.553	101.490	120.952	86.266	102.783
60	149.507	123.698	202.478	150.144	190.395	96.373	102.298	107.864	102.621	99.856	121.849
70	149.507	195.853	122.448	105.989	95.943	110.539	233.411	304.709	240.305	195.601	194.093

**Table A.21 : Sucrose Concentration of Coconut Sugar B Versus Temperature**

Time	Exposure Temperature for Coconut Sugar B			
	40	50	60	70
0	149.507	149.507	149.507	149.507
30	64.973	142.352	123.698	195.853
60	60.126	152.352	202.478	122.448
90	88.671	149.316	150.144	105.989
120	79.354	147.738	190.395	95.943
150	97.469	142.908	96.373	110.539
180	131.329	126.553	102.298	233.411
210	103.160	101.490	107.864	304.709
240	170.449	120.952	102.621	240.305
270	179.984	86.266	99.856	195.601
300	182.226	102.783	121.849	194.093