UNIVERSITI PUTRA MALAYSIA

SUGAR RECOVERY FROM BAKERY WASTE BY ENZYMATICHYDROLYSIS

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SUGAR RECOVERY FROM BAKERY WASTE BY ENZYMATIC HYDROLYSIS

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ABSTRACT

Bakery industry is one of the largest industry in food production that known as global daily need. However, there is an environmental issue regarding bakery waste. Bakery waste can be hydrolyzed to obtain its sugar to alleviate environmental issue. In this study, enzymatic hydrolysis is used to recover sugar from bakery waste. The raw material used were croissant and doughnut and and amyloglucosidase was the enzyme used for enzymatic hydrolysis treatment. The objectives were to analyze the effects of enzyme concentration (60U/L to 600U/L) and process temperature (45 to 65°C) on sugar yield. Then, optimization was done on the process parameters in order to achieve higher yield of sugar. The optimal conditions for enzymatic hydrolysis was obtained at 578U/L concentration of amyloglucosidase and temperature of 53 °C for croissant, which yield of 29865mg/L of total sugar. For doughnut, optimal conditions was at 375 U/L concentration of amyloglucosidase and temperature of 50 °C that yield 15105mg/L. In general, process temperature and concentration of enzyme bring significant effect to sugar yield for the treatment using enzymatic hydrolysis.

ABSTRAK

Industri roti adalah salah satu industri terbesar dalam pengeluaran makanan yang dikenali sebagai keperluan harian dunia. Walau bagaimanapun, terdapat isu alam sekitar mengenai sisa roti. Sisa roti boleh dihidrolisiskan untuk mendapatkan gula untuk mengurangkan masalah alam sekitar. Dalam kajian ini, hidrolisis enzimatik digunakan untuk memulihkan gula dari sisa roti. Bahan mentah yang digunakan ialah croissant dan donat dan dan amyloglucosidase adalah enzim yang digunakan untuk rawatan hidrolisis enzimatik. Objektifnya ialah untuk menganalisis kesan kepekatan enzim (60U/L to 600U/L) dan suhu proses (45 hingga 65 °C) terhadap hasil gula. Kemudian, pengoptimuman dilakukan pada parameter proses untuk mencapai kadar gula yang lebih tinggi. Keadaan optimum untuk hidrolisis enzimatik diperoleh pada kepekatan 578U/L amyloglucosidase dan suhu 53 °C untuk croissant, yang menghasilkan 29865mg/L jumlah gula. Bagi donat, keadaan optimum ialah 375 U/L kepekatan amyloglucosidase dan suhu 50 °C yang menghasilkan 15105mg/L. Secara umum, suhu proses dan kepekatan enzim membawa kesan yang signifikan kepada hasil gula untuk rawatan menggunakan hidrolisis enzimatik.

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LIST OF ABBREVIATIONS

FAO – Food and Agriculture Organization of United Nations

DP – Degrees of polymerization

GHG – Green house gas

NS – Nelson-Somogyi

DNS – Dinitrosalicylic acid

HPLC – High performance liquid chromatography

RSM – Response Surface Methodolgy

CHAPTER 1

INTRODUCTION

1.1 Overview

Bakery industry is one of the largest industry in food production and it varies in term of production scales and types of products. Baking is a traditional activity and it has been established for several thousand years. Bakery industry produces various of bakery products such as bread, muffin, croissant, pie, doughnut and biscuits. These products are known as global daily need due to its acceptabilities in all generations from youngsters to adults.

The general processes of bakery production include milling, mixing, fermentation, baking, cooling and finishing which is illustrated in Figure 1. For the market of bakery industry, annual industry sales were $14.7 billion, $16.6 billion, and $17.7 billion in 1998, 2000, and 2002, respectively; the average

weekly unit sales were $9,890, $10,040, and $10,859 during the same periods. More than 80% of the market's supply is contributed by large scales of processing plant (K.Wang Lawrence et al., 2006).

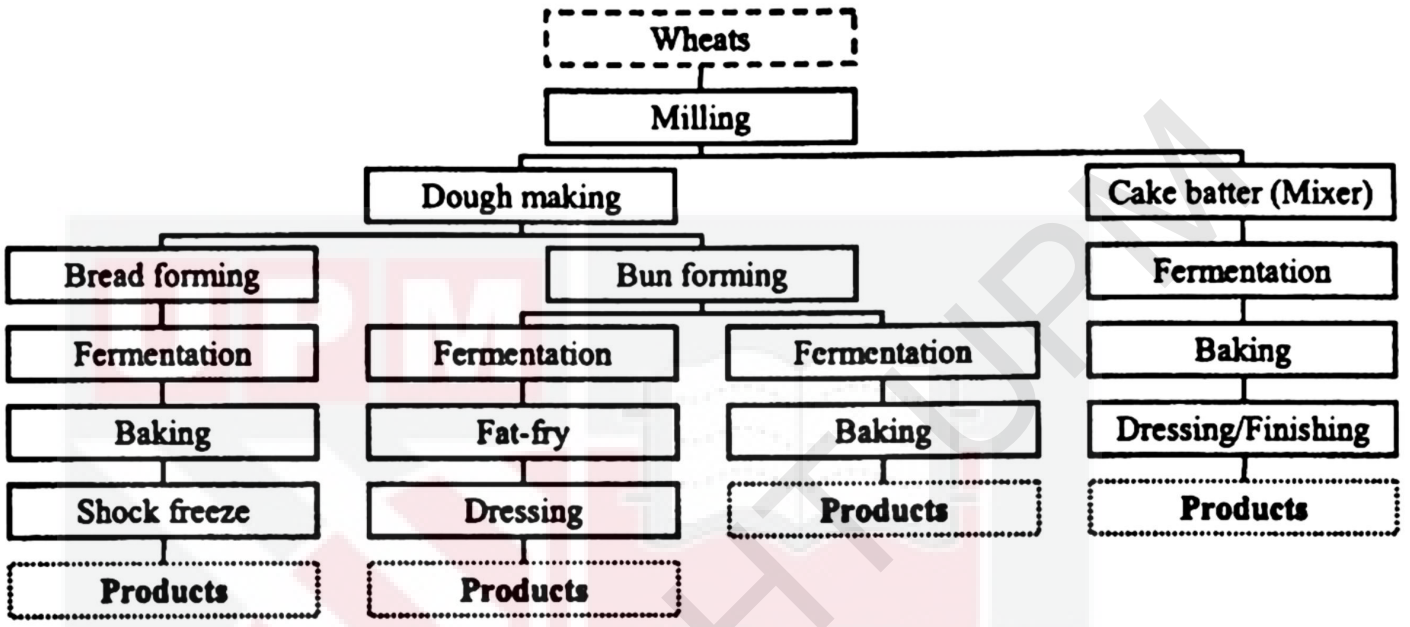


Figure 1 : General Processes Diagram in Bakery Industries

Bakery waste can be resulted due to several reasons. For instance, bakery waste becomes unsold after only 24hours and they are removed from the food market to be discarded as waste. Besides, they can result from inappropriate sizing. In a similar way, bakery waste can result from inappropriate size of production batch and result in wasted dough. They can be low quality products which are default size or texture, burnt products (Chandrasekaran, 2013). Improper handling of bakery waste could lead to environmental and financial issues.

Bakery waste can be utilized because it has rich in nutrient especially carbohydrates. Carbohydrates can be divided into four main groups which are monosaccarides, disaccharides, oligosaccharides and polysaccharides. Each of the groups are representing different degrees of polymers. Thus, bakery waste can be

hydrolyzed to obtain its sugar to alleviate the environmental issue rise from bakery waste.

1.2 Problem Statement

A recent studies from Food and Agriculture Organization of United Nations (FAO) states that about one-third of food produced for human consumption get wasted, which is about 1.3 billion ton per year (Gustavsson et al, 2011). It is wasted from initial production from agriculture until final household consumption. Food waste can lead to environmental issues and economical issues. Food waste can contribute to green house gas (GHG) especially carbon dioxide (CO_2) by decomposition of wasted food after disposal in landfills and eventually it brings impact to climate change (Venkat, 2012). Economically, food waste brings negative impact to both farmer and consumer.

Food waste can be utilized by extracting its nutrient whereby it is the purpose of doing the research. Bakery waste is categorized as high level of waste in the household and it is found that bakery waste even ranked to the most frequently waste food (Ratinger et al., 2015). There are rich in carbohydrates in the bakery waste. Thus, production of sugar can be done to utilize the bakery waste which can alleviate the negative impacts to human and environment.

In this research, optimization of sugar yield by using different parameter is being determined. Different temperature and concentration of the enzyme is used to hydrolyze the bakery waste into simple sugar by using enzymatic hydrolysis. The bakery waste is also being analyzed to obtain its total carbohydrates and reducing sugar as well as the pH analysis.

1.3 Objectives

The specific objectives for this study is listed below:

1. To analyze the effect of concentration and temperature on sugar yield in bakery waste
2. To characterize the total carbohydrate, reducing sugar and pH of bakery waste after the enzymatic hydrolysis and the optimization of process condition.

CHAPTER 2

LITERATURE REVIEW

2.1 Carbohydrates: Chemistry and Classification

Carbohydrates are the most abundant components which can be found in cereals, fruits, vegetables, and legumes. It is one of the most important energy source in human nutrition. The texture and flavor of foods is contributed by carbohydrates. Carbohydrates can be characterized into four class: monosaccharides, disaccharides, oligosaccharides, and polysaccharides which depends on the molecular structure and degree of polymerization (Stylianopoulos, 2013).

2.1.1 Monosaccharides

The simplest form of carbohydrate is known as monosaccharides and it cannot be hydrolyzed to smaller units. Monosaccharides are known as basic unit of complex sugar. The optical activity of monosaccharides are affected by of asymmetrical carbons in monosaccharides and functional groups. Monosaccharides are optically active, which means when polarized light is passed through a solution, the plane of light will be rotated to the left (L-form) or to the right (D-form) which shown in Figure 2. Consequently, stereoisomers are formed that have similar structures of the same compound.

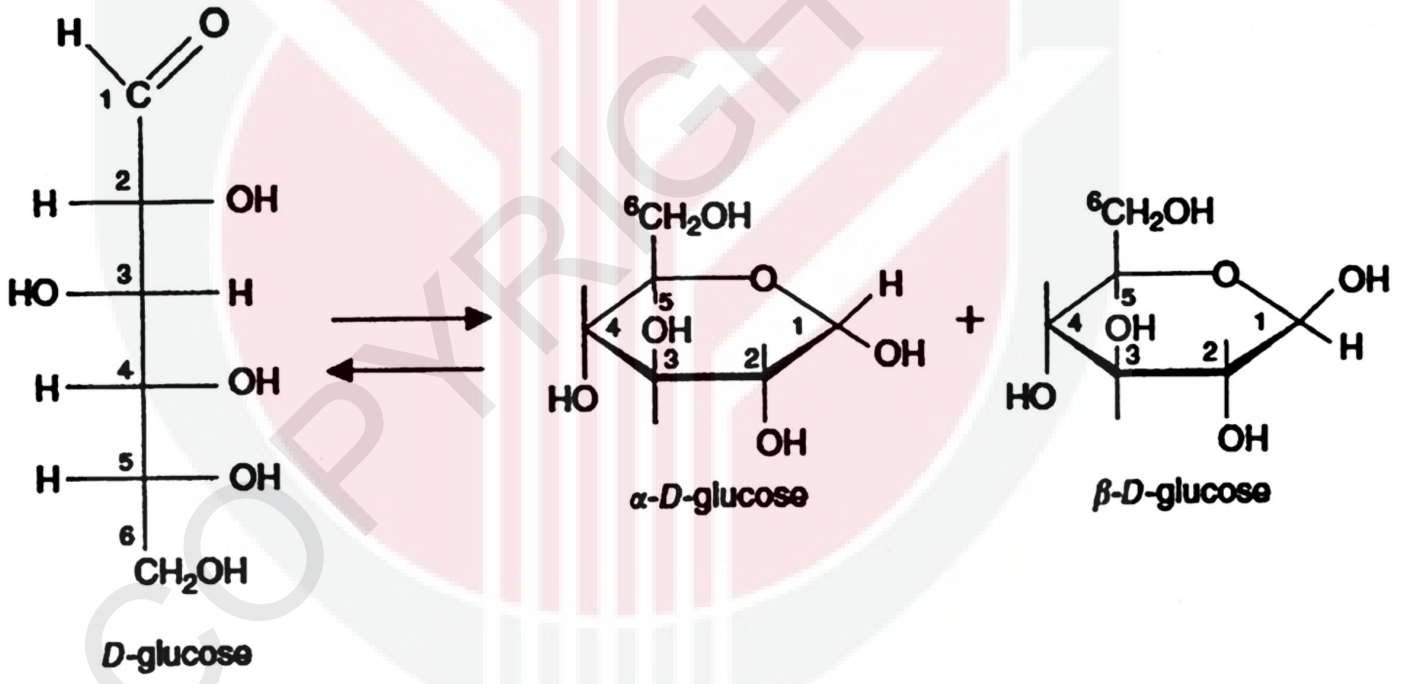


Figure 2: Glucose Molecule in Open Chain and Cyclic Pyranose Ring

(Stylianopoulos, 2013)

The typical monosaccharides and their derivatives and their functions are listed in Table 1. Table 1 shows that common monosaccharides and its derivatives that categorized by different classes.

Table 1: Some Nutritionally Common Monosaccharides and Derivatives

(Adapted from: Stylianopoulos, 2013)

Class	Species	Significance
Hexoses	D-Glucose	Major cell fuel, unbound in body fluids and tissues, building block of several polysaccharides
	D-Fructose	Cell fuel, constituent of sucrose
	D-Galactose	Cell fuel, constituent of galactose
	D-Mannose	Constituent of plant cell wall polysaccharides and gums
Pentose	L-Arabinose	Constituent of plant cell wall polysaccharides
	D-Ribose	RNA Constituent

Table 1 shows that typical monosaccharides and its derivatives that categorized by different classes.Glucose is known as the most abundant sugar in carbohydrates and give significant roles to nutrition and human's health. It is also the fundamental unit of carbohydrates. Galactose and fructose are alsofunction as cell fuel.

2.1.2 Polysaccharides

Polysaccharides is made up of long chains of simple sugar residues (10 or more) linked by glycosidic bonds. A large group of monosaccharide units give rise to polysaccharides. The characteristics of polysaccharides are affected by the types of monosaccharides, the type of bond between monosaccharides, and the extent of the chain.

Polysaccharides that bonded by α-linkages have a helical shape, for example starch molecule. For those with β-linkage,it generally the structure is linear or flat ribbon-like molecule, for example cellulose shown in Figure 3.

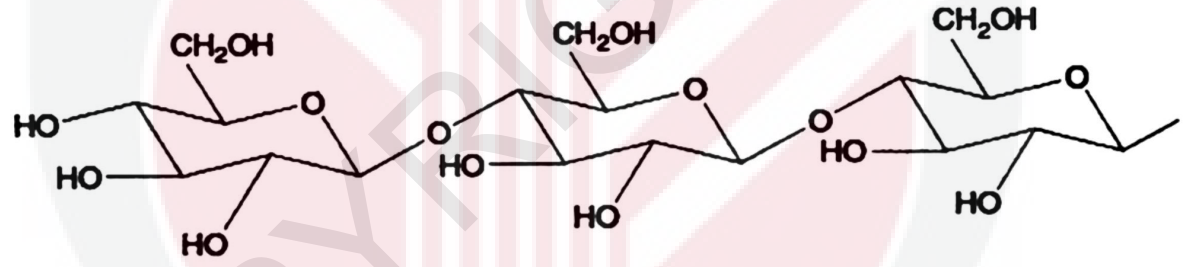


Figure 3: The structure of Cellulose

2.1.3 Reducing Sugar

The presence of aldo- and keto groups and formation of hemiacetal or acetal groups is the reason why monosaccharides have reducing properties. Important compounds formed in this way are sugar alcohols. For example, the reduction of the aldo group of glucose produces sorbitol. Disaccharides have also same reducing properties, except for sucrose. Sucrose is non-reducing sugar due to no free hemiacetal group. Polysaccharides generally consist of one reducing group at the

terminal end of the polymer. It results in lower reducing properties (Stylianopoulos, 2013).

2.1.4 Bakery and Bakery Waste

Starch is the most abundant type in carbohydrates. It was found in many food especially bakery such as muffin, doughnut, croissant and bread. Bakery industries play a crucial role in the human diet because it contains the major nutrients and known as good source of energy. Bakery products are high in starch and starch is composed of glucose units linked by α-(1,4)-glycosidic bonds and α-(1,6)-glycosidic bonds. Basically, two basic structural units in starch are amylose and amylopectin. (Blazek, 2008; Hódsági, 2011).

In bakery products, they are rich in starch where amylopectin and amylose form the starch granule, amylose and amylopectin are semi-crystalline in structure that has alternating amorphous and crystalline shells. There are minor components in starch granules between amylopection and amylose such as lipids, protein monostarch phosphate ester groups, and phospholipid (Hadnadev et al., 2009).

However, bakery products are relatively high level of wastes at the household stage. Bakery waste can be utilized because it has rich in nutrient especially carbohydrates. Food waste can contribute to green house gas (GHG) especially carbon dioxide (CO_2) by decomposition of wasted food after disposal in landfills and eventually it brings impact to climate (Venkat, 2012).

2.2 Enzyme

2.2.1 Overview

Enzyme is a biological catalyst that acts as important component in a biological reaction. The use of enzyme as catalyst is widely used since ancient time. Nowadays, enzyme is being used in various sectors in industries. They are used in detergent, paper industry, textile industry, food industry and many others industrial application.

2.2.2 Action of Amyloglucosidase of Aspergillus Niger

Amyloglucosidase is an enzyme that hydrolyze the internal of α-1,4-glycosidic bond and α-1,6-glycosidic bond in carbohydrates to yield sugar products (Pazur & Ando, 1959). Amyloglucosidase is the extracellular enzyme that converts starch to dextrins and glucose. Besides, the enzyme is used in the starch-processing industry in the commercial production of D-glucose from corn syrup (Parker et al.,2010).

2.2.3 Enzymatic Hydrolysis of Amyloglucosidase

2.2.3.1 Effect of Temperature to Enzymatic Activity

The reducing sugar yield varies with the effect of temperature. The Figure 4 below shows the effect of temperature to sugar yield by alpha-amylase and glucoamylase.

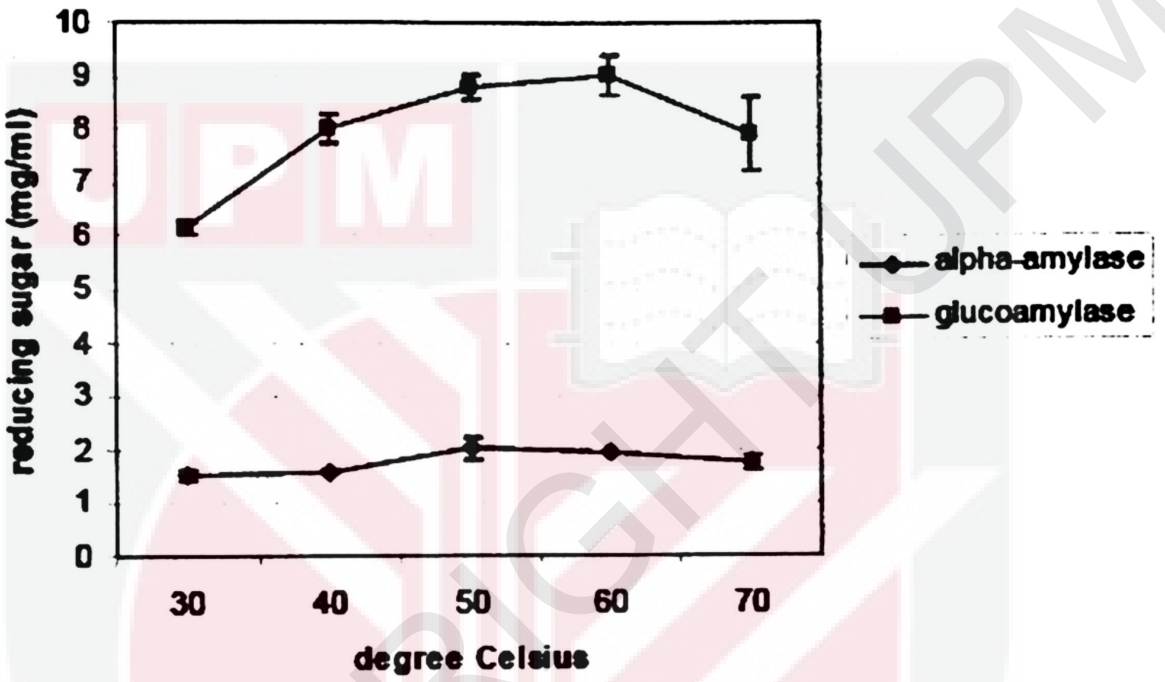


Figure 4: Effect of Temperature to Sugar Yield

(Adapted from:Chotineeranat et al.,2004)

Temperature of enzyme is one of the most significant parameter that affect the performance of the enzymatic activities for hydrolyzation. Optimum temperature is required by enzyme to have highest enzymatic activity to produce highest yield of sugar. Based on Figure 4, the graph has shown the effect of temperature of enzyme to the sugar yield from cassava pulp which is rich in carbohydrates. It can be seen that the highest yield of sugar is produced at 50°C to 60°C for Amyloglucosidase (Chotineeranat et al., 2004). Based on figure 4, apparently the reducing sugar yield hydrolyzed by Amyloglucosidase is higher compare to alpha amylase.

2.2.4 Comparison of Two Methods for Sugar Estimation

There are several types of methods for analysis of sugar have been applied in the carbohydrase activity measurement. However, Nelson-Somogyi (NS) assay and the 3,5- dinitrosalicylic acid (DNS) assay are the most popular methods being used by many researchers. Other method such as sodium 2,2 –bicinchoninate or potassium ferricyanide are seldom been used. IUPAC commission has been recommended on biotechnology to measure standard cellulase activities. DNS assay has also been used for measuring activities of other carbohydrases, such as amylases and this assay dominates in laboratories throughout the world (Gusakov et al., 2011).

DNS assay was founded by Sumner and co-worker for the determination of reducing sugar, is composed of dinitrosalicylic acid, Rochelle salt, phenol, sodium bisulfite, and sodium hydroxide. In the chemistry of testing, 3,5-dinitrosalicylic acid is reduced to 3-amino-5-nitrosalicylic acid. Meanwhile, the aldehyde groups are oxidized to carboxyl groups (Miller, 1959).

2.2.5 Total Carbohydrates Analysis

Phenol-sulphuric acid method is known as the easiest and most reliable among the calorimetric methods for carbohydrates analysis due to its sensitivity and simplicity. The sulphuric acid causes all non-reducing sugars to be converted to reducing sugars, so that this method determines the total sugars present. Other methods sensitive but are not as convenient as phenol-sulphuric acid method (Masuko et al., 2005). Figure 5 and Figure 6 shows that the influence of sulphuric acid and 5% phenol to absorbance at 490nm.

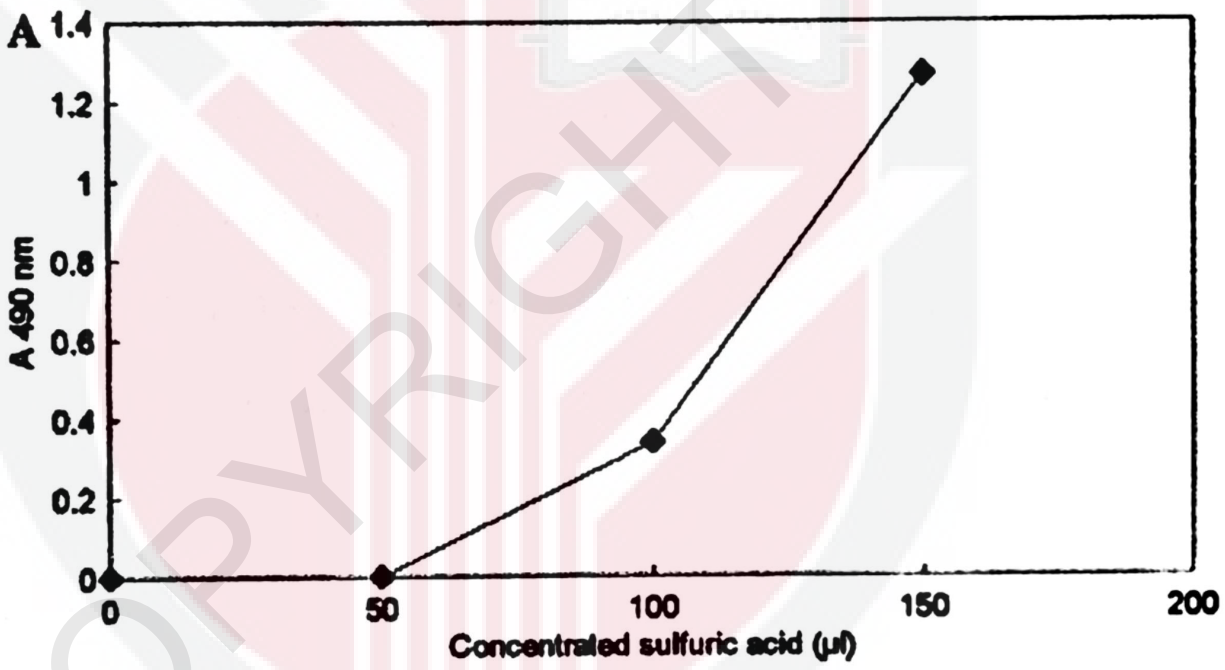


Figure 5 : Influence of Sulphuric Acid to Absorbance at 490nm

Adapted from: (Masuko et al., 2005)

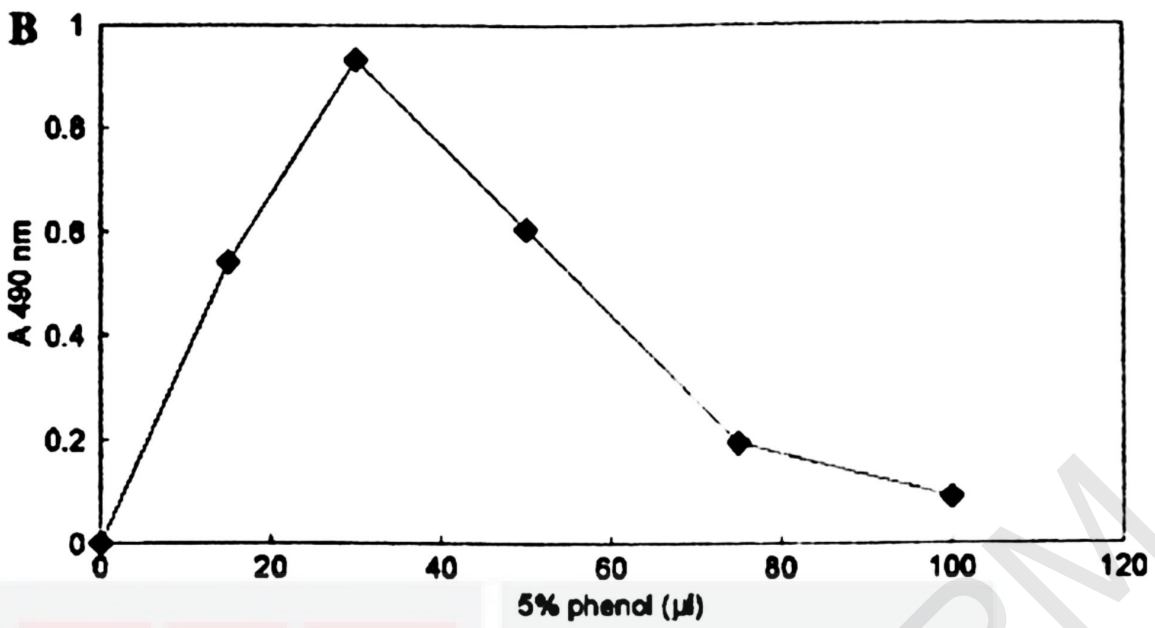


Figure 6 : Influence of 5% Phenol to Absorbance at 490nm

Adapted from: (Masuko et al., 2005)

For the optimal reaction conditions, different factors have been considered including sample size, volume of 5% phenol and volume of concentrated sulphuric acid. According to (Masuko et al., 2005), 50 µl of sample size is sufficient to allow addition of sulphuric acid and 5% phenol solutions. 150 µl of concentrated sulphuric acid and 30 µl of 5% phenol were added to 50 µl of sample immediately to get maximal absorbance as illustrated in Figure 5and Figure 6.

2.2.6 High Pressure Liquid Chromatography Separation

Liquid chromatography is a separation method in which a mixture of components is resolved into its constituent parts by passage through a chromatographic column. It is carried out by passing the mobile phase, containing the mixture of the components, through the stationary phase, which consists of a column packed with solid particles. Figure 7 shows the effect of number residue on retention time.

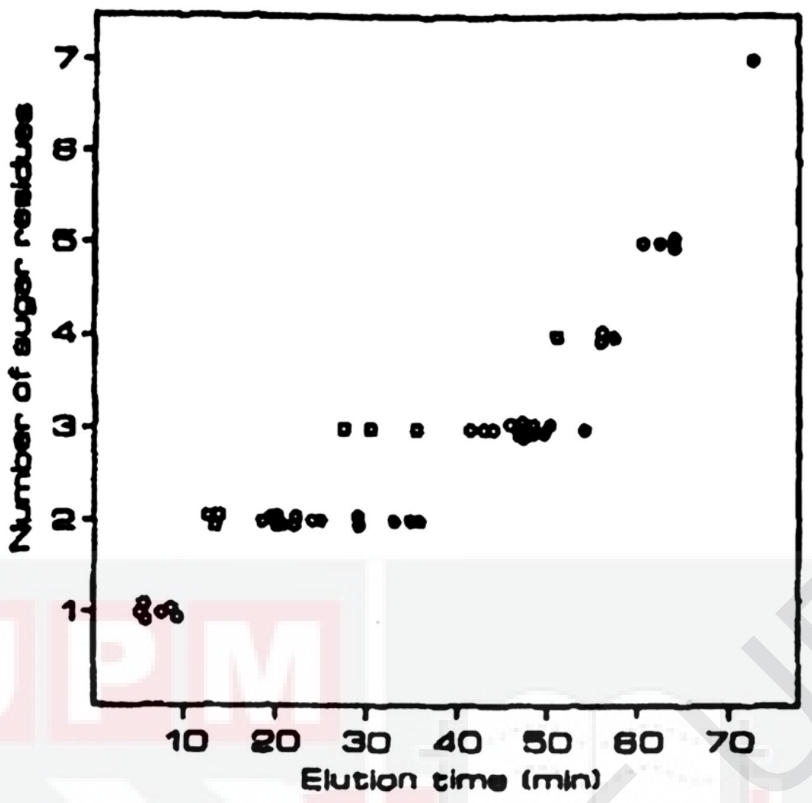


Figure 7 : Effect of Number of Residue on Retention Time

Adapted from: (Blanken et al., 1985)

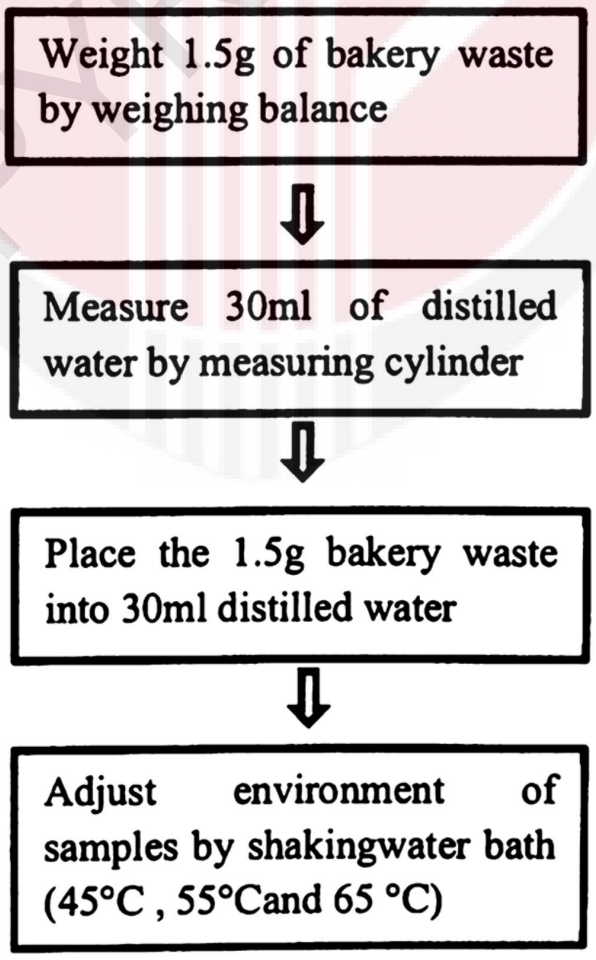
Based on the Figure 7, the larger the number of sugar residue, the longer the retention time. In average, the time for monosaccharides is 7 minutes, disaccharides is 23.1 minutes, trisaccharides is 44.4 minutes, tetrasaccharides is 56.4 minutes, pentasaccharides is 62.6 minutes and heptasaccharides is 72.7 minutes. Increase in retention time is shown by oligosaccharides having 1,6 glycosidic bond (Blanken et al., 1985).

CHAPTER 3

METHODOLOGY

3.1 Overall Process Flow Chart

Figure 8 shows the overall process flow chart for the entire experiment of enzymatic hydrolysis of bakery waste.



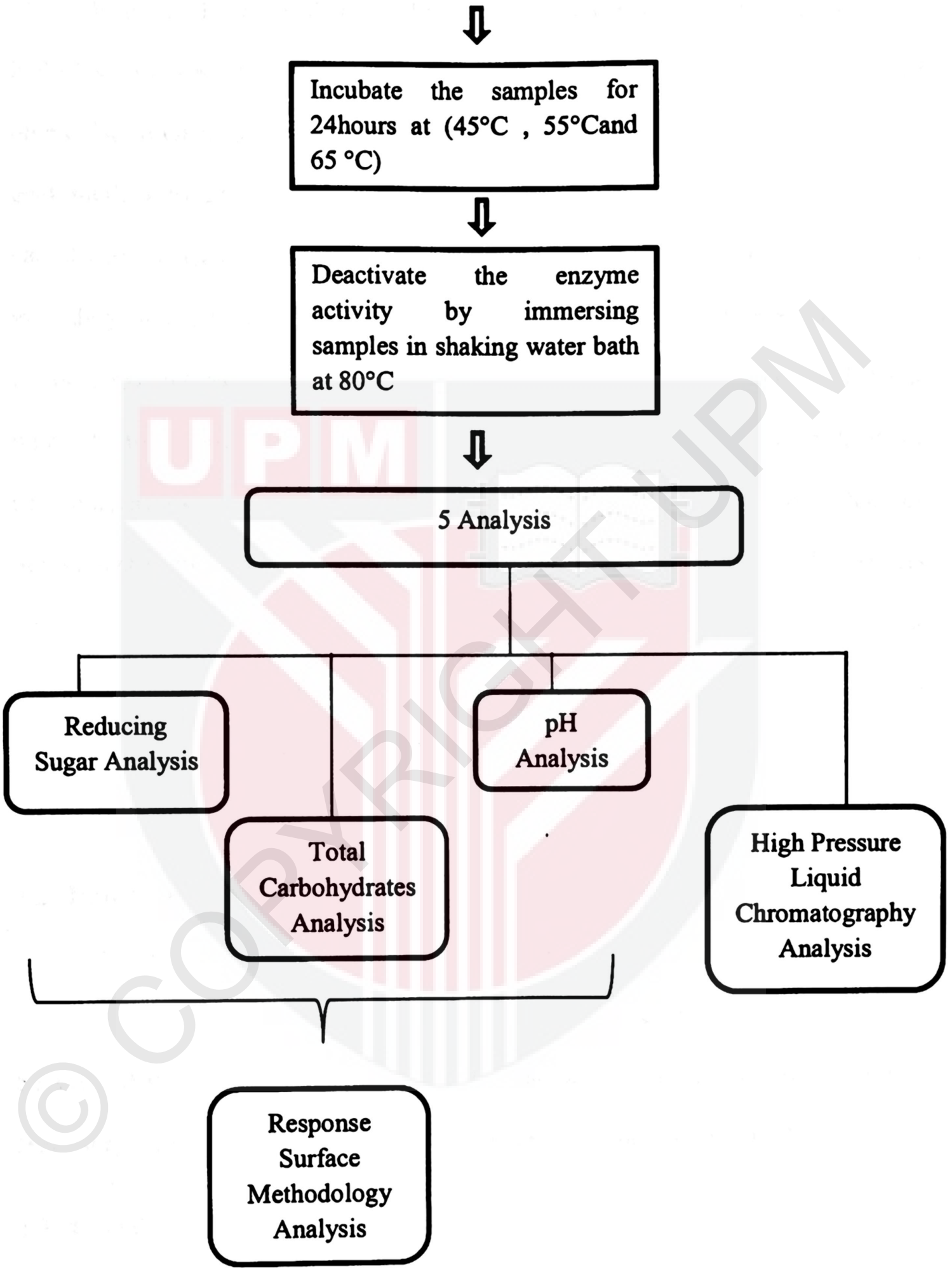


Figure 8: Process Flow Chart for Sugar Recovery from Bakery waste by Enzymatic Hydrolysis

Based on Figure 8, there are five analysis are carried out after the enzymatic hydrolysis of bakery waste by incubating the bakery waste into incubator for 24 hours. The total reducing sugar of all samples are analyzed by 3,5-dinitrosalicylic acid method to see the amount of reducing sugar for each sample. For total carbohydrates analysis, the method of analysis is phenol-sulfuric acid method which is slightly similar to 3,5-dinitrosalicylic acid method. The method detects all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides. This is different with 3,5-dinitrosalicylic acid method as the concentrated sulfuric acid breaks down any polysaccharides, oligosaccharides, and disaccharides to monosaccharides. Subsequently, the samples are tested with pH meter to see the results. The bakery waste samples are sent to high pressure liquid chromatography (HPLC) analysis to see the amount of various of sugar present in the samples. Eventually, response surface methodology is used to find the optimized condition for enzymatic hydrolysis of bakery waste in different conditions.

3.2 Raw Material

The materials which to be used in this research is croissant and doughnut which are rich in carbohydrates. The material is obtained from a local bakery in Selangor that have no sign of mold growth. The material is cut to powder form by laboratory knife mill and sealed in vacuum pack and kept in frozen at -20°C.

3.3 Chemical Reagents

Amyloglucosidase is an enzyme to hydrolyze the bakery waste into sugar during enzymatic hydrolysis. The amyloglucosidase used is 120U/mg and it is purchased via LGC Scientific Sdn. Bhd. located in Balakong, Selangor. 5% phenol

and concentrated sulphuric acid are used during analysis of total carbohydrates. DNS reagent which includes 3,4-dinitrosalicyclic acid, potassium sodium tartrate tetrahydrate and sodium hydroxide (anhydrous) are required for estimation of concentration of sugar.

3.4 Equipment

- Spectrophotometer
- Incubator
- Shaking water bath machine
- High Performance Liquid Chromatography (HPLC)

3.5 Enzymatic Hydrolysis

Physical pre-treatments are required which involve the breakdown of the bakery waste into smaller particles so that the samples are more amenable to subsequent enzymatic hydrolysis (Hodgins et al., 2001). The total hydrolysis time is set as 24 hours and the pH is set at 5.5 where pH set is fallen into effective pH range. After incubation of 24 hours, the samples are place into 80 °C to deactivate the enzyme. The substrate concentration is set at 15% (w/v) where it is suggested as the for enzymatic hydrolysis (Hudečková et al., 2017). After enzymatic hydrolysis, the samples are centrifuged at 8000rpm before analysis is made to get a clear hydrolyzates (Jamaludin et al., 2013).

3.5.1 Effect of Temperature

The effect of temperature on enzymatic hydrolysis of bakery waste is carried out at various temperature. The samples are incubated with enzymes at temperatures of 45, 55 and 65°C to determine the effect of various temperature to sugar yield.

This range is selected the enzymic activity of the product is effective in the temperature range between 40°C and 65°C based on the technical information sheet. The samples are put into waterbath with respective temperature to ensure the environment of the sample is achieved before putting into incubator for 24hours.

3.5.2 Effect of Concentration

In order to study the effect of amyloglucosidase concentration on the enzymatic hydrolysis of bakery waste, three concentration of the enzyme, ranging from 60U/L to 600U/L are used to determine the sugar yield. The range selected is to determine the yield of sugar at the minimum enzyme dosage. This is consider as preliminary test since there is no research on enzymatic hydrolysis of bakery waste.

3.6 High Pressure Liquid Chromatography

The samples with corresponding temperature and concentration are injected into vials and labeled accordingly. The column used is Agilex Hi-Plex H 300x7.7mm. The injection volume to the HPLC will be 20μL , mobile phase is distilled water, flow rate is set at 0.3ml/min , column temperature is 65°C and run for 40 minutes. The detector used is refractive index (RI). Quantitative analysis is done by preparing sugar standard curve of glucose, fructose, mannose, sucrose and galactose.

3.7 DNS Method

Prepare the bakery waste samples in different test tubes and one tube as blank by using distilled water. To each tubes, add 2mL DNS reagent to all the test tubes and mix well. Subsquently, place the tubes in boiling water and finally cool the tubes to room temperature and measure the absorbance at 540 nm by spectrophotometer.

3.8 Phenol – Sulphuric Acid Method

A 0.02mL hydrolysates of a sample solution is mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Next, 5 mL of concentrated sulphuric acid is added immediately to the mixture. The test tubes cool down to room temperature, they are vortexed for 30 seconds. The absorbance is measured at 490 nm by using spectrophotometer (Albalasmeh, Berhe, & Ghezzehei, 2013).

3.9 Response Surface Methodology

Response surface methodology (RSM) was used to determine the total carbohydrate, reducing sugar and pH for the enzymatic hydrolysis of bakery waste. The Central Composite Design (CCD) was applied to investigate a combined effect of concentration of enzyme and temperature and to obtain the optimum conditions. By applying two factors (variables) in the design which are temperature (Factor A) and concentration of enzyme (Factor B), and duplicate the experiment, 17 experimental were generated as shown in table X below and the Table Y shows the levels for the two factors.

Table 2: Process Conditions

Run	Factor A: Temperature	Factor B: Concentration
1	55	0.55
2	55	0.55
3	45	1
4	45	0.55

5	45	0.1
6	55	0.1
7	45	1
8	65	1
9	55	0.55
10	65	0.55
11	55	0.55
12	65	1
13	55	1
14	65	0.1
15	55	0.55
16	65	0.1
17	45	0.1

Table 3: Level of Two Factors

		Actual levels for each factors		
Factor	**Symbol**	**-1**	**0**	**-1**
Temperature	A	45	55	65
Concentration	B	0.1	0.5	1.0

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview

There are total 5 analysis were done after the enzymatic hydrolysis of croissant and doughnut which are analysis of monosaccharides, reducing sugar, total carbohydrates, pH and optimization of process condition of enzymatic hydrolysis.

4.2 Analysis of Monosaccharides in Bakery Waste

4.2.1 Analysis of Monosaccharides in Croissant

Figure 9 and Figure 10 show the types of sugars present in bakery waste after enzymatic hydrolysis by using high pressure liquid chromatography,

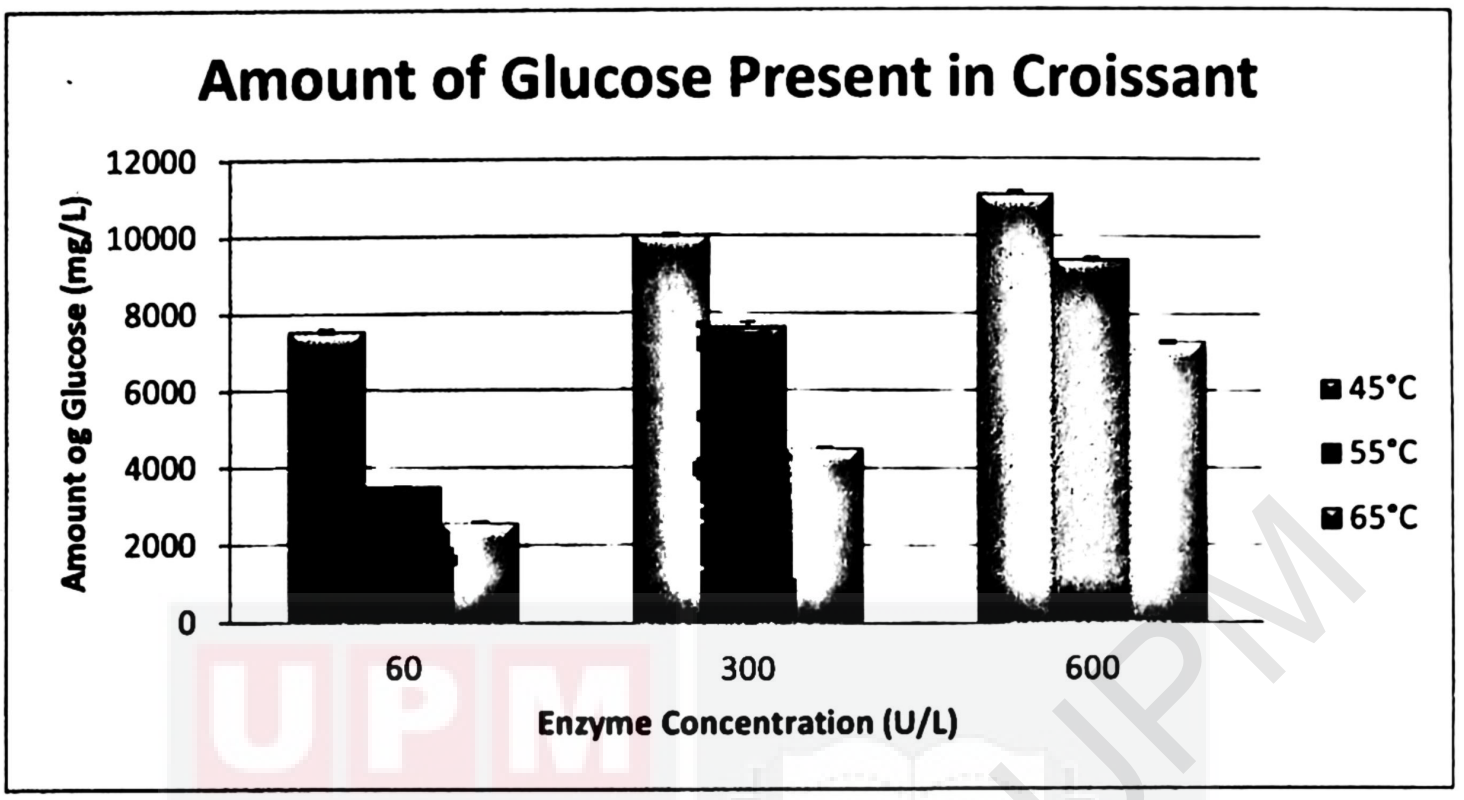


Figure 9: Amount of Glucose in Croissant

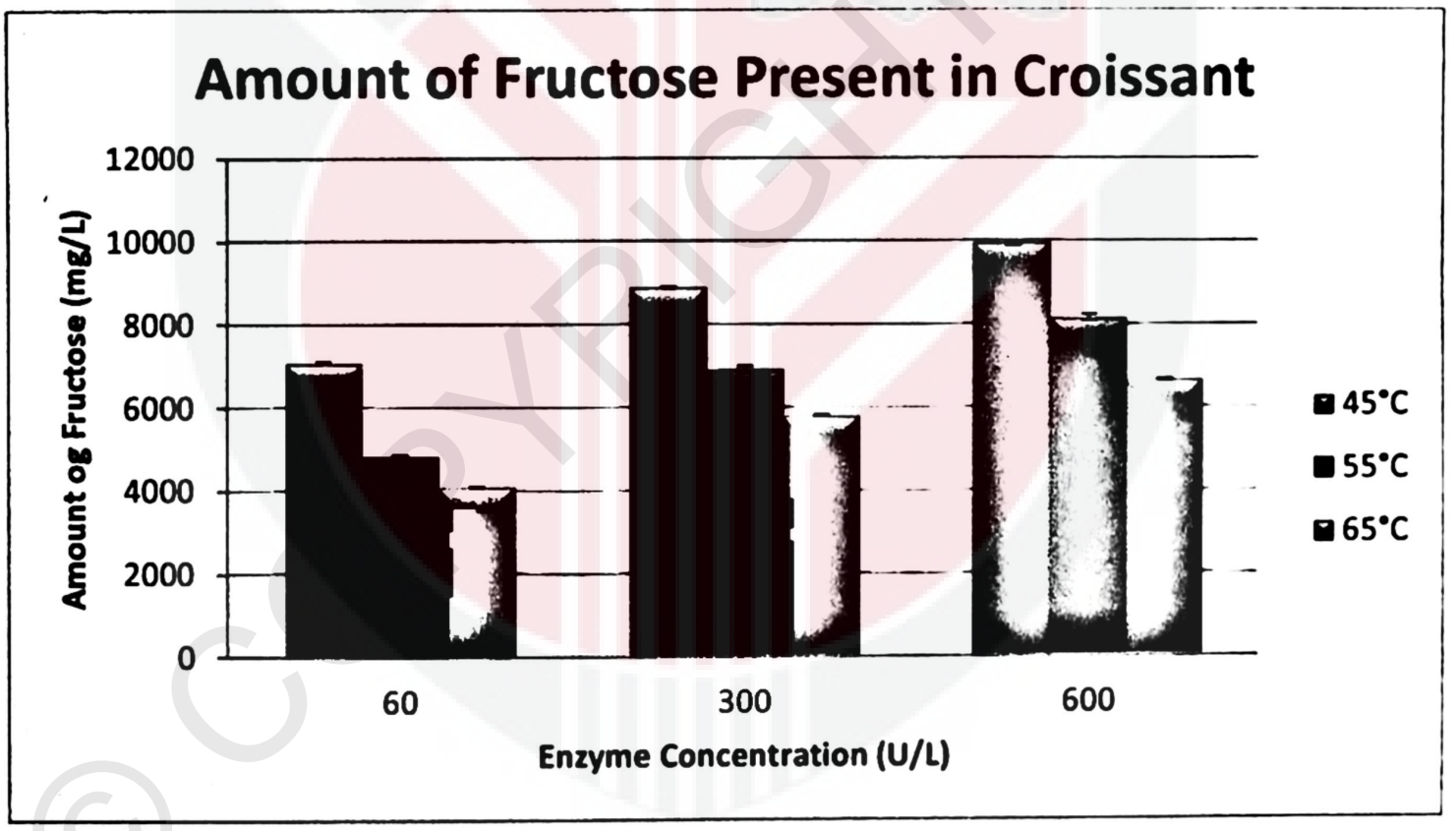


Figure 10: Amount of Fructose in Croissant

For the high pressure liquid chromatography analysis, Figure 9 and Figure 10 show the presence of monosaccharides in the croissant after enzymatic hydrolysis. The result shows that the croissant contain glucose and fructose in the croissant samples. Figure 9 shows the graph of amount of glucose present whereas Figure 10 shows the graph of amount of fructose present in the croissant.

Based on Figure 9, the amount of glucose is increasing with increase of enzyme concentration. Instead, the amount of glucose is decreasing with increase of temperature of incubation. Amount of glucose shows peak value 11122mg/L when the temperature is 45 ℃ and the concentration of enzyme is 600U/L whereas the amount of glucose shows the lowest value 2586mg/L when the temperature is 65 ℃ and the concentration of enzyme is 60U/L.

Based on Figure 10, the trend is similar to the graph of amount of glucose present in croissant. The amount of fructose is increasing with increase of concentration of enzyme whereas the amount of fructose is decreasing with increase of temperature of incubation. The highest amount fructose is obtained 9947mg/L when the temperature is 45 ℃ and the enzyme concentration is 600 U/L. The lowest amount of fructose obtained is 2586mg/L, when the temperature is 65 ℃ and the enzyme concentration is 60U/L.

Both of the graph shows that amount of sugar decreases when the incubation temperature increases and the amount of sugar increases when the enzyme concentration is increased.

4.2.2 Analysis of Monosaccharides in Doughnut

Figure 11 and Figure 12 show the type of sugar presence in doughnut after enzymatic hydrolysis by high pressure liquid chromatography.

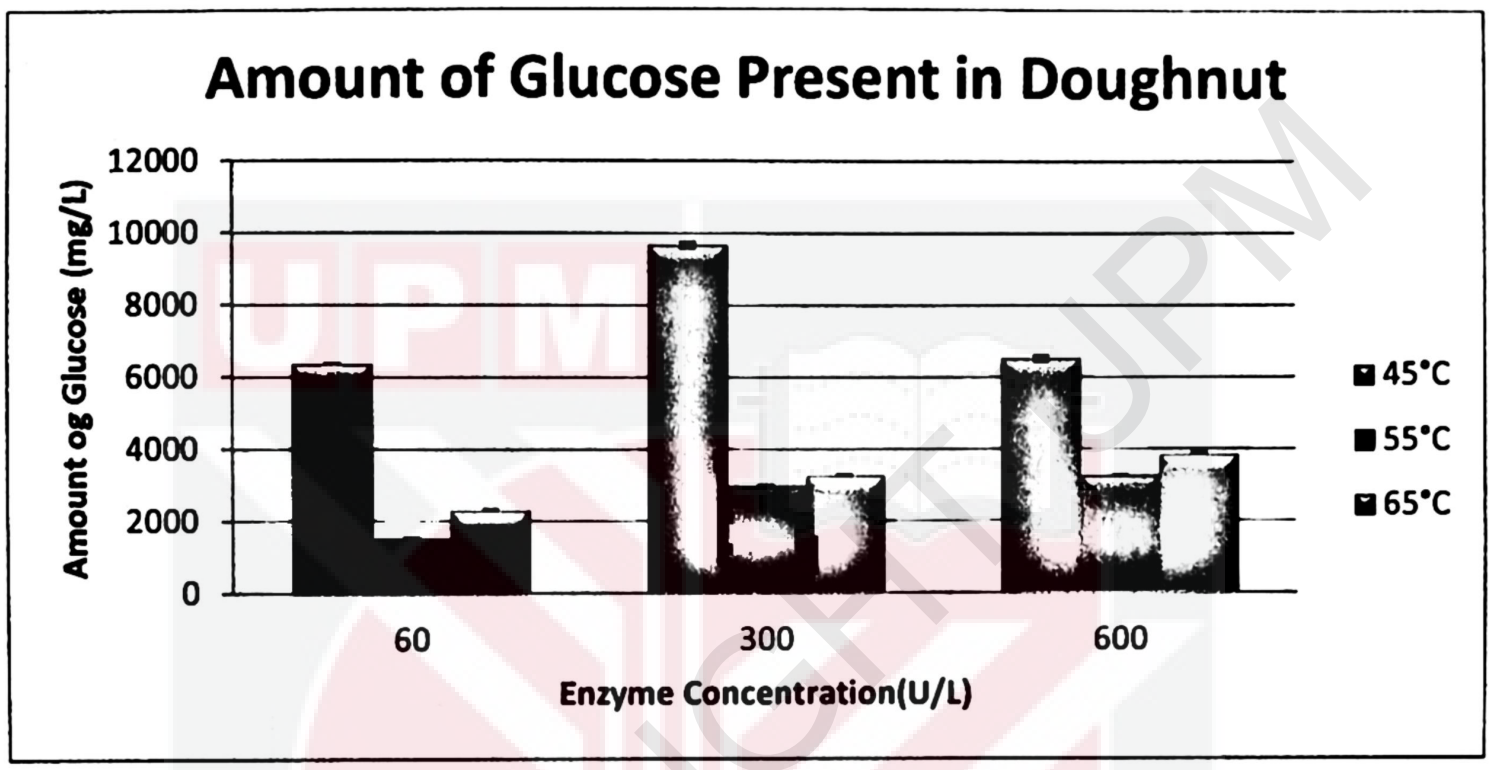


Figure 11: Amount of Glucose Present in Doughnut

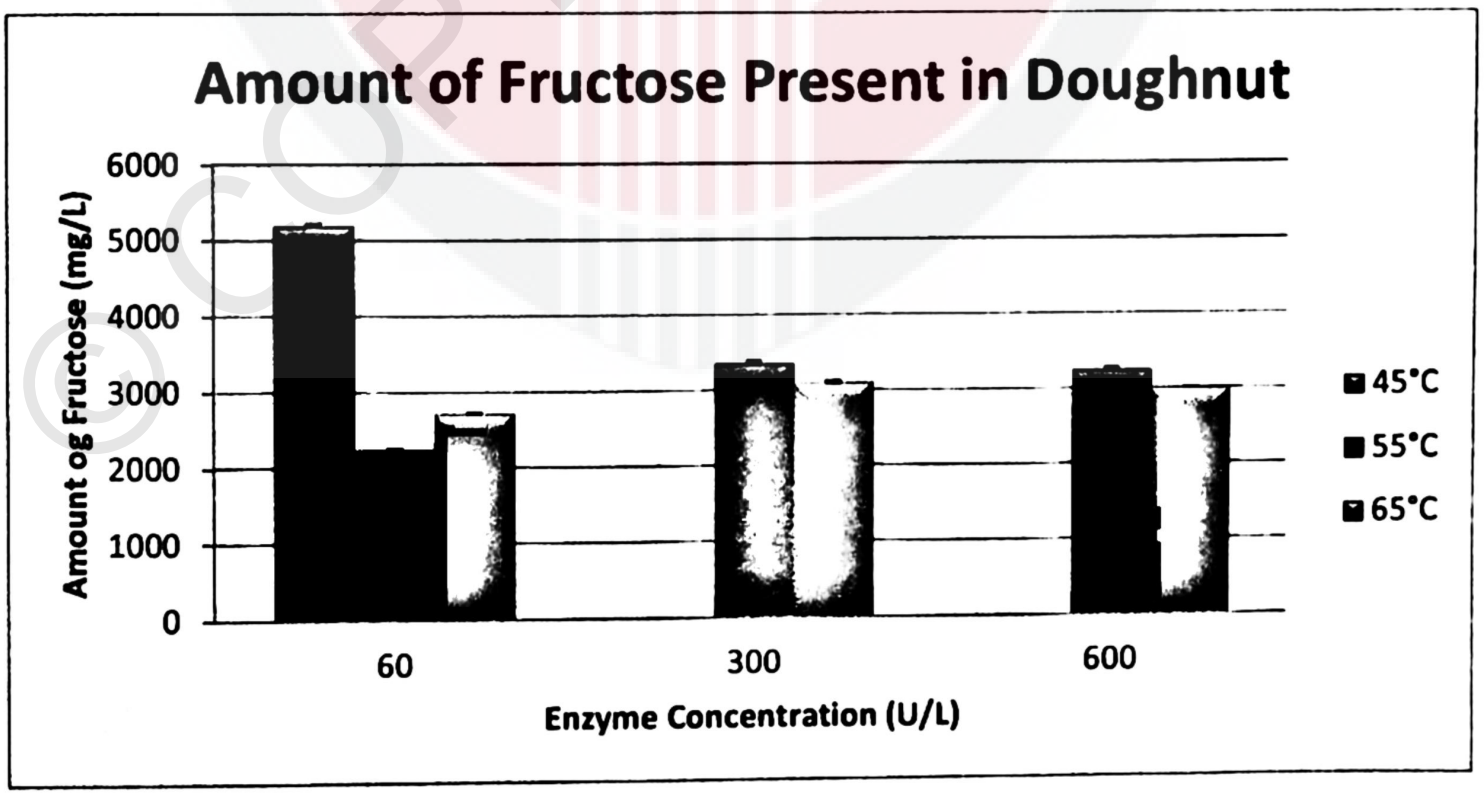


Figure 12: Amount of Fructose in Doughnut

For the high pressure liquid chromatography analysis of doughnut samples, Figure 11 and Figure 12 show the presence of monosaccharides in the doughnut after enzymatic hydrolysis. The result shows that the doughnut contains glucose and fructose in the croissant samples. Figure 11 shows the graph of amount of glucose present whereas Figure 12 shows the graph of amount of fructose present in the doughnut.

Based on Figure 11, the amount of glucose is increasing with increase of enzyme concentration. Instead, the amount of glucose is decreasing with increase of temperature of incubation. Amount of glucose yield is highest which is 9675mg/L when the temperature is 45 ℃ and the concentration of enzyme is 300U/L whereas the amount of glucose is averagely low when the temperature is 55 and 65 ℃. This is because the enzyme may undergo denaturation at high temperature and the enzyme configuration has altered.

Based on Figure 12, the trend is slightly different to the graph of amount of glucose present in doughnut. There are absence of fructose at low temperature which is 45 ℃. The highest amount fructose is obtained when the temperature is 45 ℃ which recorded 5205mg/L and the enzyme concentration is 60U/L. According to both figure, the temperature at 45 ℃ will produce highest yield of sugars whereas the concentration of enzyme is 300U/L in overall.

4.3 Reducing Sugar

The reducing sugar of the bakery waste samples can be determined by standard curve of that made of different concentration of sugar. The total reducing sugar of croissant and doughnut are plotted against enzyme concentration with 3 different temperature which is shown in Figure 9 and Figure 10 respectively.

4.3.1 Reducing Sugar for Croissant

Figure 13 shows the graph of reducing sugar in croissant against enzyme concentration by 3 different temperatures.

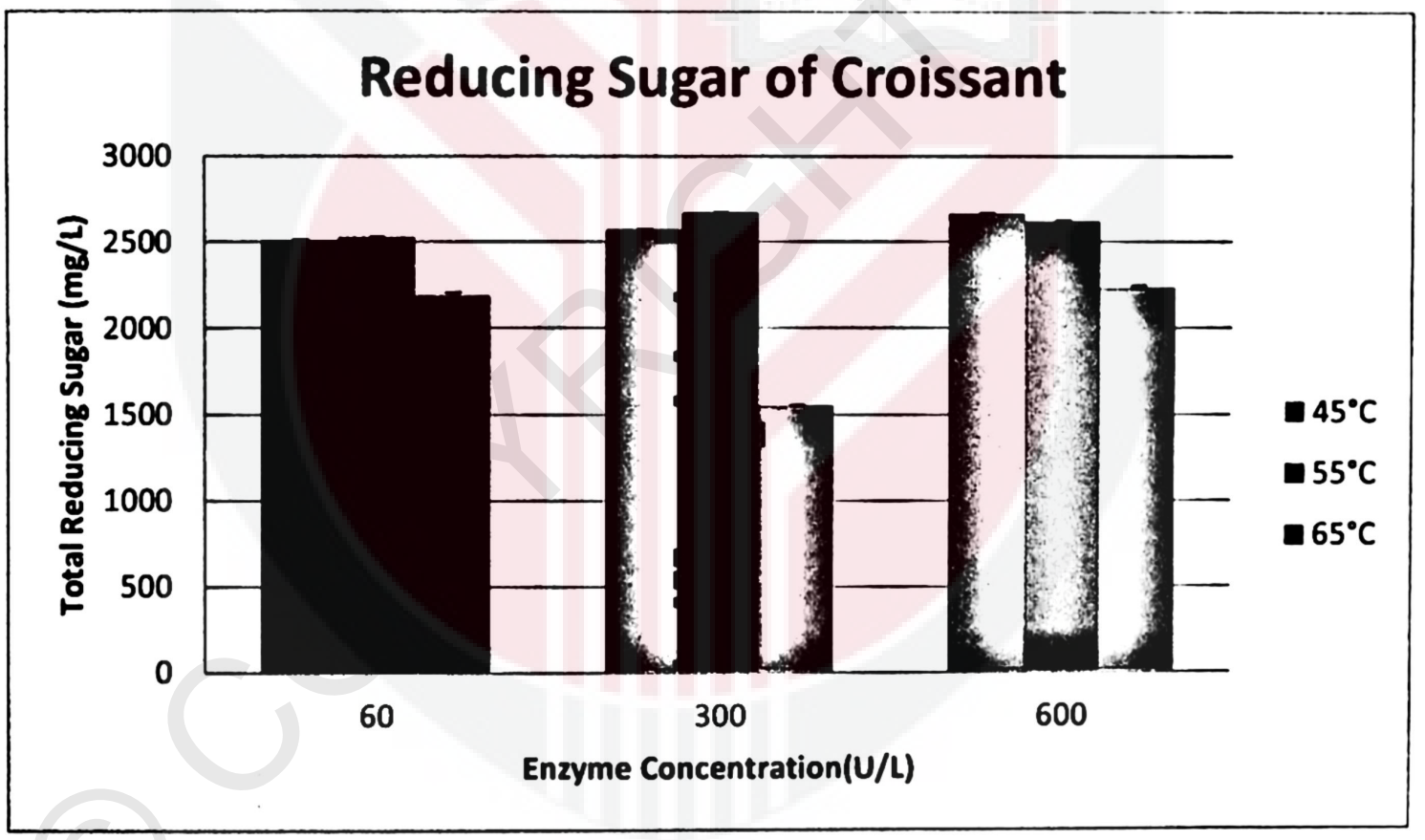


Figure 13: Reducing Sugar of Croissant

Based on Figure 13, the graph shows that the reducing sugar of croissant samples for different temperatures and enzyme concentrations. As the temperature increases, the rate of reaction will increase. The reducing sugar of the croissant is the lowest in 65℃ compared to 45 ℃ and 55 ℃. This is due to the enzyme activity is retarded at such high temperature. Meanwhile, the highest reducing sugar yield is recorded at 55 ℃. For various of enzyme concentration, generally the reducing sugar yield is increasing with increase of enzyme of concentration. However, the reducing sugar shows peak yield at temperature of 55 ℃ and 300U/L enzyme concentration instead of 600U/L. It is because the enzyme concentration become the limiting factor at this point, whereby the increase in enzyme concentration will not increase the rate of reaction. The concentration of peptide bonds available for hydrolysis may become the limiting factor (Salwanee et al., 2013).

4.3.2 Reducing Sugar for Doughnut

Figure 14 below shows the reducing sugar of doughnut after enzymatic hydrolysis for 24 hours.

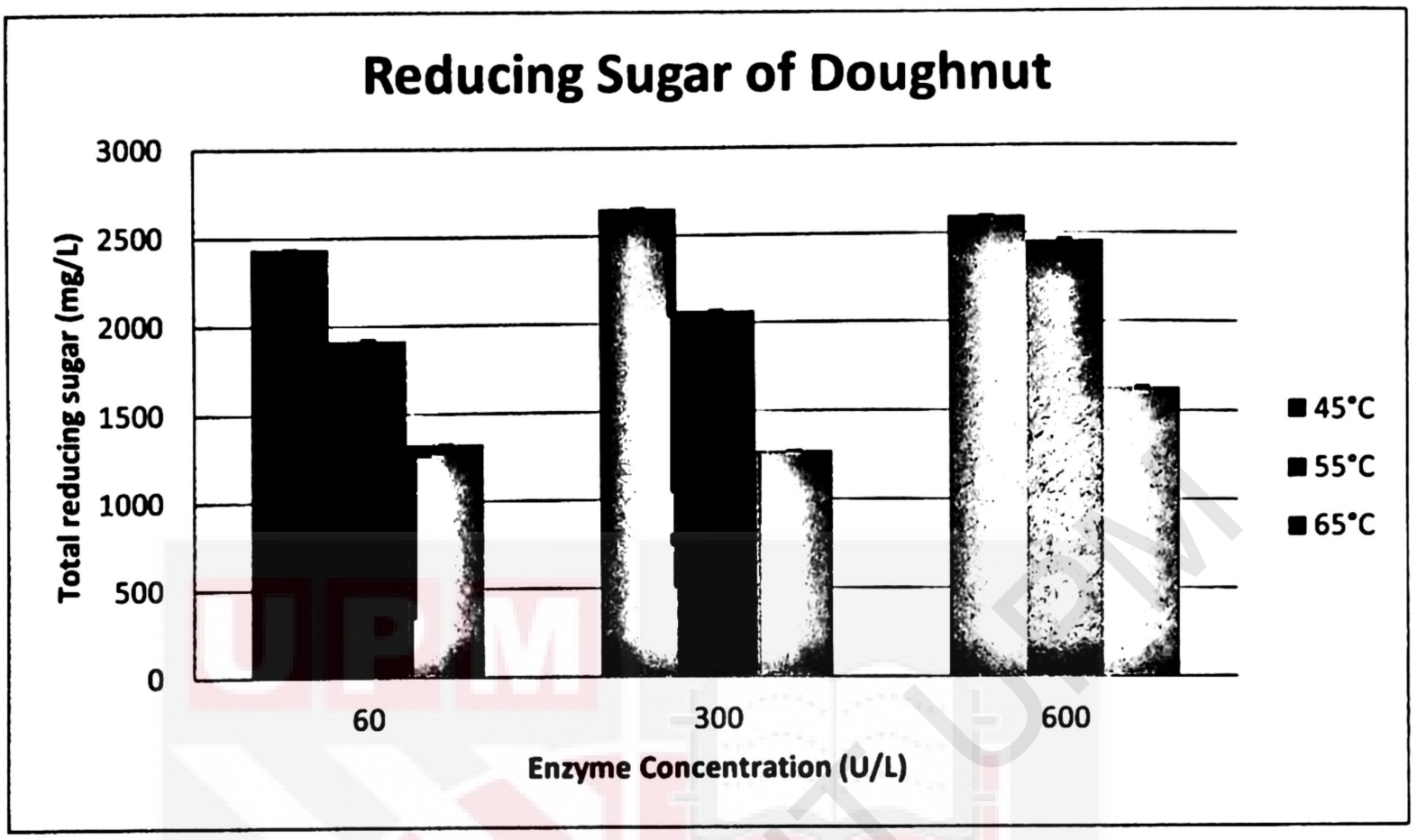


Figure 14: Reducing Sugar in Doughnut

Based on Figure 14, the graph shows that the reducing sugar of doughnut samples for different temperatures and enzyme concentrations. It is similar to croissant sample, the reducing sugar of the doughnut is the lowest in 65℃ compared to 45 ℃ and 55 ℃. This is due to the enzyme activity is retarded at such high temperature where denaturation may occur (Daniel & Danson, 2013). Meanwhile, the highest reducing sugar yield is recorded at 45 ℃. In general, the reducing sugar yield is increasing with increase of enzyme of concentration. For doughnut, the peak of sugar yield is recorded at 45 ℃ and 300U/L enzyme concentration.

However, the result of reducing sugar has deviation from the analysis of monosaccharides stated in section 4.2 which is much lower. This might due to sensitivity of high pressure liquid chromatography is higher than spectrophometric method (Xu, Liang, & Zhu, 2015) .

4.4 Total Carbohydrates of Bakery Waste

The total carbohydrates of the bakery waste samples can be determined by standard glucose curve of that made of different concentration of sugar by using phenol-sulphuric acid method.

4.4.1 Total Carbohydrates or Croissant

Total carbohydrates of bakery waste for croissant against enzyme concentration and temeperature is shown in Figure 15 below.

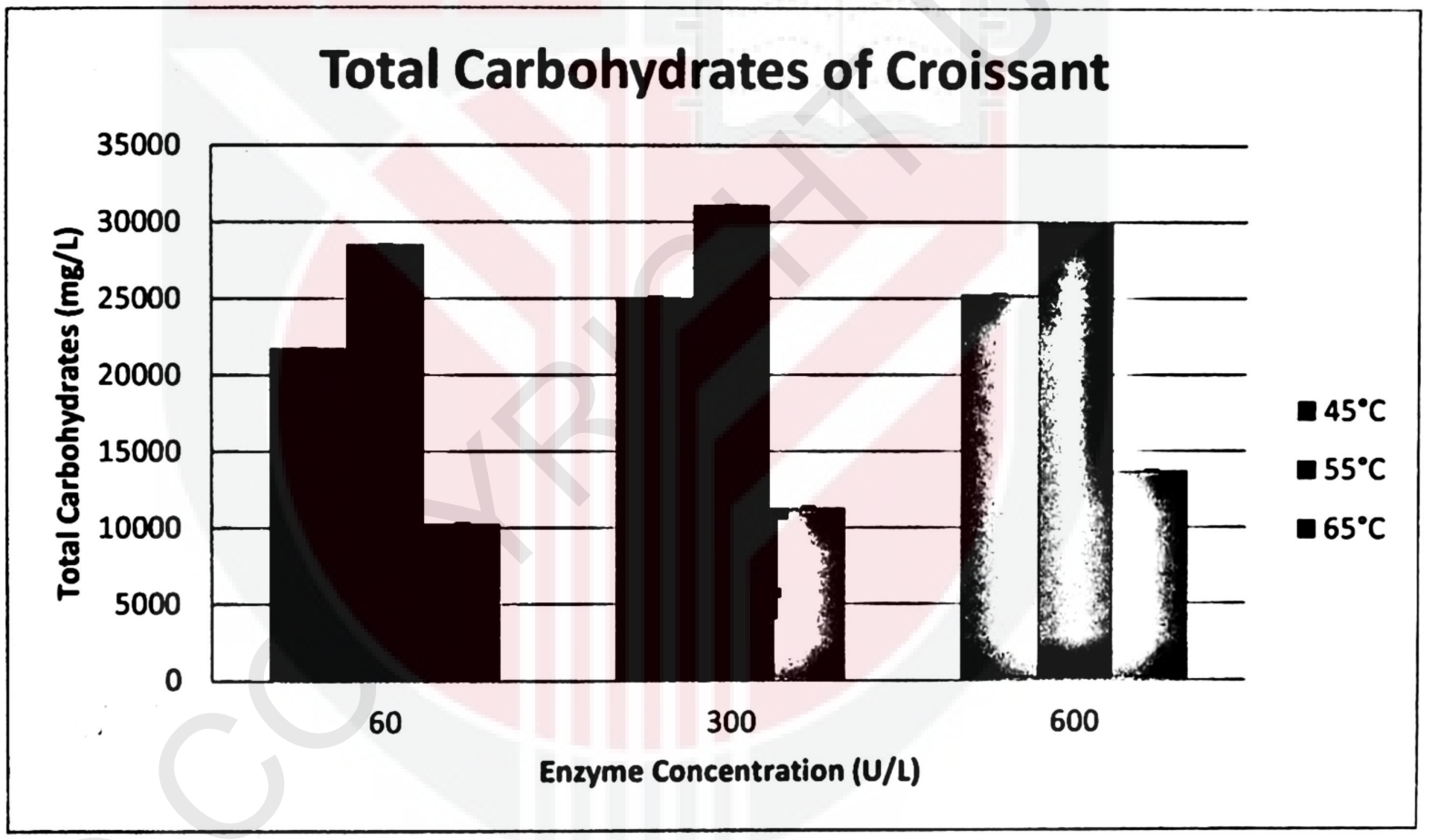


Figure 15: Total Carbohydrates of Croissant

Based on Figure 15, the graph shows the total carbohydrates present in the croissant samples. There is highest yield of total carbohydrates when the temperature is 55 °C and the enzyme concentration is 300U/L. Where the lowest total carbohydrates is in 65 °C . Increase of enzyme concentration will cause the increase of total carbohydrates as well. Generally, the total carbohydrates of croissant

increases with increasing of temperature, but it show lower value at high temperature due to inactivity of enzyme at such high temperature. The enzyme undergoes denaturation at high temperature where the bond between the enzyme is broken and the configuration has changed (Daniel & Danson, 2013). Based on Figure 11, 300U/L enzyme concentration record the highest yield of total carbohydrates. Increasing concentration will increase the enzymatic acitivity . However, 600U/L enzyme concentration does not obtain the highest yield. At this point, increasing the concentration of enzyme used may not result in higher degree of hydrolysis as the concentration of peptide bonds available for hydrolysis may become the limiting factor (Salwanee et al., 2013).

4.4.2 Total Carbohydrates for Doughnut

Total carbohydrates of bakery waste for doughnut against enzyme concentration and temperature is shown in Figure 16 below.

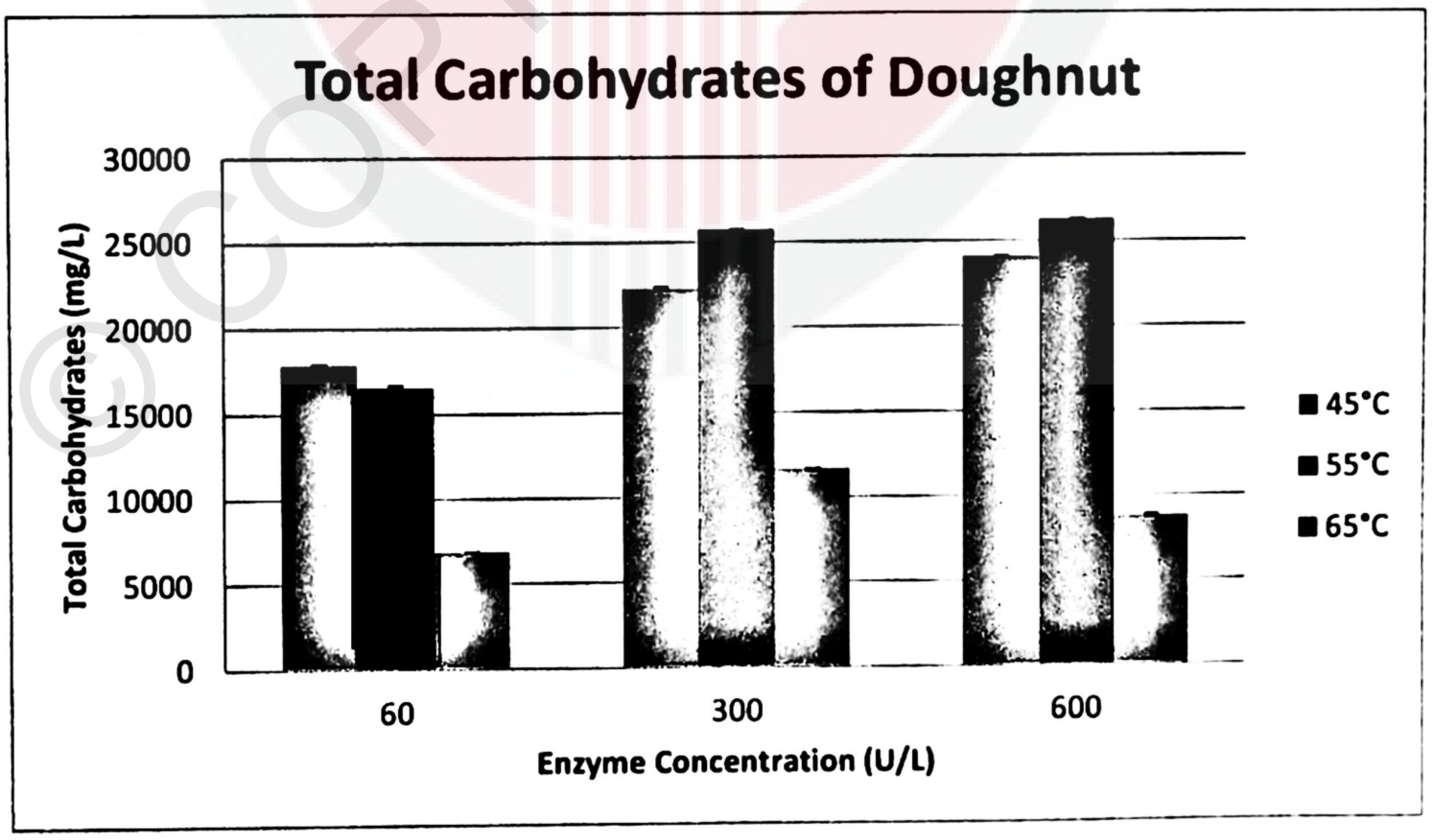


Figure 16: Total Carbohydrates of Doughnut

Based on Figure 16, the graph shows that the total carbohydrates of doughnut samples. The trend of the graph is similar to total carbohydrates in croissant that shown in Figure 15. At the low concentration of the enzyme, the total carbohydrates shows the peak yield on 45 ℃ whereas for the higher enzyme concentration, 55 ℃ is recorded as the best temperature to produce highest total carbohydrates in doughnut compared to another two temperatures. Total carbohydrates of the doughnut is the lowest in 65℃ compared to 45 ℃ and 55 ℃. This is due to the enzyme activity is retarded at such high temperature where denaturation may occur (Daniel & Danson, 2013). Increasing of enzyme concentration allows the higher degree of hydrolyzation of bakery waste. Thus, the yield of total carbohydrates increase across the enzyme concentration.

4.5 pH Analysis of Bakery Waste

The figure below shows the pH of croissant and doughnut after enzymatic hydrolysis.

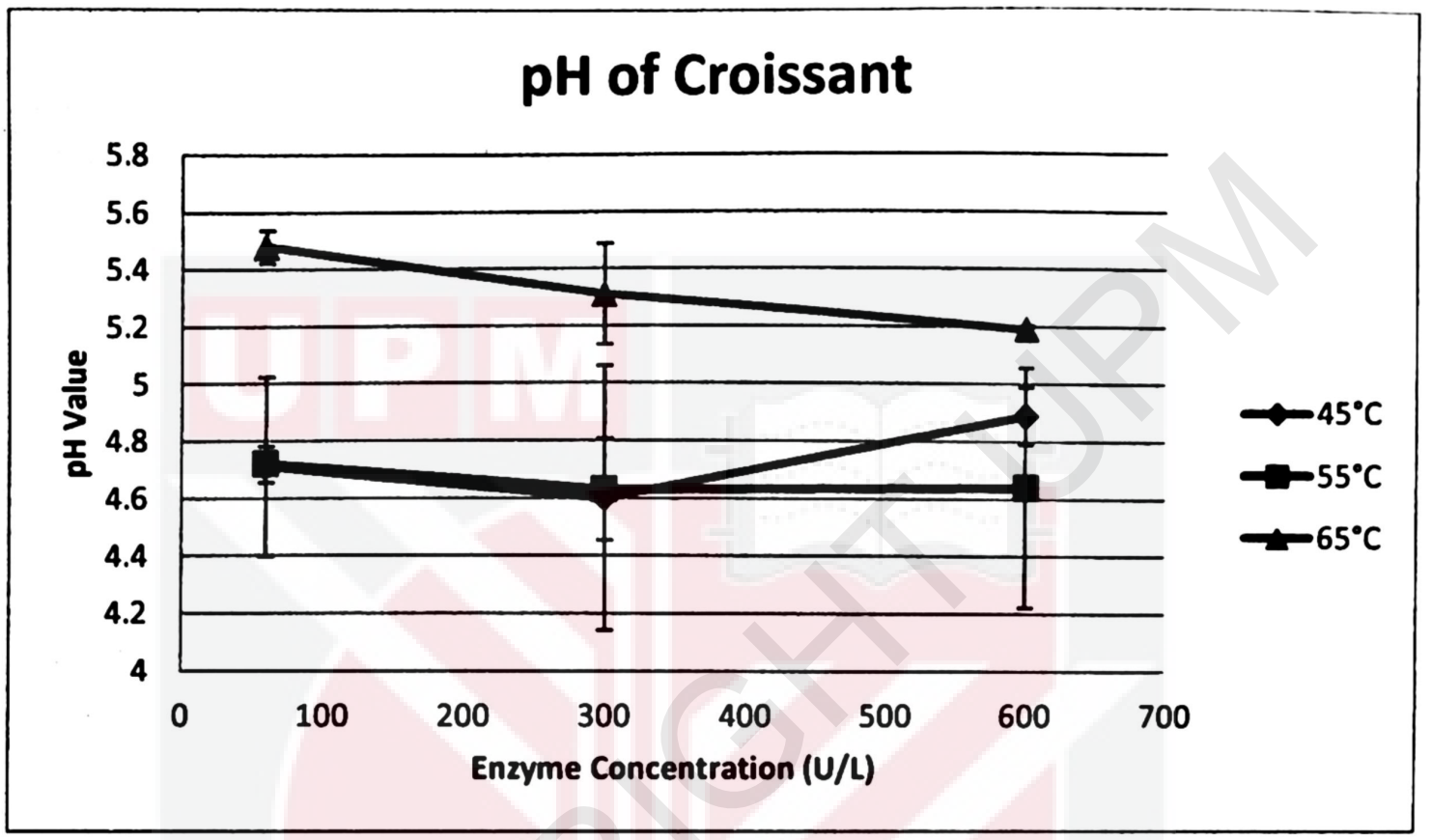


Figure 17: pH of Croissant

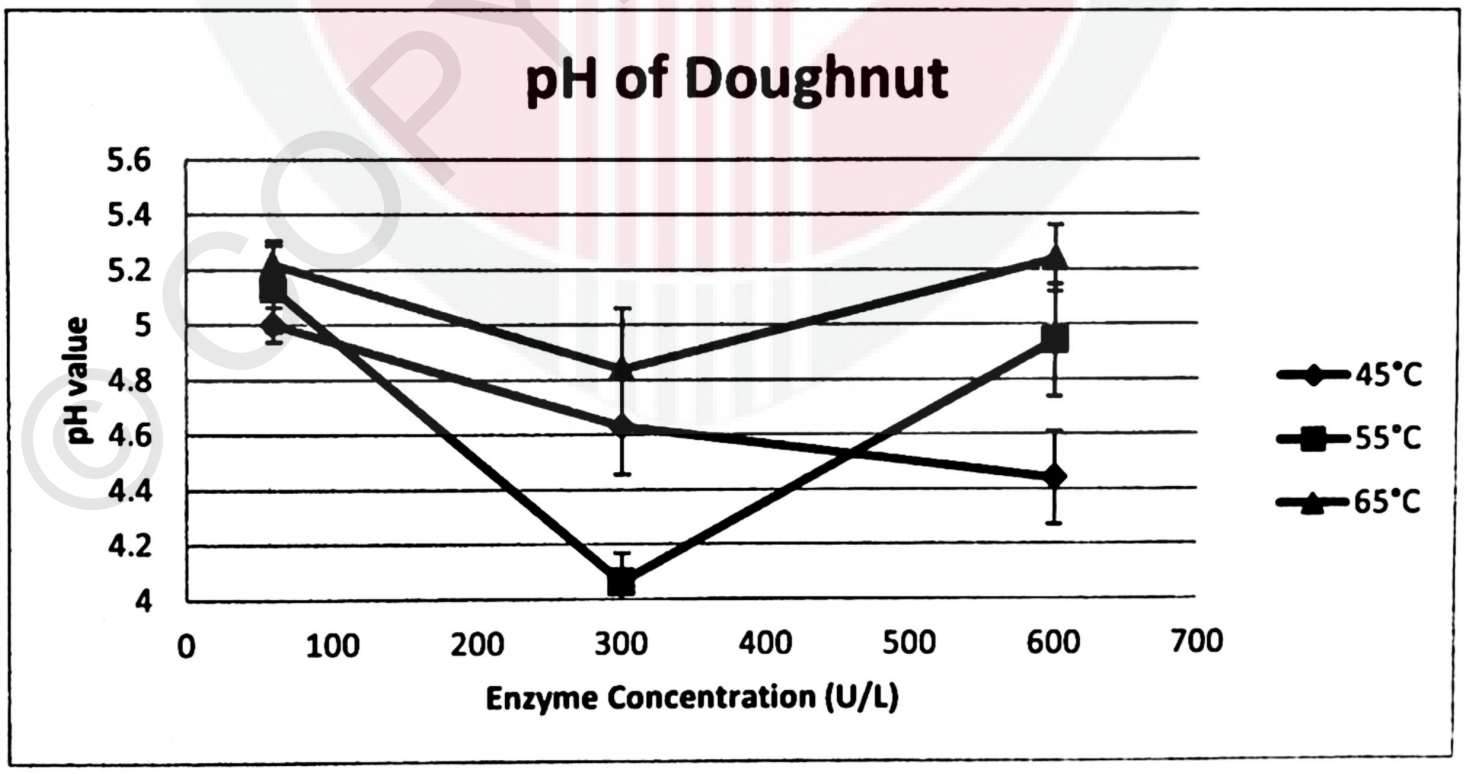


Figure 18: pH of Doughnut

Based on Figure 17 and Figure 18, the graphs show the trend of pH value of croissant samples and doughnut samples. The initial pH for both materials is 5.5. Generally, the pH shows decreasing trend for both materials across the different temperature and enzyme concentration. The decrease in pH indicates the enzymatic activity is increased (Hyde & Morrison, 1964). It means that there are more polysaccharides are being hydrolyzed into simpler sugar. The results show acidic mostly in 300U/L to 600U/L. Besides, the temperatures that shows acidic result ranges from 45 ℃ to 55 ℃. The results indicate that the higher concentration of enzyme and increasing temperature leads to better enzymatic hydrolysis. In the other hand, higher temperature which is 65 ℃ and low concentration of enzyme show no much differences from initial pH which is 5.5. This is because the degree of hydrolyzation to simpler sugar is lower at 65 ℃.

Measuring pH of foods has significant impacts to safety and quality. Besides, it gives also information about stability and preservation. It can be used to slow down the spoilage of food that could happen in the presence of some pathogens such as bacteria, molds, and yeasts. Generally, deteriorating bacteria cannot grow at pH lower than 4.5. With pH more than 4.5 are less stable. Their stabilization is achieved by heat sterilization in order to remove all pathogens, bacteria and spores (Karastogianni et al., 2015).

4.6 Optimization using Response Surface Methodology (RSM)

4.6.1 Optimization Analysis of Croissant

The Figure 19 to 21 below show the 3D plot on different responses on total carbohydrates, reducing sugar and pH of croissant by 2 factors which are different concentrations and different temperatures.

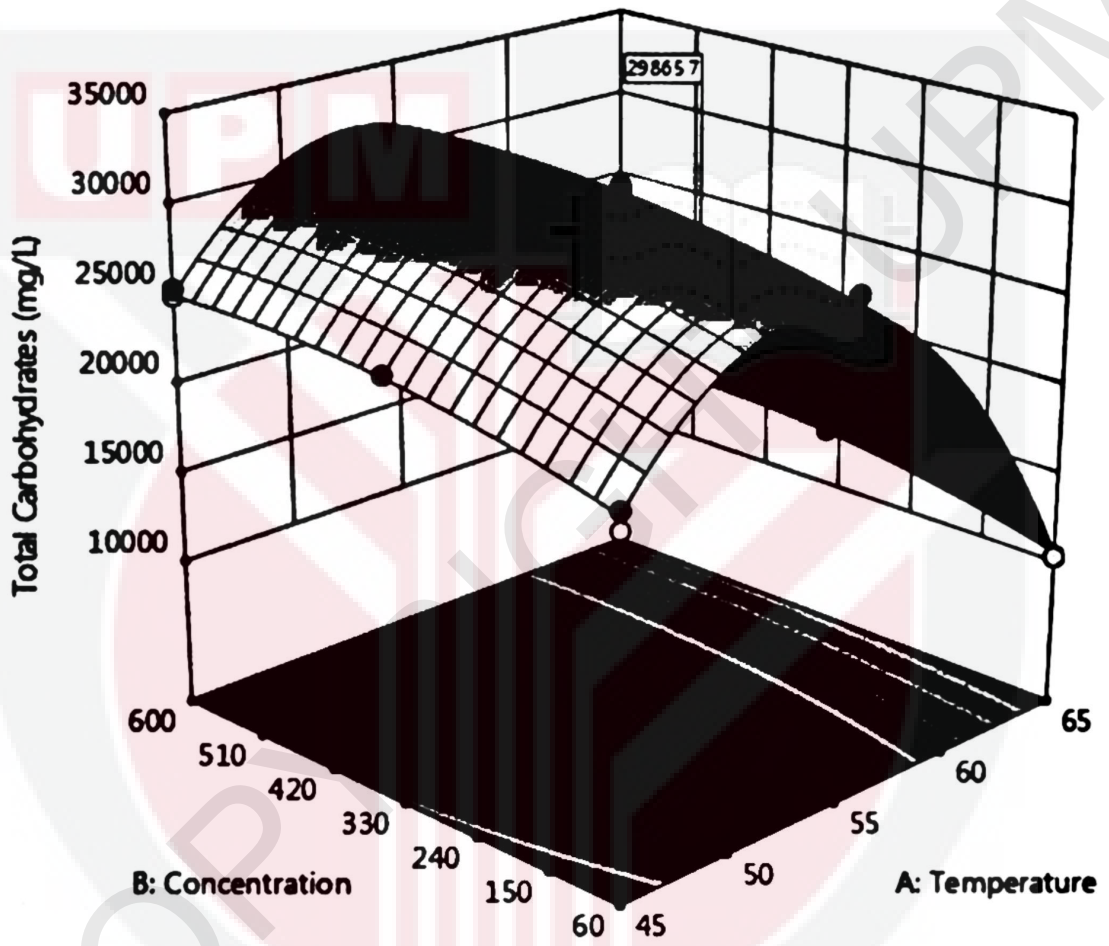


Figure 19: 3D Surface on Total Carbohydrates of Croissant

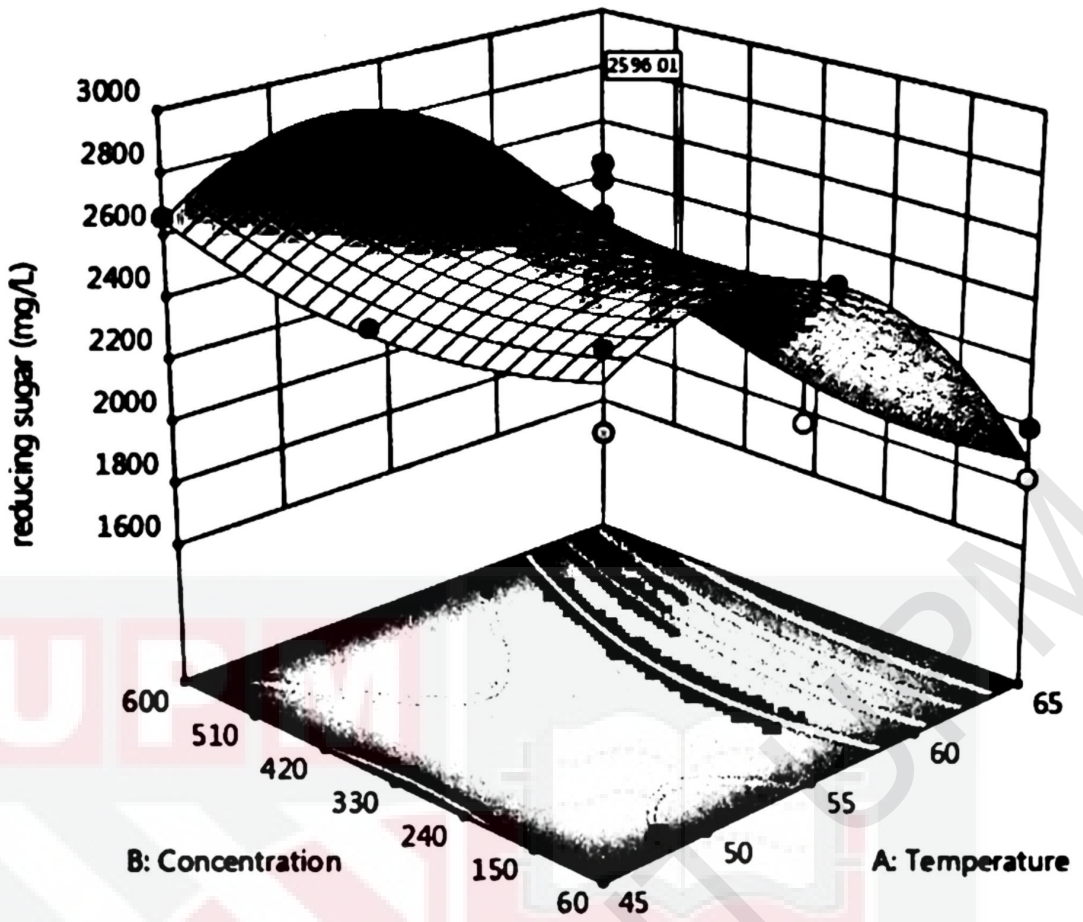


Figure 20: 3D Surface on Total Reducing Sugar of Croissant

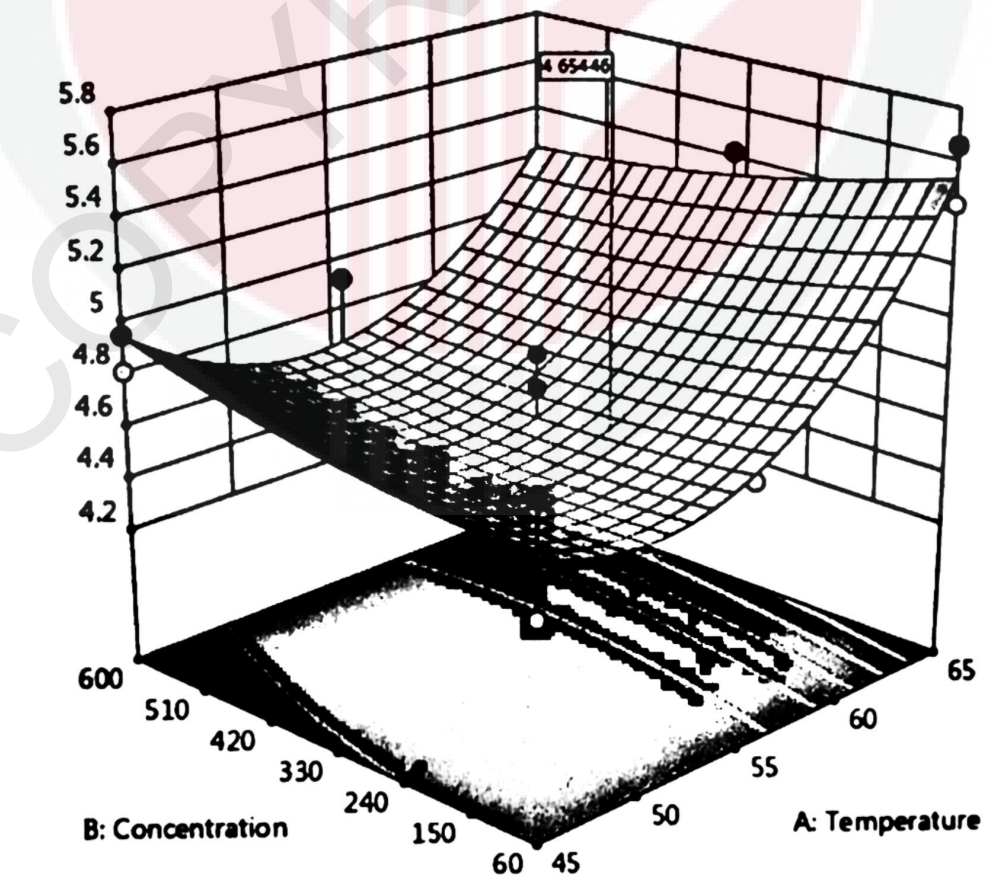


Figure 21: 3D Surface on pH of Croissant

The Figure 19 to 21 show the 3-D response surface plots of total carbohydrates, reducing sugar and pH. Looking into the 3D surface plots allowing better understanding between the factors and the optimal condition. The figure shows the interaction between incubation temperature and enzyme concentration for total reducing sugar, total carbohydrates and the pH of the croissant.

Based on Figure 19, the maximum total carbohydrates yield was observed with a optimum temperature and optimum enzyme concentration around the center point of the red region. The point is the optimal total carbohydrates which recorded 29865.7mg/L. Based on Figure 20, the graph shows the 3D surface plot for reducing sugar yield of croissant. The maximum reducing sugar was observed around the center point with optimum temperature and optimum enzyme concentration. The highest reducing sugar from the plot is 2596mg/L. For Figure 21,the graph shows the 3D surface plot on pH of croissant after enzymatic hydrolysis. The lowest pH around the center point of the plot is obtained is 4.66. Low pH indicates more carbohydrates is being hydrolyzed to simple sugar (Hyde & Morrison, 1964).

In overall optimization analysis, it can be concluded that the optimal condition to get the highest yield total carbohydrates and reducing sugar is temperature at 53° and concentration of enzyme at 578U/L.

4.6.1.1 Analysis of Variance (ANOVA) for Croissant

Response 1 (Total Carbohydrates)

Based on table 4, the F-value of the model is 457.64. Thus, the model is significant. Model terms are significant when P-values less than 0.05. Hence, A, B, A^2, B^2 are significant model terms. Thus, both temperature and concentration of enzyme give significant impact on total carbohydrates yield. The predicted R^2 of 0.9867 while adjusted R^2 of 0.9930, which is less than 0.2. Thus, the model can be used to navigate the design space. The response model can be shown in the equation below where ,

$$Y = 921.65 - 172.61A + 45.95B - 354.63A^2 - 33.31B^2$$

Y = Total Carbohydrates

A= Temperature

B= Concentration

Table 4: ANOVA for Response 1 (Total Carbohydrates)

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	8.915E+05	5	1.783E+05	457.64	< 0.0001	significant
	A-Temperature	2.979E+05	1	2.979E+05	764.74	< 0.0001	
	B-Concentration	21112.19	1	21112.19	54.19	< 0.0001	
	AB	1.51	1	1.51	0.0039	0.9514	
	A^2	3.809E+05	1	3.809E+05	977.60	< 0.0001	
	B^2	3359.76	1	3359.76	8.62	0.0135	
	Residual	4285.61	11	389.60			
	Lack of Fit	1599.96	3	533.32	1.59	0.2667	not significant
	Pure Error	2685.64	8	335.71			
	Cor Total	8.958E+05	16				

RESPONSE 2 (Reducing Sugar)

Based on table 5, the F-value of the model is 14.54. The model is significant. Model terms are significant if P-values less than 0.0500. Based on table5, only the temperature is significant to the reducing sugar yield. The predicted R^2 is 0.6543 while R^2 is 0.8088 which is less than 0.2. Thus, the model can be used to navigate the design space. The response model can be shown in the equation below where,

$$Y = 78.97 - 8.84A - 12.18A^2$$

Y = Total Reducing Sugar

A= Temperature

B= Concentration

Table 5: ANOVA for Response 2 (Reducing Sugar)

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	1346.74	5	269.35	14.54	0.0002	significant
	A-Temperature	780.75	1	780.75	42.15	< 0.0001	
	B-Concentration	56.74	1	56.74	3.06	0.1079	
	AB	26.68	1	26.68	1.44	0.2553	
	A^2	449.44	1	449.44	24.26	0.0005	
	B^2	35.59	1	35.59	1.92	0.1932	
	Residual	203.76	11	18.52			
	Lack of Fit	42.48	3	14.16	0.7024	0.5767	not significant
	Pure Error	161.28	8	20.16			
	Cor Total	1550.51	16				

RESPONSE 3 (pH)

According to table 6, F-value of 8.92 indicates the model is significant. Based on table 6, only temperature significant to the pH. Thus, the result of pH does not vary much with across the different concentration. The predicted R^2 is 0.5395 while adjusted R^2 is 0.7123, it is less than 0.2. Thus, the model can be used to navigate the design space. The response model can be shown in the equation below where ,

$$Y = 4.63 - 0.279A + 0.4284A^2$$

Y = pH

A= Temperature

B= Concentration

Table 6: ANOVA for Response 3 (pH)

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	1.79	5	0.3579	8.92	0.0013	significant
	A-Temperature	0.7784	1	0.7784	19.40	0.0011	
	B-Concentration	0.0010	1	0.0010	0.0249	0.8774	
	AB	0.1458	1	0.1458	3.63	0.0831	
	A^2	0.5558	1	0.5558	13.85	0.0034	
	B^2	0.0086	1	0.0086	0.2152	0.6517	
	Residual	0.4413	11	0.0401			
	Lack of Fit	0.1202	3	0.0401	0.9981	0.4419	not significant
	Pure Error	0.3211	8	0.0401			
	Cor Total	2.23	16				

4.6.2 Optimization Analysis of Doughnut

The Figure 22 to 24 below show the 3D plot on different responses on total carbohydrates, reducing sugar and pH of doughnut by 2 factors which are concentrations and temperatures.

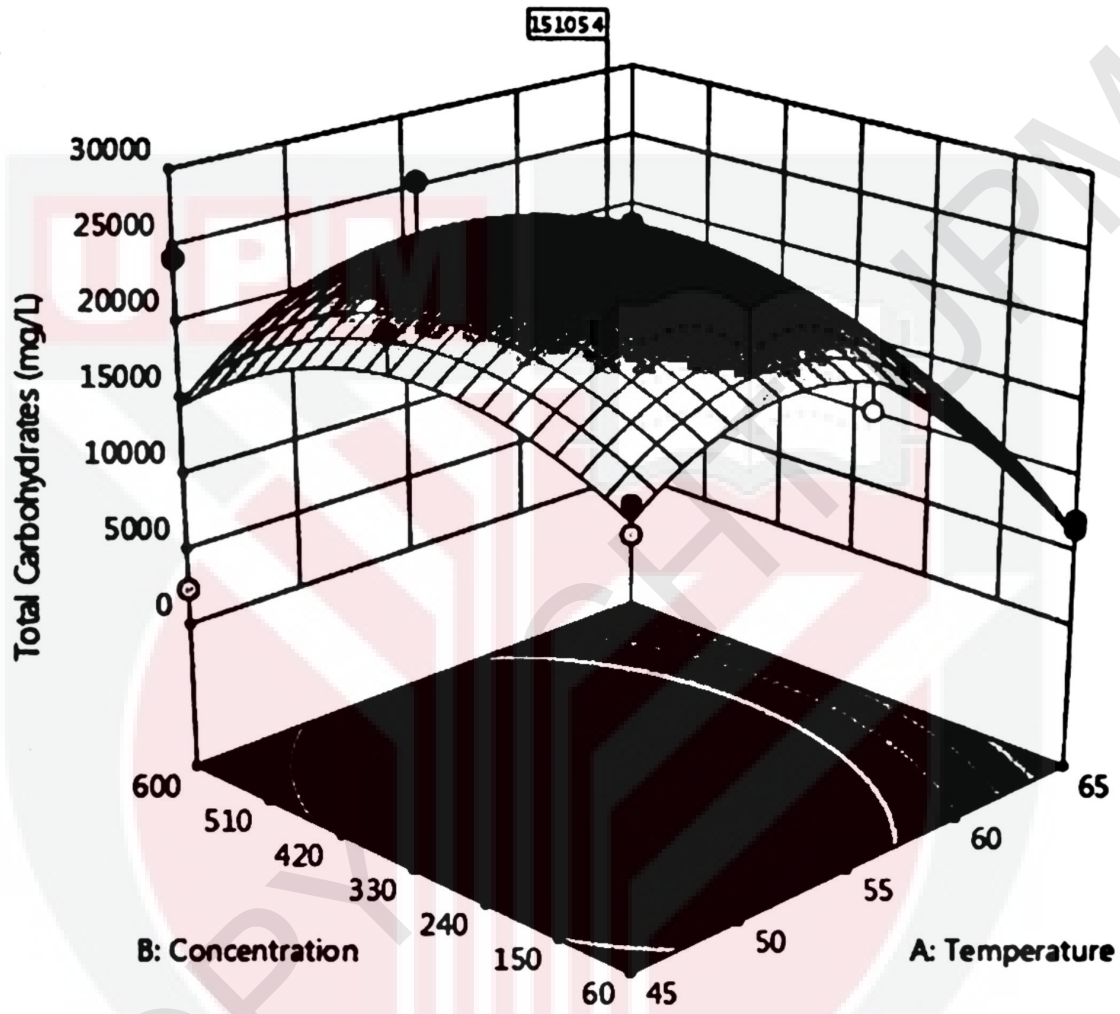


Figure 22: 3D Surface on Total Carbohydrates of Doughnut

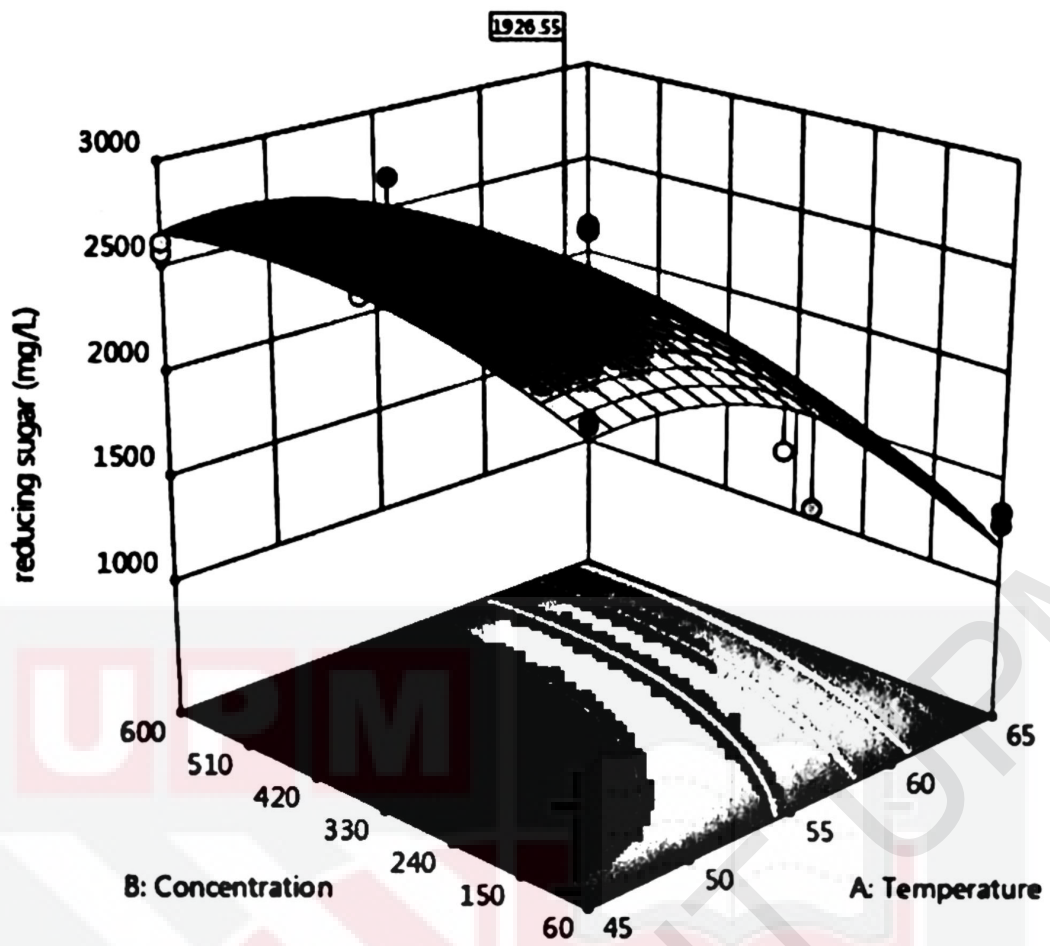


Figure 23: 3D Surface on Reducing Sugar of Doughnut

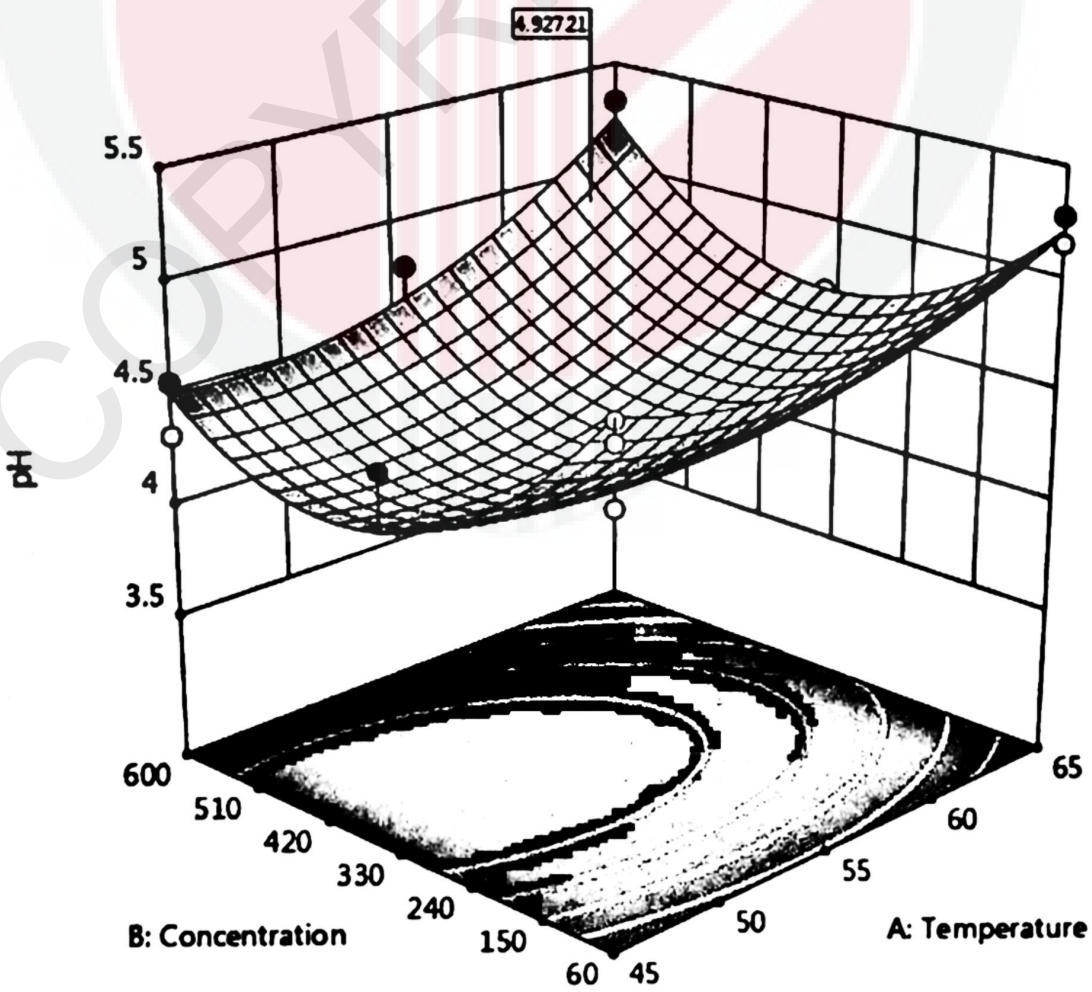


Figure 24: 3D Surface on pH of Doughnut

The Figure 22 to 24 show the 3-D response surface plots of total carbohydrates, reducing sugar and pH. Looking into the 3D surface plots allowing better understanding between the factors and the optimal condition. The figure shows the interaction between incubation temperature and enzyme concentration for total reducing sugar, total carbohydrates and the pH of the croissant.

Based on figure 22, the maximum total carbohydrates yield was observed with a optimum temperature and optimum enzyme concentration around the center point in red region. The point is the optimal total carbohydrates which recorded 15105mg/L. Based on Figure 23, the graph shows the 3D surface plot for reducing sugar yield of croissant. The maximum reducing sugar was observed around the center point with optimum temperature and optimum enzyme concentration. The highest reducing sugar from the plot is 1926mg.L. For Figure 24,the graph shows the 3D surface plot on pH of croissant after enzymatic hydrolysis. The lowest pH around the center point of the plot is obtained is 4.161. Low pH indicates more carbohydrates is being hydrolyzed to simple sugar (Hyde & Morrison, 1964).

In overall optimization analysis, it can be concluded that the optimal condition to get the highest yield total carbohydrates and reducing sugar is temperature at 50° and concentration of enzyme at 375U/L.

4.6.2.1 Analysis of Variance (ANOVA) for Doughnut

RESPONSE 1 (Total Carbohydrates)

Based on Table 7, F-value of model is 231.06, implies the model is significant. Model terms are significant when P value is less than 0.05. Table 7 shows that both of the factor, temperature and concentration bring significant result to the total yield of sugar. The predicted R^2 is 0.9750 whereas the adjusted R^2 is 0.9863, which is less than 0.2. The response model can be shown in the equation below where ,

$$Y = 780.26 - 191.5A + 66.93B - 233.41A^2 - 113.49B^2$$

Y = Total Carbohydrates

A= Temperature

B= Concentration

Table 7: ANOVA for Response 1

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	8.094E+05	5	1.619E+05	231.06	< 0.0001	significant
	A-Temperature	3.667E+05	1	3.667E+05	523.39	< 0.0001	
	B-Concentration	44798.93	1	44798.93	63.94	< 0.0001	
	AB	8371.53	1	8371.53	11.95	0.0054	
	A^2	1.650E+05	1	1.650E+05	235.50	< 0.0001	
	B^2	39005.35	1	39005.35	55.67	< 0.0001	
	Residual	7706.89	11	700.63			
	Lack of Fit	3119.51	3	1039.84	1.81	0.2227	not significant
	Pure Error	4587.38	8	573.42			
	Cor Total	8.171E+05	16				

RESPONSE 2 (Reducing Sugar)

Table 8 shows the variance analysis for reducing sugar. F-value of model is 12.02 , the model is significant. Model terms are significant when p value smaller than 0.05. There is only temperature factor brings the significant effect to the reducing sugar yield in this case. The predicted R^2 is 0.6596 and adjusted R^2 is 0.7749 which is less than 0.2. The response model can be shown in the equation below where ,

$$Y = 74.33 - 16.63A + 5.62B - 11.02A^2$$

Y = Reducing sugar

A= Temperature

B= Concentration

Table 8: ANOVA for Response 2

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	3877.03	5	775.41	12.02	0.0004	significant
	A-Temperature	2765.24	1	2765.24	42.86	< 0.0001	
	B-Concentration	315.51	1	315.51	4.89	0.0491	
	AB	9.77	1	9.77	0.1514	0.7046	
	A^2	367.70	1	367.70	5.70	0.0360	
	B^2	59.13	1	59.13	0.9164	0.3590	
	Residual	709.73	11	64.52			
	Lack of Fit	300.28	3	100.09	1.96	0.1993	not significant
	Pure Error	409.45	8	51.18			
	Cor Total	4586.76	16				

RESPONSE 3 (pH)

Based on table 9, F-value of the model is 23.25 and the model is significant. It can be explained that both of the factor, temperature and concentration bring significant effect to pH as the p value is smaller than 0.05. The predicted R^2 is 0.7865 and the adjusted R^2 is 0.8742 which is less than 0.2. The response model can be shown in the equation below where ,

$$Y = 4.24 + 0.222A - 0.1310B + 0.1425AB + 0.2272A^2 + 0.5422B^2$$

Y= pH

A=Temperature

B= Concentration

Table 9: ANOVA for Response 3

	Source	Sum of Squares	df	Mean Square	F-value	p-value	
	Model	2.77	5	0.5543	23.25	< 0.0001	significant
	A-Temperature	0.4928	1	0.4928	20.67	0.0008	
	B-Concentration	0.1716	1	0.1716	7.20	0.0213	
	AB	0.1624	1	0.1624	6.81	0.0243	
	A^2	0.1563	1	0.1563	6.55	0.0265	
	B^2	0.8902	1	0.8902	37.33	< 0.0001	
	Residual	0.2623	11	0.0238			
	Lack of Fit	0.1307	3	0.0436	2.65	0.1204	not significant
	Pure Error	0.1316	8	0.0165			
	Cor Total	3.03	16				

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, this study is to characterize total carbohydrate, reducing sugar in the bakery waste and pH of bakery waste after enzymatic hydrolysis and to analyze the effect of concentration and temperature on sugar yield (glucose and fructose) in bakery waste.

Five analytical methods for analysis were used. Analysis of monosaccharides were carried out by high pressure liquid chromatography. Reducing sugar and total carbohydrates were analysed spectrophotometrically by DNS method and phenol-sulphuric acid method respectively. The pH is analyzed by pH meter. The optimization analysis is carried out by using response surface methodology.

The sugar found from HPLC analysis are glucose and fructose.The optimal conditions of enzymatic hydrolysis for amyloglucosidase (578 U/L; 53 °C) were found for croissant. The yield of the total sugar is 29865mg/L. For doughnut, optimal

conditions for amyloglucosidase (375 U/L; 50 °C) were found and the yield of the total sugar is 15105mg/L. Both temperature and enzyme concentration have significant effect to yield of total sugar and reducing sugar. Generally, increase in temperature and enzyme concentration will increase the enzymatic activity and thus higher yield of sugar recovery. However, denaturation of enzyme occurs at extreme temperature and enzyme concentration might be the limiting factor.

In the future research, bakery waste can be utilized to produce valuable product. However, the problem is the pretreatment of the bakery waste. The pretreatment can be an issue in costing. Besides, storage of waste bakery is the major problem as it could affect the quality and safety. Spoilage may occur during storage such as mold growth. (Hudečková et al., 2017). Hence, these problems and utilization of waste bread for production of biotechnological products requires further study.

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APPENDIX A (STANDARD CURVE)

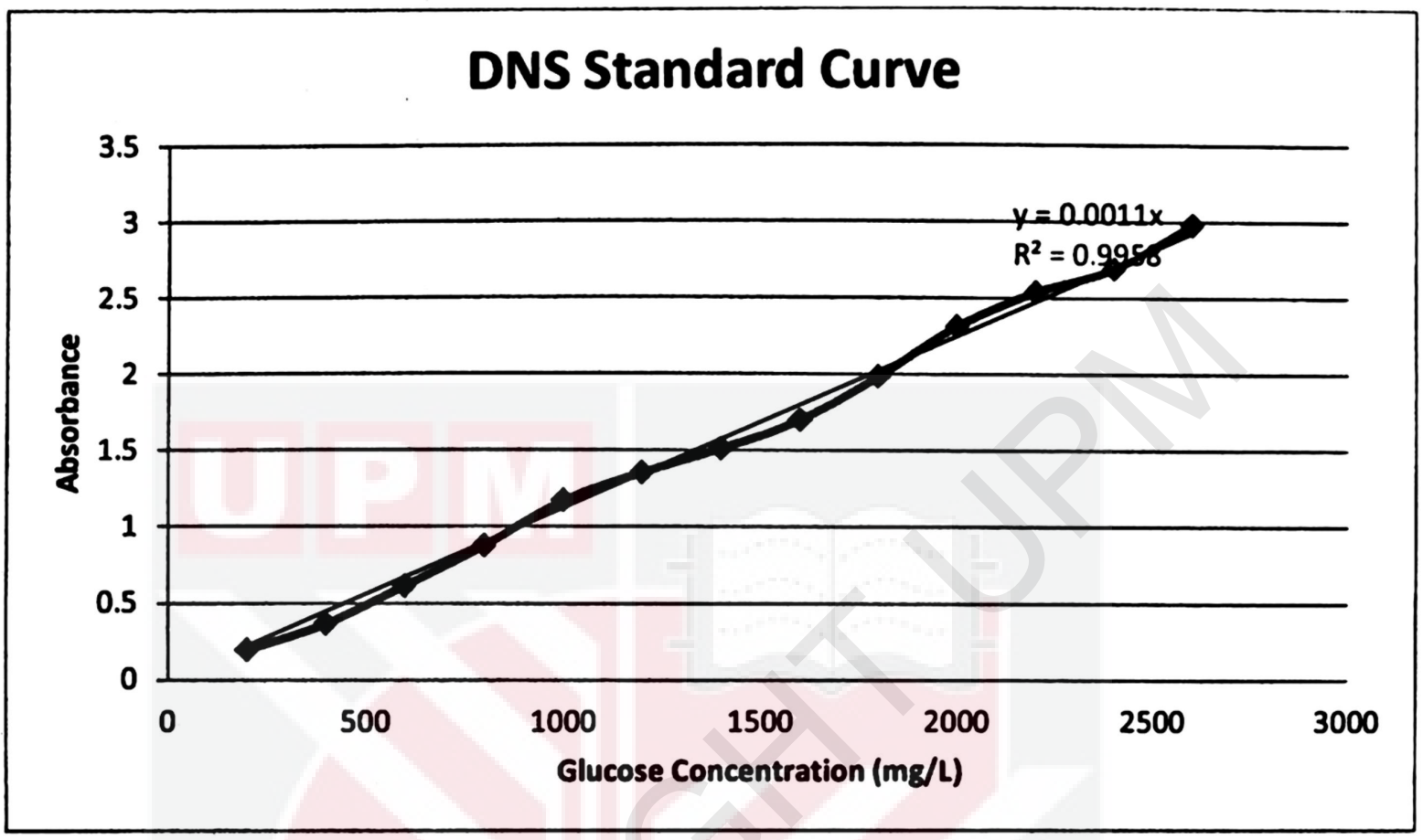

Figure 25: The Graph of Absorbance of against Glucose Concentration

Y= 0.0011X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Reducing Sugar = X x 0.03L

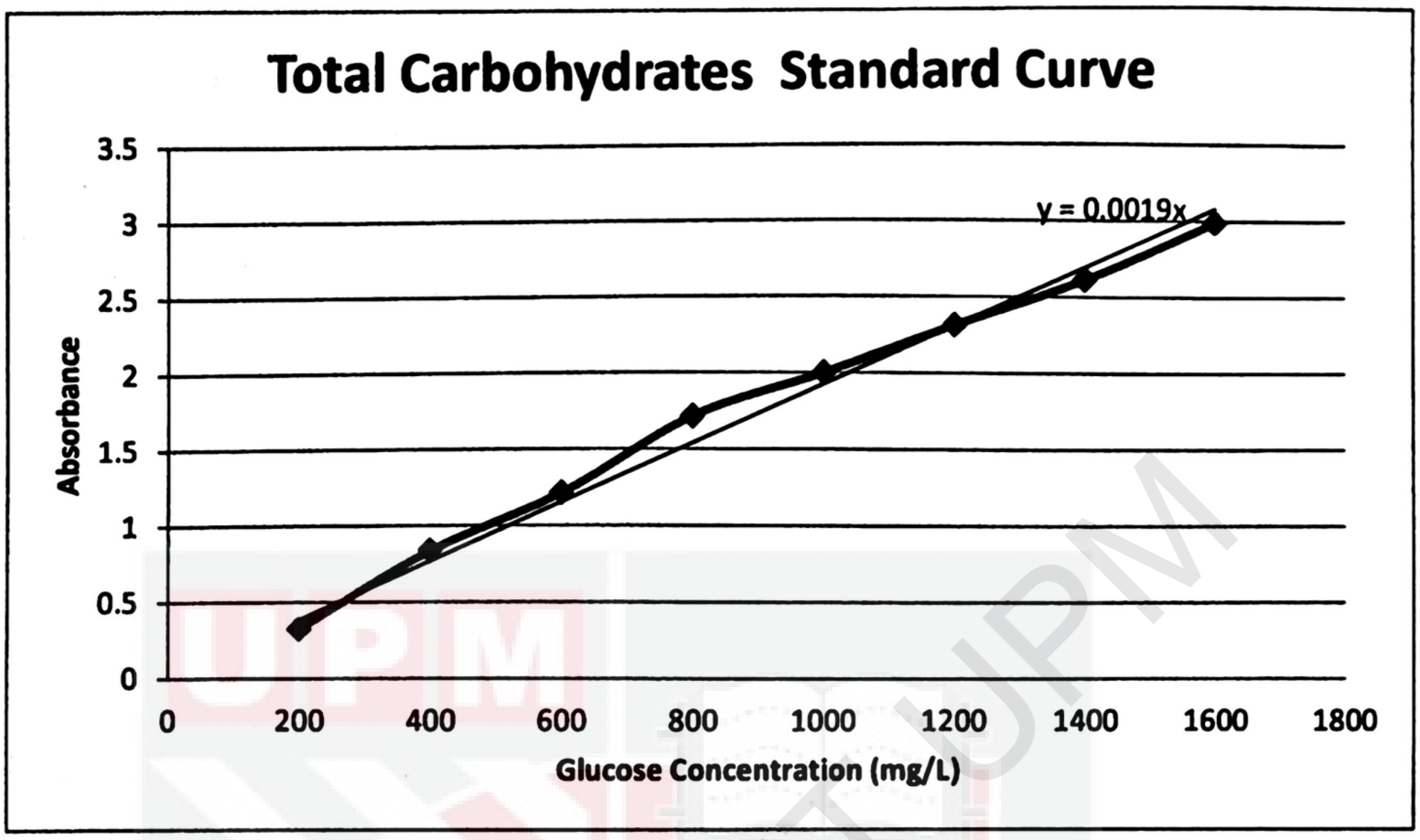

Figure 26: Standard Curve for Total Carbohydrates

Y= 0.0019X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Reducing Sugar = X x 0.03L

APPENDIX B (HPLC CALIBRATION CURVE)

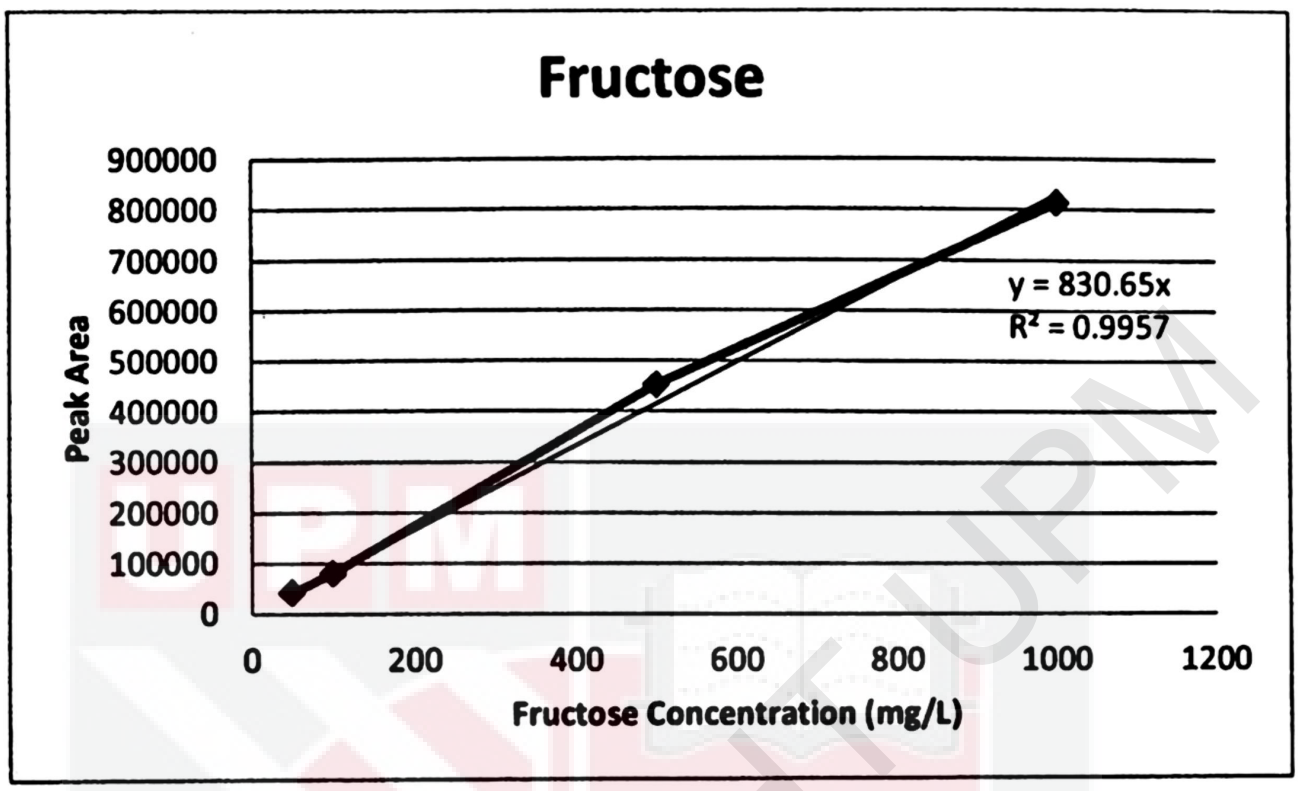

Figure 27: HPLC Calibration Curve for Fructose

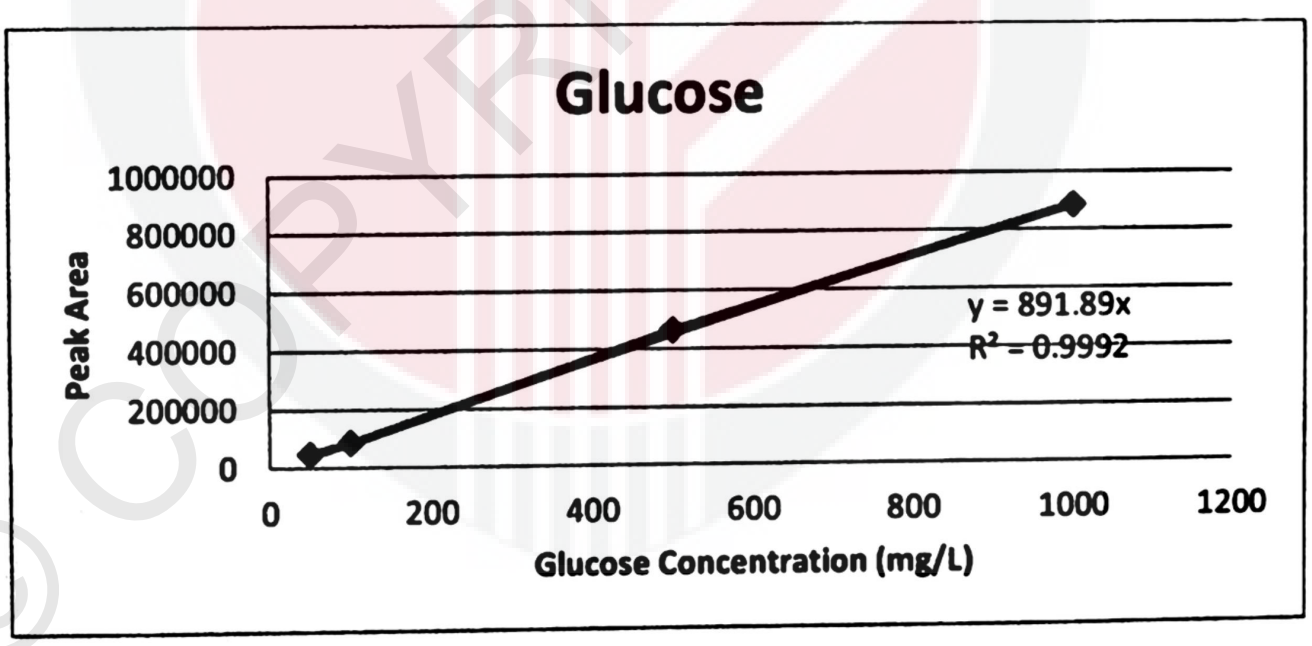

Figure 28: HPLC Calibration Curve for Glucose

APPENDIX C (CALCULATION)

REDUCING SUGAR CALCULATION (CROISSANT)

Y= 0.0011X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Reducing Sugar = X x 0.03L

Process conditions			DNS Absorbance		Reducing Sugar (mg)		
Bakery	Enzyme %	Temperature °C	1	2	1	2	Average
Croissant	0.1	45	2.528	2.989	68.95	81.52	75.23
	0.5	45	2.797	2.870	76.28	78.27	77.28
	1	45	2.933	2.930	79.99	79.91	79.95
	0.1	55	2.765	2.799	75.41	76.34	75.87

	0.5	55	2.937	2.938	80.10	80.13	80.11
	1	55	2.969	2.801	80.97	76.39	78.68
	0.1	65	2.013	2.812	54.90	76.69	65.80
	0.5	65	1.656	1.763	45.16	48.08	46.62
	1	65	2.258	2.667	61.58	72.74	67.16

Table 10: Total Reducing Sugar Calculation of Croissant

TOTAL REDUCING SUGAR CALCULATION (DOUGHNUT)

Y= 0.0011X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Reducing Sugar = X x 0.03L

Process conditions			DNS Absorbance		Reducing Sugar (mg)		
Bakery	**Enzyme %**	**Temperature °C**	**1**	**2**	**1**	**2**	**Average**
Doughnut	0.1	45	2.692	2.672	73.42	72.87	73.15
	0.5	45	2.927	2.913	79.83	79.45	79.64
	1	45	2.885	2.832	78.68	77.24	77.96
	0.1	55	1.902	2.314	51.87	63.11	57.49
	0.5	55	2.172	2.374	59.24	64.75	61.99
	1	55	2.931	2.472	79.94	67.42	73.68
	0.1	65	1.419	1.487	38.70	40.55	39.63
	0.5	65	1.422	1.377	38.78	37.55	38.17
	1	65	1.594	1.989	43.47	54.25	48.86

Table 11: Total Reducing Sugar Calculation of Doughnut

TOTAL CARBOHYDRATES CALCULATION (CROISSANT)

Y= 0.0019X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Total Reducing Sugar = X x 0.03L

Process conditions			Absorbance		Dilution Factor		Total Carbohydrates (mg)		
Bakery	Enzyme %	Temperature °C	1	2	1	2	1	2	Average
Croissant	0.1	45	2.019	2.115	20	20	637.58	667.89	652.74
	0.5	45	2.355	2.412	20	20	743.68	761.68	752.68
	1	45	2.417	2.383	20	20	763.26	752.53	757.89
	0.1	55	2.757	2.658	20	20	870.63	839.37	855.00

	0.5	55	2.941	2.965	20	20	928.74	936.32	932.53
	1	55	2.891	2.794	20	20	912.95	882.32	897.63
	0.1	65	0.982	0.973	20	20	310.11	307.26	308.68
	0.5	65	0.889	1.258	20	20	280.74	397.26	339.00
	1	65	1.221	1.389	20	20	385.58	438.63	412.11

Table 12: Total Carbohydrates Calculation of Croissant

TOTAL CARBOHYDRATES CALCULATION (DOUGHNUT)

Y= 0.0019X

Y= absorbance

X= glucose concentration (mg/L)

1 sample = 30ml

Total Carbohydrates

= X x 0.03L

Process conditions			Absorbance		Dilution Factor		Total Carbohydrates (mg)		
Bakery	**Enzyme %**	**Temperature °C**	**1**	**2**	**1**	**2**	**1**	**2**	**Average**
Doughnut	0.1	45	1.615	1.783	20	20	510.00	563.05	536.53
	0.5	45	1.957	2.278	20	20	618.00	719.37	668.68
	1	45	2.311	2.266	20	20	729.79	715.58	722.68
	0.1	55	1.837	1.304	20	20	580.11	411.79	495.95
	0.5	55	2.515	2.367	20	20	794.21	747.47	770.84
	1	55	2.418	2.558	20	20	763.58	807.79	785.68
	0.1	65	1.254	1.358	10	10	198.00	214.42	206.21
	0.5	65	2.435	1.975	10	10	384.47	311.84	348.16
	1	65	1.52	1.811	10	10	240.00	285.95	262.97

Table 13: Total Carbohydrates Calculation of Doughnut

HPLC CALCULATION

Process conditions			Type of Sugars Present	Peak Area		Amount of Sugar (mg)		
Bakery	Enzyme %	Temperature °C		1	2	1	2	Average
Croissant	0.1	45	Glucose	7874119	5602640	264.86	188.45	226.66
			Fructose	6385675	5401486	230.63	195.08	212.85
	0.5	45	Glucose	9453049	8453120	317.97	284.33	301.15
			Fructose	7637687	7133770	275.84	257.65	266.75
	1	45	Glucose	10352913	9487100	348.24	319.11	333.67
			Fructose	8686143	7829451	313.71	282.77	298.24
	0.1	55	Glucose	4535768	4401340	152.57	148.05	150.31
			Fructose	4497385	3545422	162.43	128.05	145.24
	0.5	55	Glucose	8451526	5282788	284.28	177.69	230.99
			Fructose	7258622	4253126	262.15	153.61	207.88

	1	55	Glucose	9444271	7368240	317.67	247.84	282.76
			Fructose	8011185	5549912	289.33	200.44	244.89
	0.1	65	Glucose	2386812	2226871	80.28	74.90	77.59
			Fructose	3525306	3283514	127.32	118.59	122.95
	0.5	65	Glucose	3853187	4228602	129.61	142.24	135.92
			Fructose	4974663	4677188	179.67	168.92	174.29
	1	65	Glucose	7024837	5970750	236.29	200.83	218.56
			Fructose	5762154	5331792	208.11	192.56	200.34

Table 14: Type and Amount of Sugar Present in Croissant

Process conditions			Type of Sugars Present	Peak Area		Amount of Sugar (mg)		
Bakery	Enzyme %	Temperature °C		1	2	1	2	Average
Doughnut	0.1	45	Glucose	5189462	5414331	187.42	195.55	191.48
			Fructose	4113736	4533518	148.57	163.73	156.15
	0.5	45	Glucose	7154781	10103683	240.66	339.85	290.26
			Fructose	NA	NA	NA	NA	NA
	1	45	Glucose	11613986	NA	390.65	0.00	195.33
			Fructose	NA	NA	NA	NA	NA
	0.1	55	Glucose	1496646	1233899	50.34	41.50	45.92
			Fructose	2062120	1686266	74.48	60.90	67.69
	0.5	55	Glucose	2830965	2522248	95.22	84.84	90.03
			Fructose	3169653	2433091	114.48	87.87	101.18
	1	55	Glucose	2498468	3338269	84.04	112.29	98.16

			Fructose	2461819	2961201	88.91	106.95	97.93
	0.1	65	Glucose	2115441	2061523	71.16	68.07	69.62
			Fructose	2321696	2251357	83.85	78.38	81.12
	0.5	65	Glucose	3101505	2740223	104.32	92.17	98.25
			Fructose	2806171	2365254	101.35	85.42	93.39
	1	65	Glucose	3234952	3512678	108.81	124.32	116.57
			Fructose	2436439	2364589	88.00	92.66	90.33

Table 15: Type and Amount of Sugar Present in Doughnut

APPENDIX D (HPLC RETENTION TIME)

Sugar	Concentration	Area	Retention Time
Sucrose	1000	879129	22.25
	500	485049	23.958
	100	91183	24.042
	50	47100	23.908
Fructose	1000	812658	24.2
	500	451954	24.225
	100	79851	24.175
	50	41533	24.25
Glucose	1000	883594	22.717
	500	462967	22.742
	100	86297	22.758
	50	45953	22.683
Galactose	1000	880343	23.925
	500	424283	23.933
	100	81511	23.883
	50	42423	23.925
Mannose	1000	883281	23.925
	500	448783	23.925
	100	95012	23.908
	50	48742	23.892

Table 16: Common Types of Sugars

APPENDIX E (RESULT DATA)

Process conditions			Reading 1	Reading 2	Total Reducing Sugar (mg)
Bakery	**Enzyme %**	**Temperature °C**			
Croissant	0.1	45	76.28	78.27	75.23
	0.5	45	79.99	79.91	77.28
	1	45	75.41	76.34	79.95
	0.1	55	80.10	80.13	75.87
	0.5	55	80.97	76.39	80.11
	1	55	54.90	76.69	78.68
	0.1	65	45.16	48.08	65.80
	0.5	65	61.58	72.74	46.62
	1	65	76.28	78.27	67.16

Table 17: Reducing Sugar of Croissant

Process conditions			Reading 1	Reading 2	Reducing Sugar (mg)
Bakery	**Enzyme %**	**Temperature °C**			
Doughnut	0.1	45	73.42	72.87	73.15
	0.5	45	79.83	79.45	79.64
	1	45	78.68	77.24	77.96
	0.1	55	51.87	63.11	57.49
	0.5	55	59.24	64.75	61.99
	1	55	79.94	67.42	73.68
	0.1	65	38.70	40.55	39.63
	0.5	65	38.78	37.55	38.17
	1	65	43.47	54.25	48.86

Table 18: Reducing Sugar of Doughnut

Process conditions			Total Carbohydrates (mg)		
Bakery	**Enzyme %**	**Temperature °C**	**1**	**2**	**Average**
Croissant	0.1	45	637.58	667.89	652.74
	0.5	45	743.68	761.68	752.68
	1	45	763.26	752.53	757.89
	0.1	55	870.63	839.37	855.00
	0.5	55	928.74	936.32	932.53
	1	55	912.95	882.32	897.63
	0.1	65	310.11	307.26	308.68
	0.5	65	280.74	397.26	339.00
	1	65	385.58	438.63	412.11

Table 19: Total Carbohydrates for Croissant

Process conditions			Total Carbohydrates (mg)		
Bakery	**Enzyme %**	**Temperature °C**	**1**	**2**	**Average**
Doughnut	0.1	45	510.00	563.05	536.53
	0.5	45	618.00	719.37	668.68
	1	45	729.79	715.58	722.68
	0.1	55	580.11	411.79	495.95
	0.5	55	794.21	747.47	770.84
	1	55	763.58	807.79	785.68
	0.1	65	198.00	214.42	206.21
	0.5	65	384.47	311.84	348.16
	1	65	240.00	285.95	262.97

Table 20: Total Carbohydrates for Doughnut

Bakery	Enzyme %	Temperature °C	pH Reading 1	pH Reading 2	Average
Croissant	0.1	45	4.49	4.93	4.71
	0.5	45	4.92	4.27	4.60
	1	45	4.82	4.96	4.89
	0.1	55	4.67	4.76	4.72
	0.5	55	4.5	4.75	4.63
	1	55	4.93	4.34	4.64
	0.1	65	5.44	5.52	5.48
	0.5	65	5.43	5.18	5.31
	1	65	5.18	5.2	5.19
Doughnut	0.1	45	4.95	5.04	5.00
	0.5	45	4.5	4.75	4.63
	1	45	4.32	4.56	4.44
	0.1	55	5.02	5.24	5.13
	0.5	55	4.13	3.98	4.06
	1	55	4.79	5.08	4.94
	0.1	65	5.28	5.16	5.22
	0.5	65	4.68	4.99	4.84
	1	65	5.32	5.15	5.24

Table 21: The pH of Bakery Waste

Process conditions			Type of Sugars Present	Amount of Sugar (mg)		
Bakery	Enzyme %	Temperature °C		1	2	Average
Croissant	0.1	45	Glucose	264.86	188.45	226.66
			Fructose	230.63	195.08	212.85
	0.5	45	Glucose	317.97	284.33	301.15
			Fructose	275.84	257.65	266.75
	1	45	Glucose	348.24	319.11	333.67
			Fructose	313.71	282.77	298.24
	0.1	55	Glucose	152.57	148.05	150.31
			Fructose	162.43	128.05	145.24
	0.5	55	Glucose	284.28	177.69	230.99
			Fructose	262.15	153.61	207.88
	1	55	Glucose	317.67	247.84	282.76
			Fructose	289.33	200.44	244.89
	0.1	65	Glucose	80.28	74.90	77.59
			Fructose	127.32	118.59	122.95
	0.5	65	Glucose	129.61	142.24	135.92
			Fructose	179.67	168.92	174.29
	1	65	Glucose	236.29	200.83	218.56
			Fructose	208.11	192.56	200.34

Table 22: Type and Amount of Sugar Present in Croissant

Process conditions			Type of Sugars Present	Amount of Sugar (mg)		
Bakery	Enzyme %	Temperature °C		1	2	Average
Doughnut	0.1	45	Glucose	187.42	195.55	191.48
			Fructose	148.57	163.73	156.15
	0.5	45	Glucose	240.66	339.85	290.26
			Fructose	-	-	-
	1	45	Glucose	390.65	-	195.33
			Fructose	-	-	-
	0.1	55	Glucose	50.34	41.50	45.92
			Fructose	74.48	60.90	67.69
	0.5	55	Glucose	95.22	84.84	90.03
			Fructose	114.48	87.87	101.18
	1	55	Glucose	84.04	112.29	98.16
			Fructose	88.91	106.95	97.93
	0.1	65	Glucose	71.16	68.07	69.62
			Fructose	83.85	78.38	81.82
	0.5	65	Glucose	104.32	92.17	98.25
			Fructose	101.35	85.42	93.39
	1	65	Glucose	108.81	124.32	116.57
			Fructose	88.00	92.66	90.33

Table 23: Type and Amount of Sugar in Doughnut

Run	Factor A: Temperature	Factor B: Concentration	Response 1 Total Carbohydrates	Response 2 Total Reducing Sugar	Response 3 pH
1	55	0.55	464.37	80.1	4.5
2	55	0.55	468.16	80.13	4.75
3	45	1	381.63	79.99	4.82
4	45	0.55	371.84	76.28	4.92
5	45	0.1	318.79	74.15	4.49
6	55	0.1	422.32	80.22	4.67
7	45	1	376.26	79.91	4.96
8	65	1	385.58	61.58	5.18
9	55	0.55	463.21	75.63	4.41
10	65	0.55	418.35	53.69	5.43
11	55	0.55	471.52	77.21	4.39
12	65	1	438.63	72.74	5.2
13	55	1	456.47	80.97	4.93
14	65	0.1	310.11	54.9	5.66
15	55	0.55	458.65	85.41	4.88
16	65	0.1	307.26	60.58	5.44
17	45	0.1	333.95	81.52	4.93

Table 24: Central Composite Design for Croissant

Run	Factor A: Temperature	Factor B: Concentration	Response 1 Total Carbohydrates	Response 2 Total Reducing Sugar	Response 3 pH
1	45	1	729.79	78.68	4.32
2	55	0.55	794.21	59.24	4.13
3	55	1	763.58	79.94	4.79
4	65	0.55	384.47	38.78	4.68
5	65	1	240	43.47	5.32
6	45	0.1	510	73.42	4.95
7	55	0.55	747.47	80.11	3.98
8	55	0.55	766.12	80.11	4.21
9	55	0.55	801.13	80.11	4.35
10	55	0.55	782.25	80.11	4.25
11	45	1	715.58	77.24	4.56
12	55	0.1	580.11	51.87	5.02
13	65	0.1	198	38.7	5.28
14	45	0.55	719.37	79.83	4.5
15	65	1	285.95	54.25	5.15
16	65	0.1	214.42	40.55	5.16
17	45	0.1	563.05	72.87	5.04

Table 25: Central Composite Design for Doughnut