UNIVERSITI PUTRA MALAYSIA

SUGAR RECOVERY FROM BAKERY WASTE (CHOCOLATE CAKE) VIA SUBCRITICAL WATER HYDROLYSIS

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ABSTRACT

Chocolate cakes are among the bakery products that often being disposed. These leftover chocolate cakes are mainly comprised of carbohydrates which have high potential for sugar recovery. The sugar of these bakery wastes can be recovered using subcritical water hydrolysis as this method is clean, quick and sustainable. This project was designed to evaluate the sugar recovery from waste of chocolate cake by using subcritical water hydrolysis. The leftover chocolate cakes were hydrolyzed using batch-type subcritical water reactor at process temperature of 160°C to 200°C for 5min to 15min with solid loading of 10% to 50%. For this study, the total sugar and reducing sugar yield were increased with the increase of process temperature, process time and solid loading. The optimum process conditions for sugar recovery of leftover chocolate cakes in this study were 200°C, 15min and 50%, obtaining total sugar yield of 0.895 mg/ml and 0.700 mg/ml of reducing sugar. The carbohydrates were hydrolyzed, forming oligosaccharide (sucrose) and monosaccharides (glucose, fructose, galactose, xylose and mannose). At process temperature of 200°C, yielded the highest reducing sugar content with 0.8089mg/ml. The glucose content was highest among the reducing sugars which was 0.2958mg/ml. In a nutshell, this study illustrates that subcritical water treatment is potentially used for sugar recovery of bakery leftovers.

ABSTRAK

Kek coklat adalah antara produk bakeri yang sering dibuang. Kek coklat yang tersisa ini kebanyakannya terdiri daripada karbohidrat yang berpotensi tinggi untuk pemulihan gula. Gula sisa bakeri ini dapat diperoleh kembali menggunakan hidrolisis air subkritikal kerana kaedah ini bersih, cepat dan berterusan. Projek ini dirancang untuk menilai pemulihan gula dari sisa kek coklat dengan menggunakan hidrolisis air subkritikal. Sisa kek coklat dihidrolisis menggunakan reaktor air subkritikal jenis batch pada suhu proses 160°C hingga 200°C selama 5min hingga 15min dengan muatan pepejal 10% hingga 50%. Untuk kajian ini, jumlah hasil gula dan gula pereduksi meningkat dengan peningkatan suhu proses, masa proses dan pemuatan padat. Keadaan proses yang optimum untuk pemulihan gula daripada sisa kek coklat dalam kajian ini adalah 200°C, 15min dan 50%, memperoleh jumlah hasil gula 0.895 mg/ml dan 0.700 mg/ml gula pereduksi. Karbohidrat dihidrolisiskan, membentuk oligosakarida (sukrosa) dan monosakarida (glukosa, fruktosa, galaktosa, xilosa dan manosa). Pada suhu proses 200°C, kandungan gula pereduksi tertinggi telah dihasil dengan 0.8089 mg/ml. Kandungan glukosa adalah tertinggi di antara gula pereduksi iaitu 0.2958mg/ml. Ringkasnya, kajian ini menggambarkan bahawa rawatan air subkritikal berpotensi digunakan untuk pemulihan gula sisa bakeri.

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LIST OF ABBREVIATION

FAO - The United Nation Food and Agricultural Organization

DNS - Dinitrosalicylic acid

RSM - Response Surface Methodology

CCD – Central Composite Design

HPLC – High performance liquid chromatography

FTIR – Fourier transform infrared spectroscopy

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Food waste on daily basis is discarded or disposed due to human beings living nature such as agricultural and industrial activities. The world has a staggering food waste issue which lead to global frustrations. The United Nation Food and Agricultural Organization (FAO) estimates that roughly one-third of the food produced for human consumption in the world is lost or wasted every year which approximately 1.3 billion tonnes (FAO, 2011). There is a quarter of the land of agricultural sector greater than the size of China heading for producing food that ultimately uneaten. 8 percent of the global greenhouse gas emission which contributing to global warming and climate change can be traced back to food loss and waste (Gustavsson J., et al., 2011).

According to the research of World Resources Institute (WRI, 2016), it shows that tackling the food waste and loss inefficiency is dominant for ensuring global food sustainability to cater the world population of 10 billion people by 2050. Eliminating food loss and waste is widely seen as a vital method to cut the production costs while increase the food system efficiency and improve the food nutrition and security. The Sustainable Development Goals (SDGs) has reflected food loss and waste for growing attention. For example, Zero Hunger goal (SDG 2) as a call for an end to hunger, food security achievement, improved nutrition and sustainable agriculture promotion (Abdul, J.M., 2010).

In Malaysia, the authority is encountered strenuous challenges in handling and treating the food waste. Food waste imparts the environmental issue as there is not stringent measures have been taken to address this issue. In fact, food waste is a putrescible and recyclable source in significant composition of 40% to 64% in solid waste in Malaysia. The quantity food waste is kept on escalating as the living standards of the people have improved and thus can afford more food products than before. Besides, the increment of population also increases the demands of food and more food waste is generated. Abdullah, N. and Chin, N.L (2010) reported that 90% of food waste is biodegradable and easy to recycle.

Generally, sugars can be extracted and recovered from the bakery waste such as expired bread and waste cake through hydrolysis. Researchers have made great attempts and introduced effective extraction techniques to address the challenges faced during extraction in order to implement environmentally benign methods for sugars recovery of carbohydrate-rich food products. The techniques include enzyme assisted extraction, acid assisted extraction (Suan, L., 2013) and subcritical water treatment (Zhu, G, et al., 2013). Subcritical water hydrolysis is a physical hydrolysis that attracted much attention in the field of reaction, extraction and chromatography recently (Khajavi, S.H., et al., 2005). Subcritical water hydrolysis is a short processing time and non-toxic solvent (water) requirement and considered as an eco-friendly technology (Abdelmoez, W., et al., 2014). Hence, subcritical water hydrolysis known as a good selectivity method for sugar extraction from bakery waste. The water used in subcritical water hydrolysis is maintained at liquid state and normally conducted at temperature range from 100°C to 374°C under controlled pressure which below the critical pressure of 218atm (Yoshida,

H., et al., 2014). The Figure 1.1 has depicted the phase diagram of water as a function of pressure and temperature. There is a marked and systematic decrease in permittivity, viscosity and surface tension while an increase in the diffusion rate when the temperature is escalating. As a result, the target materials at ambient temperature which are more polar and soluble in water are extracted efficiently at lower temperature.

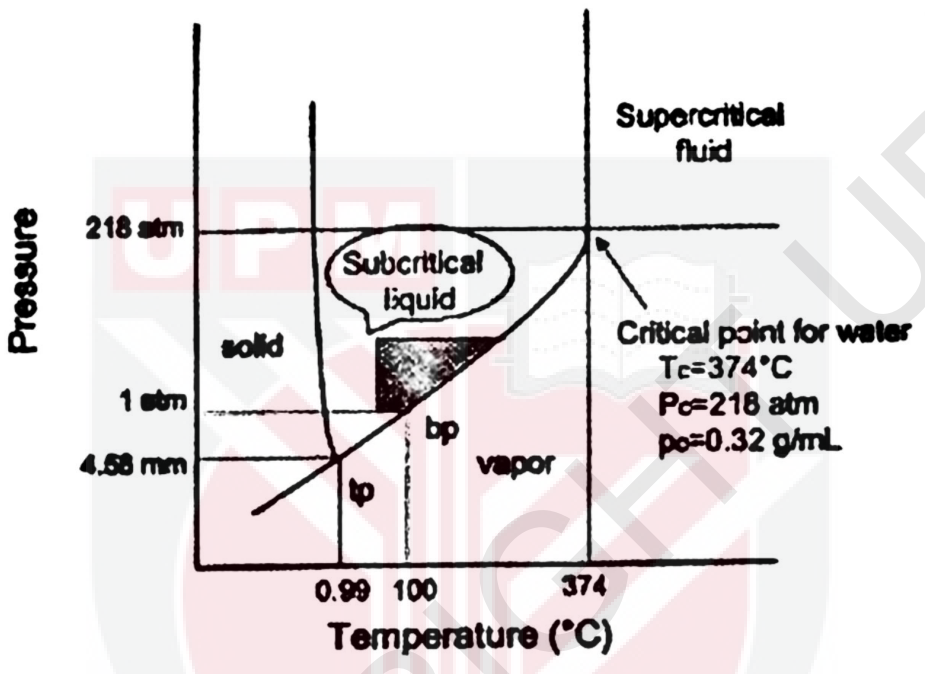


Figure 1. 1: Phase diagram of water as a function of pressure and temperature. (Adapted from Khajenoori, M., 2013).

The mechanism of subcritical water treatment commenced with desired compounds desorption from solutes at elevated pressure and temperature and followed by extracted compounds diffusion to the solvent. The extracted solution is then washed out from the cell of extraction into a collection vial. The subcritical water treatment can be classified into batch, semi-batch and continuous system (Yu,Y. and Wu, H., 2010). In a batch hydrolysis system, the specified quantity of sample and distilled water is put in the reactor. The air in the reactor is forced out by using argon gas. The proper tightened and capped reactor is then placed in a preheated oil bath at controlled process

condition. The reactor is removed from the heating bath after treatment and immersed in cooling water. In semi-batch process, the reaction medium in continuous flow is contacted with the food samples while the samples and solvent are continuously flowing into and exiting the reactor in continuous system (Lachos-Perez, D., et. al, 2017).

The hydrolysis efficiency of subcritical water is influenced by several process parameters such as reaction time, reaction temperature, solid-to-water ratio, pH value, sample particle size and solute characteristics (Ndlela, S., et al., 2012). Among the process parameters, the reaction time, reaction temperature and solid-to-water ratio have the greatest effects to the subcritical water process. Hence, these parameters needed to be optimized according to the type and amount of the samples used in order to obtain the desired products.

1.2 Problem Statement

Bread is the most wasted food in Britain which estimated to be 680000 tonnes of bakery waste produced every year (Taylor, A., 2012). There are approximately 80% of the waste come from the opened packages but not finished. The bakery wastes included mixed grain bread, wheat meal bread, pies, muffin and other bakery products. The reasons that the bakery wastes were disposed could be due to the bread or cakes are out of date, looking bad, leftover in plates, keep in the cupboard or fridge for a period of time and caused moldy (Gustavsson J., 2011). In fact, the bakery waste contains abundant of carbohydrates which has the high potential source to obtain sugar. Bakery wastes being the most promising source for the valuable food ingredients such as

monosaccharides, oligosaccharides and polysaccharides due to its richness formulations of starch and its short shelf life. The carbohydrates of the bakery waste also can be used as substrates and converted into desired products. Recently, the utilization of bakery wastes has become a research trend rather than just disposing and decomposing them. Several studies in this context have exploited that the bakery waste is renewable sources for yeast production, sugar solution or glucose syrup (Nakano, M. and Yoshida, S., 1977). However, the current methods of sugar recovery of bakery wastes bring along several side effects as the hydrolysis methods involving usage of chemicals which might harm the environment and affect the safety of the products. Besides, the process is more time-consuming and may corrode the equipment. Apart from chemicals usage, enzymatic hydrolysis method also has been considered as a way of sugar recovery due to its high sugar yield, but the high cost and toxic substances generated made not satisfactory and precise for sugar recovery. Hence, subcritical water treatment is efficient and suitable technique to recover the sugar from bakery wastes as this method is clean, quick and sustainable.

1.3 Objectives

Overall aim of this project is to evaluate the sugar recovery from waste of chocolate cake by using subcritical water hydrolysis.

The specific objectives of this study are:

i. To study the effects of the process parameters such as temperature, time and solid-to-water ratio on the recovery of sugars using subcritical water hydrolysis.

ii. To characterize the morphology residue and chemical compositions of the bakery waste hydrolysate.

1.4 Scope of the Study

To achieve the objectives of this study, proximate analysis is required to be carried out by using AOAC method (A.O.A.C, 2000). The moisture content, ash content, protein content and crude fat content of the bakery waste is measured. The carbohydrate was then calculated through the different between these contents. The subcritical water hydrolysis is carried out in a batch type reactor. There are three process parameters with distinct levels used to evaluate this study which are process temperature (160°C, 180 °C, 200°C), processing time (5min, 10min, 15min) and solid loading (10%, 30%, 50%w/v). The pH of the hydrolysate is measured after centrifugation by using pH meter. The total sugar content and the reducing sugar content of the cake waste are determined using phenol-sulphuric acid method and 3,5-dinitrosalicyclic acid (DNS) method respectively. The analysis of optimization was proceeded to determine the significance of each factors namely hydrolysis temperature, process time and solid loading. Apart from that, sugar profile analysis was carried out by using high-performance liquid chromatography (HPLC) to determine the type and concentrations of the sugars. Response surface methodology (RSM) and Fourier Transform Infrared Spectroscopy (FTIR) also employed in experimental design and analysis. The residue after the subcritical hydrolysis was analyzed using scanning electron microscopy (SEM).

1.5 Thesis Organization

This thesis consists of five chapters. Chapter one discusses the background of the research and problem statement, followed by the outline of the research objectives, the scope and the organization of the dissertation. Chapter two illustrates the literature review for the related studies that have been carried out and reported previously including general treatment for sugar extraction of food waste, fundamental of subcritical water treatment as hydrolysis technique, influential factors of subcritical water treatment, potential of cake waste as the source of sugar using subcritical water hydrolysis and the response surface methodology. Chapter three presents the methods in conducting experiments, analyzing data and results on the preliminary findings of sugar recovery through subcritical water treatment. Results and discussions are described in chapter four. The experimental results of the sugar recovery from bakery waste via subcritical water hydrolysis are presented and the result outcomes are discussed in this chapter. The conclusion and recommendations for future research is given in Chapter five.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Potential of bakery wastes as the sources of sugars recovery

Bakery industry can be considered as one of the world's major food industries. The output of the bakery industries varies widely in term of production scale and process. Bakery products can be classified into bread or bread roll products, specialty products (doughnuts, biscuits and cakes for instances) and pastry products (pastries and pies). Due to the short shelf life of the bakery products, a large quantity of by-products is generated after distribution of baked goods. The bakery wastes such as bread, dough, cakes, cracker, cereals are goods that can be recycled. It has been estimated that 2% of the bread production is wasted while others also claimed that the loss amount could be reached about 5%. Bakery in large capacity produces large volumes of goods. These manufacturing units even though only come out with small percentages of rejected products, they may amount to hundreds tons of inedible products per week due to extremely huge production rate.

The research findings of Oda, Y., et al., 1997 reported that there are approximately 80% of bakery products that are not consumed but discarded even before their expiry date. The reasons of disposal due to bakery products spoilage is simply assumed, products are out of the date, abnormal physical appearance, long storage period or grow on molds. The composition of different type of bakery wastes are illustrated in Table 2.1.

Table 2. 1: Composition of different bakery waste per 100g (Zhang, et al., 2013)

	Bread	Cake	Pastry
Moisture	22.3 g	45.0 g	34.5 g
Starch (dry basis)	59.8 g	12.6 g	44.6 g
Carbohydrate	46.8 g	62.0 g	33.5 g
Lipids	0.9 g	19.0 g	35.2 g
Sucrose	3.0 g	22.7 g	4.5 g
Fructose	—	11.9 g	2.3 g
Protein (TN × 5.7) (dry basis)	8.9 g	17.0 g	7.1 g
Total phosphorus (dry basis)	Trace	1.5 g	1.7 g
Ash (dry basis)	—	1.6 g	2.5 g

The results indicated that both pastry and cake wastes are rich in carbohydrates and thus could be the high potential source for sugar recovery. In addition, the abundance of the bakery wastes and their formulations that rich of starch and sugars permit the bakery wastes to be the most promising source for the valuable food ingredients production such as monosaccharides and oligosaccharides.

2.2 Bakery waste utilization as a source of sugars

Substantial amount of sugar components can be acquired from food wastes especially from the carbohydrate-rich food products such as bakery waste. Carbohydrates can be classified into monosaccharide, oligosaccharides and polysaccharides based on their structure, molecular weight or degree of polymerization. Monosaccharides are the simplest form of carbohydrates. They are water-soluble crystalline compounds which comprised of carbonyl and hydroxyl groups. Glucose, fructose and galactose are common monosaccharides present in foods. Oligosaccharides which known as raffinose consist of two or more monosaccharides (<20 monomers) that

joined together by O-glycosidic bonds. Most of the carbohydrates are present as polysaccharides in nature and usually act as stabilizers in food industry. The examples of polysaccharides included gums, starch and pectin (Sarko, A., et al., 1976).

2.2.1 Monosaccharides

Monosaccharides are the most basic form of carbohydrates and can be combined to create a larger polymer. Monosaccharides are used for energy storage. Human bodies create energy by breaking down the monosaccharides such as glucose and the energy released from the glycosidic bonds are harvested. Monosaccharides also used to generate long fibers that provided cellular structure for plants.

Monosaccharides have general formula of $(CH_2O)_n$ that designates central carbon bonds with two hydrogen and one oxygen. Hydroxyl group is formed when oxygen bonded with hydrogen while carbonyl group is created when the carbon in the chain form a double bond with oxygen. The monosaccharides are in aldose family if the carbonyl group is present at the end of the chain while they are in ketose family when the carbonyl group is occurred in the middle of the chain. Glucose is the common example of monosaccharides in nature and used widely in every form of life. Glucose comprised of 6 carbons with carbonyl group at first carbon and thus it is in aldose family. To form a connected and stable ring of carbons, the hydroxyl group at fifth carbon will react with the first carbon while the double bonded oxygen at the first carbon will bond with a new hydrogen. The structure of glucose is as shown in Figure 2.1.

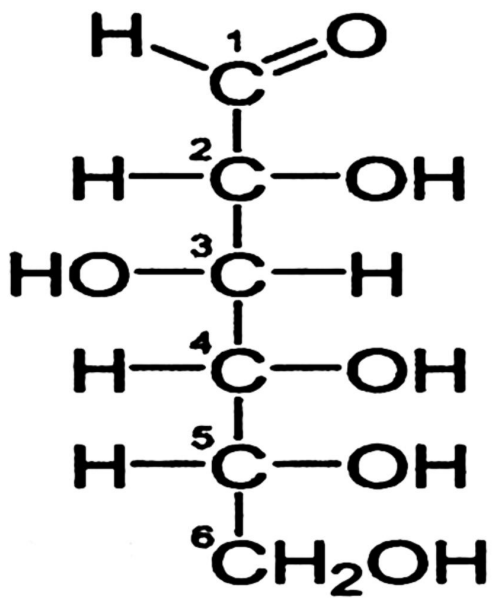


Figure 2. 1 : Structure of glucose. (Adapted from Biologydictionary.net, 2017)

Glucose can be broken down through glycolysis and provided energy for cellular respiration. Glucose will combine with other monosaccharides in order to be stored when the cells do not require that much of energy at particular moment. The chains of glucose that stored by animals are in term of polysaccharide glycogen. For plants, the excessive glucose would be stored as starch and used as energy later.

2.2.2 Oligosaccharides

Oligosaccharides are known as saccharide compounds which consisting 2 to 20 sugar units and included certain indigestible disaccharides in this case. Meanwhile, carbohydrates can also be classified into digestible and indigestible according to the physiological properties (Mussatto, S.I. & Mancilha, I.M., 2007).

Non-digestible carbohydrates may include polysaccharides and oligosaccharides which are transferred intact to the large intestines through peristalsis. The concept of the non-digestible oligosaccharides (NDO) can be observed through the present of the configuration in anometric C atom (C1 or C2) of the monosaccharide units of dietary oligosaccharides that creates osidic bounds non-digestible to the hydrolytic activity of

the human digestive enzymes (Roberfroid, M., & Slavin, J., 2000). The dietary oligosaccharides in the colon have the potential to undergo fermentation selectively and stimulate the intestinal micro biota bringing several beneficial systemic effects to the body. Hence, oligosaccharides are denoted as saccharides with prebiotic properties due to their functional properties (Moure, A., et al., 2006). Many NDO are indigestible as human body lacks the enzymes to utilized and hydrolyze the β-links formed among the units of some monosaccharide. With this property, NDOs are suitable for diabetes patients consumption and as a resource for low-calorie diet food products (Rivero-Urgell, M., & Santamaria-Orleans, A., 2001). Oligosaccharides with prebiotic properties manage to grab the researchers and the food industry interests due to their ability to be the ingredients for the formulation of functional foods (Barreteau, H., et al., 2006). The main categories of NDO in development as food ingredients comprised of the monosaccharide units such as glucose, fructose, galactose and xylose as illustrated in Figure 2.2.

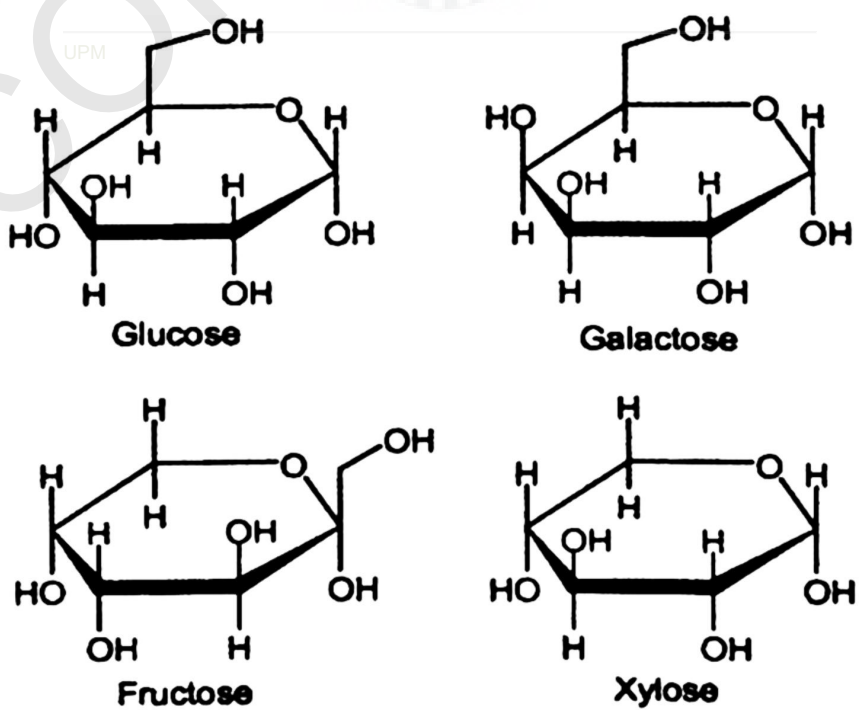


Figure 2. 2: Monosaccharide units of non-digestible oligosaccharides (Adapted from Mussatto, S.I. & Mancilha, I.M., 2007).

The specific physicochemical and physiological properties of the products containing food-grade oligosaccharides are varied with the type of mixture purchased. Oligosaccharides are water-soluble and mildly sweet. The sweetness in fact influenced by the chemical structure, the degree of present of oligosaccharides polymerization and the levels of monosaccharide and disaccharide in the mixture (Voragen, A. G., 1998). The sweetness of the sugars reduces with the increase of the oligosaccharide chain length. Their relatively low sweetness intensity made them useful in reducing sweetness of food production where the use of sucrose is restricted by its high sweetness property. In this condition, the desirable sweetness of food products can be achieved and thus enhance the food flavors.

The oligosaccharides can be used to control the intensity of browning due to Maillard reactions during heating and alter the freezing point of the frozen food. They also assist to retain the moisture to prevent drying excessively. Oligosaccharides are observed to be useful in controlling the microbial contamination due to their low water activity (Crittenden, R. A., & Playne, M., 1996). Furthermore, oligosaccharides are vital modification of the colonic microflora as they serve as low pH substrate to aid the anaerobic bacteria proliferation especially the bifidobacteria. As a result, the growth of pathogenic bacteria in the caeco-colon is inhibited (Sangeetha, P. T., et al., 2005).

2.2.3 Polysaccharides

Polysaccharides are formed when long chain of monosaccharides are linked together by glycosidic bonds. The molecular weight of the polysaccharides may reach 100,000 daltons and depends on the number of monomers linked.

Starch is form of sugars that stored in plants. It made out of amylose and amylopectin. The starch in the seeds provides food for germination and then become source of food for humans. Bodies will break down the consumed starch by salivary amylases. The amylose will be digested into smaller molecules known as maltose and glucose. The glucose as the simplest form of sugars can be absorbed easily by the body cells. On the other hand, glucose is stored as glycogen in bodies. Glycogen is made up of glucose monomers. The glycogen generally stored in liver and muscle cells and will be broken down through glycogenolysis to release glucose when blood glucose level is decreased. The figure below (Figure 2.3) depicted the structure of amylose.

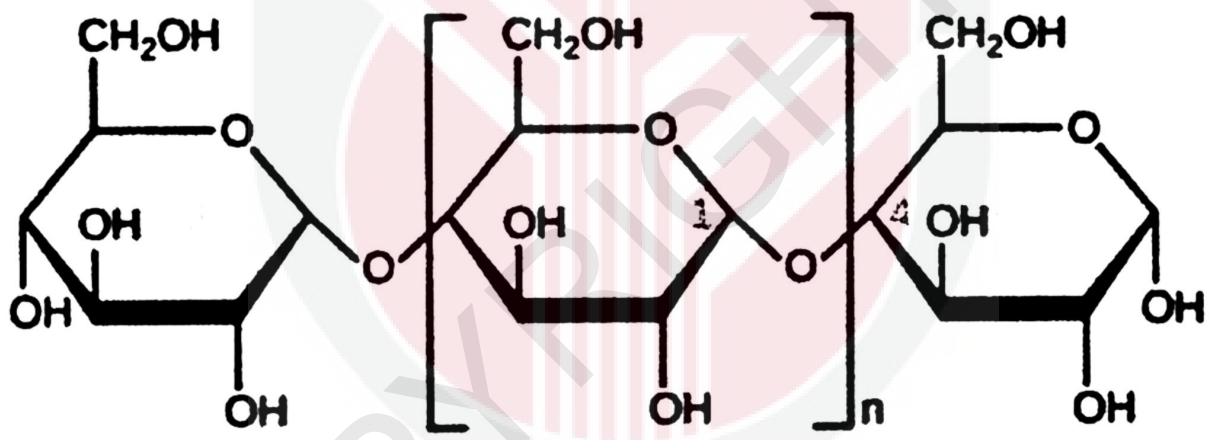


Figure 2. 3 : Structure of polysaccharide (amylose). (Adapted from Pasari, N., et al., 2017).

2.3 General treatments for the sugar extraction from bakery waste

To obtain the monosaccharides or oligosaccharides from polysaccharides, hydrolysis process is essential to be taken out to breakdown long-chain in the polysaccharides. Deshpande, M.S., et al. (1977) stated that the carbohydrate from the bakery waste could be converted into glucose or fermentable sugars through hydrolysis. The sugars can be obtained from the waste food products through hydrolysis and

extraction (Ekvall, J., et al, 2007). Bakery waste consists priory starch and minimal amounts of hemicelluloses and celluloses (Chandrasekaran, M., 2012). According to the research of Moure, A., Gullon, P., Dominguez, H. & Parajo, J.C., 2006), The hydrolysis process can be classified into acid hydrolysis, enzymatic hydrolysis and physical hydrolysis. These processes will yield different chemical properties as the chain length, monosaccharide composition, branching degree and the product purification will be influenced and varied (Mussatto, S.I. & Mancilha, I.M., 2007).

2.3.1 Acid hydrolysis

Choi, C.H. and Mathews, A.P. (1996) suggested a two-step method that yielded 92% of conversion into sugar without xylose in the hydrolysate. Acid hydrolysis is a relatively easy, cost-saving and easy-to-control process as the reaction is disturbed with neutralization of the medium (Warrand, J., & Janssen, H. G., 2007). Sulphuric acid, hydrochloric acid and trifluoroacetic acid are commonly used for polysaccharide depolymerization. The process usually optimized at temperature above 60°C and varied with time from 2 to 6 hours in average. However, acid hydrolysis may cause degradation of monosaccharide with sequential formation of toxic substances namely furfural and 5-hydroxymethylfurfural. Since acid hydrolysis is performed at high temperature and high acid conditions, a huge amount of glucosamine which also known as chitosan monomer is produced and caused difficulties in controlling the reaction progress (Ávila-Fernández, Á., et al., 2011). Hence, this method generates low yield of sugars.

2.3.2 Enzymatic hydrolysis

By referring to Barreteau, H., et al. (2006), enzymatic depolymerization of polysaccharides is the best option for greater oligosaccharides production. By applying enzymatic hydrolysis for sugar recovery, it is possible to obtain end products with desired molecular weight and minimum adverse chemical modifications (Holck, J., et al., 2011). The selective enzymes, which also known as biocatalysts, are greatly exploited and developed for more bioactive oligosaccharides production. Gluco-oligosaccharides can be produced in the trans-glycosylation method by β-glycosidase enzymes. Enzymes used for oligosaccharides hydrolysis process are microorganisms such as bacteria, fungi, endoenzymes and exoenzymes. Menezes, C. R., et als. (2009) stated that the production of these useful enzymes take place in raw material. The prebiotic activity for *Lactobacillus* and *Bifidobacterium* generation; Bacteroides and Clostridium can be illustrated by the pecticoligosaccharides (POS) that produced enzymatically (Mandalari, G., et. al., 2007). These enzyme productions required to undergo concentration or isolation with the purpose of microbiological contaminants elimination even though the enzymatic hydrolysis is efficient. Hence, the obtaining oligosaccharides through enzyme hydrolysis can be incurred higher expenses. Apart from that, enzymatic hydrolysis required necessity of a buffer to hinder the purity of the end products which could be troublesome (Burana-osot, J., et al., 2010).

2.3.3 Physical hydrolysis

Physical hydrolysis which also known as autohydrolysis carried out at high temperature from 130°C to 230°C to obtain the sugars (Vázquez, M. J., et al., 2006). It is a process involves bond cleavage by elements of water to produce hydrogen and

hydroxide ions. Subcritical water hydrolysis is a new and powerful physical hydrolysis method that generally operates at 100 °C to 374°C and under sufficient pressure to maintain the water at liquid state (Yoshida, H.; et al., 2014). There has been an increasing capacity of the literature of the subcritical water hydrolysis. This hydrolysis method has preference for wide range of application in the field of environment as it is a green and environmental-friendly technique, higher quality of extraction product, cost effective and less time consuming (Ravber, M., et al., 2015). In dynamic mode of subcritical water hydrolysis, water flows continuously through the solid samples. For static mode, on the other hand, both water and the sample are enclosed in the batch reactor. The combination of both modes is known as dynamic-static process (Jintana, W., et al., 2008).

2.4 Subcritical water extraction and hydrolysis technology

The water utilized in the subcritical water treatment gives unique properties over water at ambient conditions. Subcritical water technique has been exploited as an alternative to reduce the organic solvents usage in reaction medium (J.S.S. Pinto & F.M. Lancas, 2006). The subcritical water has low dielectric constant and high ion product. Due to the modification in the dielectric constant, the suitability of subcritical water as a solvent for dissolving organic compounds are increased. Besides, the production of ions in subcritical state is higher as compared to the ion produced in water at ambient conditions.

Water is used as a solvent for number of extraction processes focusing on the recovery of useful and potentially valuable food compounds which included bakery waste. Carbohydrates are the most common compounds that could be extracted using water as solvent due to their polarity. In general, an increase of the sugar extraction temperature will increase the rate of extraction. This could be due to the diffusion constants and solubility are elevated with the escalation of temperature. The final stage of the extraction process is controlled by diffusion and it can be improved by increasing the temperature of extraction (Rostagno, M.A. & Prado, J.M., 2013).

The hydrolysis temperature is limited to 100°C which is the normal water boiling point when water is used a solvent at ambient pressure for hydrolysis processes. In contrast, the process in pressurized systems can be performed above the boiling point of the solvent with the water remains at liquid state. In general, high-pressure hydrolysis processes using water can be carried out at temperatures up to 374°C. The characteristic of water as a solvent will be influenced when the hydrolysis temperature is escalated greater than 100°C. For instance, the dielectric constant and viscosity water are modified. Hence, the water solubility of mid-polar compounds increases with increasing temperature (Çam, M. & Hışıl, Y., 2010). Subcritical water hydrolysis usually more efficient than hydrolysis through organic solvents using conventional techniques as higher yields will be obtained for many compounds. Shitu et al., (2015) also claimed that subcritical water technology could be used for decomposition of food waste into simple sugars with high yields as compared with dilute acid pretreatment. Carbon dioxide are mostly used in reactions at supercritical conditions as catalyst. Supercritical water

treatment usually used for organic wastes treatment such as wastewater, municipal and industrial sludge (Li, Y., & Wang, S., 2019).

Generally, subcritical water hydrolysis is performed at a temperature range from 100 to 374°C under sufficient pressure (1 atm to 218 atm) to keep water in the liquid state (Yoshida et al., 2015). Subcritical water treatment aims to utilize the food waste such as bakery waste and recover the raw materials especially sugars for new products generation. In this condition, there would be a concomitant reduction in waste streams volumes. On the other hand, supercritical water processing is conducted at temperatures greater than 374°C and typically at pressure greater than 22MPa. A single non-condensable phase of water is performed at these conditions. Besides, the density of the supercritical water is close to a liquid and its transport properties similar to gas (Marrone, P.A. & Hong, G.T., 2009). Subcritical water treatment offers several advantages which including rapid reaction rates and replace the acids and bases usage with a more environmental-friendly solvent. In addition, subcritical water has gained great interest as a green alternative to replace hazardous organic solvents. This could be due to the subcritical water with tunable properties under proper temperature and pressure conditions mimic the properties of weakly polar organic solvents and hydro-organic solvent mixtures. It is also much easier to be disposed as compared to the hazardous organic solvents.

2.5 Physicochemical Properties of Water

Water at ambient temperature is a polar solvent with a density of 1000kg/m^3 and dielectric constant, ε, of 79.9. However, the hydrogen bonds of water break down when heated to higher temperature causing its dielectric constant to fall (Cheigh et al., 2015; Lu et al., 2014). Hence, the properties of water at subcritical state are significantly to be studied. The table below (Table 2.2) illustrated the properties of water at different conditions.

Table 2. 2: Water properties at different conditions and distinct state (Adapted from Jokić, S., et al., 2018).

Property	Water at normal conditions	Near-critical water	
T (°C)	25	350	400
P (bar)	1	250	500
ρ (kg m^{-3})	997.45	625.45	577.79
ε (–)	78.5	14.86	12.16
pKw (–)	14.0	11.5	11.5

Water at ambient conditions is a good solvent for polar compounds but not non-polar compounds because of its high cohesive energy. Polar compounds perform favorable dipole-dipole and hydrogen-bond interactions with the water molecules and result in overcoming its high cohesive energy. In contrast, non-polar compounds are incapable to perform such interactions thus cannot overcome the cohesive energy. Water may in fact turns into a slightly polar fluid at temperatures and pressures above its critical point. Subcritical water is considered an excellent solvent for organic non-polar compounds which are generally immiscible with the highly polar water at ambient

conditions. At such a low polarity water, the ability to dissolve the highly polar ionic compounds is disappeared but forms ion pairs or precipitate. On the other hand, subcritical water being a non-aggressive solvent that acts as an effective solvent for both polar and non-polar organic compounds.

Temperature make changes to the solvation properties and polarity of water which is usually expressed in terms of the dielectric constant. The dielectric constant of water decreases with the increase of temperature. Dielectric constants illustrate the affinity of water as a reaction media material. The dielectric constant of ethanol, methanol and pure water at room temperature and pressure are 27, 32.5 and 79.9 respectively (Singh, P.P.,2011). As indicated in Figure 2.4, the dielectric constant of the water decreases from 78.5 to 32.6 which is similar to the dielectric constant of methanol when the water is heated up to approximate 225°C at 20MPa. Subcritical water can dissolve organic compounds with low dielectric constant.

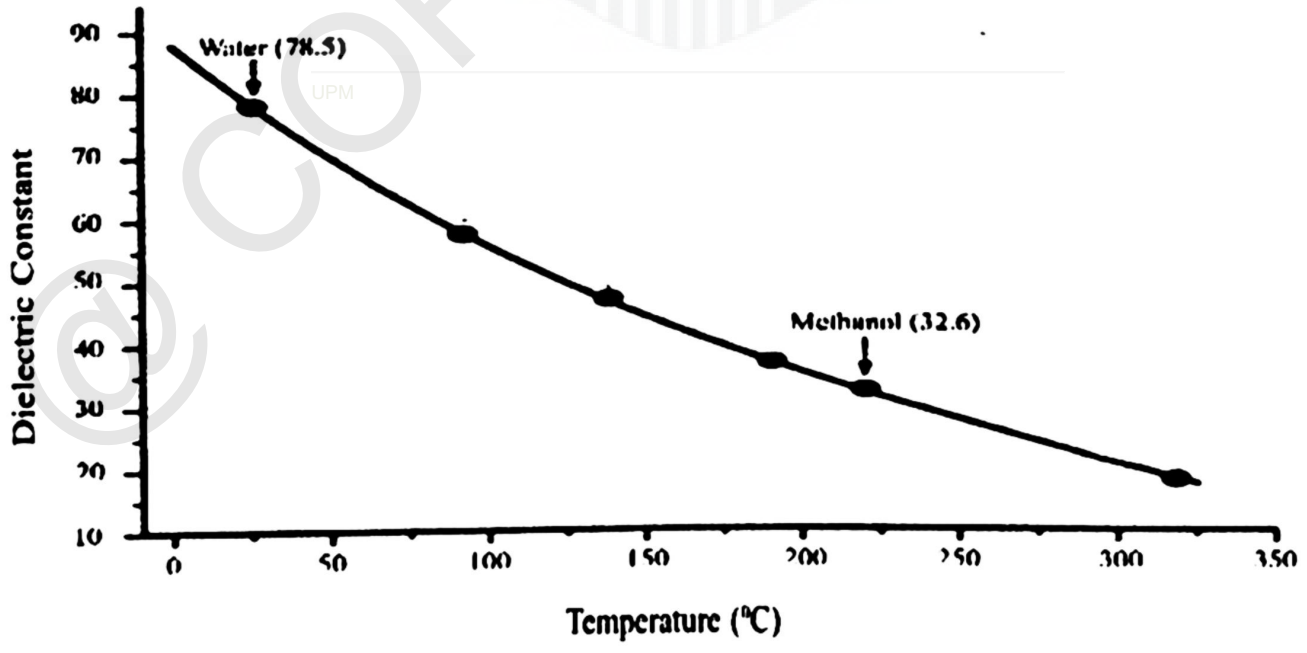


Figure 2. 4: Water dielectric constant behavior at 20 MPa (Adapted from Shitu, A., et al., 2015.)

Apart from that, other physical and chemical properties of water also change drastically with the variation of temperature and pressure. Dissociation constant of water, as an example, is significantly varied with temperature changes. Water at ambient temperature carried out auto-ionization process to provide hydronium and hydroxide ions. This process is characterized by a dissociation constant which originally is 1.0×10^{-14} at 25 °C and increased by 3 times when the experimental conditions changes from ambient to subcritical (Y. Yang & J. Sep., 2007). Meanwhile, the viscosity and density of water are reduced upon the condition changes which from ambient to subcritical. The changing trends of the thermodynamic properties of water are often redounded to the effect of temperature and pressure on its characteristic of hydrogen bond network. The presence of 4 hydrogen bonding sites in each water molecule enables water to own self-associating nature. Hence, water is potentially formed clusters of self-associating molecules with the molecules take part in varying numbers of hydrogen bonds (S.S. Xantheas, 2000; Y. Marcus & J. Mol. Liq., 1999). The hydrogen bonds are broken as the temperature of water is increased and results in alteration of hydrogen-bond network.

When water behaves as acid or base, it donates and gains protons. The interaction and reaction of water among each other produced basic hydroxide and acidic hydronium ions. The ionic constant, K_w, of water elevated with the increase of reaction temperature. Pourali, O., et al. (2009a) reported that the increment of ionic constant is about three times higher in magnitude than at room temperature. The ion product of water is defined as following equation.

$$K_w = [H+]\,[OH-] \qquad \textbf{(Eq. 2.1)}$$

The high ionization constant enables subcritical water to provide an acidic media for hydrolysis reaction. It also allows the water to behave like an acid catalyst. Figure 2.5 performed the ionization constant of water in subcritical water region.

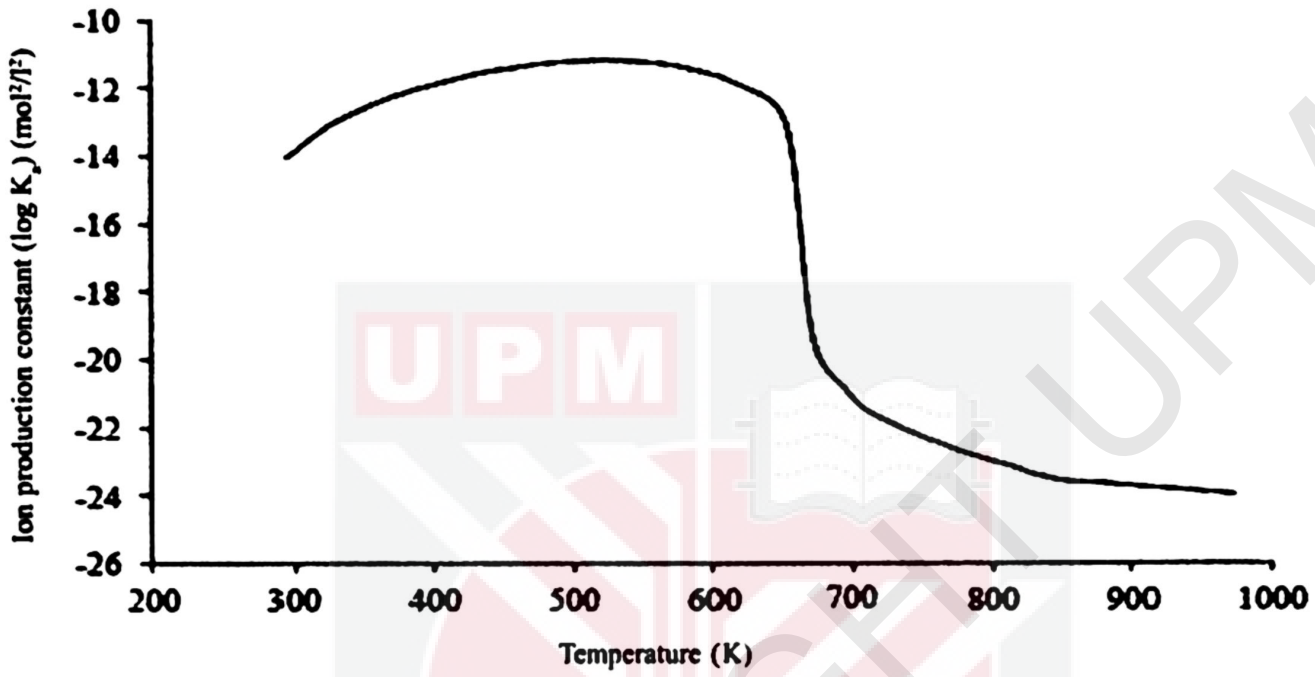
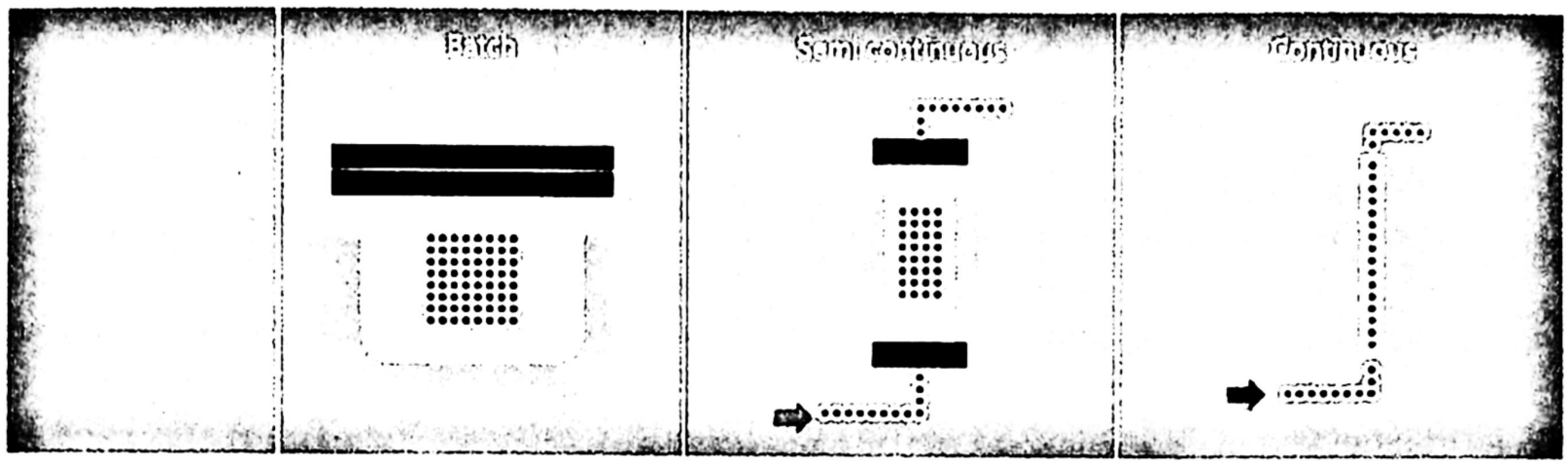


Figure 2. 5: Temperature effect on ionization constant of water (Adapted from Pourali, O., et al., 2009)

2.6 Subcritical Water Hydrolysis in Different Operation Mode

The hydrolysis using pressurized water has been intensively studied using different ways of operation modes. There are three operation modes which are batch, semi-continuous and continuous reactors (Wang, C., et al., 2012). Figure 2.6 indicated the summary of the characteristics of each reactor. Based on the reaction medium point of view, the hydrolysis of food waste such as bakery waste can be analyzed using subcritical water and supercritical water. The overview of subcritical water hydrolysis equipment setup is performed in Figure 2.7.



	Batch	Semi-continuous	Continuous
Residence time	Minutes - hours	Minutes	Milliseconds – seconds
Temperature	100 – 380 °C	170 – 310 °C	250 – 450 °C
Pressure	0.1 – 22 MPa	10 – 20 MPa	23 – 100 MPa
Process control	Low	Medium	High
Equipment requirements	Low	Medium	High

Figure 2. 6: The characteristics of batch, semi continuous and continuous reactors for subcritical water hydrolysis

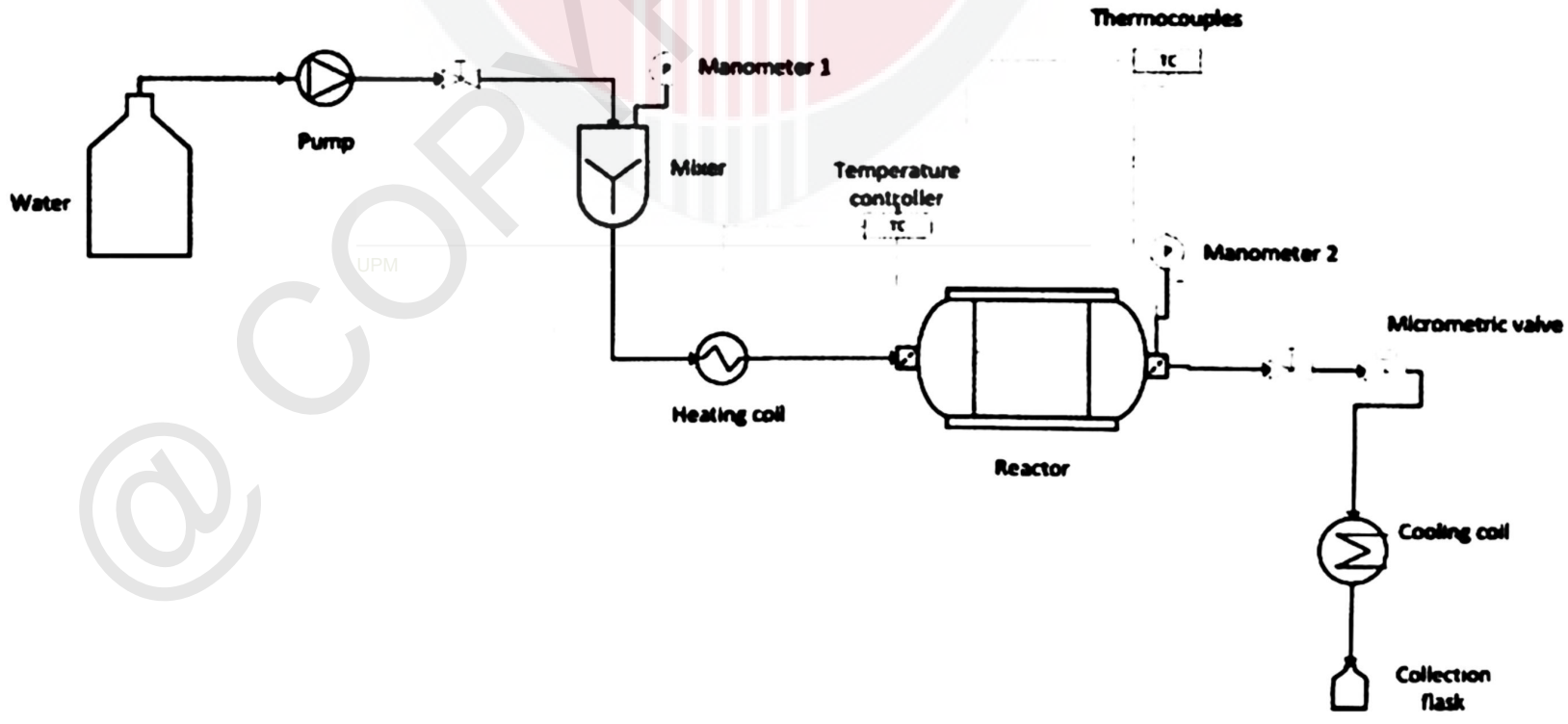


Figure 2. 7: The schematic diagram of the subcritical water hydrolysis equipment setup (Prado, J. M., et al., 2014).

2.6.1 Batch Type Reactor

The reactors in batch type allow the starch hydrolysis with a simple equipment setup. However, the process control of residence time, temperature and pressure is poor and makes difficulties in obtaining products with high selectivity. For batch type reactor, the reaction temperature is usually achieved by immersing the reactor into a bath or into a furnace (Zhao, Y., et al., 2009). This heating method is easy to set but the elevation of temperature in ramp would initialize the hydrolysis reactions before reaching the set point. Hence, the determination of the residence time is not trivial.

Investigations reviewed that there are more than three different manners of residence time counting. Start counting the residence time when the target temperature is reached could be one of the counting way of residence time (Minowa, T., et al., 1998). The reaction time in this condition initialized with the starch already degraded. Counting the residence time by considering the heating ramp and cooling time could be another manner to determine the residence time (Zhao, Y., et al., 2009). The residence time is over-estimate and it is contrary to the previous analysis method. Besides, an alternative method to determine the residence time is to consider the temperature as well. Accuracy through this method is higher than other two methods and can be applied by using the severity factor (Schacht, C., et al., 2008). In general, the batch type reactors consist of a tubing with two end caps at which the pressure measurement and control is difficult. However, some batch reactors consist of autoclave reactors and several outlets are provided thus pressure can be thoroughly measured and controlled in this situation. The obtained products after batch subcritical water hydrolysis of starch are normally analyzed into fractions such as water soluble, solids, gases and bio-oils. Typical

residence time for batch reactors is approximate 60 s at supercritical temperatures while 15 to 30 min at subcritical temperatures (Zhao, Y., et al., 2011).

2.6.2 Semi-continuous Reactor

Semi-continuous processes perform an intermediate step between batch and continuous reactors. This kind of reactors generally allows more adequate control of the pressure and residence time as compared to the batch processes. Semi-continuous reactors are advantageous as these reactors permit to keep the solid in the reactors while the soluble products of starch can be filtered by passing through a filter. There are two vital residence times in semi-continuous processes. These residence times are the solid residence time which depends on the kinetics of starch depolymerization and the liquid residence time that depends on the reactor volume and the flow (Yu,Y., & Wu, H., 2010). By reducing the residence time of the liquid phase, the degradation kinetics of the soluble products can be eliminated. In this case, an occurrence of reducing reactor volume (low amounts of treated material) while increasing the flow (more diluted products) can be observed. The hydrolysis in semi-continuous reactor undergoes at subcritical conditions.

2.6.3 Continuous Reactor

The continuous hydrolysis of starch in a subcritical or supercritical medium permits a major control upon the process. The heating could be obtained instantaneously by mixing the starch stream with hot water at the reactor inlet (Cantero, D.A., et al., 2013). The cool water injection or depressurization are used to cool the reactor outlet

stream. These heating and cooling methods make the process possible with isothermal reactors at which the starch is instantaneously heated and cooled. Hence, the residence time can be identified precisely. Moreover, the residence time is dependent on reactor volume and the flow. Hence, modifying the reactor volume and the flow may easily make changes to the residence time. In such case also means that the reactions in the continuous reactor can be controlled by simply varying the temperature, pressure and reaction time. One of the main difficulties of the continuous hydrolysis reactors will be the continuous flow of starch (solid which insoluble in water) into the reactor results in possible pump clogging. In fact, this kind of issue can be addressed in lab scale by changing the check valve system of low flow pumps. To add on, this problem also could be solved by the scaling-up using higher flows (Peterson, A.A., et al., 2008).

2.7 Process Parameters of The Subcritical Water Hydrolysis

The main factors influenced the efficiency of extraction during the subcritical water hydrolysis include the reaction temperature, time and solute characteristics. Temperature no doubt indicates an extremely significant parameter due to the fact that distinctive components are produced at different temperatures. The water at higher temperature results in improved wetting of the sample. In addition, the mass transfer kinetics is flavoured by increasing the temperature and thus faster diffusivity. When the temperature increases during the subcritical water extraction, the strong solute-matrix interactions due to hydrogen bonding, Van der Waals forces, active sites on the matrix and dipole attractions of the solute molecules can be destroyed when the temperature is increased during subcritical water hydrolysis. The high temperature weakens the

hydrogen bonding formed between the molecules. The adhesive (solute-matrix) and cohesive (solute-solute) interactions can be overcome through thermal energy by reducing the required activation energy for desorption (Richter, B. E., et al., 1996). At higher temperature, the viscosity and surface tension of subcritical water decrease and promote better penetration.

Time is another vital parameter that affects the extraction performance of subcritical water hydrolysis. The amount of end products is increased as the time prolonged. However, the treatment time also depends on the reaction temperature. When the operating temperature is significantly high, the time for treatment should be reduced to avoid decomposition of recovery material (Wang, C., et al., 2012).

Apart from temperature and time, solvent-solid ratio also one of the important parameters that influences the subcritical water hydrolysis process. The solvent-solid ratio needs to be as small as possible but sufficient to perform high extraction yield (Ravber, M.; et al., 2015).

Pressure in fact has very little effect on the properties of water. To maintain the water in liquid state at the subcritical temperature, a specific pressure is required to ensure the water is not vaporized when the temperature increased more than 100°C. The pressure elevated from 0.1MPa to 22MPa at subcritical water treatment temperature ranging from 100°C to 374°C.

The particle size of the sample used also make changes of the hydrolysis kinetics. The samples with smaller particles size may increase the surface in contact between the sample and the extractant. In this case, the rate of reaction may be higher. All these

described parameters are mandatory when analyzing the subcritical water extraction and thus the optimization of the process is desirable.

2.8 Proximate Analysis

Proximate analysis is a method used to determine the composition of foods or macronutrients values in food samples. The food components included moisture content, ash, protein, fat and carbohydrate (Gul, S., & Safdar, M., 2009). The nutritional analysis is originated since 1861 and has been developed, modified and enhanced continuously. Proximate values of the food components are generally being declared as nutritional facts that illustrated on the packages of the final food products. Therefore, it is interested in the food industry for quality control, development of food products and for regulatory purpose. Table 2.3 illustrates the methods of proximate analysis.

Table 2. 3: Methods of proximate analysis (Adapted from Roy D.C., et al., 2016).

Parameters	Methods
Moisture	Loss on drying (103-104°C for 5 hours)
Dry Matter	By calculation
Crude Protein	Crude Protein in animal and pet food (Kjeldahl method)
Crude Fat	Oil in cereal adjuncts (Petroleum ether method)
Crude Fibre	Crude Fibre in animal and pet food (F.G Crucible)
Ash	Ignition at 600°C for 2 hours

2.9 Total Sugar Yield

Carbohydrates are most abundant and significant organic components that found in many food products. The digestible carbohydrates are source of metabolic energy for body to perform normal functions. Diet without carbohydrate may lead to muscle breakdown, dehydration and ketosis (Roy, D. C., et al., 2016). Carbohydrate can be presented as isolated molecules and these molecules are classified into monosaccharides, oligosaccharides and polysaccharides with respect to the number of monomers. The quantitative estimation of total sugars that present in food products can be identified using phenol sulphuric acid method. Among the quantitative arrays, phenol sulphuric acid method is the easiest and most reliable way for total sugars estimation (Masuko, T., et al., 2005).

2.9.1 Phenol sulphuric acid method

Phenol sulphuric acid is widely used to determine the total sugar content or carbohydrate concentration present in foods. Acidic medium glucose is dehydrated to hydroxyl methyl furfural in this method. A yellow-coloured sample is formed with phenol and it has maximum absorption at 490nm. This is one of the systematic methods to estimate carbohydrates. The absorbance of the food samples can be taken using u/v spectrophotometer. The sulphuric acid is required and it can breakdown the polysaccharides into more simple form and thus the total sugar content of the food can be determined through this method. However, this method is non-stoichiometric and thus preparation of calibration curve using series of standards glucose concentration is required. The chemical reaction of food samples in phenol sulphuric acid method is illustrated in Figure 2.8.

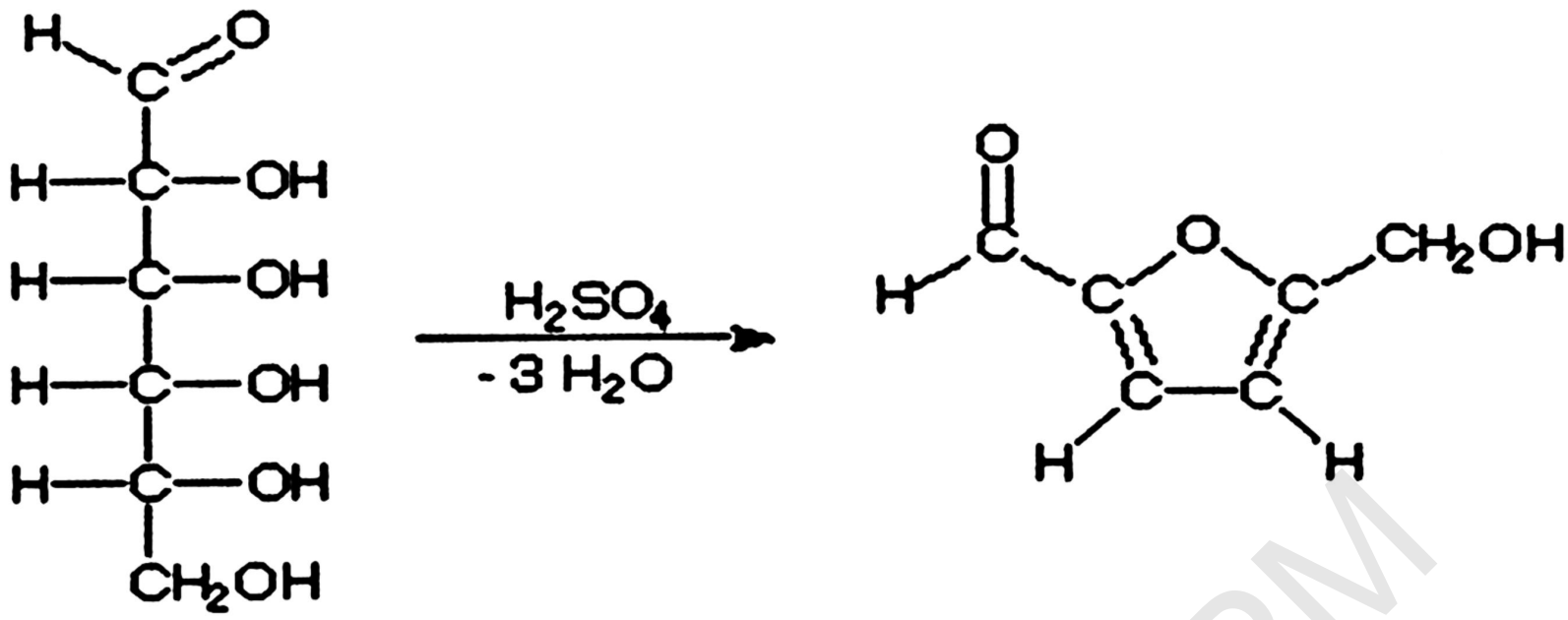


Figure 2. 8 : Chemical reaction in phenol sulphuric acid method (Jain, V. M., et al., 2017).

2.10 Reducing Sugar Yield

Reducing sugar is known as the sugar that capable to be oxidized and cause reduction of other substances. It acts as a reducing agent due to presence of free aldehyde or ketone group. All the monosaccharides are considered as reducing sugars. The common diet reducing sugar included glucose, fructose and galactose. Dinitrosalicylic acid method is one of the efficient arrays used for reducing sugars determination in foods (Aquino, et al., 2003).

2.10.1 Dinitrosalicylic Acid Method (DNS)

DNS method is a colorimetric technique which comprises of redox reaction between the reducing sugars in the food samples and 3,5-dinitrosalicylic acid. The sugars act as reducing agent and oxidized their carbonyl group into carboxyl group. On

the other hand, the DNS which is naturally yellow will be reduced to red brown 3-amino-5-nitrosalicylic acid. This can be quantified using u/v spectrometer at 540nm which wavelength of maximum absorbance. The colour intensity is proportional to the reducing sugar concentration in food samples. The oxidation-reduction reaction is as shown in Figure 2.9.

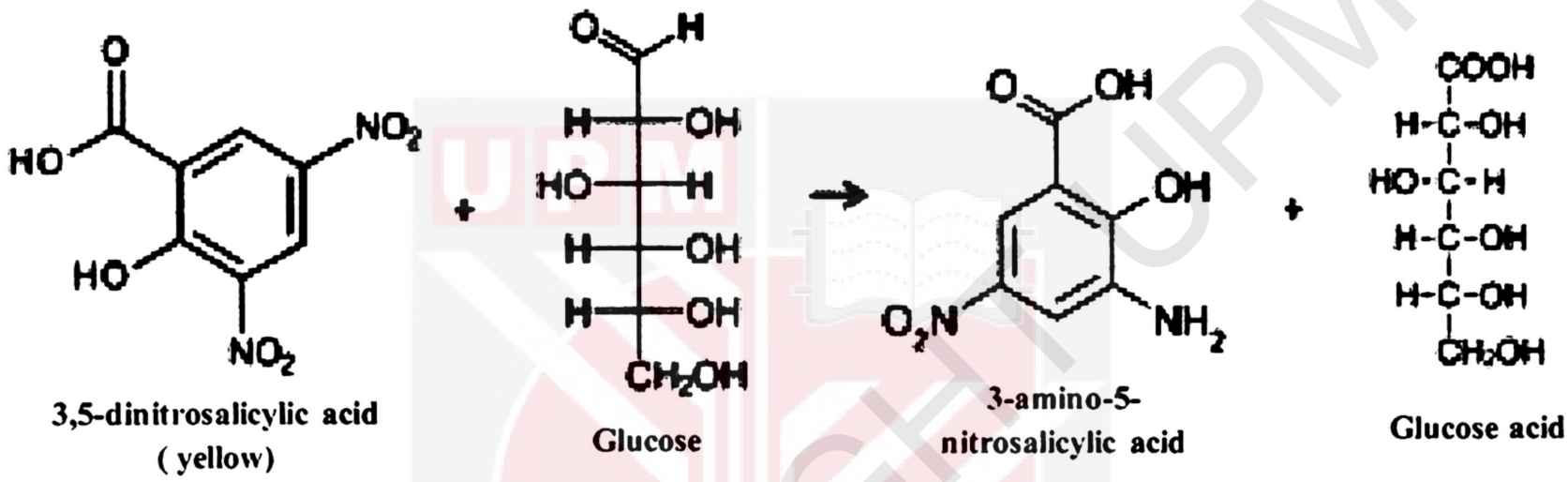


Figure 2. 9 : Oxidation-reduction reaction in DNS method (Garriga, M., et al., 2017).

2.11 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is an essential and useful technique for empirical model building through collection of statistical and mathematical techniques. The objective of RSM is to optimize the output variable (response) which effected by input variables (several independent variables). An experiment is a series of tests known as runs which modifications are made in the input variables to determine the reasons of output response changes.

RSM is developed for experimental responses modeling and then migrated into the modeling of numerical experiments. Inaccuracy can be due to measurement errors in

physical experiments while numerical noise is a result of incomplete convergence of iterative processes in computer experiments. The errors in RSM are assumed to be random. The application of RSM to design optimization is to save the expensive cost of analysis methods.

2.11.1 Central Composite Design (CCD) for Optimization

Response surface methodology consists of a number of methods to design the experimental procedures and one of the methods is Central Composite Design (CCD). Optimization using with CCD permits screening of various type of parameters as well as the role of each factor (S. Şahin, et al., 2011). Moreover, CCD also as an assistance to evaluate a single variable and the variables cumulative effect to the response. In this case, the ability of CCD is shared with the other types of experimental design such as full factorial and partial factorial method. Nevertheless, it differs in a way which reduces the experimental runs (K.G. Box, et al., 1951). For instance, with four independent variables, full factorial method requires at least 81 experimental runs and replication while CCD method only needs 31 experimental points (16 factorial points, 8 axial points and 7 center points).

2.11.2 Box-Behnken (BB) for Optimization

Box-Behnken technique is another method in response surface methodology where the optimal variables can be determined through the final outcome to achieve optimum output response. Box-Behnken method is also a design that does not involve the embedded factorial or fractional factorial point as the condition of variable stayed at the midpoint edges of the variables space as well as at the center. Other than that, the central point in fact aids to predict the pure error and allow intermediate levels calculation of the response function. Therefore, the system performance within the studied range is possible according to the replication of the central point.

CHAPTER 3

3.0 METHODOLOGY

3.1 MATERIAL

Waste cakes (chocolate cakes) are obtained from the local bakery enterprise (MFaez Food Industries Sdn. Bhd.) at Bangi, Selangor, Malaysia. The samples showed no sign of mold growth. The cakes were grinded using knife mill, vacuum-packed and stored at 4°C for further analysis. The standard sugars (sucrose, glucose, mannose and fructose) as well as other chemicals and solvents used in the experiments were of analytical grade (Fisher Scientific, UK).

3.2 METHODS

3.2.1 Proximate Analysis

The proximate analysis was carried out by using the Association of Official Analytical Chemists (AOAC) method (A.O.A.C, 2000).

3.2.1.1 Determination of Moisture Content

The oven temperature was set up to 105°C and the temperature was kept constant. The crucible and its cover were cleaned and dried in the oven for a minimum of 30 minutes. After 30 minutes, the crucible and its cover were removed with tongs and cooled down to room temperature in a desiccator (approximately 20 minutes). The

crucible and its cover were placed on a balance and then weighed rapidly and accurately. The crucible and its cover were put back into the desiccator. The weighing procedure was repeated at least twice until constant reading was obtained. The cake samples were blended into powder form by using blender. 3g of cake sample was weighed and poured into crucible mentioned previously. The crucible together with the cover and cake sample were placed into the oven and was left for at least 7 hours. The covered crucible was taken out using tongs and placed directly in the desiccator. The crucible together with the cover and cake sample were weighed after cooling for 20 minutes. The result was recorded.

The moisture content of the sample was calculated based on percentage of wet weight.

$$\text{Moisture (\%)} = \frac{W1 - W2}{W1} x\ 100 \qquad \textbf{(Eq. 3.1)}$$

Where, $W1$ = weight (g) of sample before drying

$W2$ = weight (g) of sample after drying

3.2.1.2 Determination of Ash Content

The crucibles were placed into the oven at 105°C for 1 hour. The crucibles were cooled in the desiccator. The desiccators were weighed rapidly and accurately. The cake samples were then weighed for each crucible. The crucibles with the samples were placed into the muffle furnace at 550°C for at least 2 hours or until no black particles were present. The crucibles were then cooled in the desiccator. The crucibles with the ash were weighed together.

Ash content was calculated based on percentage of dry weight.

$$\text{Ash (\%)} = \frac{(weight\ of ash + weight\ of crucible) - weight\ of crucible}{weight\ of\ the\ sample} x\ 100 \qquad \textbf{(Eq. 3.2)}$$

3.2.1.3 Determination of Protein Content

Four test tubes and four conical flasks were labelled A, B, C and D respectively. Cake sample of 0.15g was weighed accurately and placed into micro Kjeldahl test tube A, B and C. Test tube D was used for blank thus no sample. Mixed catalyst of 0.8g was added into each test tube. After that, 2.5ml of concentrated sulphuric acid of was added and the tube was swirled gently to mix the content. The tube was heated slowly on a heating coil under fume hood. The content was boiled until the solution became clear and gave blue-green colour. Conical flask was cooled to 40°C. 10ml of distilled water with was added and the digested product was transferred into distillation tube. 10 ml of 45% NaOH solution was added slowly to separate the two layers of solution The distillation tube was fixed neatly to the condenser. In conical flask, 10ml of 2% boric acid and two drops of indicator were added. The conical flask was placed at the distillate platform and the tip of distillation tube was immersed into the acid solution. The content of distillation flask was mixed by swirling it gently. Steam was purged into the flask. About 120 ml of ammonia solution was distilled into the conical flask. After mixing the distillation product by swirling the flask gently, unreacted boric acid was titrated with 0.05N H_2SO_4 until it became neutral. The same procedure was repeated for test tube D. The protein percentage of the sample was calculated by using the following formula.

$$\text{Protein (\%)} = \frac{(A-B)\ x\ N\ x\ 1.4007\ x\ 6.25}{W} \qquad \textbf{(Eq. 3.3)}$$

Where, A = volume (ml) of 0.05N H_2SO_4 used sample titration

B = weight (g) of 0.05N H_2SO_4 used in blank titration

N= normality of H_2SO_4

W= weight (g) of sample

3.2.1.4 Determination of Fat Content

Two round bottom flasks were placed into the oven (105°C) for 30 minutes. 5g of dry cake sample was weighed and put on to the filter paper and covered by using cotton wool. Then, the samples were inserted into Soxhlet apparatus. After 30 mins, the round bottom flasks were cooled in a desiccator for 15 mins. Round bottom flasks were weighed accurately and 200ml petroleum ether was added. Soxhlet was connected to the reflux and round bottom flask and the sample was refluxed continuously for more than 8 hours. The water content of water bath must be sufficient. The temperature of the heater (60°C) was controlled. Extra cautious was needed in making sure that the solvent was not evaporated or dried during refluxing. If so, more petroleum ether was added. Petroleum ether was evaporated from the round bottom flask using rotary evaporator. Round bottom flask together with the fat extracted were weighted.

Fat content was calculated by following equation.

$$\text{Fat (\%)} = \frac{weight\ of\ fat}{weight\ of\ the\ sample} x\ 100 \qquad \textbf{(Eq. 3.4)}$$

3.2.1.5 Determination of Carbohydrate

Analytical technique has been developed to measure the total amount of carbohydrate present in food sample. The percentage of carbohydrates in cake sample can be obtained by the using the following equation.

$$\text{Carbohydrate (\%)} = 100\% - \%\ of\ moisture + \%\ of\ ash + \%\ of\ protein + \%\ of\ fat \qquad \textbf{(Eq. 3.5)}$$

3.2.1.6 Determination of Yeasts and Molds

The chocolate cake sample was stored at -20°C for 72 hours before plating to kill mites and insects which may interfere the analysis. 50g of sample was transferred into a sterilized 300ml beaker. 95% ethanol-flamed forceps were used to place intact sample on the surface of solidified agar. There were 5 to 10 items per plate depending on the size of the sample. There are total 50 food items. Several forceps were used alternately to prevent overheating. 3 to 5 plates were aligned in stacks and sample number was also identified with the date of plating. Incubator stacks were left undisturbed in dark for 5 days at room temperature. The occurrence of the mold was determined in percentages. Moldiness would be 100% if the mold emerged from all 50 food items or 64% if only emerged from 32 food items. Occurrence percentage of individual mold genera and species was determined in a similar way. *Aspergillus*, *Penicillium* and most other foodborne mold genera were identified directly on medium with low power (10-30X) magnification. This test was carried out by following FDA, Food And Drug Administration (Bacteriological Analytical Manual).

3.2.2 Subcritical Water Hydrolysis

Subcritical water hydrolysis was carried out in a batch type oil bath reactor (Thomas Kagaku Co. Ltd) at 160°C, 180°C and 200°C. The pressure during the process was referred to the steam table of the saturated vapor pressure. The pressure applied were 0.6MPa, 1MPa and 1.5MPa respectively for the mentioned temperatures as in subcritical phase. At these stated temperature and specific pressure, liquid water was in equilibrium with vapor at the saturated vapor pressure in the closed system (Koretsky,

M., 2004). The reactor was equipped with heating coils and K-type thermocouple to regulate the temperature. The stainless-steel reactors were tolerant to high pressure. The cap diameter and length of the reactor was 2cm and 17cm respectively (Swagelok, Malaysia). The reactor was loaded with different solid loading ratios of cake samples leftovers and water which were 10% w/v, 30% w/v and 50% w/v. After that, argon gas was used to purge the reactor for 3 minutes to release the gas trapped in the reactor. The reactor lid was then shut and clamped tightly. The reactor was immersed in the oil bath at the specified temperature (160°C, 180°C and 200°C) and time (5min, 10min and 15min). The reactor was cooled immediately after the heating process to stop the reaction. The obtained hydrolysate was centrifuged at 900rpm for 20min by using centrifugal incubator (Hettich Universal 320/320R). After centrifugation, the hydrolysate was separated into a few layers such as oil, aqueous solution and solid residue. The hydrolysate was subsequently filtered through a Whatman No.1 filter paper in order to remove the oil layer on hydrolysate surface and separate the aqueous solution from solid residues. The filtrate was used for further analysis while the residue used for surface morphology.

3.2.3 Measurements of pH

Measurements of pH of the hydrolysate was carried out after centrifugation using pH meter (ThermoScientific).

3.2.4 Total Sugar Content

The total sugar content of the hydrolysate was determined by using phenol-sulphuric acid method (Nielsen, S. S., 2017). This method was used to detect all classes

of carbohydrates including monosaccharides, disaccharides, oligosaccharides and polysaccharides. Firstly, 1ml of hydrolysate was mixed with 1ml of 5% phenol solution and 5ml of sulphuric acid (H_2SO_4). The test tube was vortexed for 30s and placed in water bath at 25-30°C for 20 minutes. The absorption of the solution at 490nm was recorded by using an Ultrospec 3100 Pro UV/visible spectrophotometer. The readings obtained were used for standard curve preparation.

3.2.4.1 Preparation for standard curve for phenol sulphuric method

Dilutions of glucose standards with blank, 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml were prepared. 1ml of each glucose concentration were filled in different test tubes included blank sample. All the test tubes were mixed with 1ml of 5% phenol solution and 5ml of sulphuric acid (H_2SO_4). The test tubes were vortexed for 30s and placed in water bath at 25-30°C for 20 minutes. The absorption of the solution at 490nm was recorded by using an Ultrospec 3100 Pro UV/visible spectrophotometer. The standard graph of absorbance at 490nm against concentration of glucose solution was plotted.

3.2.5 Reducing Sugar Content

Reducing sugars are sugars with free aldehyde groups or ketone groups which enable them to act as reducing agents. The analytical method that used to determine the presence of reducing sugars in the cake samples was dinitrosalicylic acid (DNS) method (Garriga, M., et al., 2017). This method was used to test for presence of the free carbonyl groups (C=O) in reducing sugars. A 0.1% solution of 3,5-dinitrosalicyclic acid (DNS) was prepared by dissolving 0.25g of DNS in 50ml of 2M NaOH solution. The

solution was adjusted to 200ml using distilled water. 0.2ml of hydrolysate was subsequently added to the 2ml of 0.1% of DNS solution in a test tube. The test tube was placed in boiling water for 10 minutes and cooled immediately to ambient temperature by using of ice water. The absorbance at 540nm was recorded. Glucose was used for standard curve preparation.

3.2.5.1 Preparation for standard curve for dinitrosalicylic acid method

Dilutions of glucose standards with blank, 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml were prepared. 0.2ml of each glucose concentration were filled in different test tubes included blank sample. All the test tubes were added to the 2ml of 0.1% of DNS solution in a test tube. The test tubes were placed in boiling water for 10 minutes and cooled immediately to ambient temperature by using a container of ice and water. The absorbance at 540nm was recorded by using an Ultrospec 3100 Pro UV/visible spectrophotometer. The standard graph of absorbance at 540nm against concentration of glucose solution is plotted.

3.2.6 Experiment Design and Analysis for Optimization

With the MINITAB software, the statistical analysis in term of ANOVA (Analysis of Variance) was carried out using Response Surface Methodology (RSM). RSM was used to determine the significance of each factor which are hydrolysis temperature, process time and solid loading; to elucidate the relationship between the factors and their response under different condition and to obtain the optimization of the responses. The effects of the hydrolysis temperature, process time and solid loading

were evaluated, and the empirical model was performed according to the relationship. The central composite design was used.

In this study, total of three independent variables were process temperature, process time and solid loading. The variables to be studied were process temperature between 160°C to 200°C, 5min to 25 min process time and 10% to 50% solid loading. A total of 40 runs (with duplicate) were sequenced using central composite design.

3.2.7 Sugar Profile Analysis

Sugar analysis profile was carried out to identify the type and concentration of sugars in the obtained hydrolysate by using high performance liquid chromatography (HPLC) (Jasco, Japan). The HPLC was equipped with an auto sampler, a pump, a vacuum degasser, network chromatography interface (NCI) 900 and connected with both refractive index (RI) and UV/Vis detectors. An Agilex Hi-Plex H column (300 x 7.7 mm) was used. The column temperature was set to 65 °C. 20µl of the samples was injected at a flow rate of 0.3 ml/min of distilled water for chromatographic measurements, which was used as the mobile phase. A quantitative analysis was carried out by preparing standard curves of sucrose, xylose, glucose, fructose, mannose and galactose. The monosaccharides yields were calculated using following equation.

$$\text{Monosaccharide yields (mg/g substrate)} = \frac{M_{mono}\left(\frac{mg}{L}\right) x\, V_{aq}\ (L)}{W_{substrate}\ (g)} \qquad \textbf{(Eq. 3.6)}$$

Where, M_{mono} = concentration of monosaccharide

V_{aq} = volume of aqueous phase

$W_{substrate}$ = mass of substrate

3.2.8 Fourier Transform InfraRed (FTIR) Spectroscopy

The Fourier Transform InfraRed (FTIR) is a method of infrared spectroscopy in which radiation passes through the sample (Collado, et al., 2018). The samples absorbed some infrared radiation while the rest was transmitted. The spectra represented molecular absorption and transmission. There will not have two unique molecular structures produced the same infrared spectrum by using FTIR. Hence, FTIR was useful for various analysis. FTIR were used for identification of chemical composition and structure changes of treated samples. 20mg of the cake sample was combined with 30 mg of spectroscopic grade dried KBr. The samples were analyzed after mixing by using FTIR spectroscopy over the wave number range of 4000 to 400 cm^{-1} with resolution of 2cm^{-1} for 30 scans per sample.

3.2.9 Scanning Electron Microscopy (SEM)

SEM is a test process that used for observation of specimen surface. It scans a sample with electron beam to generate a magnified image for analysis (Goldstein, J. I., et al., 2017). The surface morphology of leftover chocolate cake samples before and after hydrolysis was qualitatively identified using scanning electron microscope (HITACHI). The chocolate cake samples were fixed with a conducting adhesive carbon tape on an aluminium stub and sputtered with gold coating 2 to 3 times. The samples were observed in SEM which operating at 10kV (Kumar, S., et al., 2010).

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS

4.1 Proximate and Bacteriological Analysis

Proximate analysis is a method for quantitative analysis of macronutrients. This analysis was developed in 1860 by Henneberg and Stohman in Germany (Henneberg, W. and Stohman, F., 1860). This technique is used to determine the amount of moisture content, ash, protein, fat and carbohydrate. On the other hand, bacteriological analysis is a collection of procedures preferred by U.S. Food and Drug Administration laboratories analysts for the pathogen detection in food products such as yeast and mold. The results of proximate and bacteriological analysis of leftover chocolate cake used in this study is presented in Table 4.1. Carbohydrate was found to be the main component for leftover chocolate cake which was 47.4%, followed by moisture (23.4%), fat (20.1%), protein (6.9%) and ash (2.2%) while the yeast and mold were detected less than 100 CFU/g.

Table 4. 1 : Proximate and bacteriological analysis of leftover chocolate cake.

Physiochemical properties	Concentration (g/100g)
Protein	6.9
Moisture	23.4
Ash	2.2
Fat	20.1
Carbohydrate	47.4
Yeast and Mold	<100 CFU/g

The analysis depicted that the leftover chocolate cake has high carbohydrate content which was 47.4g/100g. With this high composition, the chocolate cake is suitable for sugar recovery. Carbohydrate being the essential source for the valuable

food ingredients like monosaccharide, oligosaccharide and polysaccharides. Besides, carbohydrates act as energy storage molecules in human bodies. They also used as substrates and converted into others desired products (Moore, K. J., & Hatfield, R. D., 1994).

The moisture content of the leftover chocolate cake was recorded second highest with 23.4/100g. The typical cake has moisture content between 15% to 30% (Mohamad, R. A., et al., 2015). Moisture plays a vital role in the food industry with safety, quality, shelf-life, processing, texture and sensory properties implications (Fontana, A., 2006). An increase of water activity in confectionery product is almost always accompanied with the increase of water content. Hence, the moisture content of the chocolate cake samples needed to be recognized to predict the growth of microorganisms and the way to keep the samples during the experiment period.

The leftover chocolate cake had 20.1g/100g of fat content. Fat is an essential source that provides energy when carbohydrates are not available. It also allows vitamin absorption like vitamins A, D, E and K which know as fat-soluble vitamins. These vitamins cannot function without consuming adequate fat. Fat cells which stored in adipose tissue assists in insulating and sustaining body temperature (Anne Melodie, 2018).

The protein content of the leftover chocolate cake was 6.9g/100g. Protein poses a major role in the growth and assists in enzymatic activity. It also provides essential amino acids for body muscle growth (Wu, G., et al., 2014).

The ash content of the leftover chocolate cake was 2.2g/100g. The ash content indicates the content of mineral in the chocolate cake samples. The ash is the inorganic

residues which obtained after the water was being removed from the leftover chocolate cake through heating.

The amount of yeast and mold were less than 100 CFU/g. According to microbiological guidelines for food, the leftover chocolate cake was considered satisfactory (Centre for Food Safety Food and Environmental Hygiene Department, 2014). The test results indicating good microbiological quality which is less than 100 CFU/g.

4.2 pH Value Measurements

Temperature plays an important role on pH measurements. According to Figure 4.1(a), the pH of the leftover chocolate cake hydrolysate decreased distinctly from 160°C to 200 °C at 50% solid loading for 15 min. The pH of leftover chocolate cake hydrolysate was recorded 6.24 ± 0.01 at 160 °C and dropped to 4.47 ± 0.03 when the temperature reached 180 °C. The leftover chocolate cake pH decreased continuously to 3.62 ± 0.03 at 200 °C. This could be due to molecular vibrations increase with the temperature which results in water ionization and more hydrogen ions were produced. Hence, the pH of the leftover chocolate cake hydrolysate was undergone a change in value of pH through temperature change. The dissociation of the water that contributing formation of hydrogen and hydroxide ions can be performed as H_2O (l) ⇌ H^+ (aq) + OH^- (aq). According to Le Chatelier's Principle, the position of equilibrium will shift to counter the condition changes in a reaction in dynamic equilibrium (Vilar, V. J., et al., 2005). As a result, the equilibrium will shift to lower the temperature when the temperature is increased. This also means that the water will absorb the heat and more hydrogen ions and hydroxide ions will be formed through forward reaction. In addition,

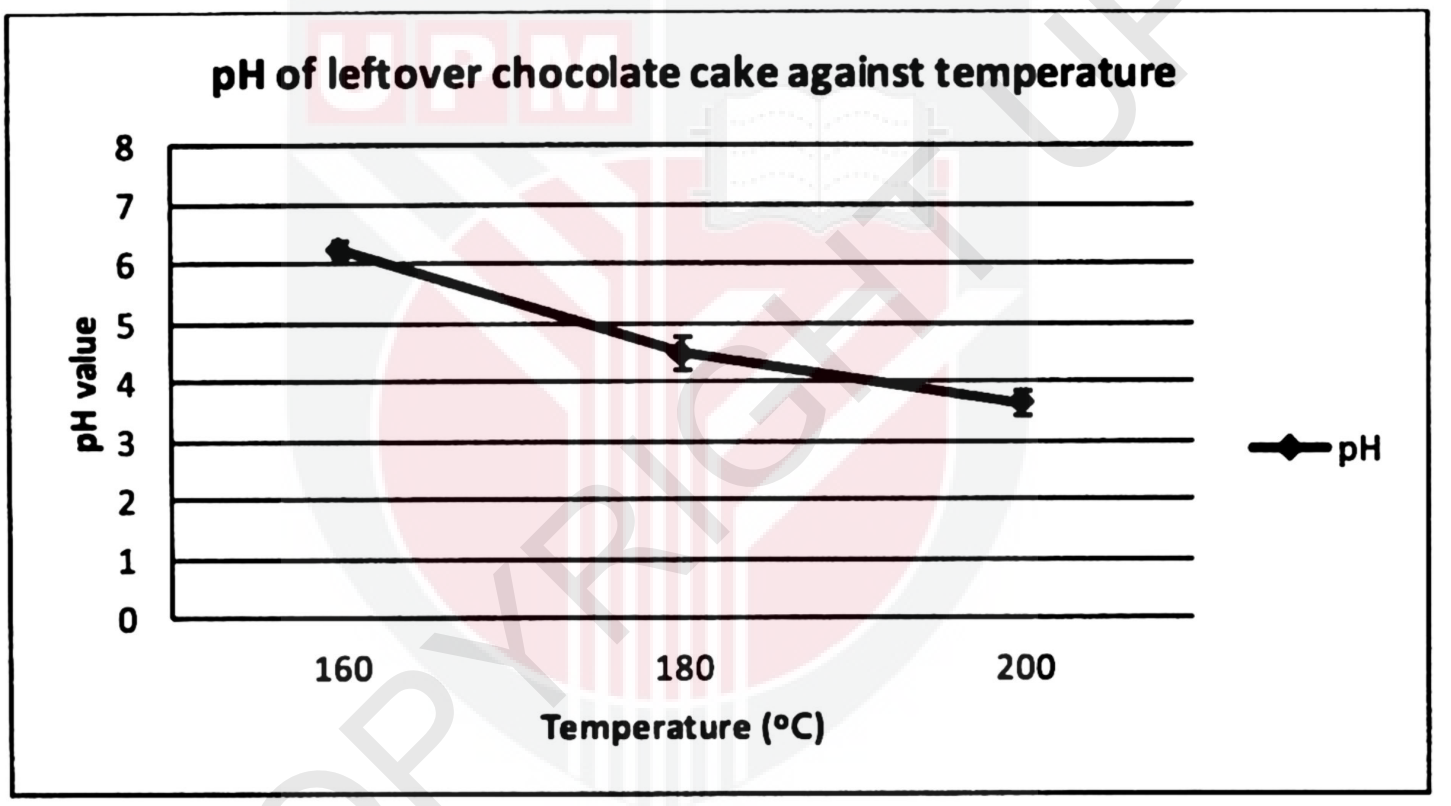
low pH value of the hydrolysate indicates more acidic component formation which essential for subcritical water hydrolysis reactions (Shimanouchi, T., et al., 2019). Acetic acid, glycolic acid and formic acid are the common examples of acids that formed during subcritical water hydrolysis. Apart from that, the protein of the leftover chocolate cake sample will be broken down into amino acids while the fat converted into fatty acids and glycerol after hydrolysis treatment. Hence, the pH value of the chocolate cake hydrolysate decreased with the increase of process temperature. Zakaria, S. M., & Kamal, S. M. (2016) reported that these acid components can be acted as catalysts for subcritical water hydrolysis and thus increasing the efficiency of the process.

Based on Figure 4.1(b), the pH value of the leftover hydrolysate which undergone subcritical water hydrolysis decreased with the increase of processing time from 5 min to 10 min at 200°C with solid loading of 50%. The pH value of the hydrolysate dropped gradually from 4.74 ± 0.04 at 5 min to 3.62 ± 0.02 at 15 min of processing time. The decreasing trend of pH value of the hydrolysate could be due to more sample solution will be hydrolyzed after a period of time. In the situation with longer processing time, more ions were produced as more deionized water and hydrolyzed sample were obtained through subcritical water hydrolysis. The contents of the leftover chocolate cake like lactic acid and cocoa will lower down its pH value after undergone hydrolysis (Marsiglia, D., et al., 2016).

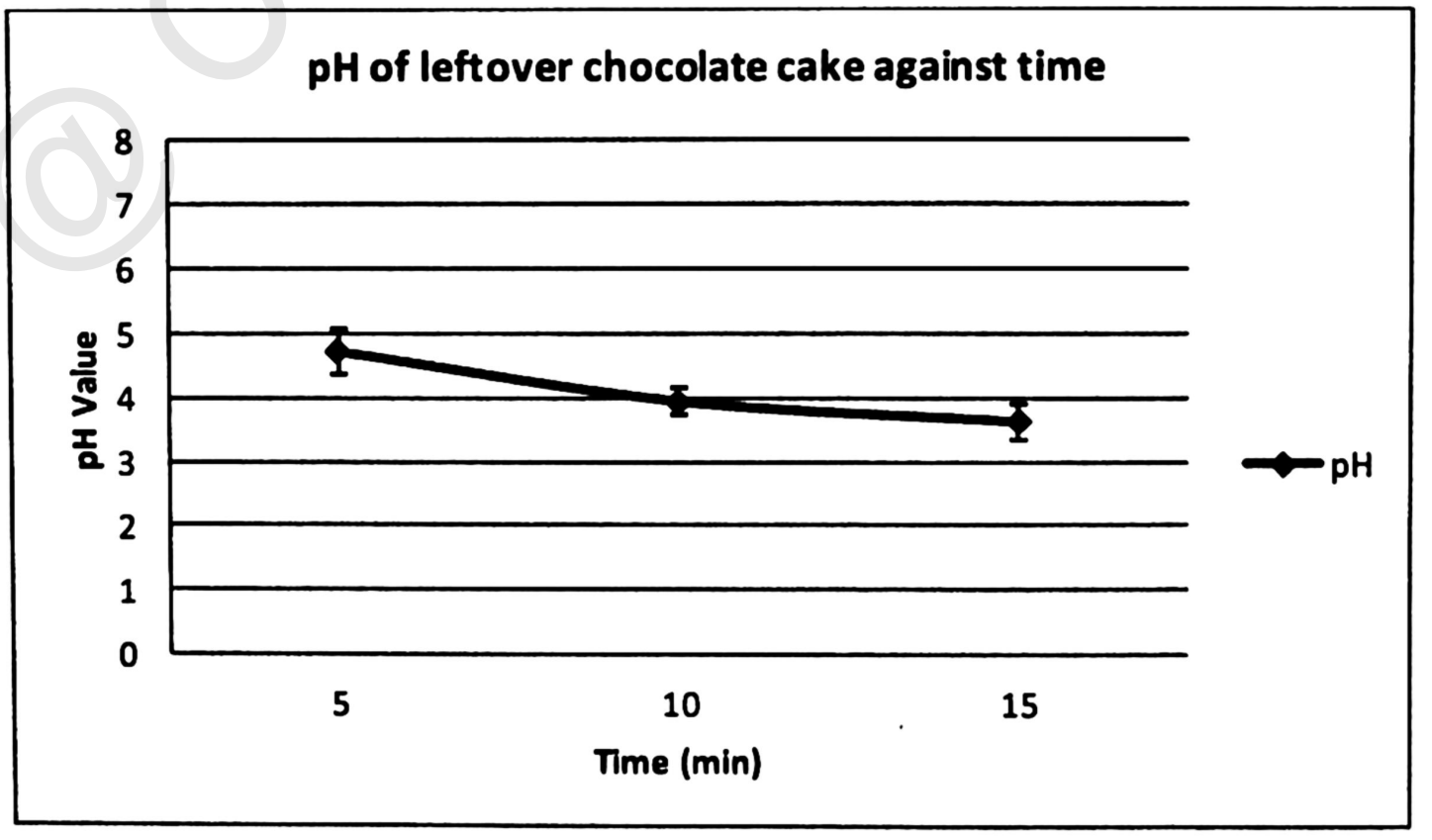
The pH value of the leftover chocolate cake also varied with the change of the solid loading. Figure 4.1 (c) showed that the pH value decreased when the solid loading of the chocolate cake sample increased from 10% to 50% at 200°C for 15 min. The pH value was 4.2 ± 0.02 at 10% solid loading and dropped to 3.62 ± 0.03. The chocolate

cake sample with higher solid loading lead to higher concentration of the hydrogen ions presented in the hydrolysate thus lowest pH value was obtained at 50% solid loading as compared to 10% and 30%. However, the percentage of solid loading need to be optimum as the hydrolysis process may be disrupted once the solid loading is too high (Mohan, M., et al., 2015).

(a)



(b)



(c)

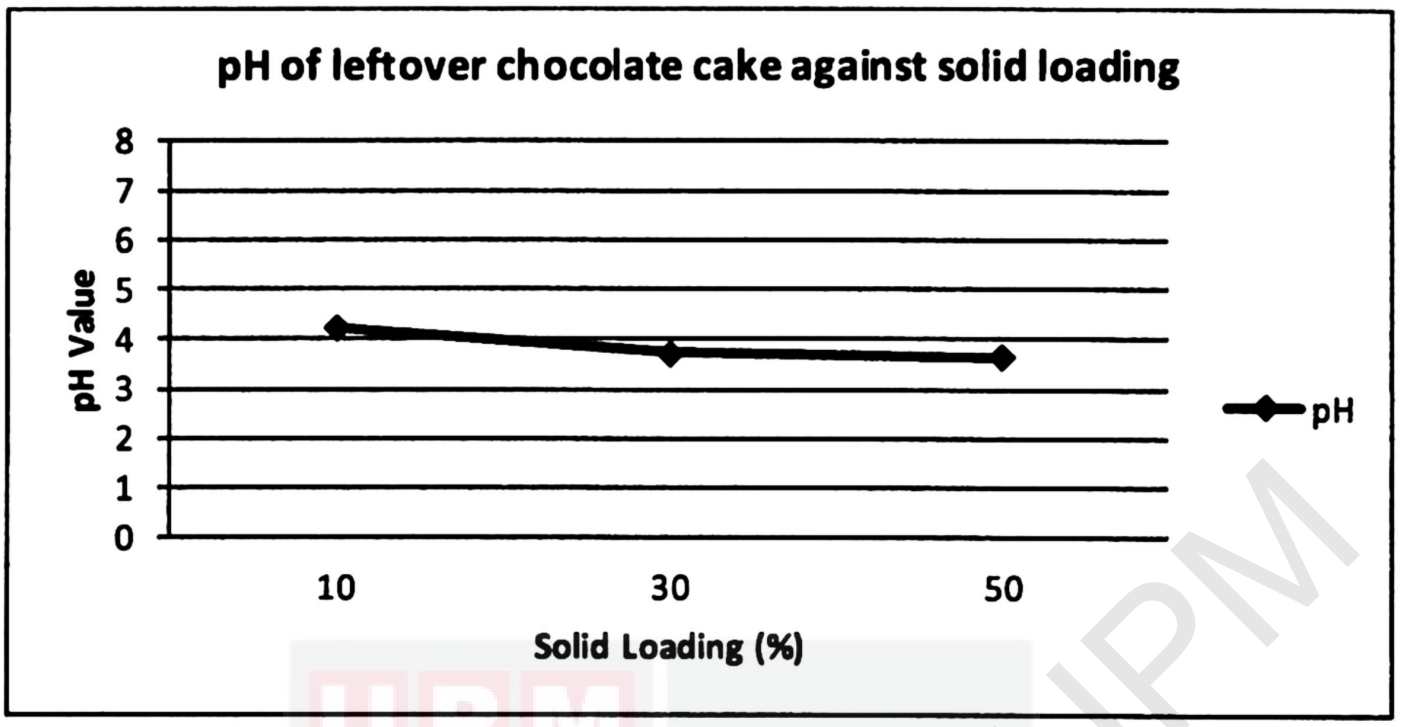


Figure 4. 1: pH value of leftover chocolate cake hydrolysates prepared through subcritical water hydrolysis under different process conditions. (a) Process temperature (160°C to 200°C at 15 min with 50% solid loading), (b) Process time (5min to 15 min at 200°C with 50% solid loading), (c) Solid loading (10% to 50% at 200°C for 15 min)

4.3 Total Sugar Yield and Reducing Sugar Yield by Subcritical Water Hydrolysis

4.3.1 Model Fitting of Total Sugar Yield

A fitted line plot regression model was applied for the experimental data using the least square technique. There were several main parameters that required to be taken into consideration in order to evaluate the statistical results. For examples, the coefficients of regression, the standard error of coefficient and the P value of the effects of factors. Table 4.2 depicted the ANOVA of total sugar yield by subcritical water hydrolysis. P value is the probability that determine the association of each term in the model against the null hypothesis. Stronger evidence against the null hypothesis will be

provided with lower p value. Hence, the results of this study indicated that the factors and interaction were highly significant which with P value approximate to zero. Value for R^2 of 0.9785 and R^2 (adjusted) of 0.9721 were considered very high which designated that 97.85% of sample variation in the response was attributed to the independent variables (process temperature, process time and solid loading). The standard deviation of the differences between the data value and the fitted value was denoted as S and it was measured in the units of the response. The smaller the value of S, the better the model for response description (Mathews, P. G., 2005). For this study, the S value was 0.0330816 which considered small value and thus this model was suitable to be used to investigate the design space. The complete design matrix and response values for total sugar yields of leftover chocolate cake through subcritical water hydrolysis were given in **Appendix B1**.

Table 4. 2: Analysis of variance (ANOVA) of total sugar yield by subcritical water hydrolysis of chocolate cake.

Term	Notation	Coefficient	Std. Error of coefficient	P value
Constant	-	0.40714	0.008042	0.000
Temperature	X_1	0.06300	0.007397	0.000
Time	X_2	0.14950	0.007397	0.000
Solid Loading	X_3	0.19350	0.007397	0.000
Temperature*Temperature	X_1*X_1	-0.03659	0.014106	0.015
Time*Time	X_2*X_2	0.05091	0.014106	0.001
Solid Loading*Solid Loading	X_3*X_3	-0.02909	0.014106	0.048
Temperature*Time	X_1*X_2	-0.02312	0.008270	0.009
Temperature*Solid Loading	X_1*X_3	0.04063	0.008270	0.000
Time*Solid Loading	X_2*X_3	0.10187	0.008270	0.000
S = 0.0330816	R^2 = 0.9785		R^2 (adjusted) = 0.9721	

By employing a regression model for the experimental data, the suggested response Y for the yield of the total carbohydrate could be obtained. Equation 4.1

represents the regression model for the total sugar yield of chocolate cake through subcritical water hydrolysis. The regression model can be used to analyze and calculate the effect of factor on subcritical water hydrolysis performance.

$$Y = 0.40714 + 0.06300 (X_1) + 014950 (X_2) + 0.19350 (X_3) - 0.03659 (X_1^2) + 0.05091 (X_2^2) - 0.02909 (X_3^2) - 0.02312 (X_1X_2) + 0.04063(X_1X_3) + 0.10187 (X_2X_3) \quad \textbf{(Eq. 4.1)}$$

Where Y represents the total carbohydrate yields, X_1, X_2 and X_3 are the decoded values of hydrolysis temperature, process time and solid loading respectively.

4.3.2 Model Fitting of Reducing Sugar Yield

A fitted line plot regression model was also applied for the experimental data using the least square technique. The coefficients of regression, the standard error of coefficient and the P value of the effects of factors were taken into consideration so as to evaluate the statistical results. Table 4.3 stipulated the ANOVA of reducing sugar yield by subcritical water hydrolysis. The result of this study indicated that the factors and interaction were highly significant with zero or low P value except for X_2^2, X_3^2 and X_1X_2 which with P value of 0.180, 0.111 and 0.372 respectively. Value for R^2 of 0.9573 and R^2 (adjusted) of 0.9445 were considered very high which designated that 95.73% of sample variation in the response was attributed to the independent variables (process temperature, process time and solid loading). R^2 is used for model fitting response determination. The higher the value of R^2, the better the model fits to the experimental data. For this study of reducing sugar yield, the S value was 0.0388652 and hence this model was suitable to be used to investigate the design space. The complete design

matrix and response values for reducing sugar yields of leftover chocolate cake through subcritical water hydrolysis were given in **Appendix B2**.

Table 4. 3: Analysis of variance (ANOVA) of reducing sugar yield by subcritical water hydrolysis of chocolate cake.

Term	Notation	Coefficient	Std. Error of coefficient	P value
Constant	-	0.301100	0.009448	0.000
Temperature	X_1	0.088950	0.008691	0.000
Time	X_2	0.114950	0.008691	0.000
Solid Loading	X_3	0.155550	0.008691	0.000
Temperature*Temperature	X_1*X_1	-0.037750	0.016572	0.030
Time*Time	X_2*X_2	-0.022750	0.016572	0.180
Solid Loading*Solid Loading	X_3*X_3	0.027250	0.016572	0.111
Temperature*Time	X_1*X_2	-0.008812	0.009716	0.372
Temperature*Solid Loading	X_1*X_3	0.040688	0.009716	0.000
Time*Solid Loading	X_2*X_3	0.063187	0.009716	0.000
S = 0.0388652	$R^2 = 0.9573$		R^2 (adjusted) = 0.9445	

By employing a regression model for the experimental data, the suggested response Y for the yield of the reducing sugar could be obtained. Equation 4.2 represents the regression model for the reducing sugar yield of chocolate cake through subcritical water hydrolysis. The regression model can be used to analyze and calculate the effect of factor on subcritical water hydrolysis performance.

$$Y = 0.3011 + 0.08895(X_1) + 0.11495(X_2) + 0.15555(X_3) - 0.03775(X_1^2) + 0.040688(X_1X_3) + 0.0063187\ (X_2X_3) \quad \textbf{(Eq. 4.2)}$$

Where Y represents the reducing sugar yields, X_1, X_2 and X_3 are the decoded values of hydrolysis temperature, process time and solid loading respectively.

4.3.3 Effects of Process Temperature, Process Time and Solid Loading on Total Sugar Yields and Reducing Sugar Yields

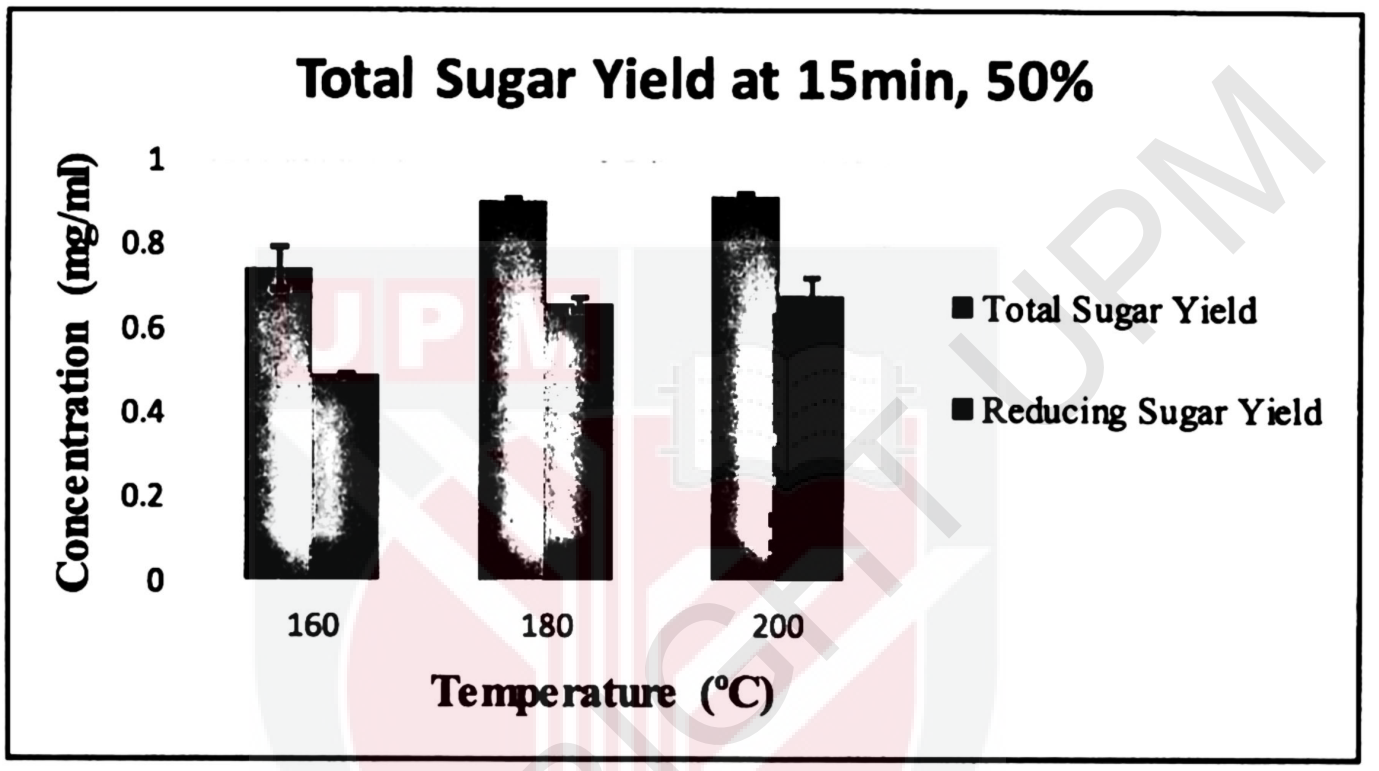
The design matrix of central composite design obtained from response surface methodology (RSM) and experimental data of the total sugar yield and reducing sugar yield for subcritical water hydrolysis of leftover chocolate cake were designated in Table 4.4. The total sugars produced and reducing sugar were achieved using standard curve with various concentration of glucose by phenol sulphuric acid method and 3,5-Dinitrosalicylic Acid (DNS) method respectively. The complete runs of the experiments data of the total sugar yield and reducing sugar yield for subcritical water hydrolysis of leftover chocolate cake were illustrated in **Appendix C3 and Appendix C4.**

Table 4. 4 : The design matrix of central composite design obtained from response surface methodology (RSM) and experimental data of the total sugars yield for subcritical water hydrolysis of leftover chocolate cake.

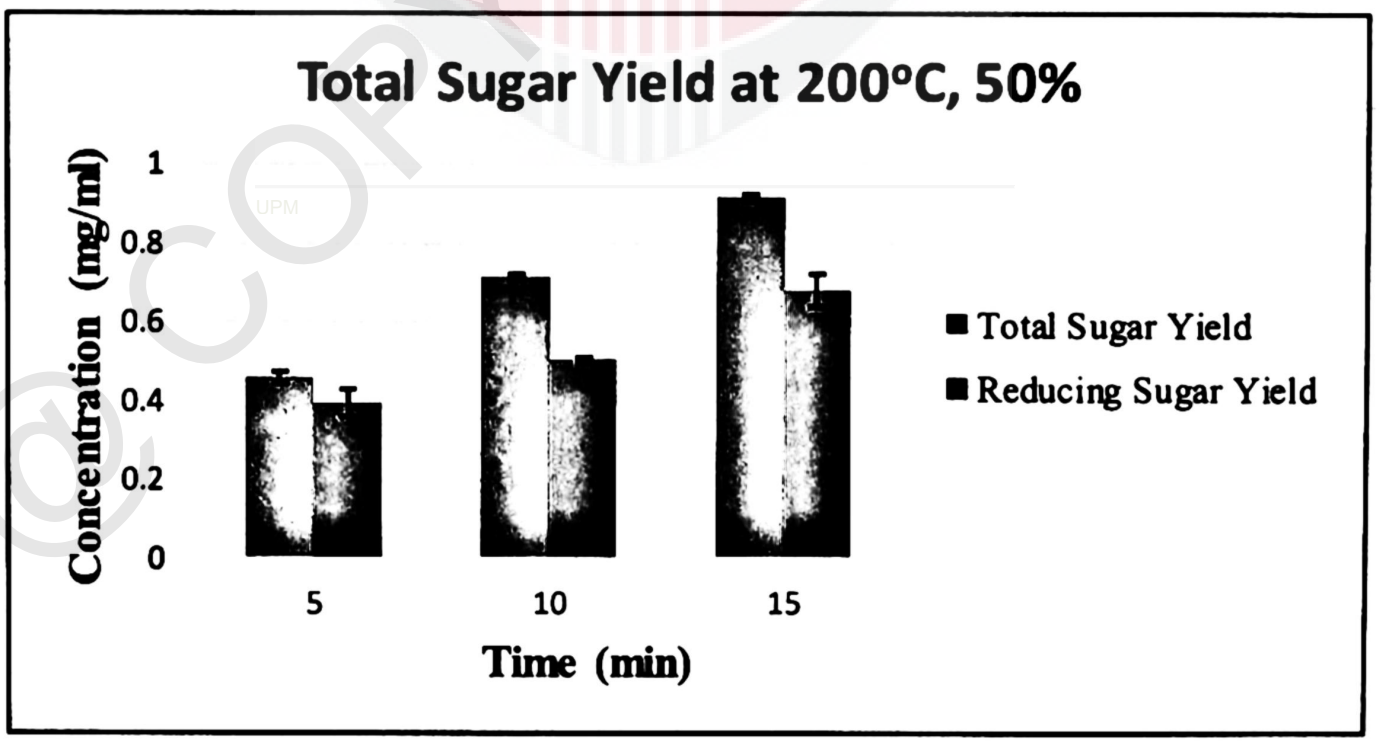
Factor 1 Process Temperature (°C)	Factor 2 Process Time (min)	Factor 3 Solid Loading (%)	Average total sugars yield (mg/ml)	Average reducing sugar yield (mg/ml)
160	5	10	0.105 ± 0.007	0.045 ± 0.007
160	5	50	0.240 ± 0.014	0.125 ± 0.035
160	10	30	0.290 ± 0.014	0.115 ± 0.007
160	15	10	0.245 ± 0.007	0.113 ± 0.004
160	15	50	0.745 ± 0.049	0.493 ± 0.004
180	5	30	0.270 ± 0.014	0.105 ± 0.007
180	10	10	0.215 ± 0.007	0.175 ± 0.007
180	10	30	0.403 ± 0.017	0.305 ± 0.007
180	10	50	0.555 ± 0.007	0.485 ± 0.021
180	15	30	0.660 ± 0.085	0.455 ± 0.007
200	5	10	0.205 ± 0.007	0.105 ± 0.007
200	5	50	0.460 ± 0.014	0.395 ± 0.035
200	10	30	0.465 ± 0.007	0.415 ± 0.007
200	15	10	0.210 ± 0.014	0.185 ± 0.007
200	15	50	0.915 ± 0.007	0.680 ± 0.042

The subcritical water hydrolysis was carried out at 160°C, 180°C and 200°C for 5 to 15 minutes with solid loading of 10% to 50%. The total sugar and reducing sugar recovered were depicted in Figure 4.2.

(a)



(b)



(c)

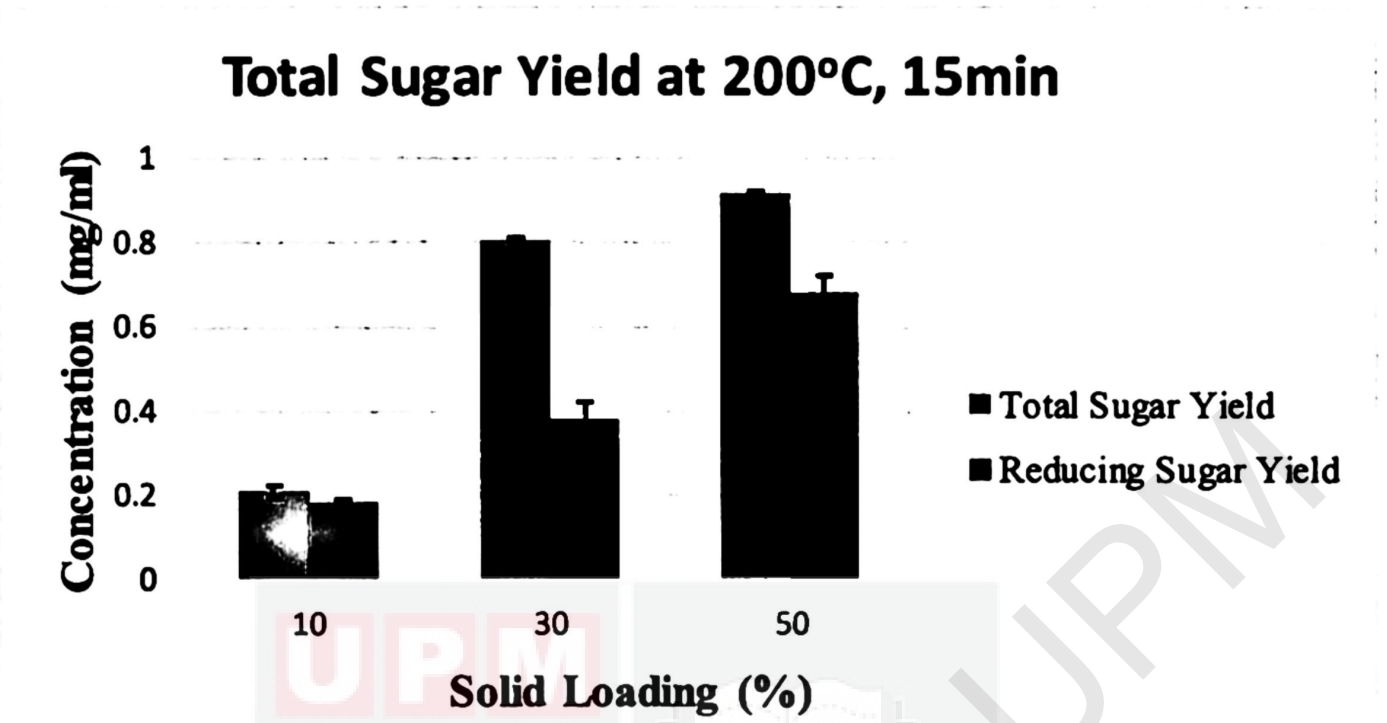


Figure 4. 2: Total sugar and reducing sugar yield of leftover chocolate cake hydrolysates prepared through subcritical water hydrolysis under different process conditions. (a) Process temperature (160°C to 200°C at 15 min with 50% solid loading), (b) Process time (5min to 15 min at 200°C with 50% solid loading), (c) Solid loading (10% to 50% at 200°C for 15 min).

Moreover, contour plots were used for better illustration of the mutual effects of process temperature, process time and solid loading on the response (total sugar yield and reducing sugar concentration in the hydrolysates). The contour plots were generated based on the design of experiments through MINITAB. Figure 4.3 stipulated the contour plot of the effect of process temperature and time on the total sugar with constant 50% solid loading while Figure 4.4 depicted the contour plot of the effect of solid loading and process temperature as well on the total sugar with constant 15min process time. On the other hand, Figure 4.5 showed the contour plot of the effect of process temperature and time on the reducing sugar yield with constant 50% solid loading meanwhile the contour plot of the effect of solid loading as well as process temperature on the reducing sugar yield with constant 15min process time was illustrated in Figure 4.6.

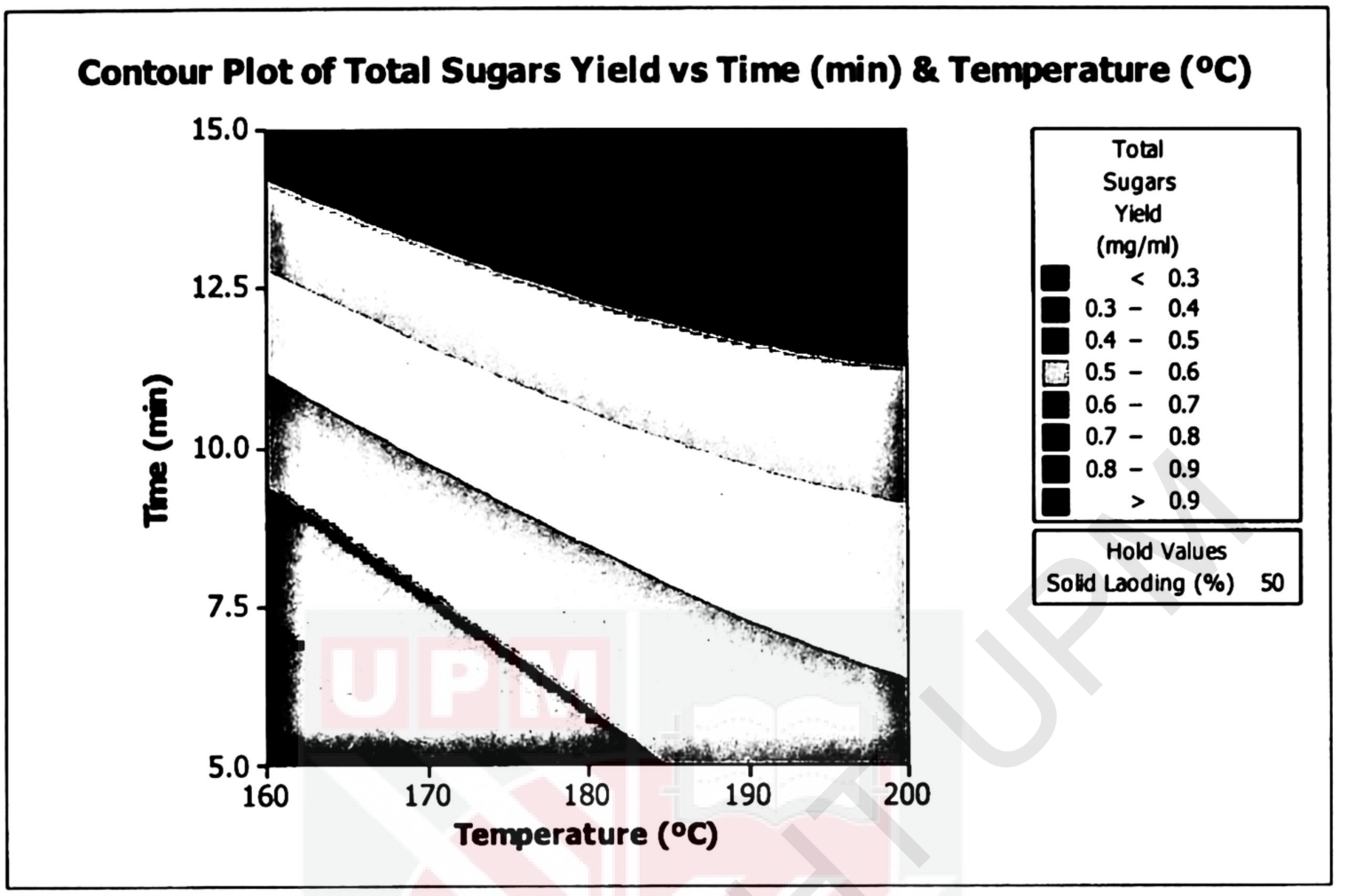


Figure 4. 3: Contour plot for the effect of process temperature and process time on the total sugar yield in subcritical water hydrolysis

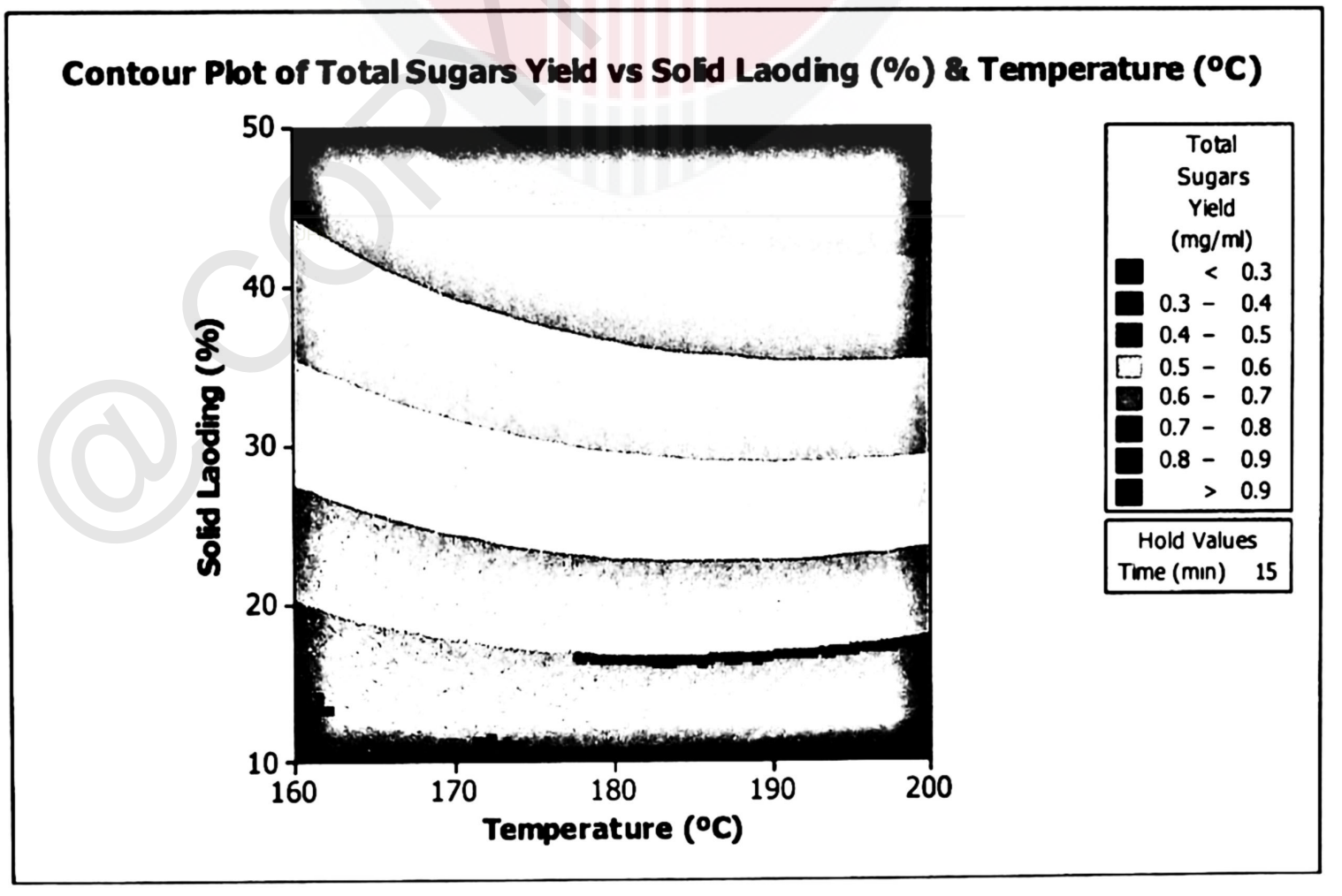


Figure 4. 4: Contour plot for the effect of process temperature and solid loading on the total sugar yield in subcritical water hydrolysis

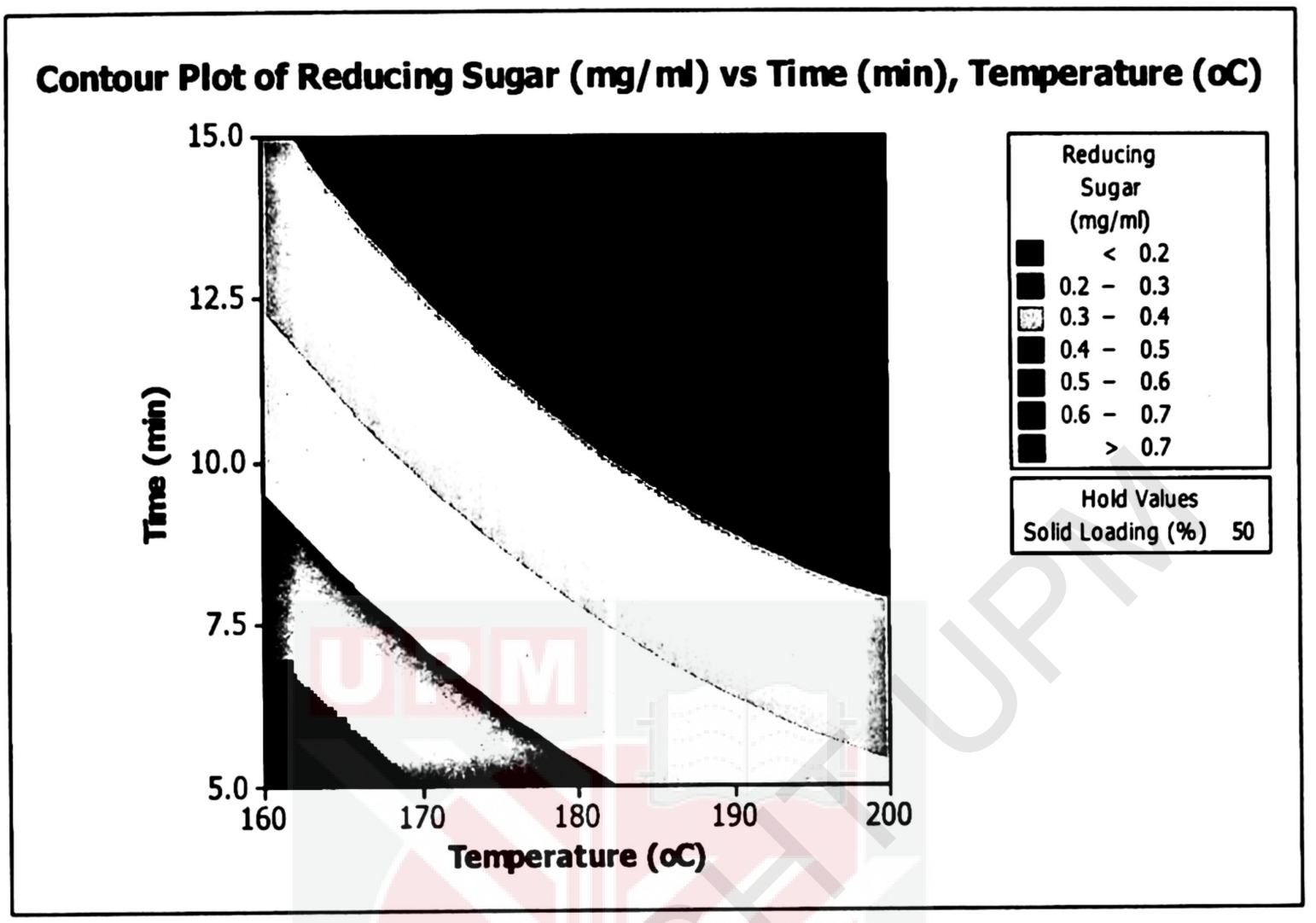


Figure 4. 5: Contour plot for the effect of process temperature and process time on the reducing sugar yield in subcritical water hydrolysis

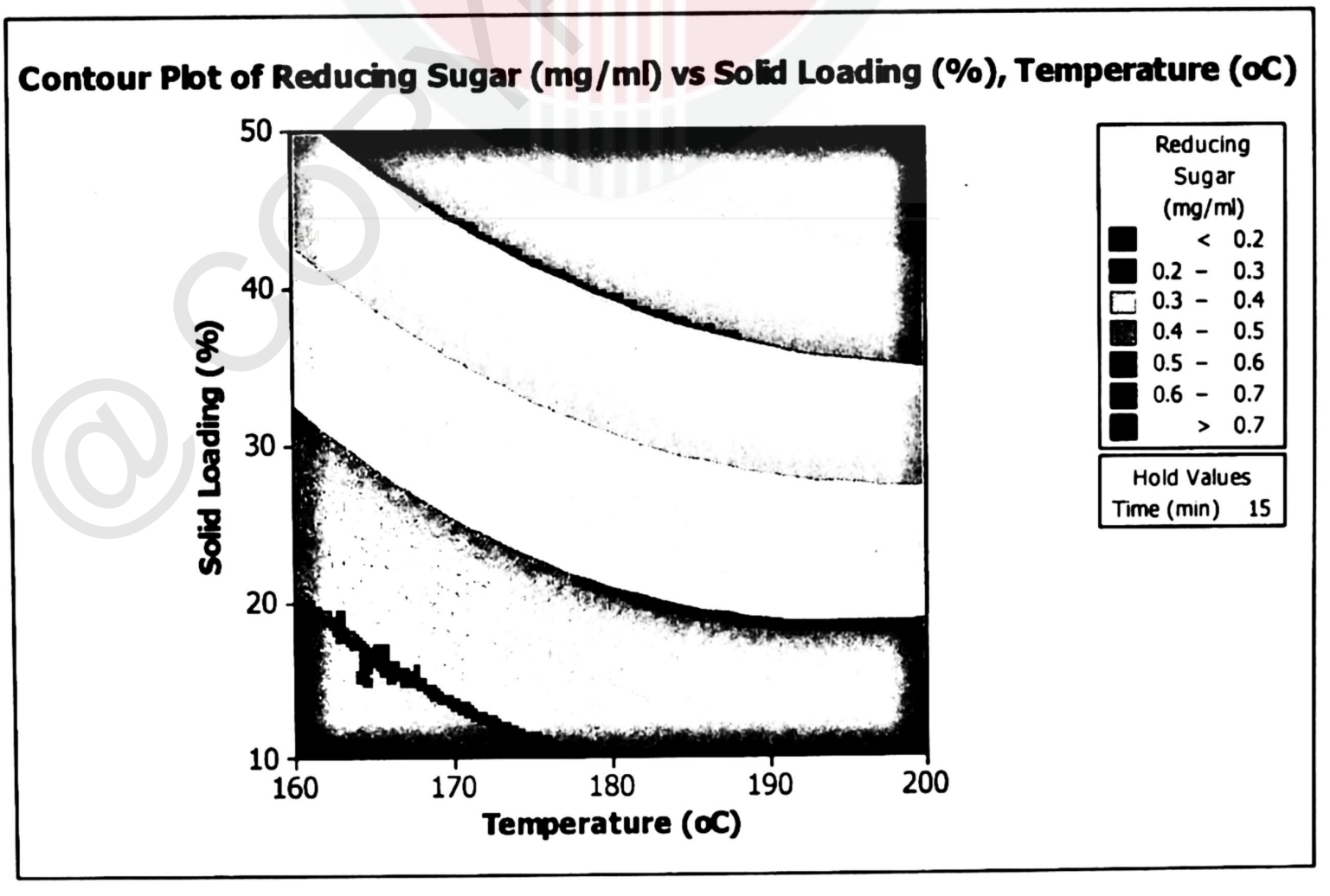


Figure 4. 6: Contour plot for the effect of process temperature and solid loading on the reducing sugar yield in subcritical water hydrolysis

4.3.3.1 Effect of Process Temperature

According to the plot as shown in Figure 4.3, the yield of total sugar increase with the increase of hydrolysis temperature from 160°C to 200°C. The same trend was recorded for the yield of reducing sugar as shown in Figure 4.5. Table 4.4 as well as Figure 4.2(a) illustrated the highest total sugar and reducing sugar yield were achieved at 200°C of 50% solid-to-water ratio for 15min with 0.915 ± 0.007 mg/ml and 0.68 ± 0.042 mg/ml respectively. The increase in the production of total sugar and reducing sugar could be due to the changes of dielectric constant (ε) of the subcritical water. Cheigh, C.I., et al. (2015) stated that water is a polar solvent with dielectric constant of 79.9 and density of 1000 kg/m3 at ambient temperature. When the water was heated to higher temperature, for example temperature of 200°C, the hydrogen bonds will be broken down and reduced the dielectric constant to a range of 27 to 32.5. This indicated that the affinity of water to be a source of reaction media as the polarity of the water was reduced. The dielectric constant of water under this condition is nearly similar to common solvents such as methanol (ε=33) and ethanol (ε=25) at room temperature. Hence, polysaccharides were enabled to be hydrolyzed effectively with similar degree to that in organic solvents (Fernández, M. A., 2018). Besides, more collisions were occurred between the substrate molecules when the reaction temperature was increased (Salwanee, S., et al., 2013). However, increase of hydrolysis temperature beyond the permitted value will cause the degradation of total sugars and reducing sugars. Furan derivatives like furfural (F) and 5-hydroxymethylfurfural (5-HMF) will be formed as the decomposition of polysaccharides at extremely high temperature. These compounds are

the main degradation products of carbohydrate dehydration reactions and result in a lower sugar yield.

4.3.3.2 Effect of Process Time

The study on effect of process time during hydrolysis of leftover chocolate cake is vital as it may affect the equilibrium concentration during subcritical water hydrolysis. Based on Figure 4.3 and Figure 4.5, the increase of process time from 5 min to 15min at constant temperature and solid loading had a positive effect on recovery of both total sugar and reducing sugar. For this study, the highest total sugar and reducing sugar were achieved at 15min of processing time. As designated in Table 4.4 and Figure 4.2(b), the total sugar at 200°C of 50% solid loading was increased from 0.46 ± 0.014 mg/ml (5min processing time) to 0.915 ± 0.007 mg/ml (15min processing time). On the other hand, the increase of reducing sugar yield from 0.395 ± 0.035 mg/ml (5min processing time) to 0.68 ± 0.042 mg/ml (15min processing time) at 200°C of 50% solid loading. The increase of both total sugar and reducing sugar yields could be due to optimum processing time is significant to maximize the yield of sugar recovery as sufficient time was required to break down the glycosidic bonds of the polysaccharides (Mayanga-Torres, P. C., et al., 2017). However, a long contact time may cause sugar degradation. The sugar yield will be initially increased to a maximum value but it degraded after a period of process time (Cantero, D. A., et al., 2013). In fact, the process time and process temperature were closely related in the case of sugar hydrolysis. Hence, the process temperature and process time should be optimized for each individual case with the desired product properties being taken into consideration.

4.3.3.3 Effect of Solid Loading

The solid loading which also known as solid-to-water ratio is another factor affecting subcritical water hydrolysis in terms of sugar recovery. In this study, subcritical water hydrolysis was carried out with solid loading of 10%, 30% and 50%. Figure 4.4 and 4.6 illustrated the increasing trend in the total sugar and reducing sugar contents respectively from 10% to 50%. Figure 4.2(c) also depicted the sugar recovery of leftover chocolate cake at different solid loading. The highest total sugar yield was achieved at 50% for each process temperature and process time. For instance, at 200°C of 15min process time, the total sugar yield increased from 0.21 ± 0.014 mg/ml with solid loading of 10% to 0.915 ± 0.007 mg/ml with solid loading of 50%, as well as reducing sugar, the yield of reducing sugar increased from 0.1845 ± 0.006 mg/ml to 0.68 ± 0.042 mg/ml when the solid loading increased from 10% to 50%. The increasing trend could be due to more amount of chocolate cake sample was available to be hydrolyzed in order to maximize sugar recovery. Gong, Y., et al. (2015) reported at certain high volume of water decreases the quantity of sugar recovery because the amount of solid available is restricted while the water is in excess for subcritical water hydrolysis. However, this is not applicable for every hydrolyzed material. When the volume of water is too low (high concentration of solid particles), water and solid particles simply overflow, thus resulting in low reaction efficiency and rate of hydrolysis (Chao, Z., et al., 2013). Hence, during subcritical water hydrolysis, solid loading needs to be optimized depended the on type of the raw material used and the desired products.

4.3.4 Optimization of The Operating Conditions on Total Sugar and Reducing Sugar Yield by Subcritical Water Hydrolysis

To obtain the best condition for the production of highest yield of total sugar and reducing sugar from leftover chocolate cake by subcritical water hydrolysis, optimization is an important element to be considered to maximize the production of the sugar. In this study, optimization plots were used to depict the optimization of the response. The complete optimization responses for both production of total sugar and reducing sugar were provided in **Appendix D1** and **Appendix D2** respectively.

Figure 4.7 and 4.8 depicted the optimization plots of total sugar and reducing sugar recovery by subcritical water hydrolysis. Since the objective was to maximize the production of total sugar, the target value was set as the highest value obtained in experimental data which was 0.92mg/ml. The composite desirability was calculated as 0.99724 which indicated that all parameters were within the target to maximize the total sugar yield. On the other hand, for reducing sugar yield to reach 0.68 mg/ml, the lower values or the minimum acceptable value was set at 0.04 mg/ml since the objective was to maximize the recovery of reducing sugar. The target values which is the highest reducing sugar yield was set at 0.68 mg/ml. The optimum total sugar and reducing sugar with value of 0.9177 mg/ml and 0.7224 mg/ml respectively can be achieved at temperature of 200°C with 50% solid loading and process time of 15min. The desirability of optimization of reducing sugar was calculated as 1 which indicated the settings had achieved favorable results.

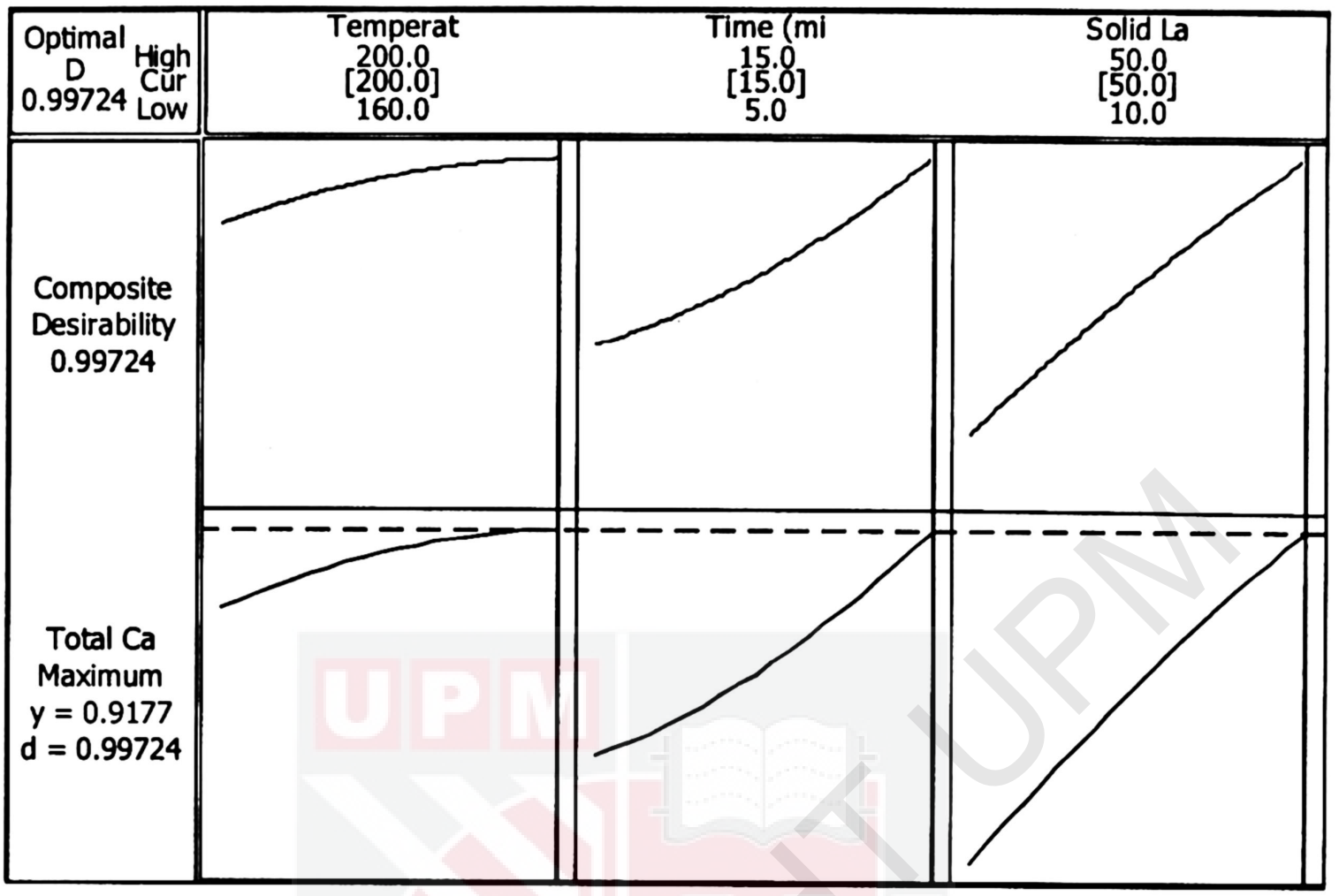


Figure 4. 7 : Optimization plot for total sugar recovery through subcritical water hydrolysis

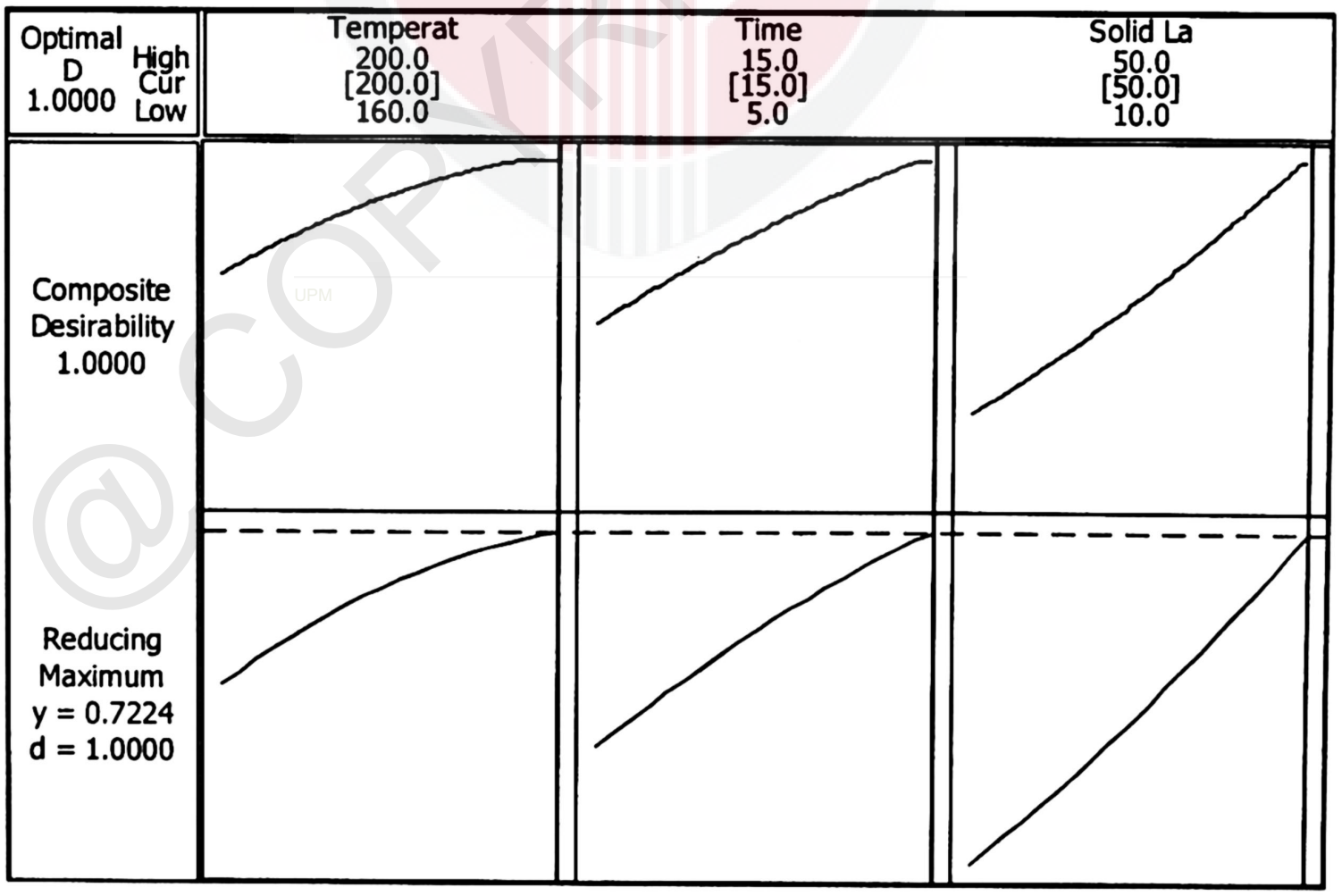


Figure 4. 8 : Optimization plot for reducing sugar recovery through subcritical water hydrolysis

To validate the optimization of the response, the experiment was carried out under the recommended optimum condition at 200°C with 50% solid loading and process time of 15 min. The suggested and actual response values of optimized process were presented in Table 4.5. For optimization process, the actual experimental data of total sugar yield obtained was 0.895 mg/ml and 0.700 mg/ml for reducing sugar. The results illustrated that there were no significant variation of the total sugar and reducing sugar yields between the corresponding experimental response and the suggested value.

Table 4. 5: Suggested and experimental response value of optimized process.

	Process Temperature (°C)	Process Time (min)	Solid Loading (%)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)
Suggested	200	15	50	0.9177	0.7224
Experimental	200	15	50	0.8950	0.7000
Difference (%)	-	-	-	2.44	3.10

4.4 High Performance Liquid Chromatography (HPLC)

HPLC is a common method used to analyze the amount of monosaccharides and oligosaccharides that present in food. In this study, HPLC was used for sugar profile analysis. The amount of these sugars generated at different process temperature for 15min with constant 50% solid loading was as shown in Table 4.6 below.

Table 4. 6: Amount of sucrose and reducing sugars produced through HPLC method at different process temperature (15 min with constant 50% solid loading).

Process Temperature (°C)	160	180	200
Sucrose contents (mg/ml)	0.1713	0.1828	0.0486
Reducing sugars contents (mg/ml)			
Glucose	-	0.2546	0.2958
Xylose	-	0.1586	0.1409
Galactose	0.0242	-	0.2322
Mannose	0.0154	0.1404	0.2487
Fructose	0.0150	0.0504	0.1003
Total reducing sugars	0.0546	0.6040	0.8089

According to Table 4.6, the greatest amount of sucrose at 15 min process time with constant 50% was achieved at process temperature of 180°C with the value of 0.1828mg/ml. On the other hand, the amount of reducing sugars that recovered from the leftover chocolate cake was recorded the highest with 0.8089mg/ml at process temperature of 200°C. The decrease of sucrose amount at 200°C could be due to the breaking of glycosidic bonds of sucrose at certain higher temperature and lead to formation of fructose and glucose. Thus, more reducing sugars were obtained at 200°C. Gao, D., et al., (2013) also stated that sucrose may turn into fructose and glucose through sucrose hydrolysis in subcritical water. Based on the analysis of HPLC, the

hydrolysate of leftover chocolate cake consisted of sucrose and monosaccharides such as glucose, xylose, galactose mannose and fructose. At 200°C process temperature, the glucose contents were highest among the reducing sugars with 0.2958mg/ml. The HPLC sugar profile chart of leftover chocolate cake hydrolysates at different process temperature can refer to **Appendix E1 to Appendix E3**.

4.5 Determination of Functional Group of Hydrolysate of Leftover Chocolate Cake

Fourier transform infrared spectroscopy (FTIR) was used to investigate the chemical changes in the chocolate cake hydrolysate. The FTIR spectra of chocolate cake samples treated at temperature of 160°C, 180°C and 200°C by subcritical water hydrolysis was depicted in Figure 4.9.

According to Mohan et al. (2015), the peak in the region of 3247 to 3476.80cm^{-1} was due to present of O-H stretching with strong and broad peak intensity. Since the spectra in Figure 4.9 showed peak 3399.76cm^{-1}(at 160°C), 3399.81cm^{-1}(at 180°C) and 3401.34cm^{-1}(at 200°C), the hydroxyl groups were presented. The hydroxyl group was the functional group of the sugars (glucose, galactose etc). It was also noticed that the spectra had peak absorption between 2108.41cm^{-1} to 2112.13cm^{-1} at temperature 160°C to 200°C. This indicated weak monosubstituted alkyne groups of CΞC stretching were present. Besides, the absorption peaks at 1642.19cm^{-1}(at 160°C) to 1644.75cm^{-1}(at 200°C) were arisen from medium C=C stretching. Czegledy (2014) stated that absorption band at 1640cm^{-1} is an aldehyde functional group. Hence, it can be deduced

that the hydrolysates comprised of functional group of sugar which was aldehyde group. The present of aldehyde group may due to the present of glucose, xylose etc. The infrared spectrum peak for C=O groups were between 1640cm^{-1} to 1810cm^{-1}. This indicated that the ketone as functional group for fructose could be present.

The peak from 1363.83cm^{-1} to 1366.26 cm^{-1} corresponded to the bending mode of C-H (alkane group). According to Smets, J. et al. (1995), the peak from 1200cm^{-1} to 1310cm^{-1} represented the aromatic ether with C-O stretching. Based on Figure 4.8, the peaks ranged from 1025.18cm^{-1} to 1030.87cm^{-1} were assigned to C-O-C stretching which was the functional group of sucrose. The peak at region 665cm^{-1} to 840cm^{-1} corresponded to the alkene group, C=C bending.

As temperature increased from 160°C to 200°C, higher peak was obtained. The changes attributed to overlapping was considered negligible. According to Collado et al (2018), low transmittance indicated there was high population of bonds that have vibrational energies corresponding to the incident light. From Figure 4.8, the greatest different of the spectra of each temperature could be observed between 1500cm^{-1} to 3000cm^{-1}. This showed that the alkyne groups (CΞC) were reduced as the temperature increased due to breakdown of bond for more molecules. There was also variation depicted at region of 665cm^{-1} to 840cm^{-1} when temperature was risen from 160°C to 200°C. More bending of alkene groups can be detected at 200°C with lower transmittance as the carbon-carbon double bonds became weaker at higher process temperature (Hellier, P. et al., 2018).

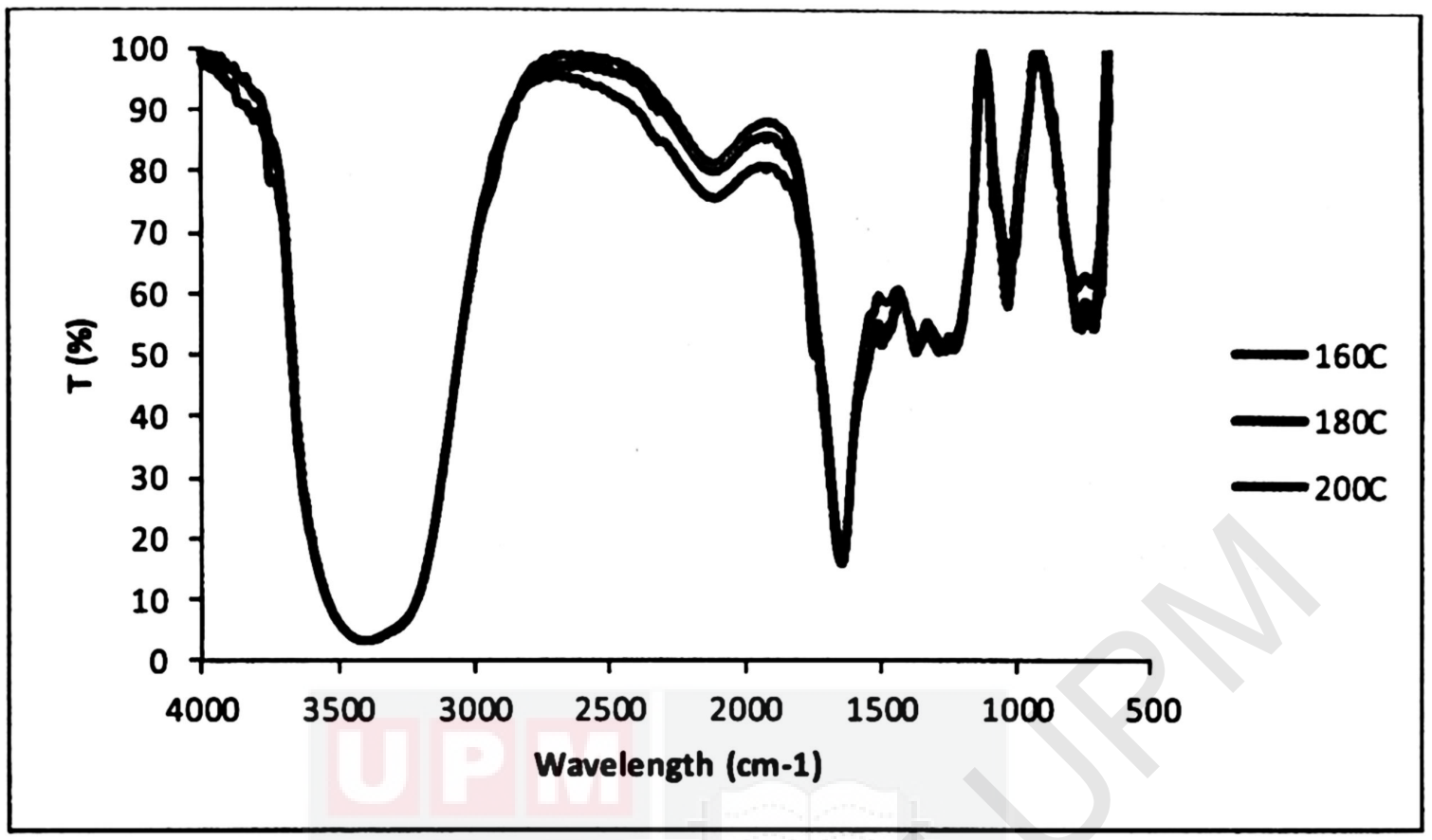


Figure 4. 9 : FTIR spectra of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 160°C, 180°C and 200°C under subcritical water hydrolysis.

4.6 Surface Morphology of The Leftover Chocolate Cake Before and After Subcritical Water Hydrolysis by Scanning Electron Microscopy (SEM)

Figure 4.10 designated the surface morphology of subcritical water hydrolysis untreated and treated leftover chocolate cake samples. The treated samples at each different process temperature (160°C, 180°C, 200°C) with solid loading of 50% and process time of 15min were taken for SEM. From the images, it can be deduced that the untreated chocolate cake sample appeared intact and agglomerated, forming a smoother lump surface. At temperature of 160°C, the structure of the sample started to break and the surface became rougher as compared to untreated sample. After subcritical water hydrolysis at 180°C and 200°C, the structure of the chocolate cake samples looked ruptured and segregated into particulate cells. Roughest surface of sample structure could be observed at 200°C which with most uneven surface. The strength of hydrogen

bonding existing in water decreased with the increasing of process temperature and resulted in dielectric constant reduction (Rodríguez-Meizoso, I., et al., 2010). Moreover, rising of process temperature also flavored water ionization and increased the hydrogen ions concentration that allowed water to be the acid catalyst during subcritical water hydrolysis (Thani, N. M., et al. 2019). Hence, when the process temperature was increased, nonpolar compounds in chocolate cake samples would be dissolved in water and lead to segregation and disruption of the chocolate cake samples.

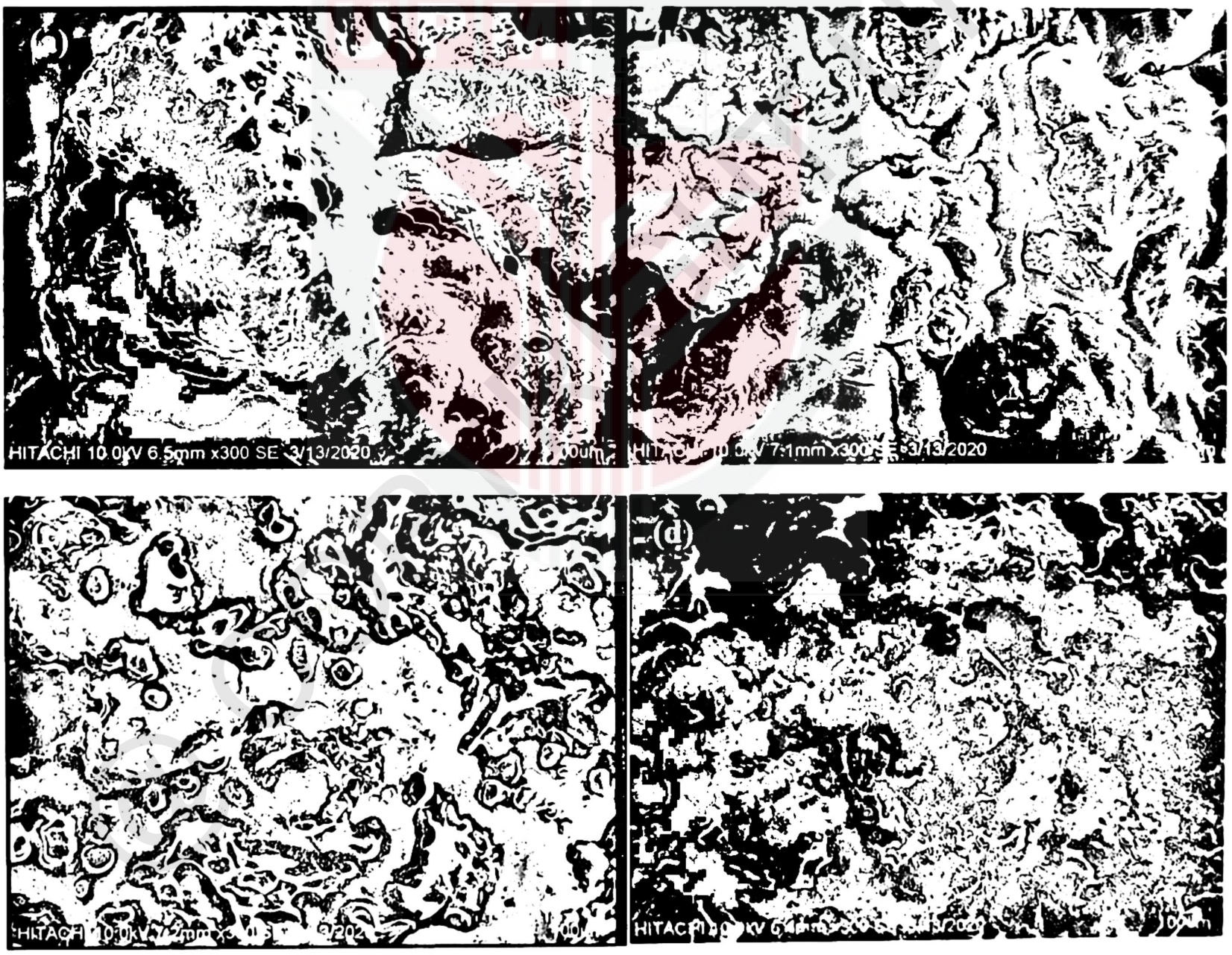


Figure 4. 10: SEM images of untreated (a) and subcritical water treated chocolate with solid loading of 50% and process time of 15min samples at 160°C (b), 180°C(c) and 200°C (d) with magnification of 300 SE.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

In this project, subcritical water hydrolysis has designated as an efficient and green approach for leftover bakery waste sugar recovery. Process temperature and the process time had affected the total sugars and reducing sugars yield significantly while solid loading had less effects towards sugar recovery as compared to process temperature and time. The amount of sugar obtained through subcritical water hydrolysis was increased with the increased of process temperature as breakdown of bonds was occurred at higher temperature and better for sugar recovery. The most desirable process temperature, process time and solid loading for sugar recovery of leftover chocolate cakes in this study were 200°C, 15min and 50% respectively, obtaining total sugar yield of 0.895 mg/ml and 0.700 mg/ml of reducing sugar.

The HPLC had shown leftover chocolate cake hydrolysates contained sucrose, glucose, xylose, galactose, mannose and fructose. The greatest amount of monosaccharides (0.8089 mg/ml) was achieved at process temperature of 200°C. The functional group of the sugar of the leftover chocolate cake waste were determined using FTIR. The aldehyde groups represented the functional group of galactose, glucose, xylose and mannose while ketone groups represented the functional group of fructose. The presence of sucrose was indicated by the presence of ester group through FTIR.

The objectives of evaluating the sugar recovery from bakery waste of chocolate cakes were achieved. The effects of the process parameters such as temperature, time and solid-to-water ratio on the recovery of sugars using subcritical water hydrolysis had

been studied and the morphology residue and chemical compositions of the bakery waste hydrolysate also characterized. This proposed further study on the subcritical water hydrolysis potential for other type of food wastes.

There are several recommendations being suggested to continue this research for future studies. The parameters of subcritical water hydrolysis such as the process temperature, process time and solid loading could be altered over a wide range to obtain the maximum sugar recovery. Besides, different type of cake wastes could be used to compare the sugar recovery among the cakes. Other techniques such as enzymatic hydrolysis and supercritical water hydrolysis can also be studied and thus making comparison to figure out the best method for sugar recovery.

REFERENCES

Abdelmoez, W., Nage, S. M., Bastawess, A., Ihab, A., & Yoshida, H. (2014). Subcritical water technology for wheat straw hydrolysis to produce value added products. *Journal of Cleaner Production*, *70*, 68-77

Abdul Jalil, M. (2010). Sustainable development in Malaysia: A case study on household waste management. *Sustainable Development in Malaysia: A Case Study on Household Waste Management. OIDA International Journal of Sustainable Development*, *1*(1), 23-34.

Abdullah, N. and Chin, N.L. (2010). Simplex-centroid mixture formulation for optimized composting of kitchen waste. Bioresource Technology 101: 8205-8210.

Anne, Melodie. (2018). Three Functions of Fat in the Body. Healthy Eating | SF Gate. Retrieved from http://healthyeating.sfgate.com/three-functions-fat-body-3402.html

A.O.A.C, (2000). Official Methods of Analysis of AOAC International , 17th edition. AOAC International, Maryland, pp: 794-796.

Aquino, A. C. M. M., Jorge, J. A., Terenzi, H. F., & Polizeli, M. D. L. T. M. (2003). Studies on a thermostable α-amylase from the thermophilic fungus Scytalidium thermophilum. *Applied microbiology and biotechnology*, *61*(4), 323-328.

Ávila-Fernández, Á., Galicia-Lagunas, N., Rodríguez-Alegría, M. E., Olvera, C., & López-Munguía, A. (2011). Production of functional oligosaccharides through limited acid hydrolysis of agave fructans. *Food Chemistry*, *129*(2), 380-386.

Barreteau, H., Delattre, C. & Michaud, P. (2006). Production of oligosaccharides as promising new food additive generation. Food Technology and Biotechnology, 44, 323–333.

Biologydictionary.net Editors. (2017). Monosaccharide. Retrieved from *https://biologydictionary.net/monosaccharide/*

Burana-osot, J., Soonthornchareonnon, N., Hosoyama, S., Linhardt, R. J., & Toida, T. (2010). Partial depolymerization of pectin by a photochemical reaction. *Carbohydrate Research*, *345*(9), 1205-1210.

Çam, M., & Hışıl, Y. (2010). Pressurised water extraction of polyphenols from pomegranate peels. *Food chemistry*, *123*(3), 878-885.

Cantero, D. A., Bermejo, M. D., & Cocero, M. J. (2013). High glucose selectivity in pressurized water hydrolysis of cellulose using ultra-fast reactors. *Bioresource technology*, *135*, 697-703.

Centre for Food Safety Food and Environmental Hygiene Department. (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items).

Chandrasekaran, M. (2012). Future Prospects and The Need for Research. In *Valorization of Food Processing By-Products* (pp. 786-801). CRC Press.

Chao, Z., Ri-fu, Y., & Tai-qiu, Q. (2013). Ultrasound-enhanced subcritical water extraction of polysaccharides from Lycium barbarum L. *Separation and Purification Technology*, *120*, 141-147.

Cheigh, C. I., Yoo, S. Y., Ko, M. J., Chang, P. S., & Chung, M. S. (2015). Extraction characteristics of subcritical water depending on the number of hydroxyl group in flavonols. *Food chemistry*, *168*, 21-26.

Choi, C.H. & Mathews, A.P. (1996). Two step acid hydrolysis process kinetics in the saccharification of low-grade biomass: 1. Experimental studies on the formation and degradation of sugars. *Bioresource Technology* 58:101-106.

Collado, C. V. M., Drakontis, C. E., Filippou, S., & Gulezian, S. (2018). Evaluation of Subcritical Water Hydrolysis for Sugar Extraction from Malt Bagasse.

Crittenden, R. A., & Playne, M. (1996). Production, properties and applications of food-grade oligosaccharides. *Trends in food science & technology*, *7*(11), 353-361.

Czegledy, N. (2014). Art as a Catalyst. *Leonardo*, *47*(5), 492-492.

Deshpande, M.S., Rale, V.B., & Lynch, J.M. (1992). *Aureobasidium pullulans* in applied microbiology: A status report. *Enzyme Microbiology Technology* 14:514-527.

Ekvall, J., Stegmark, R., & Nyman, M. (2007). Optimization of extraction methods for determination of the raffinose family oligosaccharides in leguminous vine peas (Pisum sativum L.) and effects of blanching. *Journal of food composition and analysis*, *20*(1), 13-18.

FAO (2011)- Food and Agriculture Organization of the United Nations. Global food losses and food waste: extent, causes and prevention in *Save Food! Global Food Losses and Food Waste*.

Fernández, M. A., Rissanen, J., Nebreda, A. P., Xu, C., Willför, S., Serna, J. G., & Grénman, H. (2018). Hemicelluloses from stone pine, holm oak, and Norway spruce with subcritical water extraction– comparative study with characterization and kinetics. *The Journal of Supercritical Fluids*, *133*, 647-657.

Fontana, A. (2006). Water activity for confectionery quality and shelf-life. *Senior Research Scientist*, 1-20.

FDA, Food And Drug Administration (Bacteriological Analytical Manual Online). (2012). Chapter 18.

Gao, D., Kobayashi, T., & Adachi, S. (2013). Kinetics of sucrose hydrolysis in a subcritical water-ethanol mixture. *Journal of Applied Glycoscience*, jag-JAG.

Garriga, M., Almaraz, M., & Marchiaro, A. (2017). Determination of reducing sugars in extracts of Undaria pinnatifida (harvey) algae by UV-visible spectrophotometry (DNS method). *DESARROLLO E INNOVACIÓN EN INGENIERÍA*, 444.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J., & Joy, D. C. (2017). *Scanning electron microscopy and X-ray microanalysis*. Springer.

Gong, Y., Zhang, X., He, L., Yan, Q., Yuan, F., & Gao, Y. (2015). Optimization of subcritical water extraction parameters of antioxidant polyphenols from sea buckthorn (Hippophaë rhamnoides L.) seed residue. *Journal of food science and technology*, *52*(3), 1534-1542.

Gul, S., & Safdar, M. (2009). Proximate composition and mineral analysis of cinnamon. *Pakistan Journal of Nutrition*, *8*(9), 1456-1460.

Gustavsson, J., Cederberg, C., Sonesson, U., van Otterdijk, R., & Meybeck, A. (2011). Global Food Losses and Food Waste: Extent. *Causes and Prevention*, *29*.

Hellier, P., Talibi, M., Eveleigh, A., & Ladommatos, N. (2018). An overview of the effects of fuel molecular structure on the combustion and emissions characteristics of compression ignition engines. *Proceedings of the Institution of Mechanical Engineers, Part D: Journal of Automobile Engineering*, *232*(1), 90-105.

Henneberg, W. and Stohman, F. 1860. Begründung einer rationellen Ftterung der Wiederkäuer. Vol. 1. Schwetschtke u. Sohn, Braunschweig, Germany.

Holck, J., Hjernø, K., Lorentzen, A., Vigsnæs, L. K., Hemmingsen, L., Licht, T. R.,. & Meyer, A. S. (2011). Tailored enzymatic production of oligosaccharides from sugar beet pectin and evidence of differential effects of a single DP chain length difference on human faecal microbiota composition after in vitro fermentation. *Process Biochemistry*, *46*(5), 1039-1049.

Jain, V. M., Karibasappa, G. N., Dodamani, A. S., & Mali, G. V. (2017). Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method. *Journal of education and health promotion*, *6*.

Jintana, W., & Shuji, A. (2008). Extraction of functional substances from agricultural products or by-products by subcritical water treatment, Food Sci. Tech. Res., 14(4): 319–328.

Jokić, S., Aladić, K., & Šubarić, D. (2018). Subcritical Water Extraction Laboratory Plant Design and Application.

Pinto, J. S. S., & Lanças, F. M. (2006). Hydrolysis of corn oil using subcritical water. *Journal of the Brazilian Chemical Society*, *17*(1), 85-89.

Box, G. E., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the royal statistical society: Series b (Methodological)*, *13*(1), 1-38.

Khajavi, S.H., Kimura, Y., Oomori, T., Matsuno, R., Adachi, S., (2005). Kinetics on sucrose decomposition in subcritical water. LWT Food Sci. Technol. 38(3), 297-302.

Khajenoori, M. (2013). Subcritical Water Extraction of Essential Oils from Matricaria Chamomilla L. *International Journal of Engineering*, *26*(5), 489-494.

Koretsky, M. D. (2004). Engineering and Chemical Thermodynamics. John Wiley& Sons. *Inc*, *457*, 458.

Kumar, S., Gupta, R., Lee, Y. Y., & Gupta, R. B. (2010). Cellulose pretreatment in subcritical water: effect of temperature on molecular structure and enzymatic reactivity. *Bioresource technology*, *101*(4), 1337-1347.

Liang, J., Chen, X., Wang, L., Wei, X., Qiu, F., Lu, C., (2016). Hydrolysis behaviors of sugarcane bagasse pith in subcritical carbon dioxide-water. RSC Adv. 6(101), 99322-99330.

Li, Y., & Wang, S. (2019). Supercritical water oxidation for environmentally friendly treatment of organic wastes. In *Advanced Supercritical Fluids Technologies*. IntechOpen.

Lu, J., Feng, X., Han, Y., & Xue, C. (2014). Optimization of subcritical fluid extraction of carotenoids and chlorophyll a from Laminaria japonica Aresch by response surface methodology. *Journal of the Science of Food and Agriculture*, *94*(1), 139-145.

Mandalari, G., Palop, C. N., Tuohy, K., Gibson, G. R., Bennett, R. N., Waldron, K. W. & Faulds, C. B. (2007). In vitro evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel. *Applied Microbiology and Biotechnology*, *73*(5), 1173-1179.

Marrone, P.A., Hong, G.T., (2009). Corrosion control methods in supercritical water oxidation and gasification processes. J. Supercrit. Fluids. 51(2), 83-103.

Marsiglia, D., Ojeda, K., Ramirez, M., & Sánchez, E. (2016). Pectin extraction from cocoa pod husk (Theobroma Cacao L.) by hydrolysis with citric and acetic acid. *International Journal of Chem Tech Research*, *9*(7), 497-507.

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical biochemistry*, *339*(1), 69-72.

Mayanga-Torres, P. C., Lachos-Perez, D., Rezende, C. A., Prado, J. M., Ma, Z., Tompsett, G. T., & Forster-Carneiro, T. (2017). Valorization of coffee industry residues by subcritical water hydrolysis: recovery of sugars and phenolic compounds. *The Journal of Supercritical Fluids*, *120*, 75-85.

Menezes, C. R., Silva, Í. S., Pavarina, É. C., Dias, E. F. G., Dias, F. G., Grossman, M. J., & Durrant, L. R. (2009). Production of xylooligosaccharides from enzymatic hydrolysis of xylan by the white-rot fungi Pleurotus. *International Biodeterioration & Biodegradation*, *63*(6), 673-678.

Minowa, T., Zhen, F., and Ogi, T., (1998). Cellulose decomposition in hot-compressed water with alkali or nickel catalyst. The Journal of Supercritical Fluids. 13(1–3): p. 253- 259.

Mohamad, R. A., Taip, F. S., Kamal, S. M. M., & Bejo, S. K. (2015). Color and volume development of cake baking and its influence on cake qualities. *J Appl Sci Agric*, *10*, 63-68.

Mohan, M., Timung, R., Deshavath, N. N., Banerjee, T., Goud, V. V., & Dasu, V. V. (2015). Optimization and hydrolysis of cellulose under subcritical water treatment for the production of total reducing sugars. *RSC Advances*, *5*(125), 103265-103275.

Moore, K. J., & Hatfield, R. D. (1994). Carbohydrates and forage quality. *Forage quality, evaluation, and utilization*, 229-280.

Moure, A., Gullon, P., Dominguez, H. & Parajo, J.C. (2006). Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. Process Biochemistry, 41, 1913–1923.

Mussatto, S.I. & Mancilha, I.M. (2007). Non-digestible oligosaccharides: a review. Carbohydrate Polymers, 68, 587–597.

Nakano, M., & Yoshida, S., (1977). Patent No. JP52117446197710O1. Japan.

Ndlela, S. C., De Moura, J. M. L. N., Olson, N. K., & Johnson, L. A. (2012). Aqueous extraction of oil and protein from soybeans with subcritical water. *Journal of the American Oil Chemists' Society*, *89*(6), 1145-1153.

Nielsen, S. S. (2017). Total Carbohydrate by Phenol-Sulfuric Acid Method. In *Food Analysis Laboratory Manual* (pp. 137-141). Springer, Cham.

Oda, Y., Park, B. S., Moon, K. H., & Tonomura, K. (1997). Recycling of bakery wastes using an amylolytic lactic acid bacterium. *Bioresource Technology*, *60*(2), 101-106.

Pasari, N., Adlakha, N., Gupta, M., Bashir, Z., Rajacharya, G. H., Verma, G., ... & Yazdani, S. S. (2017). Impact of module-X2 and carbohydrate binding module-3 on the catalytic activity of associated glycoside hydrolases towards plant biomass. *Scientific reports*, *7*(1), 1-15.

Peterson, A. A., Vogel, F., Lachance, R. P., Fröling, M., Antal Jr, M. J., & Tester, J. W. (2008). Thermochemical biofuel production in hydrothermal media: a review of sub-and supercritical water technologies. *Energy & environmental science*, *1*(1), 32-65.

Pourali, O., Asghari, F. S., & Yoshida, H. (2009). Simultaneous rice bran oil stabilization and extraction using sub-critical water medium. *Journal of Food Engineering*, *95*(3), 510-516.

Prado, J. M., Forster-Carneiro, T., Rostagno, M. A., Follegatti-Romero, L. A., Maugeri Filho, F., & Meireles, M. A. A. (2014). Obtaining sugars from coconut husk,

defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water. *The Journal of Supercritical Fluids*, *89*, 89-98.

Prado, J.M., Vardanega, R., Nogueira, G.C., Forster-Carneiro, T., Rostagno, M.A., Filho, F.M., Meireles, M.A.A., (2017). Valorization of residual biomasses from agri-food industry by subcritical water hydrolysis assisted by CO2. Energy Fuels. 31(3), 2838-2846.

Ravber, M., Knez, Ž., & Škerget, M. (2015). Simultaneous extraction of oil-and water-soluble phase from sunflower seeds with subcritical water. *Food chemistry*, *166*, 316-323.

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L. (1996). "Accelerated solvent extraction: A te- chnique for sample preparation", Analytical Chemistry, 68: 1033-1039

Rivero-Urgell, M., & Santamaria-Orleans, A. (2001). Oligosaccharides: application in infant food. *Early human development*, *65*, S43-S52.

Roberfroid, M., & Slavin, J. (2000). Nondigestible oligosaccharides. *Critical Reviews in Food Science and Nutrition.*, *40*(6), 461-480.

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Señoráns, F. J., Cifuentes, A., & Ibáñez, E. (2010). Subcritical water extraction and characterization of bioactive compounds from Haematococcus pluvialis microalga. *Journal of pharmaceutical and biomedical analysis*, *51*(2), 456-463.

Rogalinski, T., Ingram, T., Brunner, G., (2008). Hydrolysis of lignocellulosic biomass in water under elevated temperatures and pressures. J. Supercrit. Fluids. 47(1), 54-63

Rostagno, M.A., Prado, J.M., (2013). Natural product extraction: principles and applications. Royal Society of Chemistry

Roy, D. C., Pakhira, M. C., & Roy, M. (2016). Estimation of Amino Acids, Minerals and Other Chemical Compositions of Azolla. *Advanced in Life Sciences*, *5*(7).

Salwanee, S., Mustapha, W. A. W., Mamot, S., Maskat, M. Y., & Ibrahim, S. (2013). Effects of enzyme concentration, temperature, pH and time on the degree of hydrolysis of protein extract from viscera of tuna (Euthynnus affinis) by using alcalase. *Sains Malaysiana*, *42*(3), 279-287.

Sangeetha, P. T., Ramesh, M. N., & Prapulla, S. G. (2005). Recent trends in the microbial production, analysis and application of fructooligosaccharides. *Trends in food science & technology*, *16*(10), 442-457.

Sarko, A., Southwick, J., & Hayashi, J. (1976). Packing analysis of carbohydrates and polysaccharides. 7. Crystal structure of cellulose IIII and its relationship to other cellulose polymorphs. *Macromolecules*, *9*(5), 857-863.

Saurav, J., Kumar, N., Raja, R. B., & Arunachalam, D. K. (2010). Qualititative analysis of organically farmed pulse varieties using FTIR (fourier transform infrared) spectroscopy. *African Journal of Microbiology Research*, *4*(12), 1314-1318.

Schacht, C., Zetzl, C., & Brunner, G. (2008). From plant materials to ethanol by means of supercritical fluid technology. *The Journal of Supercritical Fluids*, *46*(3), 299-321.

Shimanouchi, T., Mano, R., Yoshioka, Y., Fukuda, A., Park, K. M., & Kimura, Y. (2019). Kinetic pH titration to predict the acid and hydrothermal conditions for the hydrolysis of disaccharides: use of a microcapillary system. *Journal of Chemistry*, *2019*.

Shitu, A., Izhar, S., Tahir, T.M., 2015. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: a review of recent work. Global J. Environ. Sci. Manage. 1(3), 255-264.

Singh, P. (2011). Subcritical water extraction of functional ingredients and glycoalkaloids from potato peel.

Smets, J., Adamowicz, L., & Maes, G. (1995). Matrix-Isolation FT-IR Studies and Ab-Initio Calculations of Hydrogen-Bonded Complexes of Molecules Modeling Cytosine or Isocytosine Tautomers. 2. 4-Aminopyridine and 4-Aminopyrimidine Complexes with H2O in Ar Matrixes. *The Journal of Physical Chemistry*, *99*(17), 6387-6400.

Xantheas, S. S. (2000). Cooperativity and hydrogen bonding network in water clusters. *Chemical Physics*, *258*(2-3), 225-231.

Suan, L., (2013). A review on plant-based rutin extraction methods and its pharmacological activities, J, Ethnopharmacology, 150(3): 805-817.

Taylor, A., (2012). BBC Food. Retrieved 2019 from BBC News: https://www.bbc.com/news/magazine-17353707 10.1094/PDIS-11-11-0999-PDN

Thani, N. M., Kamal, S. M. M., Taip, F. S., Sulaiman, A., & Omar, R. (2019). Effect of sub-critical water hydrolysis on sugar recovery from bakery leftovers. *Food and Bioproducts Processing*, *117*, 105-112.

Vázquez, M. J., Alonso, J. L., Domínguez, H., & Parajó, J. C. (2006). Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredients. *Industrial Crops and Products*, *24*(2), 152-159.

Vilar, V. J., Botelho, C. M., & Boaventura, R. A. (2005). Influence of pH, ionic strength and temperature on lead biosorption by Gelidium and agar extraction algal waste. *Process Biochemistry*, *40*(10), 3267-3275.

Voragen, A. G. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science & Technology*, *9*(8-9), 328-335.

Wang, C., Zhou, F., Yang, Z., Wang, W., Yu, F., & Wu, Y. (2012). Hydrolysis of cellulose into reducing sugar via hot-compressed ethanol/water mixture. *biomass and bioenergy*, *42*, 143-150.

Warrand, J., & Janssen, H. G. (2007). Controlled production of oligosaccharides from amylose by acid-hydrolysis under microwave treatment: Comparison with conventional heating. *Carbohydrate polymers*, *69*(2), 353-362.

WRI-World Resources Institute, (2016). Food Loss and Waste Accounting and Reporting Standard. *FLW Protoc.*

Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J., & Wu, Z. (2014). Amino acid nutrition in animals: protein synthesis and beyond. *Annu. Rev. Anim. Biosci.*, *2*(1), 387-417.

Yoshida, H., Izhar, S., Nishio, E., Utsumi, Y., Kakimori, N., & Feridoun, S. A. (2014). Recovery of indium from TFT and CF glasses in LCD panel wastes using sub-critical water. *Solar energy materials and solar cells*, *125*, 14-19.

Yu, Y., & Wu, H. (2010). Understanding the primary liquid products of cellulose hydrolysis in hot-compressed water at various reaction temperatures. *Energy & Fuels*, *24*(3), 1963-1971.

Yang, Y. (2007). Subcritical water chromatography: A green approach to high-temperature liquid chromatography. *Journal of separation science*, *30*(8), 1131-1140.

Zakaria, S. M., & Kamal, S. M. (2016). Subcritical water extraction of bioactive compounds from plants and algae: applications in pharmaceutical and food ingredients. Food Eng Rev 8 (1): 23–34.

Zhang, A. Y. Z., Sun, Z., Leung, C. C. J., Han, W., Lau, K. Y., Li, M., & Lin, C. S. K. (2013). Valorisation of bakery waste for succinic acid production. *Green chemistry*, *15*(3), 690-695.

Zhao, Y., Lu, W. J., & Wang, H. T. (2009). Supercritical hydrolysis of cellulose for oligosaccharide production in combined technology. *Chemical Engineering Journal*, *150*(2-3), 411-417.

Zhao, Y., Wang, H. T., Lu, W. J., & Wang, H. (2011). Combined supercritical and subcritical conversion of cellulose for fermentable hexose production in a flow reaction system. *Chemical Engineering Journal*, *166*(3), 868-872.

Zhu, G., Zhu, X., Xiao, Z., Zhou, R., Zhu, Y., & Wan, X. (2014). Kinetics of peanut shell pyrolysis and hydrolysis in subcritical water. *Journal of Material Cycles and Waste Management*, *16*(3), 546-556.

APPENDICES

Appendix A: Proximate analysis of leftover chocolate cake.

UKM UNIPEQ

Ref : ULUKM/18630/19
Date : 12/12/2019
Page : 1 of 1

CERTIFICATE OF ANALYSIS

Name of Customer : DEPARTMENT OF PROCESS AND FOOD ENGINEERING
Address : Faculty of Engineering, Universiti Putra Malaysia, 43400 Selangor.
(Attn : Dr Siti Mazlina Mustapa Kamal)
Tel No. : 013-302 1497 / 017-266 0319
Sample Description : One (1) sample described as Chocolate Cake
Sample Ref. No : U2877/19
Date of Receipt : 29/11/2019
Test Performance Date : 03/12/2019 – 10/12/2019

ANALYSIS RESULTS
(As per sample)

Parameter, Unit	Result	Test Method
Protein, g/100g	6.8	In house method No. STP/Chem/A03 based on AOAC 18th Ed 981.10
Total Fat, g/100g	20.1	In house method No. STP/Chem/A02 based on AOAC 18th Ed 991.36
Total Carbohydrate, g/100g	47.4	In house method No. STP/Chem/A05 based on Pomeranz Food Analysis: Theory and Practice 2nd Ed. pg 637)
Ash, g/100g	2.2	In house method No. STP/Chem/A06 based on AOAC 18th Ed. 923.03
Moisture, g/100g	23.4	In house method No. STP/Chem/A04 based on AOAC 18th Ed 950.46
Energy, kcal/100g	386 (1623kJ)	In house method No. STP/Chem/AC1 based on Pearson's The Chemical Analysis of Foods (9th Edition, page 578)
Yeast & Mould, (CFU/g)	<100	FDA - Food and Drug Administration (Bacteriological Analytical Manual Online) 2012, Chapter 18

Remarks:
- % Total Carbohydrate = 100 - (%Ash + %Moisture + %Protein + %Fat)
- Energy factor: 0.25
- CFU: Colony Form Unit
- < : Less than
- Query / Reference samples will be discarded two weeks after issuance of Certificate of Analysis

SAC-MRA

STANDARDS
ISO/IEC 17025
TESTING
SAMM NO. 339

Prof. Madya Ir. Dr. Siti Mazlina Mustapa Kamal
Head of Unit

This report refers to the received sample only. Sampling is not conducted by our organization. All analysis are carried out to the best of our knowledge and ability and our responsibility is limited to the correctness of the result. This report is issued on the understanding that it does not in any manner relieve any person from any responsibility, liability or obligation. Duplication of this report is not permitted except in full without written approval of the laboratory.

Bridge To A Better Product UPEQ Sdn. Bhd. (811500-D) Block A, UKM - MTDC Technology Centre, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia

Tel : 603 8921 5555 Fax : 603 8925 2118 Email : unipeq@ukm.edu.my Web : www.unipeq.com.my

Appendix B1: Response surface regression for total sugar yields.

The analysis was done using coded units.

Estimated Regression Coefficients for substrate conc.

Term	Coef	SE Coef	T	P
Constant	0.40714	0.008042	50.628	0.000
Temperature	0.06300	0.007397	8.517	0.000
Time	0.14950	0.007397	20.210	0.000
Solid Laoding	0.19350	0.007397	26.158	0.000
Temperature*Temperature	-0.03659	0.014106	-2.594	0.015
Time*Time	0.05091	0.014106	3.609	0.001
Solid Laoding*Solid Laoding	-0.02909	0.014106	-2.062	0.048
Temperature*Time	-0.02312	0.008270	-2.796	0.009
Temperature*Solid Laoding	0.04063	0.008270	4.912	0.000
Time*Solid Laoding	0.10187	0.008270	12.318	0.000

S = 0.0330816 PRESS = 0.0638529
R-Sq = 97.85% R-Sq(pred) = 95.83% R-Sq(adj) = 97.21%

Analysis of Variance for total carbohydrate conc.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1.49667	1.49667	0.166296	151.95	0.000
Linear	3	1.27523	1.27523	0.425077	388.41	0.000
Temperature	1	0.07938	0.07938	0.079380	72.53	0.000
Time	1	0.44700	0.44700	0.447005	408.45	0.000
Soliad Laoding	1	0.74885	0.74885	0.748845	684.26	0.000
Square	3	0.02042	0.02042	0.006806	6.22	0.002
Temperature*Temperature	1	0.00552	0.00736	0.007364	6.73	0.015
Time*Time	1	0.01024	0.01425	0.014255	13.03	0.001
Soliad Laoding*Soliad Laoding	1	0.00465	0.00465	0.004655	4.25	0.048
Interaction	3	0.20102	0.20102	0.067006	61.23	0.000
Temperature*Time	1	0.00856	0.00856	0.008556	7.82	0.009
Temperature*Soliad Laoding	1	0.02641	0.02641	0.026406	24.13	0.000
Time*Soliad Laoding	1	0.16606	0.16606	0.166056	151.73	0.000
Residual Error	30	0.03283	0.03283	0.001094		
Lack-of-Fit	5	0.01881	0.01881	0.003761	6.70	0.000
Pure Error	25	0.01403	0.01403	0.000561		
Total	39	1.52950				

Obs	StdOrder	substrate conc.	Fit	SE Fit	Residual	St Resid	
1	21	0.100	0.106	0.021	-0.006	-0.22	
2	1	0.110	0.106	0.021	0.004	0.17	
3	25	0.230	0.208	0.021	0.022	0.87	
4	5	0.250	0.208	0.021	0.042	1.64	
5	29	0.300	0.308	0.016	-0.008	-0.26	
6	9	0.280	0.308	0.016	-0.028	-0.96	
7	3	0.240	0.247	0.021	-0.007	-0.28	
8	23	0.250	0.247	0.021	0.003	0.11	
9	7	0.780	0.757	0.021	0.023	0.91	
10	27	0.710	0.757	0.021	-0.047	-1.82	
11	31	0.260	0.309	0.016	-0.049	-1.69	
12	11	0.280	0.309	0.016	-0.029	-0.99	
13	33	0.220	0.185	0.016	0.035	1.23	
14	13	0.210	0.185	0.016	0.025	0.89	
15	15	0.400	0.407	0.008	-0.007	-0.22	
16	39	0.410	0.407	0.008	0.003	0.09	
17	16	0.390	0.407	0.008	-0.017	-0.53	
18	36	0.420	0.407	0.008	0.013	0.40	
19	17	0.400	0.407	0.008	-0.007	-0.22	
20	35	0.370	0.407	0.008	-0.037	-1.16	
21	40	0.430	0.407	0.008	0.023	0.71	
22	19	0.410	0.407	0.008	0.003	0.09	
23	18	0.400	0.407	0.008	-0.007	-0.22	
24	38	0.410	0.407	0.008	0.003	0.09	
25	20	0.410	0.407	0.008	0.003	0.09	
26	37	0.380	0.407	0.008	-0.027	-0.85	
27	34	0.550	0.572	0.016	-0.022	-0.75	
28	14	0.560	0.572	0.016	-0.012	-0.40	
29	12	0.720	0.608	0.016	0.112	3.91	R
30	32	0.600	0.608	0.016	-0.008	-0.26	
31	2	0.210	0.197	0.021	0.013	0.52	
32	22	0.200	0.197	0.021	0.003	0.13	
33	6	0.450	0.461	0.021	-0.011	-0.44	
34	26	0.470	0.461	0.021	0.009	0.34	
35	30	0.460	0.434	0.016	0.026	0.92	
36	10	0.470	0.434	0.016	0.036	1.27	
37	4	0.220	0.246	0.021	-0.026	-1.00	
38	24	0.200	0.246	0.021	-0.046	-1.78	
39	8	0.920	0.918	0.021	0.002	0.09	
40	28	0.910	0.918	0.021	-0.008	-0.30	

R denotes an observation with a large standardized residual.

Appendix B2: Response surface regression for reducing sugar yields.

The analysis was done using coded units.

Estimated Regression Coefficients for substrate conc.

Term	Coef	SE Coef	T	P
Constant	0.301100	0.009448	31.871	0.000
Temperature	0.088950	0.008691	10.235	0.000
Time	0.114950	0.008691	13.227	0.000
Solid Laoding	0.155550	0.008691	17.899	0.000
Temperature*Temperature	-0.037750	0.016572	-2.278	0.030
Time*Time	-0.022750	0.016572	-1.373	0.180
Solid Laoding*Solid Laoding	0.027250	0.016572	1.644	0.111
Temperature*Time	-0.008812	0.009716	-0.907	0.372
Temperature*Solid Laoding	0.040688	0.009716	4.188	0.000
Time*Solid Laoding	0.063187	0.009716	6.503	0.000

S = 0.0388652 PRESS = 0.0927256
R-Sq = 95.73% R-Sq(pred) = 91.26% R-Sq(adj) = 94.45%

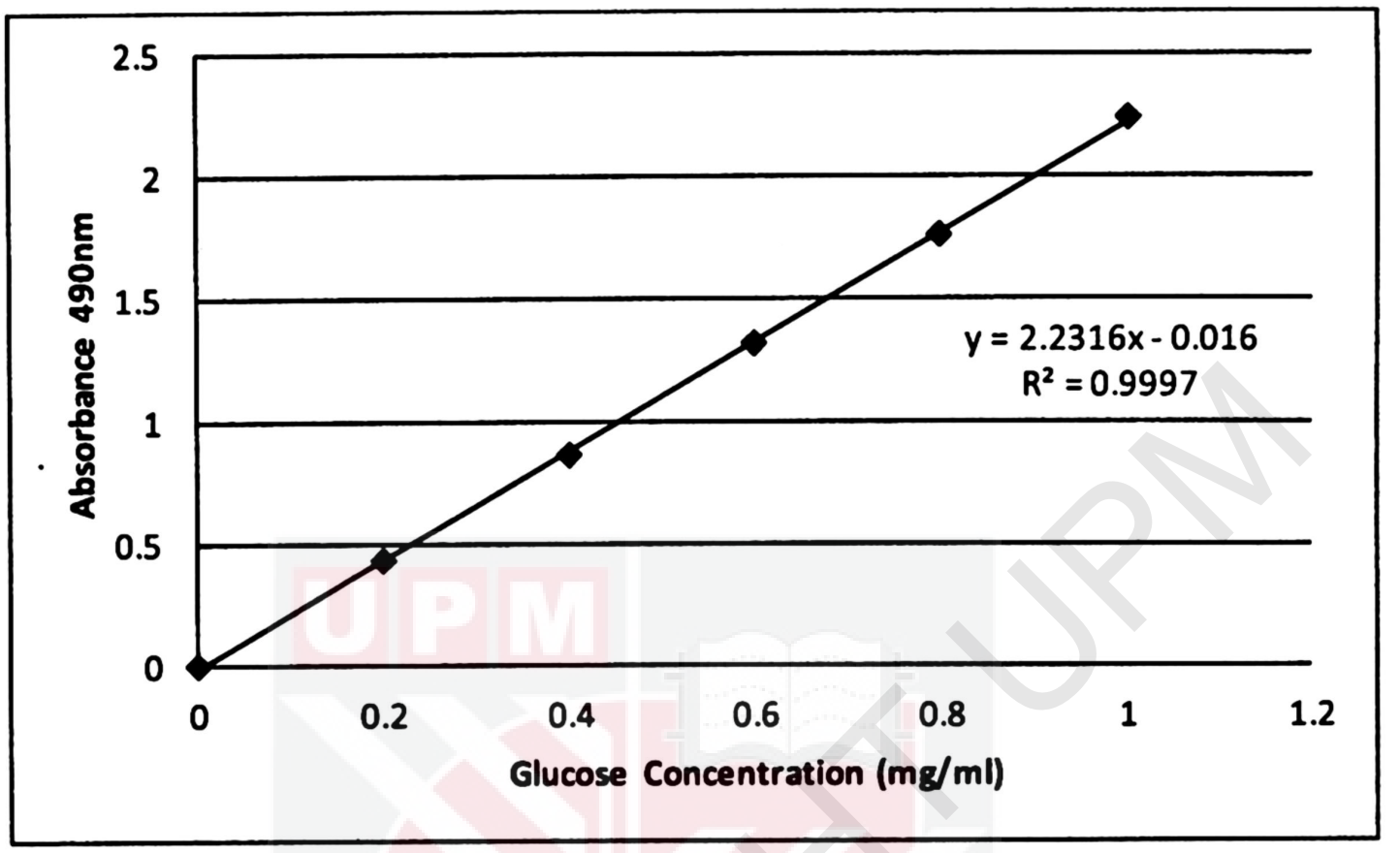
Analysis of Variance for substrate conc.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1.01541	1.01541	0.112824	74.69	0.000
Linear	3	0.90643	0.90643	0.302143	200.03	0.000
Temperature	1	0.15824	0.15824	0.158242	104.76	0.000
Time	1	0.26427	0.26427	0.264270	174.96	0.000
Solid Laoding	1	0.48392	0.48392	0.483916	320.37	0.000
Square	3	0.01737	0.01737	0.005791	3.83	0.020
Temperature*Temperature	1	0.01229	0.00784	0.007838	5.19	0.030
Time*Time	1	0.00101	0.00285	0.002847	1.88	0.180
Solid Laoding*Solid Laoding	1	0.00408	0.00408	0.004084	2.70	0.111
Interaction	3	0.09161	0.09161	0.030538	20.22	0.000
Temperature*Time	1	0.00124	0.00124	0.001243	0.82	0.372
Temperature*Solid Laoding	1	0.02649	0.02649	0.026488	17.54	0.000
Time*Solid Laoding	1	0.06388	0.06388	0.063883	42.29	0.000
Residual Error	30	0.04532	0.04532	0.001511		
Lack-of-Fit	5	0.03895	0.03895	0.007790	30.59	0.000
Pure Error	25	0.00637	0.00637	0.000255		
Total	39	1.06073				

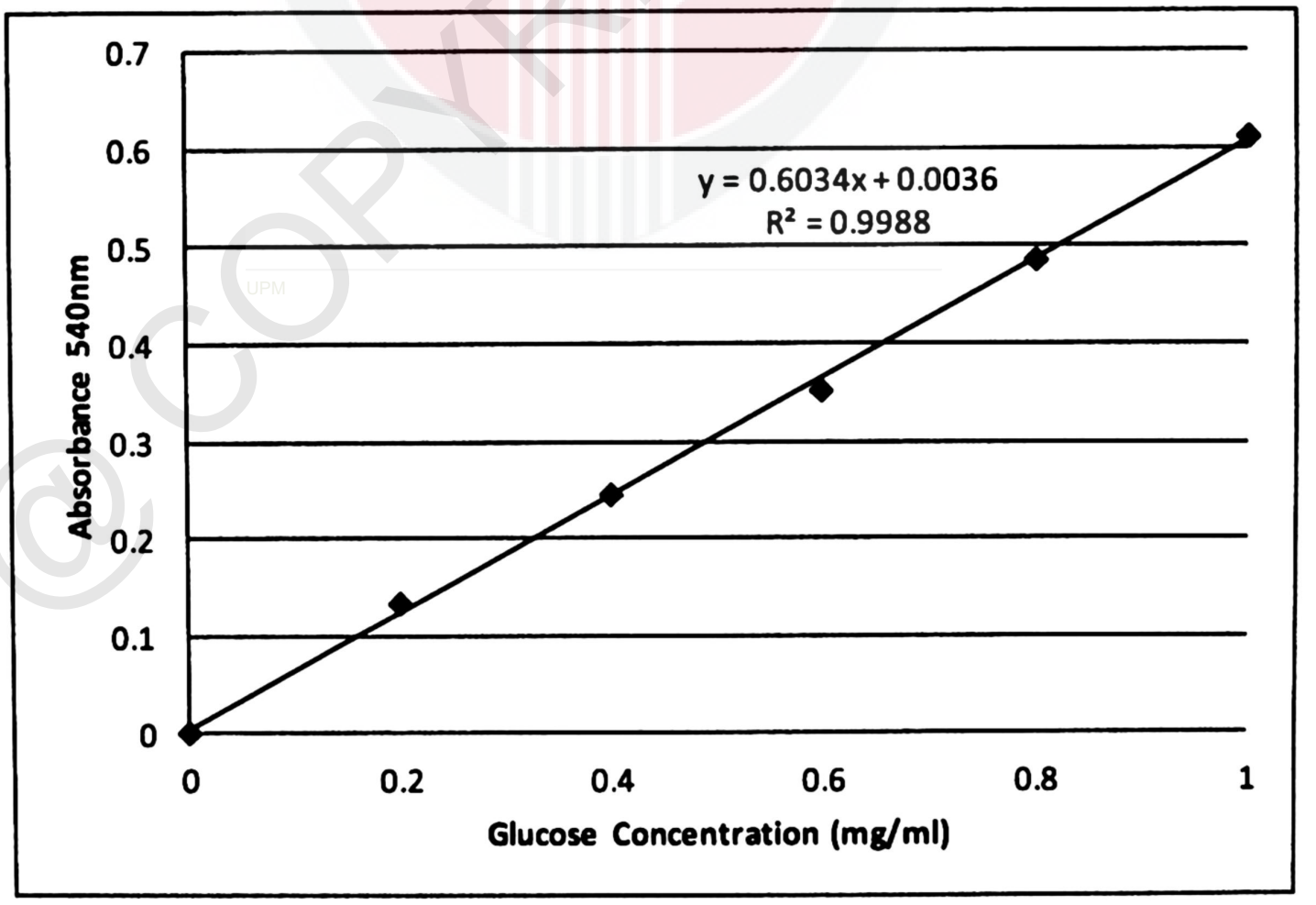
Obs	StdOrder	substrate conc.	Fit	SE Fit	Residual	St Resid
1	21	0.040	0.003	0.024	0.037	1.21
2	1	0.050	0.003	0.024	0.047	1.54
3	25	0.100	0.107	0.024	-0.007	-0.23
4	5	0.150	0.107	0.024	0.043	1.43
5	29	0.110	0.174	0.019	-0.064	-1.91
6	9	0.120	0.174	0.019	-0.054	-1.61
7	3	0.115	0.125	0.024	-0.010	-0.32
8	23	0.110	0.125	0.024	-0.015	-0.48
9	7	0.495	0.481	0.024	0.014	0.47
10	27	0.490	0.481	0.024	0.009	0.31
11	31	0.100	0.163	0.019	-0.063	-1.88
12	11	0.110	0.163	0.019	-0.053	-1.58
13	33	0.170	0.173	0.019	-0.003	-0.08
14	13	0.180	0.173	0.019	0.007	0.21
15	15	0.310	0.301	0.009	0.009	0.24
16	39	0.300	0.301	0.009	-0.001	-0.03
17	16	0.290	0.301	0.009	-0.011	-0.29
18	36	0.300	0.301	0.009	-0.001	-0.03
19	17	0.280	0.301	0.009	-0.021	-0.56
20	35	0.320	0.301	0.009	0.019	0.50
21	40	0.300	0.301	0.009	-0.001	-0.03
22	19	0.310	0.301	0.009	0.009	0.24
23	18	0.300	0.301	0.009	-0.001	-0.03
24	38	0.300	0.301	0.009	-0.001	-0.03
25	20	0.290	0.301	0.009	-0.011	-0.29
26	37	0.300	0.301	0.009	-0.001	-0.03
27	34	0.470	0.484	0.019	-0.014	-0.41
28	14	0.500	0.484	0.019	0.016	0.48
29	12	0.450	0.393	0.019	0.057	1.68
30	32	0.460	0.393	0.019	0.067	1.98
31	2	0.100	0.118	0.024	-0.018	-0.58
32	22	0.110	0.118	0.024	-0.008	-0.25
33	6	0.370	0.384	0.024	-0.014	-0.45
34	26	0.420	0.384	0.024	0.036	1.20
35	30	0.410	0.352	0.019	0.058	1.71
36	10	0.420	0.352	0.019	0.068	2.01 R
37	4	0.189	0.204	0.024	-0.015	-0.48
38	24	0.180	0.204	0.024	-0.024	-0.78
39	8	0.710	0.722	0.024	-0.012	-0.41
40	28	0.650	0.722	0.024	-0.072	-2.40 R

R denotes an observation with a large standardized residual.

Appendix C1: Standard curve for total carbohydrate yield by phenol sulphuric acid method



Appendix C2: Standard curve for reducing sugar yield by DNS method



Appendix C3: Total carbohydrate produced designed by MINITAB (based on total carbohydrate standard curve)

Run Order	Process Temperature (°C)	Process Time (min)	Solid Loading (%)	Absorbance	Total carbohydrate yield (mg/ml)	Average total carbohydrate yield (mg/ml)
9	160	5	10	0.207	0.10	0.105 ± 0.007
11	160	5	10	0.229	0.11	
8	160	5	50	0.501	0.23	0.240 ± 0.014
31	160	5	50	0.542	0.25	
1	160	10	30	0.653	0.30	0.290 ± 0.014
26	160	10	30	0.609	0.28	
16	160	15	10	0.521	0.24	0.245 ± 0.007
18	160	15	10	0.542	0.25	
3	160	15	50	1.727	0.78	0.745 ± 0.049
14	160	15	50	1.655	0.71	
24	180	5	30	0.564	0.26	0.270 ± 0.014
25	180	5	30	0.609	0.28	
12	180	10	10	0.475	0.22	0.215 ± 0.007
36	180	10	10	0.453	0.21	
15	180	10	30	0.877	0.40	0.403 ± 0.017
20	180	10	30	0.899	0.41	
22	180	10	30	0.854	0.39	
27	180	10	30	0.921	0.42	
28	180	10	30	0.876	0.40	
30	180	10	30	0.810	0.37	
33	180	10	30	0.944	0.43	
34	180	10	30	0.898	0.41	
35	180	10	30	0.877	0.40	
37	180	10	30	0.898	0.41	
38	180	10	30	0.901	0.41	
39	180	10	30	0.832	0.38	
29	180	10	50	1.211	0.55	0.555 ± 0.007
40	180	10	50	1.234	0.56	
13	180	15	30	1.591	0.72	0.660 ± 0.085
23	180	15	30	1.323	0.60	
4	200	5	10	0.455	0.21	0.205 ± 0.007
19	200	5	10	0.432	0.20	
10	200	5	50	0.988	0.45	0.460 ± 0.014
17	200	5	50	1.055	0.47	
2	200	10	30	1.053	0.46	0.465 ± 0.007
6	200	10	30	1.084	0.47	
5	200	15	10	0.475	0.22	0.210 ± 0.014
32	200	15	10	0.431	0.20	
7	200	15	50	2.037	0.92	0.915 ± 0.007
21	200	15	50	2.015	0.91	

Appendix C4: Reducing sugar produced designed by MINITAB (based on reducing sugar standard curve)

Run Order	Process Temperature (°C)	Process Time (min)	Solid Loading (%)	Absorbance	Reducing sugar yield (mg/ml)	Average total carbohydrate yield (mg/ml)
9	160	5	10	0.028	0.04	0.045 ± 0.007
11	160	5	10	0.034	0.05	
8	160	5	50	0.064	0.10	0.125 ± 0.035
31	160	5	50	0.094	0.15	
1	160	10	30	0.070	0.11	0.115 ± 0.007
26	160	10	30	0.076	0.12	
16	160	15	10	0.073	0.115	0.113 ± 0.004
18	160	15	10	0.070	0.11	
3	160	15	50	0.302	0.495	0.493 ± 0.004
14	160	15	50	0.299	0.49	
24	180	5	30	0.064	0.10	0.105 ± 0.007
25	180	5	30	0.070	0.11	
12	180	10	10	0.106	0.17	0.175 ± 0.007
36	180	10	10	0.112	0.18	
15	180	10	30	0.191	0.31	0.305 ± 0.007
20	180	10	30	0.185	0.30	
22	180	10	30	0.179	0.29	
27	180	10	30	0.185	0.30	
28	180	10	30	0.173	0.28	
30	180	10	30	0.197	0.32	
33	180	10	30	0.186	0.30	
34	180	10	30	0.191	0.31	
35	180	10	30	0.185	0.30	
37	180	10	30	0.186	0.30	
38	180	10	30	0.180	0.29	
39	180	10	30	0.185	0.30	
29	180	10	50	0.287	0.47	0.485 ± 0.021
40	180	10	50	0.305	0.50	
13	180	15	30	0.275	0.45	0.455 ± 0.007
23	180	15	30	0.281	0.46	
4	200	5	10	0.064	0.10	0.105 ± 0.007
19	200	5	10	0.070	0.11	
10	200	5	50	0.227	0.37	0.395 ± 0.035
17	200	5	50	0.257	0.42	
2	200	10	30	0.251	0.41	0.415 ± 0.007
6	200	10	30	0.257	0.42	
5	200	15	10	0.118	0.19	0.185 ± 0.007
32	200	15	10	0.112	0.18	
7	200	15	50	0.432	0.71	0.68 ± 0.042
21	200	15	50	0.396	0.65	

Appendix D1: Response optimization for total carbohydrate yield.

Parameters

	Goal	Lower	Target	Upper	Weight	Import
substrate conc.	Maximum	0.1	0.92	0.92	1	1

Global Solution

Temperature	=	200
Time	=	15
Soliad Laoding	=	50

Predicted Responses

substrate conc. = 0.917739 , desirability = 0.997242

Composite Desirability = 0.997242

Appendix D2: Response optimization for reducing sugar yield.

Parameters

	Goal	Lower	Target	Upper	Weight	Import
Reducing Sugar	Maximum	0.04	0.71	0.71	1	1

Starting Point

Temperature	=	160
Time	=	5
Solid Laoding	=	10

Global Solution

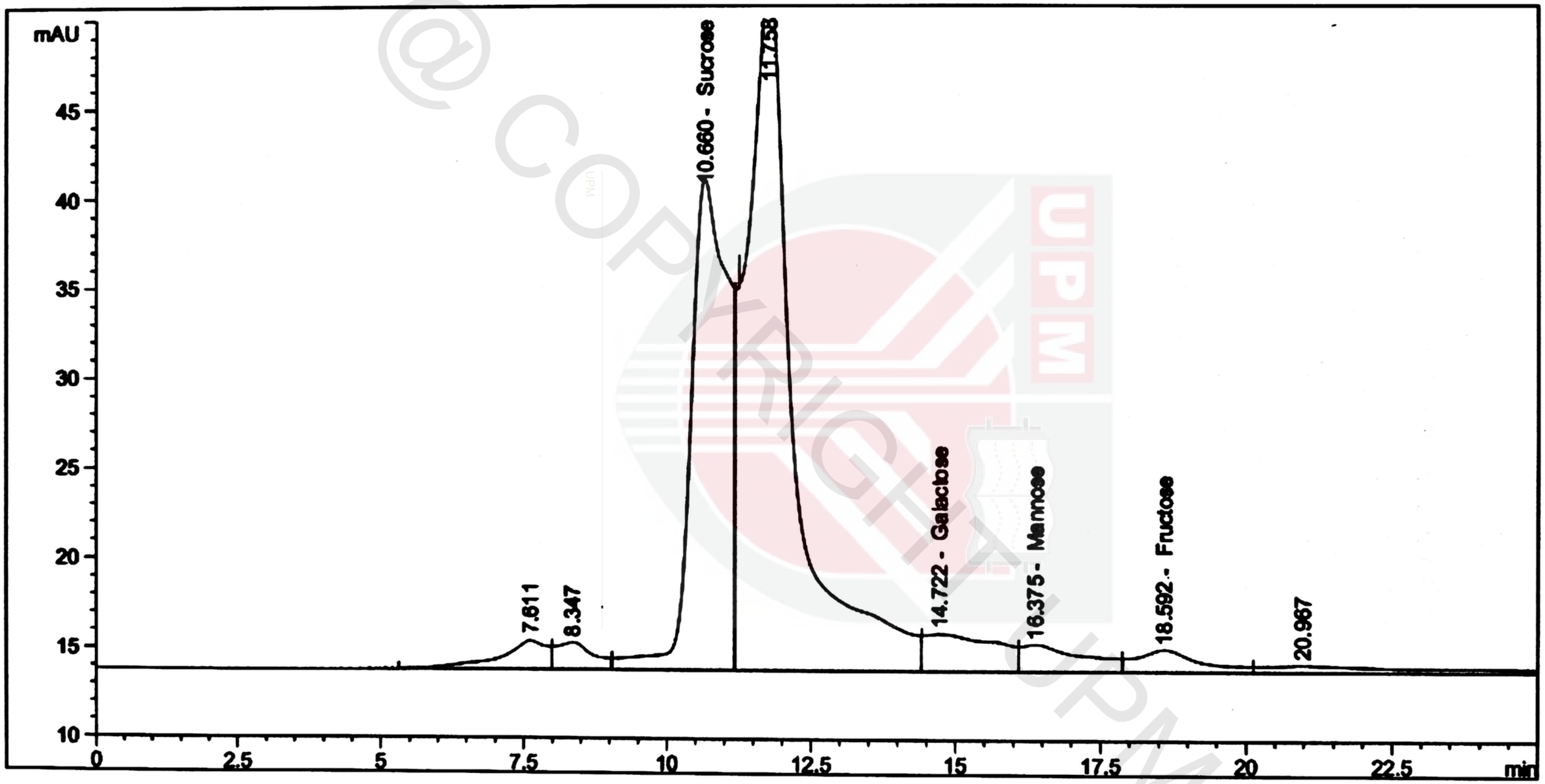
Temperature	=	200
Time	=	15
Solid Laoding	=	50

Predicted Responses

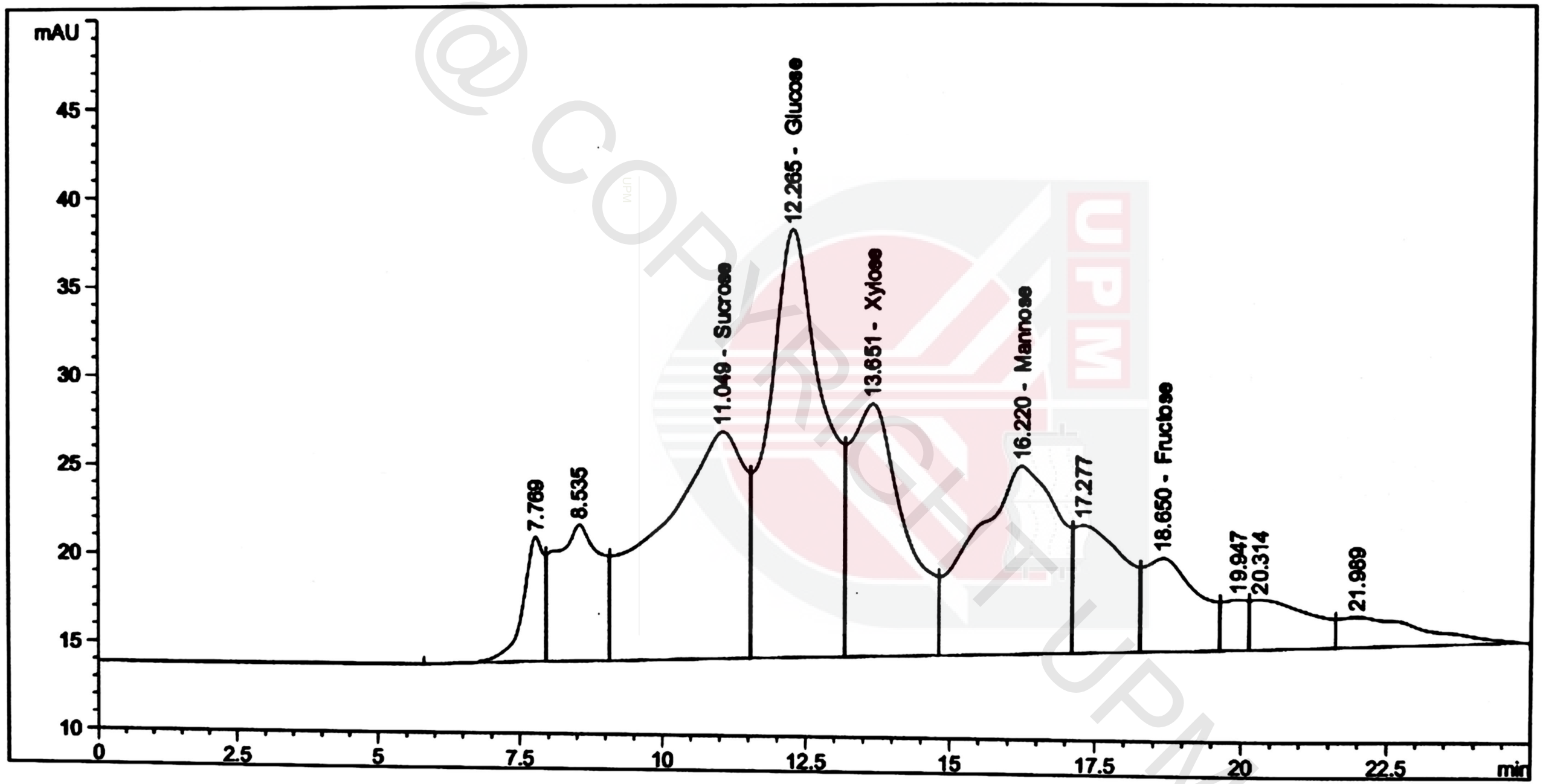
Reducing Sugar = 0.722362 , desirability = 1.000000

Composite Desirability = 1.000000

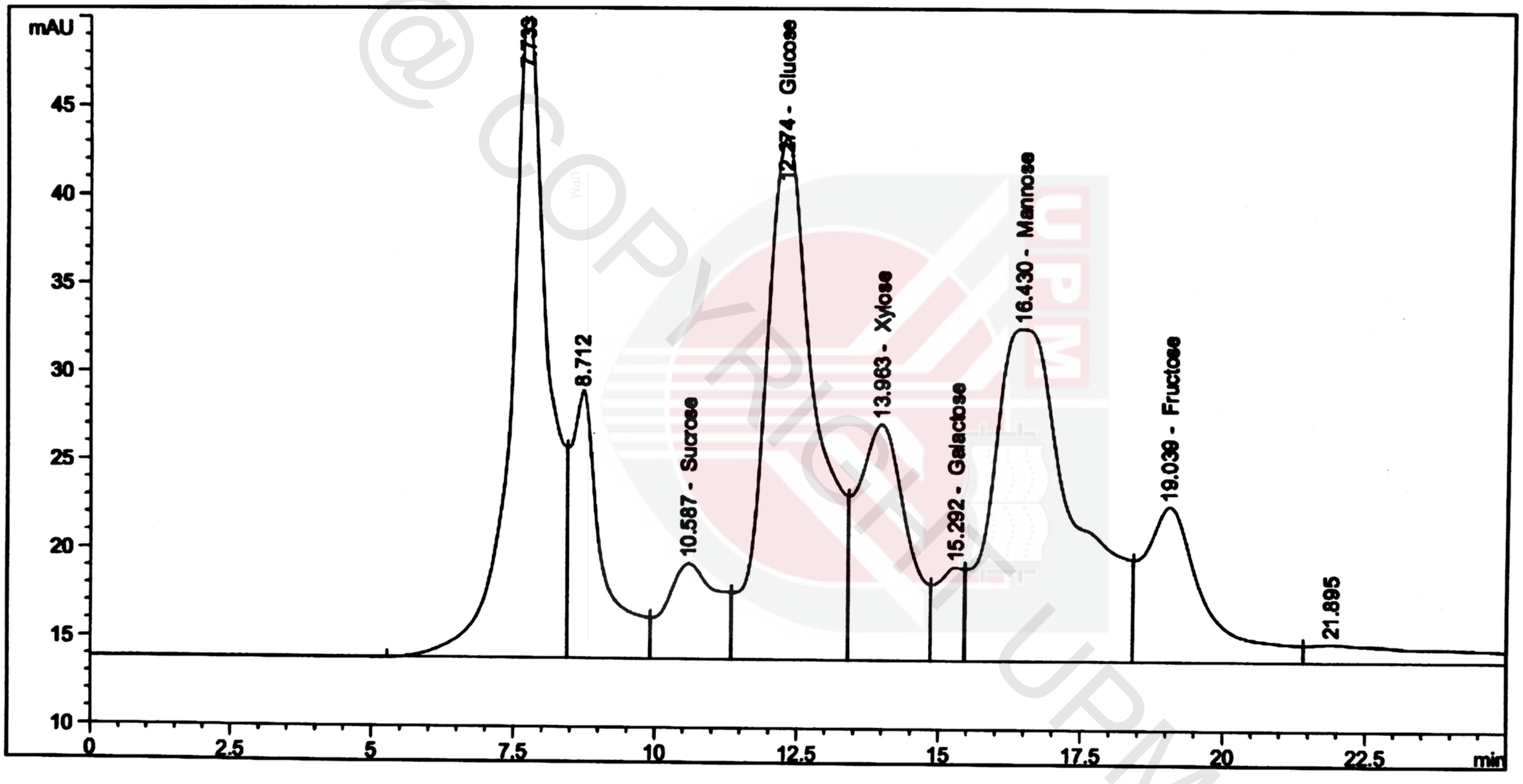
Appendix E1: HPLC of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 160°C under subcritical water hydrolysis.



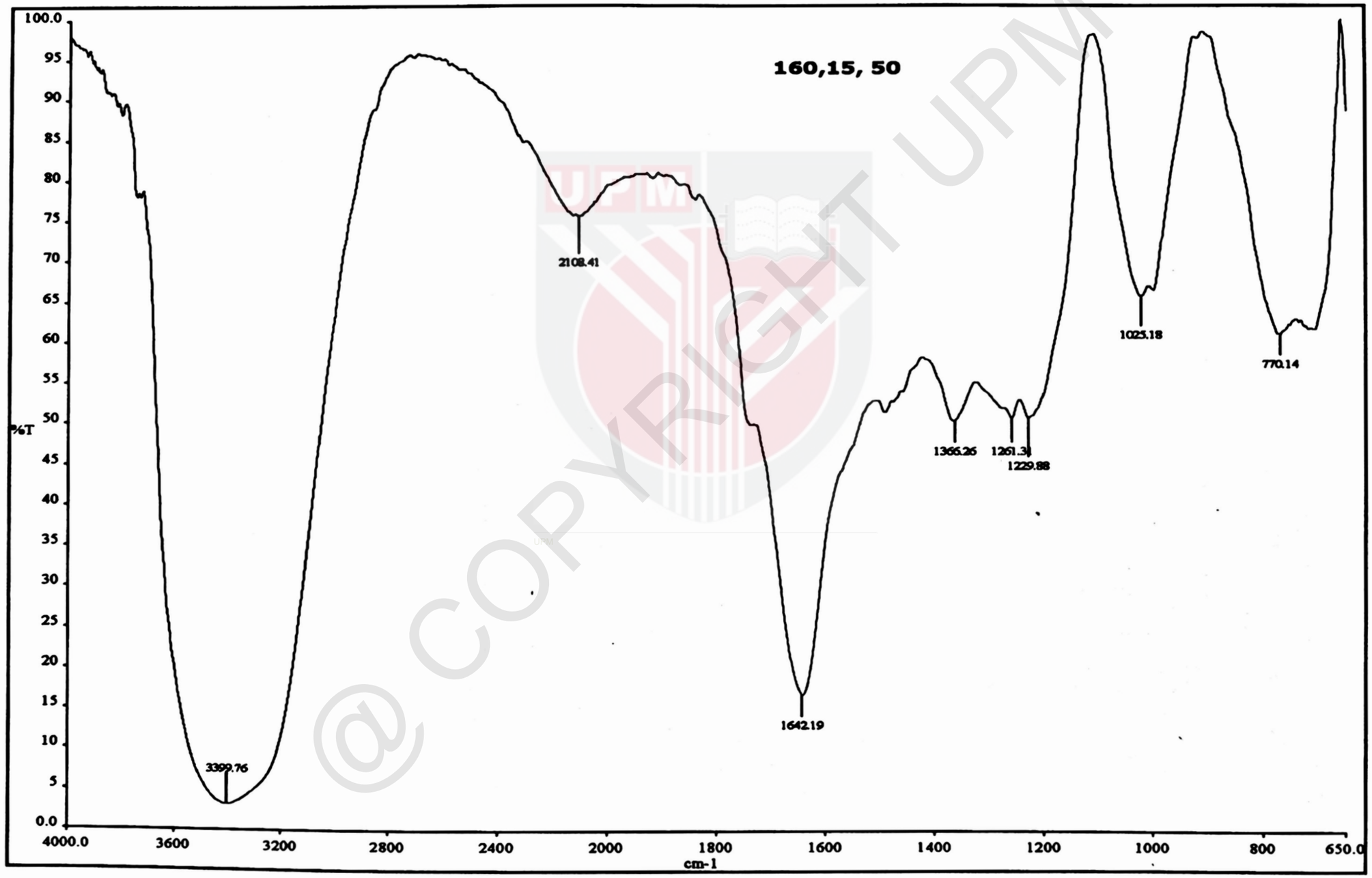
Appendix E2: HPLC of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 180°C under subcritical water hydrolysis.



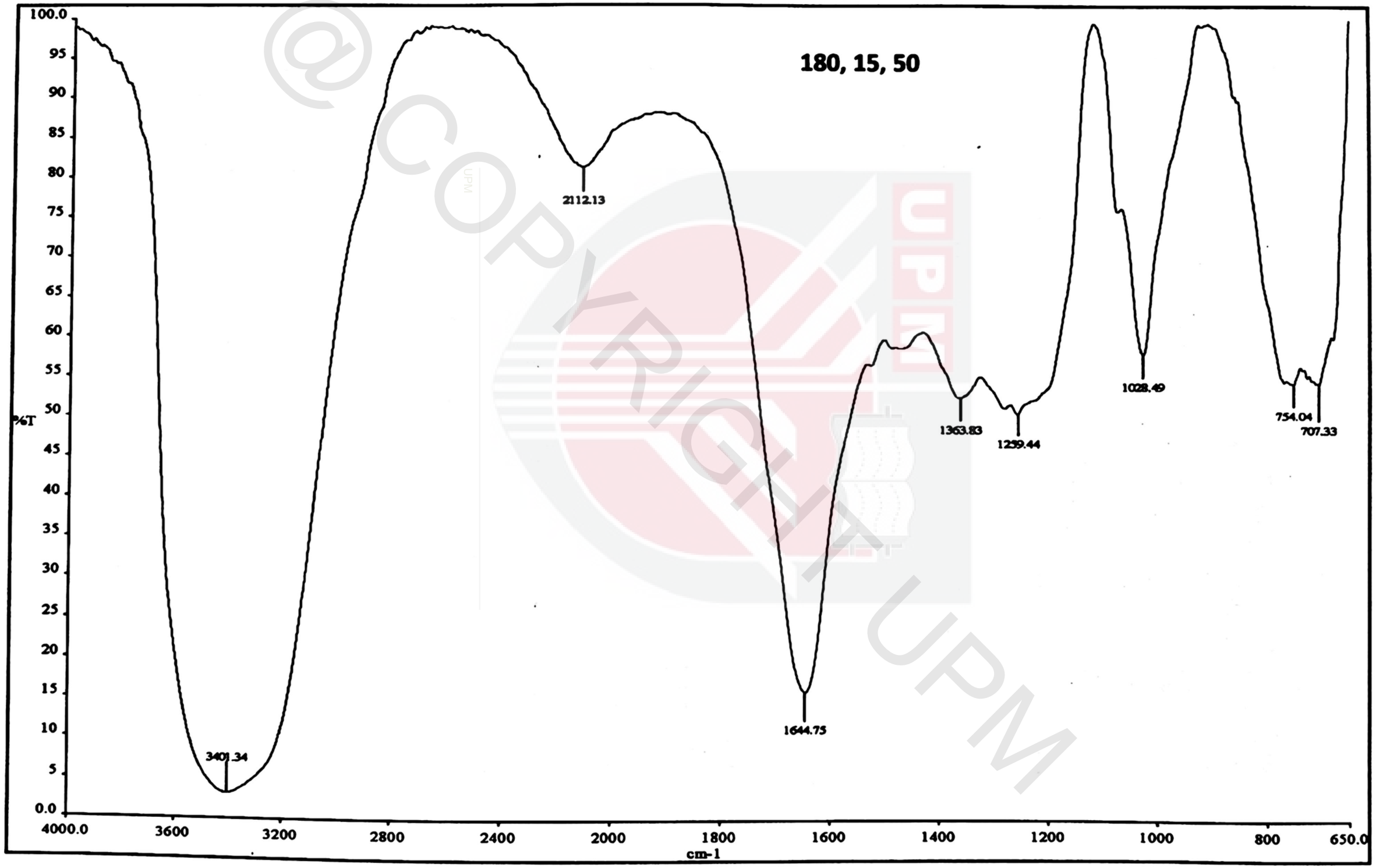
Appendix E3: HPLC of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 200°C under subcritical water hydrolysis.



Appendix F1: FTIR spectra of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 160°C under subcritical water hydrolysis.



Appendix F2: FTIR spectra of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 180°C under subcritical water hydrolysis.



Appendix F3: FTIR spectra of leftover chocolate cake samples (50% solid loading & 15min process time) treated at 200°C under subcritical water hydrolysis.