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PREDICTION OF IN VITRO GLYCEMIC RESPONSE AND SUGAR COMPOSITION ON SELECTED TYPES OF COMMERCIAL SUGARS IN MALAYSIA

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A project submitted as a partial fulfillment of the requirement for the of Bachelor of Science (Nutrition and Community Health) form the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

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ABSTRACT

PREDICTION OF *IN VITRO* GLYCEMIC RESPONSE AND SUGAR COMPOSITION ON SELECTED TYPES OF COMMERCIAL SUGARS IN MALAYSIA

Fikratul Najihah Mohamad Nawawi

The prevalence of diabetes and obesity have risen in Malaysia due to increase consumption of food with high sugar content or high glycemic index food. Sugar act as a significant basic ingredient in a food product by imparting the sweetness and the function of sugar. The objective of this study was to determine the *in vitro* glycemic response and sugar composition on selected types of commercial sugars in Malaysia. Eight types of commercial sugar samples were analyzed using HPLC to determine the sugar composition. While the estimated glycemic response of all sugar samples was determined using the *in vitro* method and indicated as dialyzable glucose concentration at 120 minutes. The *in vitro* method used about 0.25g total carbohydrate available carbohydrate of the sugar samples, which was determined by clegg anthrone method. All sugar samples contained a high amount of sucrose content compared to fructose and glucose, which was highest in icing sugar (99.84%). Palm sugar had the highest fructose (1.50%) and glucose (2.19%) content compared to other types of sugar samples. The dialyzable glucose concentration was highest in sugar in sachet (3.47mg glucose) but lowest in sugar with stevia (0.49mg glucose). There was no significant correlation between the estimated *in vitro* glycemic response and sugar composition of selected types of sugar samples. These results might be influenced by the sources of sugar, the processing of raw sugar and the polyphenol compounds in the raw sugar. In general, the study indicated that sugar composition on a selected type of sugar sample does not affect the glycemic response. Further studies related to the glycemic index of these sugars are needed especially using *in vivo* method to achieved a more presentable and reliable data.

ABSTRAK

RAMALAN TINDAK BALAS GLISEMIK *IN VITRO* DAN KOMPOSISI GULA PADA JENIS-JENIS GULA KOMERSIAL TERPILIH DI MALAYSIA

Fikratul Najihah Mohamad Nawawi

Kelaziman diabetes dan obesiti telah meningkat di Malaysia kerana peningkatan penggunaan makanan yang mengandungi gula yang tinggi atau makanan indeks glisemik yang tinggi. Gula bertindak sebagai bahan asas yang penting dalam produk makanan dengan memberi rasa manis dan fungsi gula dalam makanan. Objektif kajian ini adalah untuk menentukan tindak balas glisemik *in vitro* dan komposisi gula terhadap jenis-jenis gula komersial yang terpilih di Malaysia. Lapan jenis sampel gula komersial telah dianalisis menggunakan kaedah *HPLC* untuk menentukan komposisi gula. Ramalan terhadap tindak balas glisemik untuk semua sampel gula ditentukan dengan menggunakan kaedah *in vitro* dan ditunjukkan sebagai kepekatan *dialyzable* glukosa pada 120 minit. Kaedah *in vitro* ini telah menggunakan sebanyak 0.25g jumlah karbohidrat yang terdapat dalam sampel gula yang telah ditentukan oleh kaedah *anthrone clegg*. Semua sampel gula mengandungi kandungan sukrosa yang tinggi berbanding dengan kandungan fruktosa dan glukosa di mana jumlah sukrosa yang paling tinggi adalah dalam gula aising (99.84%). Gula melaka mempunyai kandungan fruktosa (1.50%) dan glukosa (2.19%) yang tertinggi berbanding dengan jenis gula yang lain. Seterusnya, kepekatan *dialyzable* glukosa yang paling tinggi adalah gula dalam paket kecil (3.47mg glukosa) dan yang paling rendah adalah gula dengan stevia (0.49mg glukosa). Tiada korelasi yang signifikan diantara ramalan tindak balas glisemik dan komposisi gula terhadap jenis-jenis gula komersial yang terpilih. Komposisi gula dan ramalan tindak balas glisemik mungkin dipengaruhi oleh sumber gula, pemprosesan gula dan kandungan polifenol dalam gula. Secara umumnya, kajian ini menunjukkan bahawa komposisi gula pada sampel gula yang dipilih tidak mempengaruhi tindak balas glisemik. Kajian lanjut yang berkaitan dengan indeks glisemik gula amatlah perlu terutama menggunakan kaedah *in vivo* untuk mencapai data yang lebih rapi dan boleh dipercayai.

CHAPTER 1

INTRODUCTION

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1.1 Background of study

Sugar presents as a significant part in a food product by imparting the sweetness and the function of sugar. Sugars can be defined as carbohydrate building blocks that compose of monosaccharides, disaccharides, and polyols, which is sugar alcohol (Amarra, Khor & Chan, 2016). These carbohydrates supply energy for the body in the form of glucose. Naturally, sugars like fructose and glucose came from the fruits and honey while sucrose that contains both glucose and fructose can be found from the sugar cane and beets extract (Das, 2015).

Amarra, Khor, and Chan (2016) reported that about 30 grams of sweetened condensed milk and 21 grams of table sugar are available in common beverages of Malaysian such as tea and coffee. This result in daily sugar intake among Malaysian at approximately 37 grams. WHO recommends for sugar intake should be below 50 grams per day in order to decrease the risk of chronic diseases.

The glycemic response can be estimated by measuring the glycemic index and glycemic load using *in vitro* or *in vivo* methods (Argyri et al., 2016). The glycemic index is a system that can classify carbohydrate-containing foods according to how fast they are digested and absorbed during the postprandial period (Almousa et al., 2013). Based on their direct impact on blood glucose levels during two hours after the meal, the glycemic index can be considered as the tool that measures the quality of carbohydrate.

High glycemic index consumption is related to health risks such as diabetes mellitus, metabolic syndrome, cardiovascular disease, and some forms of cancer. Thus, according to Augustin et al. (2015), it is significant to develop a list of glycemic index values of foods for health professionals and the public to use it, as a diet guide or personal use to increase the intervention in preventing the health risks.

Based on the international table of glycemic index values, different ingredients and food processing methods can affect the glycemic index values for diverse types of the same food (Foster-Powell, Holt & Brand-Miller, 2002). Thus, the food industry can develop a greater range of low glycemic index foods by using this information from the table of glycemic index values.

Moreover, food products that contain low glycemic index can benefit consumer's health and decrease the prevalence of food-related diseases in the country. This is in accordance to the result of study from Camps et al. (2017) who reported that people who apply low GI diet might be able to improve their glycemic response by enhancing the fat oxidation rather than carbohydrate oxidation which help to control their weight gain as well as preventing obesity that leads to other health risks such as cardiovascular disease and diabetes mellitus.

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Today, the concept of the glycemic index is very important in order to characterize food products from the industry. Usually, the use of the glycemic index in the food products will be stated at nutritional labeling. Mitchell (2008) found out that from 38 countries surveyed, Malaysia was one of the six countries (Canada, United States, Brazil, Australia, New Zealand) that have mandatory nutrition labeling of foods which are known as Malaysian Food Regulations 1985 but these regulations do not include the glycemic index information. Research on Nutrition Labelling in Malaysia by Tee (2017) stated that based on Food Regulation 1985, nutrition labelling in Malaysian food products must declare four main nutrients on the label which are energy, protein, carbohydrate and fat as well as other nutrients permitted to be declared on the label such as vitamins, mineral, dietary fibre, fatty acids and cholesterol. This show that glycemic index is not mandatory labeling in the country.

Furthermore, the glycemic index also can be correlated with the sugar composition of the food. According to Cummings et al. (2007), sugar composition can be categorized into three classes which are monosaccharide (glucose, fructose, galactose), disaccharides (sucrose, lactose, maltose) and polyols or sugar alcohol. Glucose has been used as a standard in determining the glycemic index values. According to the list of sugar composition, maltose has the highest glycemic index value (105) followed by glucose (100), sucrose (65), lactose (46), and fructose (19) (Australian Standard, 2007). Thus, sugar composition in the foods might affect the value of the glycemic index.

Recognizing the significance of etiology and prevention of disease in Malaysia, the existing of Food Composition Data need to be updated with information on the glycemic index and sugar composition of the food. Thus, the objective of this study was to determine the *in vitro* glycemic response based on dialyzable glucose

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concentration and sugar composition on selected types of commercial sugars in Malaysia.

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1.2 Problem statement

Studies on the glycemic index of foods in Malaysia are growing but the nutrient content of the glycemic index in various types of sugar are not well studied. Based on the Malaysian Adult Nutrition Survey 2003, it was stated that almost 58% of the Malaysian population used sugar in their daily life (Amarra, Khor, & Chan, 2016). About half percent of Malaysian adults, their sources of energy intake came from sugar-containing food (Fokeena, Jamaluddin, & Khaza'ai, 2015). For example, sugar or condensed milk are added into the beverages like a cordial syrup, tea, coffee and also the Malaysian local *kuih* especially the starchy traditional cakes. Usually, traditional sweet *kuih* in Malaysia used sugar, brown sugar and palm sugar as one of the basic ingredients (Raji et al., 2017). Since the sources of food in Malaysia mostly contain sugar, there is a need to put interest to study the glycemic index of various types of sugar.

Based on the 4th edition of Nutrient Composition of Malaysian Food (Tee, 1997), there were only a few data have been published for nutrient content in sugars in Malaysia. These data also only include macronutrient, micronutrient as well as vitamins and minerals without information of glycemic index. Tony (2010) stated that there is no new edition of comprehensive Malaysian Food Composition Data has been published for the past several years. Thus, research on the glycemic index for various types of sugars in Malaysia is significant and should be initiated so that data can be included in the Malaysian Food Composition Data in future.

Moreover, the prevalence of obesity and diabetes in Malaysia from 2006 to 2011 has raised a serious public health threat to Malaysians (NHMS, 2015). These indicate that the potential of Malaysian consumers exposed to health risks are increasing and the lack of nutritional composition data related to the glycemic index might be one of the factors. According to WHO (2018), increase the consumption of sugar-sweetened beverages among children will increase the risks of obesity. Besides, research on sugar intake in India reported that high consumption of sugar might be related to increased insulin resistance, hyperglycemia, abdominal adiposity and atherosclerosis (Gulati & Misra, 2014). Therefore, this study is significant in order to provide glycemic index data of commercial sugars in Malaysia that hopefully can assist in the selection of sugar of the consumer.

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1.3 Significance of research

This study is significant as it provides the initial trend of the *in vivo* glycemic index value and assists future study using *in vivo* method. Actual *in vivo* study on the glycemic index of food should be done to provide data or guidelines on a diet related to low glycemic index food which can assist the health professional such as dietitian and nutritionist in assessing their patient's diet and perhaps increase awareness of the public. According to Augustin et al. (2015), they are convinced that dietary glycemic index and glycemic load need to be introduced to the public and health professional through dietary guidelines, country-specific glycemic index databases, food composition table and food labels in order to be concern about the enhancement of disease related to glucose metabolism.

The results of this study also provide some ideas on the ranking of sugars as it affects the glucose response. The glycemic index and glycemic load have been introduced as two empirical metrics to rank the food according to the effect on glucose response (Scazzina et al., 2016). According to Amarra, Khor and Chan (2016), sugar act as a significant ingredient in food and beverage production. Thus, information regarding the rank of estimated glycemic index values for various types of sugars might be useful for the public as well as industrial food as it creates a new and better recipe or formulation of food products.

Furthermore, most of the glycemic index study using *in vivo* method, not at the same time determine the sugar composition. For example, a previous study that determined the glycemic index of selected Malaysian traditional foods such as curry puff, Chinese carrot cake and nasi lemak, did not measure the types of sugar content in the foods that might influence the results of the glycemic index (Sun et al., 2015).

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Thus, a study that combines glycemic response and sugar composition may provide more meaningful information especially related to the glycemic index value of food.

The used of an *in vitro* method to determine the glycemic index values of sugars can be one of the advantages of the study. According to Bellmann et al. (2018), American Association of Cereal Chemists (AACC) encourage the development of *in vitro* method as a new effective tool to determine the glycemic response of carbohydrate-containing food. Although *in vivo* method that uses human or animal as a subject has been widely used in the previous studies of glycemic carbohydrate, this study also found that it is no difference in the result of predicting the blood glucose curve after consumption of carbohydrate-containing food between the *in vitro* method and the *in vivo* method. Therefore, this method of *in vitro* can be one of the alternative methods to assist the growth of research related to glycemic carbohydrate response.

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1.4 Objectives

1.4.1 General objective

To determine the *in vitro* glycemic response based on dialyzable glucose concentration and sugar composition on selected types of commercial sugars (refined sugar, aromatic brown sugar, soft brown sugar, better brown sugar, icing sugar, sugar with stevia, sugar in sachet and *gula melaka)* in Malaysia.

1.4.2 Specific objectives

i. To determine and compare sugar composition (eg: glucose, maltose, fructose, sucrose, lactose) for selected types of commercial sugars in Malaysia.

ii. To determine and compare the estimated glycemic response indicated as dialyzable glucose concentration for selected types of commercial sugars in Malaysia using *in vitro* method.

iii. To correlate the estimated glycemic response indicated as dialyzable glucose concentration and sugar composition on selected types of commercial sugars in Malaysia.

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1.5 Hypothesis

i. There is a significant difference of sugar composition (eg: glucose, maltose, fructose, sucrose, lactose) for selected types of commercial sugars in Malaysia.

ii. There is a significant difference of the estimated glycemic response indicated as dialyzable glucose concentration for selected types of commercial sugars in Malaysia using *in vitro* method.

iii. There is a significant correlation of the estimated glycemic response indicated as dialyzable glucose concentration and sugar composition on selected types of commercial sugars in Malaysia.

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CHAPTER 2

LITERATURE REVIEW

2.1 Sugar

Sugar is a sweetly flavored food substance that can be obtained from various plants such as sugar cane, sugar palm, and sugar beet. By following many procedures in extracting the sugar juice, many types of sugar can be produced. Based on the Sugar Association (2018), the process of sugar making involves cleaning, crystallizing and drying with different level of molasses to generate various types of sugar. Sugar also can be packed in various packagings such as granulated sugar, sugar in sachet, sugar cube and powdered sugar.

Ministry of International Trade and Industry Malaysia stated that Malaysia only contributes about 1% of sugar production and another 99% has been imported to meet the high local demand for the commodity. According to USDA (2018), World Market of Sugar reported that almost every year the net of sugar import in Malaysia had risen gradually and Malaysia has been in the seventh rank of highest sugar

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importer. Examples of sugar available in Malaysia are refined sugar, brown sugar, icing sugar, palm sugar as well as stevia. The amount of molasses remaining or added to sugar crystal also can determine colors of sugar, delightful flavors and change the moisture of sugar. Besides that, sugar color and flavor also can be altered by the process of heating in order to produce a caramel taste (Nor Shuhada, 2010).

In general, sugars have a sweet taste, crystalline texture and soluble in water (Kamal & Klein, 2011). Sugars are suitable for different types of foods and beverages because of various crystal sizes of sugar which create unique functional properties and sweetness. In the same terminology from WHO (2015) and Scientific Advisory Committee on Nutrition (2015), the term of free sugar and added sugars refers to sugar and syrup that are used for food processing and preparation. Based on Recommended Nutrient Intake (2017), carbonated drinks, fruit juices, desserts, cakes, biscuits, and candies are the sources of added sugar in the Malaysian diet.

Sugar has been used extensively as an ingredient in food preparation. Increase intake of sugar consumption among Malaysian might be due to the Malaysian staple drink such as tea, coffee as well as ABC and cendol (Ramli et al., 2016). This is because, during the preparation of these beverages, sugar or condensed milk are added to enhance the taste of the drinks (Gupta et al., 2013). Thus, sugar was important as an ingredient in Malaysian food preparation. Moreover, according to Raji et al. (2017), most of the Malaysian local *kuih* such as *kuih keria* and *kuih lapis* also used refined sugar or *gula melaka* as an essential ingredient.

Next, sugar is the part of carbohydrate and according to FAO/WHO (1998), the term of sugar is conventionally used to describe monosaccharide and disaccharides. Wan Shakira et al. (2015) report that the pattern of carbohydrate intake among

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Malaysian after a decade has decreased from 220 grams in 2003 and 195 grams in 2014. While, sugar has been recognized as the top ten food items since about half of the Malaysian population did consume sugar in their daily life from 2003 to 2014 (Noraida et al., 2018). NHMS 2015 also stated that as compared to NHMS 2011, results shown the prevalence of overweight and obesity were increased. The issues of overweight and obesity in Malaysia are still rising and these might be due to high sugar content in almost carbohydrate food choices among Malaysian. A study involving cross-sectional study has established that almost 13% of energy intake among Malaysian is contributed by sugar consumption which has a significant relationship to the weight status (Safii & Yuin, 2013).

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2.1.1 Refined sugar

Refined sugar is also known as a table or white sugar contains up to 99.9% of sucrose and commonly being extracted from sugar cane or sugar beet (Seguí et al.,2015). The extraction process of juice for the production of sugar might degenerate the natural fiber and nutrient content of sugar cane and sugar beet. According to Wojtczak, Antczak and Lisik (2014), the refining process of white cane sugar involve several main stages such as melting, filtration, evaporation, and crystallization. Thus, the impacts of refining process lead to color removal and minimize the contamination of soluble and insoluble. Furthermore, this process of refining sugar also produced icing sugar that more smooth and soft texture of powdered sugar and made it suitable for baking products or dessert (Chen & Chou, 1993).

Demands on refined cane sugar are higher compared to other sugar due to their purity and color (Nayaka et al., 2009). Refined sugar typically sold as granulated sugar and people believe that the white color of refined sugar ensuring the cleanliness but they did not notice about the nutrients inside of sugar. Varzakas and Chryssanthopoulos (2012) state that white sugar has a little amount of nutritional value and composed much amount of calories because of high purity. Increasing the intake of refined sugar might lead to health problems such as obesity, cardiovascular disease, diabetes as well as dental caries among children that have been explained in the previous study (Moynihan & Kelly, 2014).

2.1.2 Brown sugar

Brown sugar has a considerable place in the market since it has been used extensively in many countries and regions such as South America, South Asia, and Africa (Asikin et al.,2016). According to Orlandi et al. (2017), the brown sugar is the raw, moist and dark sugar obtained after sugar cane dehydration and does not go through a large number of the chemical process. Moreover, several studies found that brown sugar contains antioxidant, cytoprotective, and antiangiogenesis activities which can provide some beneficial biofunctions (Singh et al., 2015; Jaffé, 2015; Tian et al., 2018). Many consumers accepted brown sugar as organic and low-processed food products. Thus, Malaysia is one of the countries that produced this kind of sugar for consumers to compete and seize market opportunities.

As a sucrose sugar product, unique brown color of this sugar makes its different from refined or white sugar. Ducat et al. (2015) explain that the presence of molasses influence the brown sugar color and provide various nutrients such as sucrose, glucose, amino acids, vitamins, metal ions, and other components. Brown sugar can create a sweet, burnt, pungent and caramel-like aroma in baked foods. This is accordance with the findings by Asikin et al. (2014) state that brown sugar consists of many acids and heterocyclic compounds that can contribute to various types of flavor for the foods.

Example of brown sugar available in Malaysia is soft brown sugar. This brown sugar is known as refined crystal substances that consist a dark brown colored, soft and slightly moist texture. This sugar usually used in preparing the Malaysian traditional *kuih* and foods such as *laksa* and *beef rendang*. Through the process of cooling the high concentrated cane syrup, the small agribusinesses used artisanal way

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in order to produce the soft brown sugar. This process preserved most of the components of sugar cane including the biological value such as sucrose, glucose, and fructose. Therefore, this brown sugar considered a high nutritional value compared to other types of sugar (Guerra & Mujica, 2010).

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2.1.3 Palm sugar

Palm sugar can be derived into two sources of palm which are coconut palm (*Cocos nucifera*) and Nipah palm (*Nypa fructicans*). The process of sugar production from these palms are similar. Young shoots of the palm have been tapped to obtain the palm sap and then boiled for several hours to remove the water.

The coconut palm sugar will produce *gula Melaka* and pour into the bamboo tubes to be shaped as a solid and hard cylindrical cake. While, the Nipah palm sugar will produce *gula anau* as a viscous fluid (Hoyle & Santos, 2010). In Malaysia, these two sugar have been produced only by a few homesteads or small business enterprised and not in industrial scale although there are many coconuts and Nipah palm were planted. A study by Ishak et al. (2013) stated that Malaysia has a thousand farmers who earn a living by tapping sugar palm sap especially in the areas such as Kuala Pilah and Jempol in Negeri Sembilan, Kuala Lipis in Pahang and Tawau in Sabah.

Besides used as an ingredient in making a dessert, cake and food coating, palm sugar also can be eaten fresh (Ho et al., 2008). The sweet taste of this sugar also very popular in making the Malaysian traditional *kuih* such as *kuih bingka*, *dodol* and *onde-onde* (Raji et al. 2017). Comparing to others sugar, palm sugar is considered as more natural and healthier sugar. This is according to the results of the study on antioxidant activity in cane sugar and palm sugars from Southeast Asia which found that *gula anau* from palm sugar contains the highest level of antioxidant activity compared to cane sugar.

2.1.4 Stevia

Stevia is referred to different forms of sweetener which has been used as a natural sugar substitute and flavoring ingredient. The sweetness of this sweetener can be found in the leaves of the stevia plant. Through the process of drying the stevia leaves, this sugar is usually being applied for tea sweetener as well as a sweet herb for medicines (Lemus-Mondaca et al., 2012).

According to Ashwell (2015), about 95% or more steviol glycosides have been found in the high purity of stevia leaf extract and only this specification is approved by World Health Organization (WHO). Furthermore, the study also concludes that stevia can assist people in lower their energy intake as well as preventing the rising of obesity. More people who are conscious of healthy food believe that this sugar as a zero-calorie sweetener and naturally sourced.

Another study by Shivanna et al. (2013) found that stevia can provide benefits to protect people against diabetes, oxidative stress, and kidney damage. This finding was also supported by Abo Elnaga et al. (2016), who reported that the blood glucose level of the rats in their study was decreased due to the consumption of stevia sweetener. Although many studies have approved the benefits of stevia on health, JECFA (2016) reports that acceptable daily intake (ADI) of steviol is only 0-4 mg/kg body weight.

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2.2 Glycemic index and Glycemic load

According to Scazzina et al. (2016), the glycemic index (GI) was developed as a tool that can categorize the food systematically. The use of the glycemic index is significant in identifying the food classes regarding the nutrients content provided in the food. However, the concept of the glycemic index should be used correctly by referring to the glycemic load (GL) concept. This is because the glycemic index only shows how fast a particular carbohydrate converted into sugar. While, the glycemic load was created to determine the amount of carbohydrate in the food (Salmerón et al., 1997).

According to Dan Ramdath (2015), GL can be defined as a product of carbohydrate content per serving of food and its GI. Moreover, the glycemic load was proposed to be calculated by multiplying the carbohydrate in grams with the glycemic index. Therefore, the glycemic load is significant in representing both qualitative and quantitative measures of food or diet. Apart from that, both the glycemic index and glycemic load can be used as a tool to estimate the glycemic response in nutritional epidemiology using in-vitro or in-vivo methods (Argyri et al., 2016).

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According to Beals (2005), a study on The Glycemic Index: Research Meets Reality by United States Potato Board, glycemic index values can be categorized as in Table 2.1 below;

Table 2.1: Glycemic Index Categories

Category	**Glycemic Index Value**
Low	Less than 55
Medium	55-70
High	More than 70

Source: Beals (2005)

While, based on the Ministry of Health Malaysia (2016), the list of foods according to the level of glycemic index values stated in Table 2.2.

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Table 2.2: The List of Foods with Glycemic Index Levels

Starch	Vegetables	Fruits	Dairy	Snack
Low GI (55 or below)				
Barley	Green peas	Apple	Milk, full fat	Chocolate milk
Yam	Cabbage	Watermelon	Milk, skimmed	Chocolate bar
Oat bran, raw	Broccoli	Pisang berangan	Yoghurt	Pizza
	Tomatoes	Pear	Soy milk, drinks	Curry puff
		Durian	Custard	Ice cream
Moderate GI (56 to 69)				
Basmati	Boiled potato	Papaya		Doughnut
Tosai with chutney		Sultanas		
Nasi lemak		Raisins (Canada)		
Digestive biscuit				
High GI (70 and above)				
Fried meehoon/ macaroni		Dates, dries		Waffles
Roti canai and dhal curry		Pineapple		Sago porridge
Glutinous and white rice		Lychee, canned in syrup		
Sweet potato				

Source: Ministry of Health Malaysia (2016)

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Next, the concept of low GI and GL diet has been widely used in preventing non-communicable disease such as diabetes mellitus. This is in accordance with the finding of the study by Augustin et al. (2015), where type 2 diabetes and total body fat mass could be reduced by low GI diets. Thus, the low GI concept was recommended in ensuring the context of a healthy diet. Moreover, the previous study which stated that fast, high and longstanding postprandial hyperglycemia that affected by the high glycemic index food, resulting to burden in controlling and preventing the complication of diabetes (Campbell et al., 2015). This is because the high GI food has the potential to increase insulin resistance rather than the low GI carbohydrates (Shumoy et al., 2018). These showed that the low GI concept in the diet was much better than high GI concept.

A study on the glycemic index and glycemic load on cancer concluded that there is no association between the glycemic index and cancer but low glycemic index diets might provide a rich amount of fiber, micronutrients, and phytochemicals that could be benefits for health (Sieri & Krogh, 2017). Although strategy on lowering the glycemic index (GI) and glycemic load (GL) might be a better choice in improving the quality of diet, without consideration from other health professionals such nutrition and dietetics, we cannot merely recommend for the high intake of low glycemic index. This is because of the high consumption of low glycemic index also favorable to increase the chance of high saturated fatty acid and sodium intake (Murakami & Sasaki, 2018). Thus, it is important for the public to refer the health professionals first before they decide to use or make low glycemic index foods as a tool to improve their diets.

However, the American Diabetes Association did not recommend the concept of GI in dietary counseling. This is because they raised their concern on the difficulties

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to apply the concept of GI which might affect to unfavorable effect on fat intake and food choices. The use of the GI concept in ensuring a healthy diet should be focusing on the recommended low GI diet. Therefore, there are several associations such as the European Association for the Study of Diabetes, the Canadian Diabetes Association, and the Dietitians Association of Australia that still believes that the low GI foods with high fiber can improve the postprandial glycemia and weight control of diabetes patients (Foster-Powell et al., 2002).

Comparing to the high glycemic index (GI) food, food with low glycemic index quality will be digested and absorbed slowly, thus produce low glycemic index response. This can be referred to the blood glucose level condition after consumed the low or high GI food (Figure 2.1);

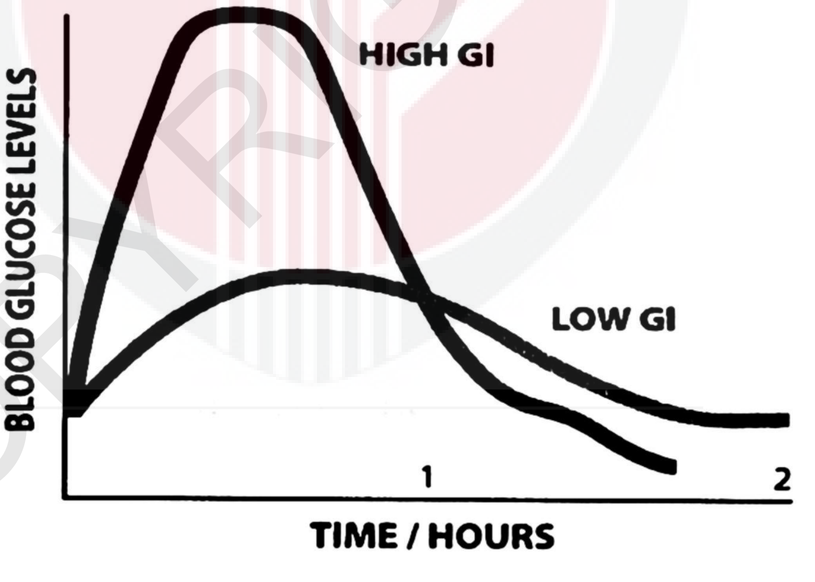

Figure 2.1: Blood glucose level for the high and low GI foods.

Source: Sydney University's Glycemic Index Research Services (SUGiRs) (2017)

2.3 Sugar composition

According to Cummings et al. (2007), sugar composition related to three classes of sugar which are monosaccharide (glucose, fructose, galactose), disaccharides (sucrose, lactose, maltose) and polyols which is sugar alcohol. The sources of these sugar such as glucose, fructose, and sucrose can be found in fruits and honey while galactose and lactose in a milk product and maltose in malt product. Different types of sugar might have a different level of sweetness. When the sweetness of food is compared, sucrose or table sugar is used as a reference standard. Furthermore, different concentration, temperature, and present of other ingredients might affect to varies of sugar sweetness. Fructose has been recognized as the highest relative sweetness followed by sucrose, galactose, glucose, maltose, and lactose (Canadian Sugar Institute, 2014).

In relation to the glycemic index, glucose has been used as a standard. The levels of glycemic index values for sugar composition vary. The highest glycemic index values were maltose, followed by glucose, sucrose, lactose, and fructose (Australian Standard, 2007). This showed that sucrose which is the most types of sugar consumed by people has a medium glycemic index value but raises the amount of consumed could lead to a high glycemic index of foods and beverages.

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2.4 *In vivo* and *in vitro* methods

In vivo and *in vitro* are the two main methodological classes for the bioaccessibility assessment. These methods have been used to determine the response of the glycemic index. According to Argyri et al. (2016), measurement of glycemic response using *in vivo* method might be regarded as complicated, expensive and time-consuming because it requires medical personnel for blood drawing and many clinical procedures.

However, *in vitro* method has been known as an alternative method to determine the glycemic index response in low cost, time and energy demanding. Thus, they offer a more practical way of bioaccessibility assessment. Moreover, they also can be conducted in a well-controlled experiment to ensure a higher quality of validation and precision of the methods. Although, the validation of *in vitro* results still depend on *in vivo* studies, in vitro methods seems to be more simple to conduct because it does not involve any ethical issues that related to human or animals studies (Cardoso, Afonso, & Lourenco, 2014).

In this study, we decided to used *in vitro* method by Argyri et al. (2016) because it has been developed and validated to predict the glycemic index response. Moreover, the *in vitro* method in this study showed to be the difference from other studies. Firstly, the used of dialysis membrane with an elastic band to a cylindrical insert in a six-well plate which required a much smaller amount of food sample compared to previously proposed methods.

Next, they determine the dialyzable glucose spectrophotometrically at 120 minutes after the second phase of *in vitro* digestion to promote more sophisticated prediction of the glycemic response. The chewing process of this method also used homogenizer and treatment of α-amylase to reduce the variation between human which might limit the ability to produce a good quality of in vitro findings. Lastly, the time of pepsin incubation in this method was up to 120 minutes to retain the advantage of simultaneous prediction of glycemic response and mineral bioavailability such as zinc and iron, in one experimental conduct.

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2.5 High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) has been recognized as the most effective and innovative method for analysis of sugar or carbohydrate (Khan et al., 2015). This method has been used widely in separating and quantifying the sugar content in the foods. This is because of its potentialities in providing a high degree of accuracy, precision, and separation (Charez, Castellote, & Lopez, 2004).

Different concentration of the standard solution of different sugar such as sucrose, glucose, fructose, lactose, and maltose need to be prepared in HPLC method to create the calibration curve of peak area versus concentration of each sugar before quantitative and qualitative sugar determination (Pecchio et al., 2014). Based on Yadav et al. (2011), the comparison between the retention time with the standard will identify the chromatographic peak responding to each sugar in sample and sugar concentration for each sample will quantify according to the linear calibrate curve.

Moreover, this HPLC method used refractive index detector to determine the sugar content of the samples. However, there are limitations in using this detector which limits the sensitivity, poor baseline stability and depends on the flow rate (Li, Chen, & Zhu 2007). Despite these disadvantages, the HPLC method still considers as a simple, economical, and rapid method to determine the sugar composition.

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CHAPTER 3

METHODOLOGY

3.1 Sample collection

There are eight different types of commercial sugars used as samples in this study which are refined sugar, sugar in a sachet, aromatic brown sugar, soft brown sugar, better brown sugar, *gula melaka,* icing sugar and sugar with stevia. All sugar samples have been received from Central Sugars Refinery (CSR), Malaysia except *gula melaka* which has been purchased from a supermarket in Kajang, Selangor.

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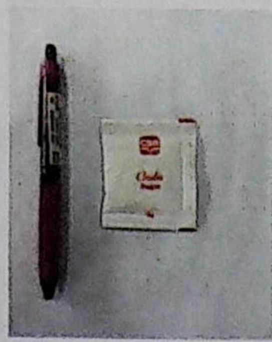
Fine Sugar



Fine sugar
(in sachet)

Aromatic Brown
Sugar

Soft Brown
Sugar

Better brown sugar

Gula Melaka

Icing sugar

Sugar with Stevia

Figure 3.1: Photograph of actual commercial sugars analyzed

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3.2 Determination of sugar composition

Determination of sugar composition was based on the HPLC method (Waters, 2012). Firstly, 3g of samples weighed into a 5mL centrifuge tube to extract each sample before injecting to HPLC instrument. Next, the sample added with 25mL mixtures of water and acetonitrile with a ratio of 50:50 and homogenized in the tube. Then, the mixtures centrifuged for about 30 minutes at 3200rpm using a centrifuge. The result of supernatant collected, filtered by using 0.45μm membrane filters and stored in a vial.

Table 3.1: Working condition of Alliance HPLC system

Working Condition	
System	: Alliance HPLC
Detector	: Refractive Index
Column	: Xbridge BEH Amide XP
Column Size	: 4.6mm x 100mm, 2.5μm
Mobile phase	: Acetonitrile : distilled water (90:10), TEA
Flow Rate	: 0.8mL/ min
Temperature	: 40°C

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For the standard, five sugars (fructose, glucose, sucrose, maltose, and lactose) were mixed with five different concentration to produce a calibration curve. Before the analysis, 10μL of supernatant from the vial injected in Alliances HPLC system.

The total sugar content of each sample was determined as formula below:

Total sugar content (%) = Glucose (%) + Fructose (%) + Maltose (%) +

Lactose (%) + Sucrose (%)

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3.3 Determination of total available carbohydrate

Clegg Anthrone method was used to determine the total available carbohydrate in the samples (AOAC, 2000). Firstly, the sample undergoes extraction process.

About 1g of dry sample weighed and transferred into a 100mL graduated cylinder. Then, the sample was added 10mL of water and the solution stir using a glass rod to disperse the sample thoroughly. Next, solution added 13mL of 52% perchloric acid and stir frequently for 20 minutes. The content diluted to 100mL with water and filtered into a 250mL graduated flask. The cylinder washed with water and the washing collected in the same graduated flask. The content diluted to the mark and mixed thoroughly.

Next, the extracted sample undergoes the determination process. About 10mL of the extracted sample taken and diluted to 100mL with water. Next, 1mL of the diluted solution transferred into a test tube using a pipette. At the same time, 1mL of glucose standard solution at different concentration (0.1M, 0.15M, 0.2M, 0.25M) were used to construct a standard calibration graph. After that, 5mL of Anthrone reagent was rapidly transferred into all the test tubes and the content mixed thoroughly. All the test tube placed in a boiling water bath for 12 minutes and then cool down to room temperature. Finally, the solutions were transferred into glass cuvettes and the absorbance of the samples and the standards were read at 630nm against the blank. The concentration of the sample was obtained by using the equation of the straight line from the calibration graph.

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The calculation of total available carbohydrate:

Total available carbohydrate content (% glucose by weight) = (25 x b) / (a x w)

a: absorbance of glucose standard working solution

b: absorbance of sample extract

w: weight of sample taken (g)

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3.4 Determination of glycemic response (dialyzable glucose concentration)

Determination of glycemic index by using *in vitro* digestion protocol was based from Argyri et al. (2016). *In vitro* digestion involved two phases.

In the first phase, a certain amount of sugar samples selected and homogenized by using Ultra Turrax T25 with an equal amount of water. Then, the solution of samples incubated with α-amylase(185U/g available carbohydrate, -amylase from human saliva, type XIII-A A1031-1KU, Sigma-Aldrich, Taufkirchen, Germany) for 15 minutes at 37°C shaking incubator ES-20/60 with speed at 110rpm to imitate the oral digestion. Next, the pH adjusted to 2.5 with 0.1M HCl and water added if the volume of the solution is less than 2mL. Each sample then transferred 2mL into six well plates. In each well, 0.1mL of pepsin (porcine pepsin preparation, suspended in 4g/100 mL in 0.1M HCl, porcine pepsin, P-7000, Sigma-Aldrich, Taufkirchen, Germany) added and the plates placed on a shaking incubator at 37 °C for 2 hours. These will be stimulating the gastric phase on the human digestion. After 2 hours, a cylindrical insert with a piece of dialysis membrane placed at the top of each well in such a way that the membrane is in contact with the digest.

Then, each ring filled with 2mL 0.1M PIPES buffer pH 6.5 (piperazine-1,4-bis (2-ethane-sulfonic acid) disodium salt (P3768, Sigma-Aldrich, Taufkirchen, Germany)), simulating the gradual increase of pH in the human small intestine. The plates incubated for another 30 min, shaking at 37 °C

Phase 1

Sugar sample selected with certain quantity

Sample homogenized with Ultra Turrax T25 Basic with equal amount of water

Solution of sample incubated with α-amylase for 15 minutes at 37°C shaking incubator ES-20/60

The pH adjusted to 2.5 with 0.1M HCl

(add water if solution is less than 2ml)

Each sample transferred 2mL into 6 well plates

Each plate added 0.1mL pepsin

The well plates placed on a shaking incubator at 37°C for 2 hours

Cylindrical insert with a piece of dialysis membrane placed in top of each well

Each ring filled with 2mL of 0.1M PIPES buffer pH 6.5

Then, the well plates incubated on shaking incubator at 37°C for 30 minutes

Figure 3.2: Flow chart for the first phase of *in vitro* digestion protocol

The second phase of the *in vitro* digestion started after the end of this incubation period and lasted for 120 min. An aliquot (0.2mL) taken from the supernatant (t =0 minute). Subsequently, the insert carefully removed and 10μL amyloglucosidase (3260U/mL amyloglucosidase from Aspergillus niger E-AMGDF, Megazyme Inc., Chicago, IL, USA) and 0.5mL pancreatin- bile salt

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mixture (0.2g porcine pancreatin from porcine pancreas, P-1750 Sigma, and 1.2g bile extract, B-8631 Sigma, suspended in 100mL 0.1 M $NaHCO3$) added to the well. The cylindrical insert placed back, and incubation continued in a shaking incubator for 2 hours.

Within this 2 hours, 0.2mL of an aliquots taken out from the dialysate for every 30 minutes to determine the glucose (t = 30 min, t = 60 min, t = 90 min, t = 120 min, where t = 0 min was set at the start of the second phase of the in vitro digestion procedure). The digested samples (0.2 mL aliquots) mixed immediately with 0.8mL ethanol in a microcentrifuge tube and 30 min later the tubes centrifuged for 10 min at 5000rpm at 20°C to clarify the ethanol supernatant fraction before analysis of sugars. Dialyzable glucose, i.e., the concentration of glucose in the soluble and low molecular weight fraction of the digest, was tested as an index for the prediction of glycemic response.

Glucose determination performs spectrophotometrically using the dinitrosalicylic method (DNS 98%, 12,884-8, Sigma) in a 96-well plate at 562nm.

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Phase 2

About 0.2mL of an aliquot from supernatant was taken for t =0 minute

(t= 0, 30, 60, 90, and 120 minutes)

Insert of cylindrical removed and mixture of 10μL amyloglucosidase and 0.5mL pancreatin- bile salt added to the well

Cylindrical insert was placed back and incubated for 2 hours

(0.2mL aliquot will take out for every 30 minutes)

Each aliquot mixed with 0.8mL ethanol in microcentrifuge tube

After 30 minutes, the mixtures centrifuged for 10 minutes at 5000rpm at 20°C

(Mikro 200R, Hettich)

Glucose was determined by using dinitrosalicyclic (DNS) method

Figure 3.3: Flow chart for the second phase of *in vitro* digestion protocol

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3.5 Statistical analysis

Data analyzed by using SPSS Software 23 (IBM version). The results of a particular test were expressed in mean (± SEM) for estimated glycemic response value and sugar composition value by using one-way ANOVA method. Determination of significant correlation between the estimated glycemic response and sugar composition among different types of commercial sugars used Pearson Correlation Test. Values of $p<0.05$ defined the statistical significance in the association.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Sugar composition on sugar samples

Sugar composition of a selected type of sugar samples was determined by using the High-Performance Liquid Chromatography (HPLC) method. In this method, the individual sugars included were fructose, glucose, sucrose, maltose, and lactose. The total sugar content for samples was calculated by addition of all sugars in each of the sample. Based on the chromatogram of sugar standards at concentrations of 0.1mg/ml, 0.2mg/ml, 0.5mg/ml, 0.8mg/ml, and 1.0mg/ml (Appendix F), a standard curve was plotted between peak area in RIU and the concentration of standard sugar (mg/ml).

Table 4.1 shows sucrose was significantly higher in all sugar samples compared to fructose and glucose. This is related to the source of sugar samples where mostly are from sugar cane. Sucrose was mainly produced from sugar cane or sugar beet (Lu, Thomas & Schmidt, 2017). Among all the studied sugar samples, sucrose content

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was significantly highest in icing sugar (99.84%) followed by sugar in sachet (99.62%), sugar with stevia (97.73%), better brown sugar (97.31%), refined sugar (96.53%), aromatic brown sugar (96.35%), soft brown sugar (95.95%) and palm sugar (80.44%).

Next, the amounts of fructose (%) and glucose (%) contents in all sugar samples were lower than 30% and considered as a very low amount. Fructose and glucose contents were significantly higher in palm sugar at 1.50% and 2.19% respectively. While, refined sugar had low fructose content (0.36%) and glucose content (0.29%). No glucose and fructose were detected in better brown sugar.

The total sugar content for all sugar samples was significantly highest in icing sugar (100.64%) followed by sugar in sachet (100.30%), aromatic brown sugar (98.70%), sugar with stevia (98.41%), soft brown sugar (97.63%), better brown sugar (97.31%), refined sugar (97.18%), and palm sugar (84.14%). The total sugar content of samples was contributed mainly by sucrose available in the samples.

In Table 4.1, all studied samples contained multiple sugars which are glucose, fructose, and sucrose. However, no lactose and maltose were detected in the samples as samples were either from sugar cane and palm. The extraction process of sugar cane juice produces various types of sugar such as refined sugar, brown sugar, powdered sugar, sugar cube and sugar in sachet (Segui et al., 2015).

In this study, a sample of sugar with stevia contains a high amount of sucrose (97.73%). This might be due to the combination of refined sugar with stevia. Stevia can be partially used with sucrose for certain food product to achieve maximum consumer acceptance and quality (Saniah & Samsiah, 2012).

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For the palm sugar, sucrose content in the sample of this study was similar to a previous study by Chong et al. (2019) who reported that palm sugar contains about 84.6% of sucrose. According to Srikaeo, Sangkhiaw, and Likittrakulwong (2019), palm sugar contains high fructose and glucose content with better antioxidant properties due to the presence of phenolic and flavonoid compounds. Thus, this supports a higher amount of fructose and glucose content in palm sugar compared to other sugar samples.

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Table 4.1 : Sugar content on selected types of sugars in Malaysia

Sugar sample	Fructose (%±SD)	Glucose (%±SD)	Sucrose (%±SD)	Maltose (%±SD)	Lactose (%±SD)	Total sugar (%±SD)
Refined sugar	0.36 ± 0.02^{a}	0.29 ± 0.02^{a}	96.53 ± 3.33^{bc}	-	-	97.18 ± 3.36^{b}
Sugar in sachet	0.37 ± 0.06^{a}	0.31 ± 0.08^{a}	99.62 ± 1.92^{cd}	-	-	100.30 ± 1.78^{bc}
Icing sugar	0.44 ± 0.05^{b}	0.37 ± 0.05^{a}	99.84 ± 0.87^{d}	-	-	100.64 ± 0.77^{c}
Sugar with stevia	0.37 ± 0.05^{a}	0.31 ± 0.05^{a}	97.73 ± 1.91^{bcd}	-	-	98.41 ± 1.81^{bc}
Soft brown sugar	0.81 ± 0.03^{c}	0.86 ± 0.01^{b}	95.95 ± 0.16^{b}	-	-	97.63 ± 0.12^{bc}
Aromatic brown sugar	1.09 ± 0.02^{d}	1.26 ± 0.01^{c}	96.35 ± 1.05^{b}	-	-	98.70 ± 1.02^{bc}
Palm sugar	1.50 ± 0.01^{e}	2.19 ± 0.00^{d}	80.44 ± 0.09^{a}	-	-	84.14 ± 0.10^{a}
Better brown sugar	-	-	97.31 ± 0.00^{bc}	-	-	97.31 ± 0.00^{b}

Values are expressed as mean ± SD, n=3. Valued in the same columns with same superscript letters were not significantly different ($p>0.05$, ANOVA, Duncan). The total sugar is the sum of fructose, glucose and sucrose. (-) sign : "Not detected"

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4.2 Estimated glycemic response of sugar samples (dialyzable glucose concentration)

In this study, *in vitro* method has been used to estimate the glycemic response for each type of sugar samples. The estimated glycemic response was indicated as a dialyzable glucose concentration at 120 minutes (mg glucose/ 0.25g CHO sample). Glucose solution was used as a positive control in comparing the sugar samples.

Based on Figure 4.1, among all the studied sugar samples, sugar in sachet (3.47mg glucose) yielded the highest dialyzable glucose concentration, while sugar with stevia (0.49mg glucose) showed the lowest dialyzable glucose concentration. Next, the dialyzable glucose concentration was higher in palm sugar (3.19mg glucose) compared to icing sugar (3.17mg glucose) and refined sugar (1.68mg glucose). Moreover, the dialyzable glucose concentration was higher in aromatic brown sugar (1.51mg glucose) compared to soft brown sugar (1.11mg glucose) and better brown sugar (0.66mg glucose).

The particle size of sugar in sachet and icing sugar was smaller than in common refined sugar (0.5mm). Most of the icing sugar has a particle size of around 20μm (Barringer, 2013). The small particle size of sugar was due to sugar processing which can contribute to high glycemic response. According to Butardo and Sreenivasulu (2016), the high processing step of milling and refining of food sample increase the glycemic index value. Generally, the glycemic response might increase due to the small particle size of food sample as the surface area increase for a digestive enzyme to attack and metabolize the food (Glycemic Index Foundation, 2017).

A previous study by Aziz and Crude (2006) reported that palm sugar was considered as a low glycemic index compared to white sugar. However, results in

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this study showed that palm sugar contained high dialyzable glucose concentration based on the *in vitro* method compared to the granulated sugar (icing sugar and refined sugar). Based on the book by Wardlaw et al. (2009) stated that the glycemic index of palm sugar might rate a little higher due to some varieties are mixed with cane sugar and not pure.

Apart from that, results in this study showed that sugar with stevia had the lowest dialyzable glucose concentration among all of the sugar samples. Sugar with stevia contains bioactive compounds of plants such as alkaloids, flavonoids, tannins and phenolic that are able to improve the glucose response (Khiraoui & Guedira 2018). According to Edwards et al. (2016), phytochemical compounds such as polyphenols in sugar contribute to the low glycemic response.

On the other hand, aromatic brown sugar which more refined and free from dust compared to the soft brown sugar might contain some molasses (British Sugar, 2015). This could be related to the refining process of aromatic brown sugar which was more extensive compared to soft brown sugar and almost all the antioxidant such as polyphenols in the aromatic brown sugar were removed from the processing. Therefore, this can be the reason of why glycemic index which expressed as dialyzable glucose concentration at 120 minutes in aromatic brown sugar was higher compared to soft brown sugar.

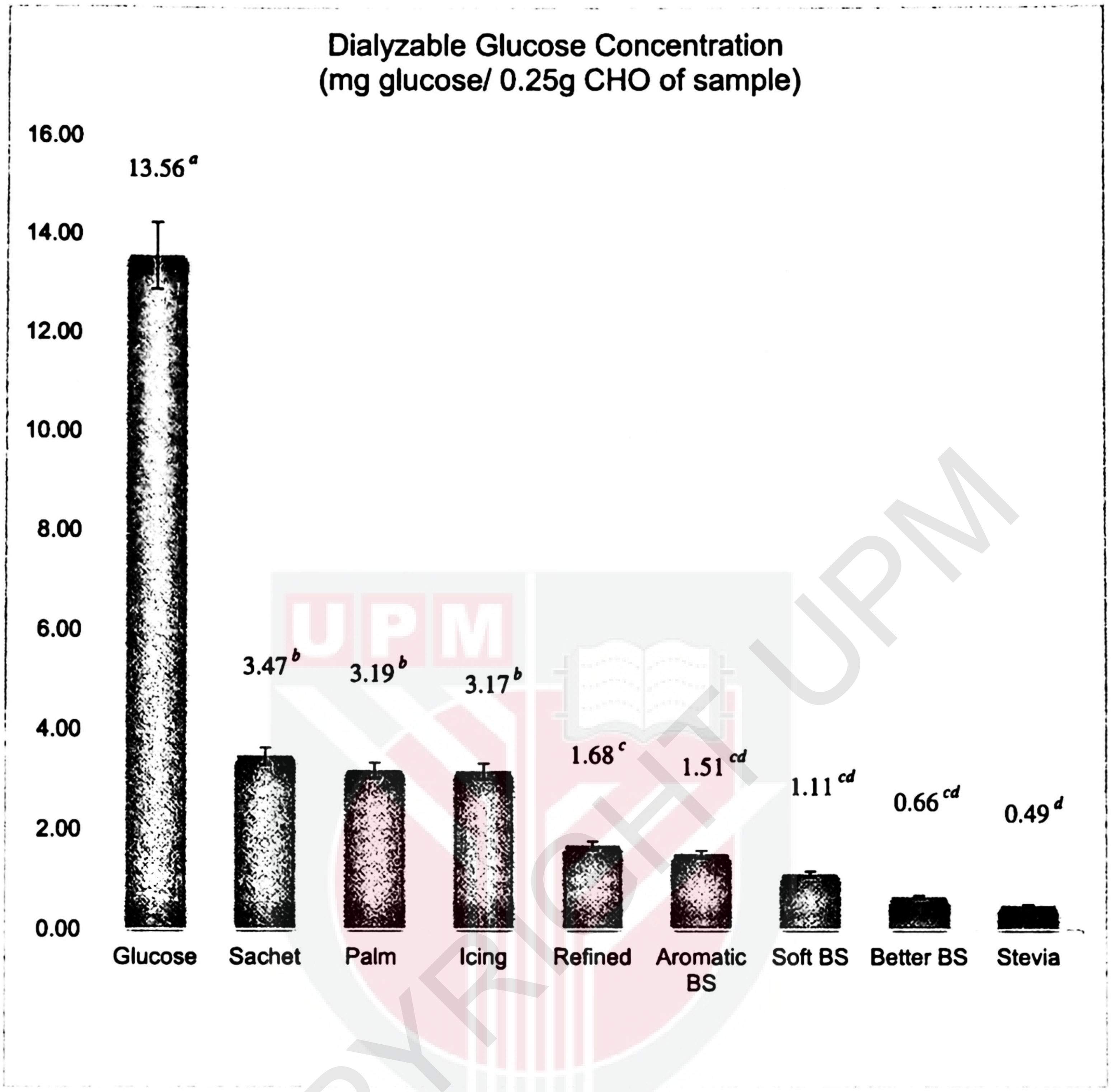

Figure 4.1: The dialyzable glucose concentration at 120 minutes on selected types of sugar samples in descending order.

Values are expressed as mean ± SD, n=3. Glucose solution used as a positive control.

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4.3 Correlation of estimated glycemic response and sugar composition on selected types of sugars in Malaysia.

Table 4.2: Correlation between dialyzable glucose concentration and sugar composition on selected types of sugar samples

Sugar composition	Dialyzable glucose concentration (mg glucose/ 0.25g CHO of sample)	
	r	*p*
Fructose	0.143	$p > 0.05$
Glucose	0.193	
Sucrose	-0.279	
Total sugar	-0.258	

The correlation was determined by conducted the Pearson's Correlation analysis.

* Correlation is at p<0.05.

Based on Table 4.2, there was no significant correlation (p>0.05) between the dialyzable glucose concentration (estimated glycemic response) and sugar composition for all selected types of commercial sugars studied. This shows that sugar composition on selected type of sugar sample does not affect the glycemic response. However, there is a trend for fructose, glucose, sucrose and total sugar with the dialyzable glucose concentration. Increase fructose and glucose composition of sugar samples might increase the dialyzable glucose concentration value. While increase sucrose and total sugar content of sugar samples might decrease the dialyzable glucose concentration value. Total sugar content was mainly contributed by the amount of sucrose in sugar samples.

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A study that seeks a correlation between glycemic index value and sugar composition is still scarce. To this date, there is also no published local study being conducted on this. Hence, the comparison between current findings on the relationship between glycemic index value and sugar composition cannot be done. Therefore, there is a need for future study to confirm the findings.

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CHAPTER 5

CONCLUSION, LIMITATION AND RECOMMENDATION

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Sugar composition such as fructose, glucose, sucrose, maltose, and lactose on selected types of sugars in Malaysia was analyzed in this study. All sugar samples contain high sucrose content (80.44%-99.84%) and total sugar content (84.14%-100.64%). Icing sugar (99.84%) had the highest sucrose content compared to other types of sugar samples. While palm sugar had the highest fructose content (1.50%) and glucose content (2.19%) compared to other types of sugar samples. The presence of sugar type sugar samples might be depending on the sources of sugar.

For the estimated glycemic response, sugar in sachet had the highest dialyzable glucose concentration and sugar with stevia had the lowest dialyzable glucose concentration. These estimated glycemic responses might be influenced by the phytochemical compounds available in the raw sugar samples.

The correlation between estimated glycemic response and sugar composition was not significant ($p>0.05$). Generally, the result of the study showed that sugar composition on selected type of sugar sample does not affect the glycemic response. However, the consumption of sugar needs to be controlled in order to prevent non-communicable diseases such as diabetes and heart disease.

In this study, findings related to the estimated glycemic response of selected types of commercial sugars were expressed as dialyzable glucose concentration at 120 minutes and cannot be compared with previous *in vivo* studies related to the glycemic index. Validation of *in vitro* method on glycemic index value still depends on the *in vivo* method. So, this study was not presenting the actual glycemic index but only predict the trend between the sugar samples. For future, the exact values of glycemic index for both *in vitro* and *in vivo* methods are needed to ensure the more presentable and reliable results can be achieved.

Moreover, previous studies related to *in vitro* and *in vivo* glycemic index value of the raw sugars are still limited in both local and foreign countries. Thus, there is a need to increase this kind of studies in Malaysia for future use and add more data on Malaysian Food Composition Database.

As a recommendation, sugar with stevia and better brown sugar were the best sugar samples to be selected by the consumer due to the lowest estimated glycemic response. Polyphenolic content of sugars might provide benefits to improve the glycemic response. Future study should focus to characterized antioxidant present in better brown sugar and stevia. This study has shown that the estimated glycemic response of better brown sugar and stevia were among the lowest compared to other types of sugars. Low glycemic index sugar consumption might be helpful in reducing the issues related to diet and health in the long term.

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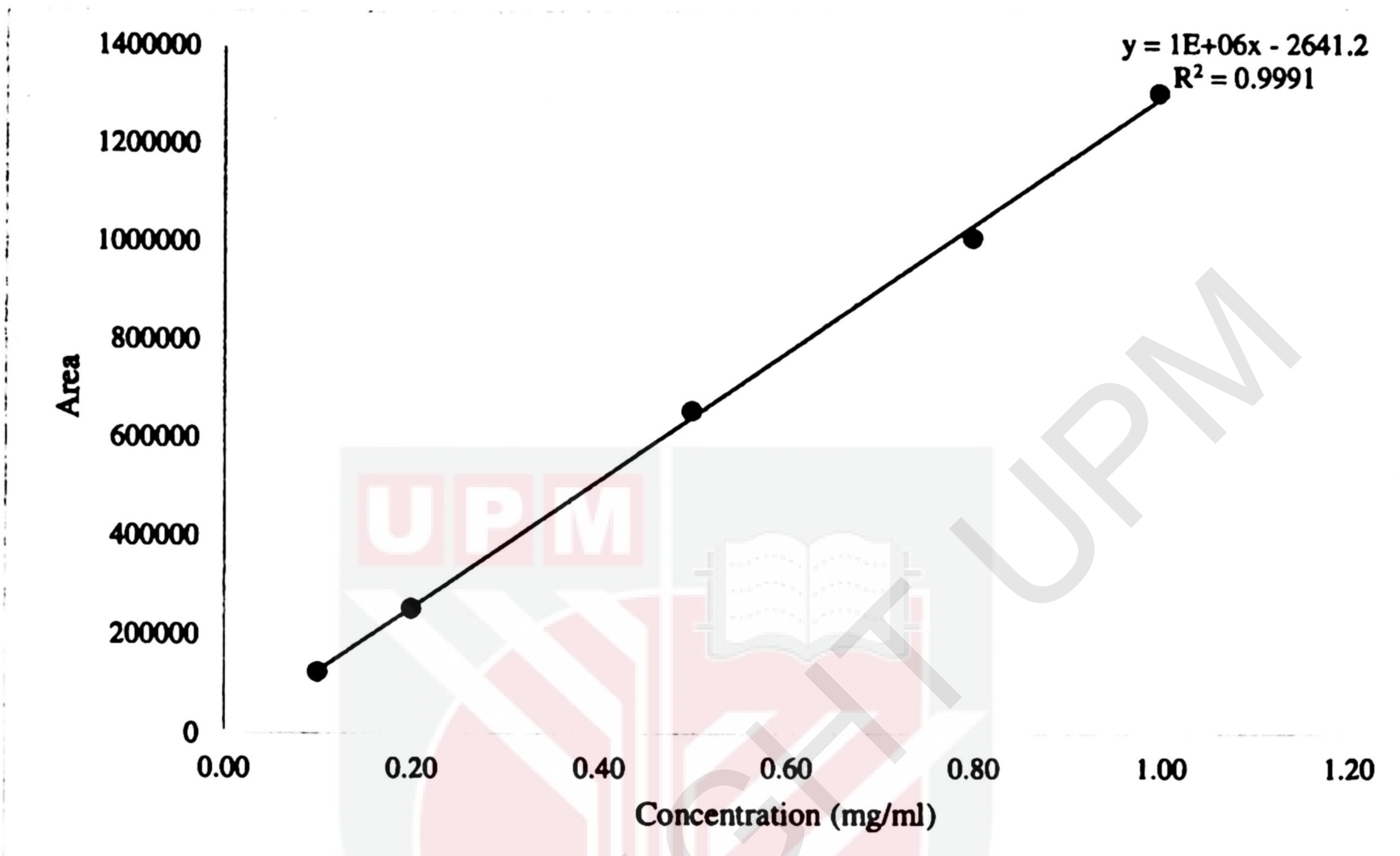
APPENDIX A

Figure 1: Sucrose standard curve by HPLC-RI



Peak: Sucrose

Sample Name	Peak Name	RT	Area	% Concentration	Height	Concentration
Std 1	Sucrose	9.562	125152	20.40	9620	0.100
Std 2	Sucrose	9.568	254360	20.32	19316	0.200
Std 3	Sucrose	9.591	655817	20.28	49093	0.500
Std 4	Sucrose	9.606	1005388	19.79	72177	0.800
Std 5	Sucrose	9.618	1300273	20.84	91344	1.000

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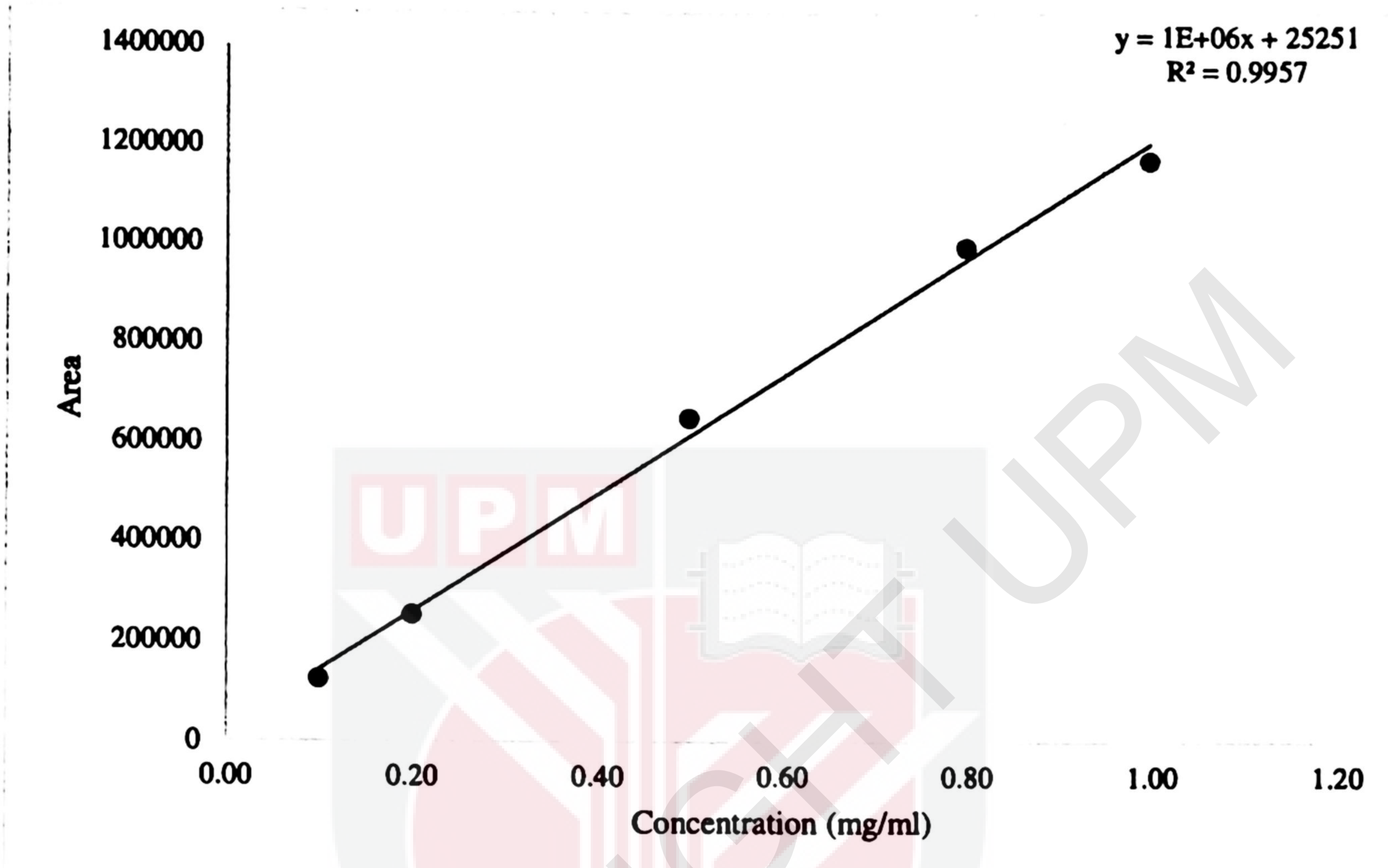
APPENDIX B

Figure 2: Maltose standard curve by HPLC-RI



Peak: Maltose

Sample Name	Peak Name	RT	Area	% Concentration	Height	Concentration
Std 1	Maltose	11.067	124592	20.31	6694	0.100
Std 2	Maltose	11.082	254168	20.31	13433	0.200
Std 3	Maltose	11.132	652767	20.18	33506	0.500
Std 4	Maltose	11.172	996524	19.62	50011	0.800
Std 5	Maltose	11.195	1173110	18.80	58359	1.000

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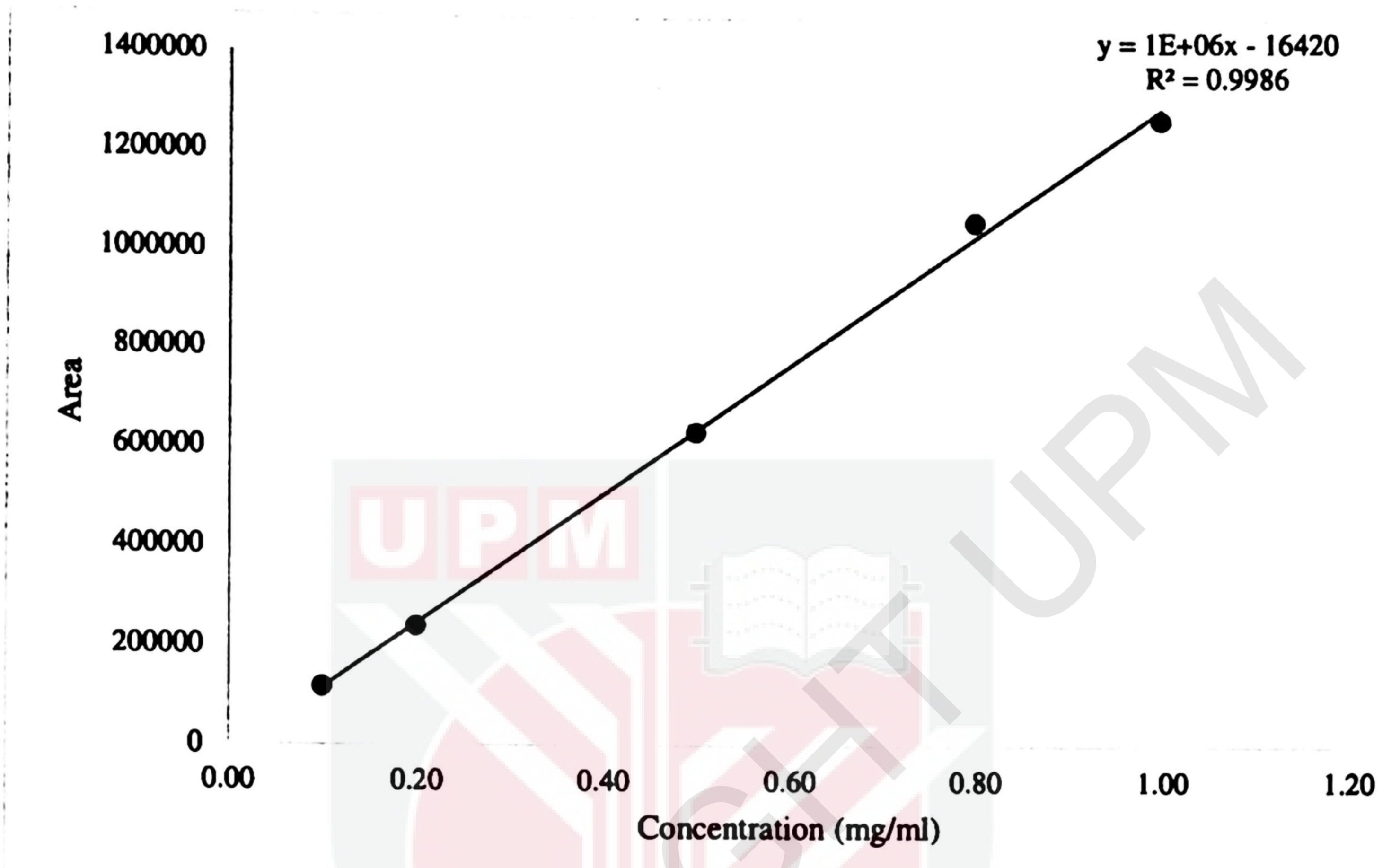
APPENDIX C

Figure 3: Fructose standard curve by HPLC-RI



Peak: Fructose

Sample Name	Peak Name	RT	Area	% Concentration	Height	Concentration
Std 1	Fructose	6.387	115369	18.81	11872	0.100
Std 2	Fructose	6.391	238620	19.07	24417	0.200
Std 3	Fructose	6.405	632222	19.55	63888	0.500
Std 4	Fructose	6.415	1059135	20.85	104193	0.800
Std 5	Fructose	6.418	1268602	20.33	123349	1.000

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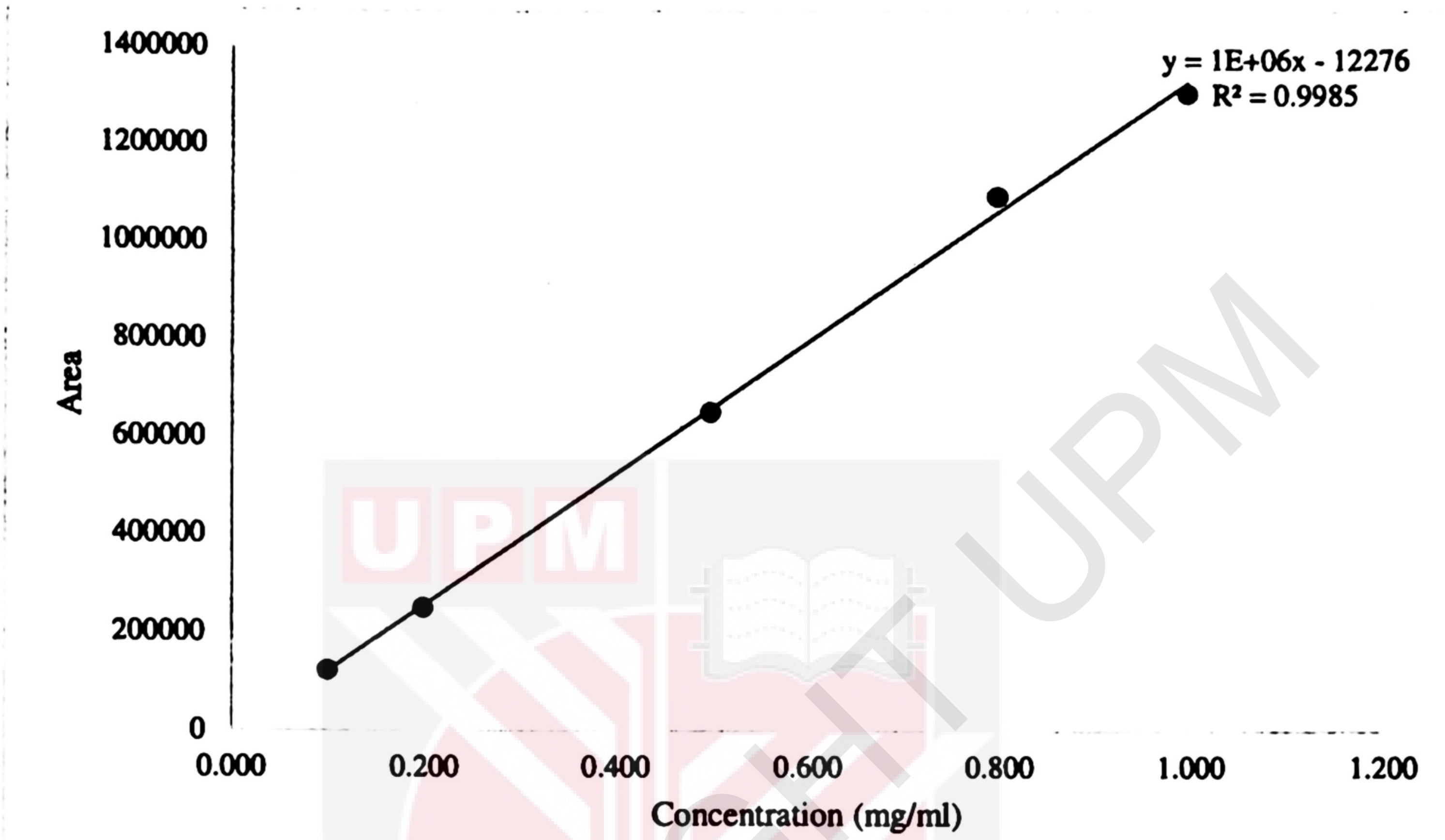
APPENDIX D

Figure 4: Glucose standard curve by HPLC-RI



Peak: Glucose

Sample Name	Peak Name	RT	Area	% Concentration	Height	Concentration
Std 1	Glucose	7.298	122891	20.03	10123	0.100
Std 2	Glucose	7.302	250163	19.99	20454	0.200
Std 3	Glucose	7.318	650809	20.12	52595	0.500
Std 4	Glucose	7.331	1091580	21.49	86197	0.800
Std 5	Glucose	7.337	1305269	20.92	101834	1.000

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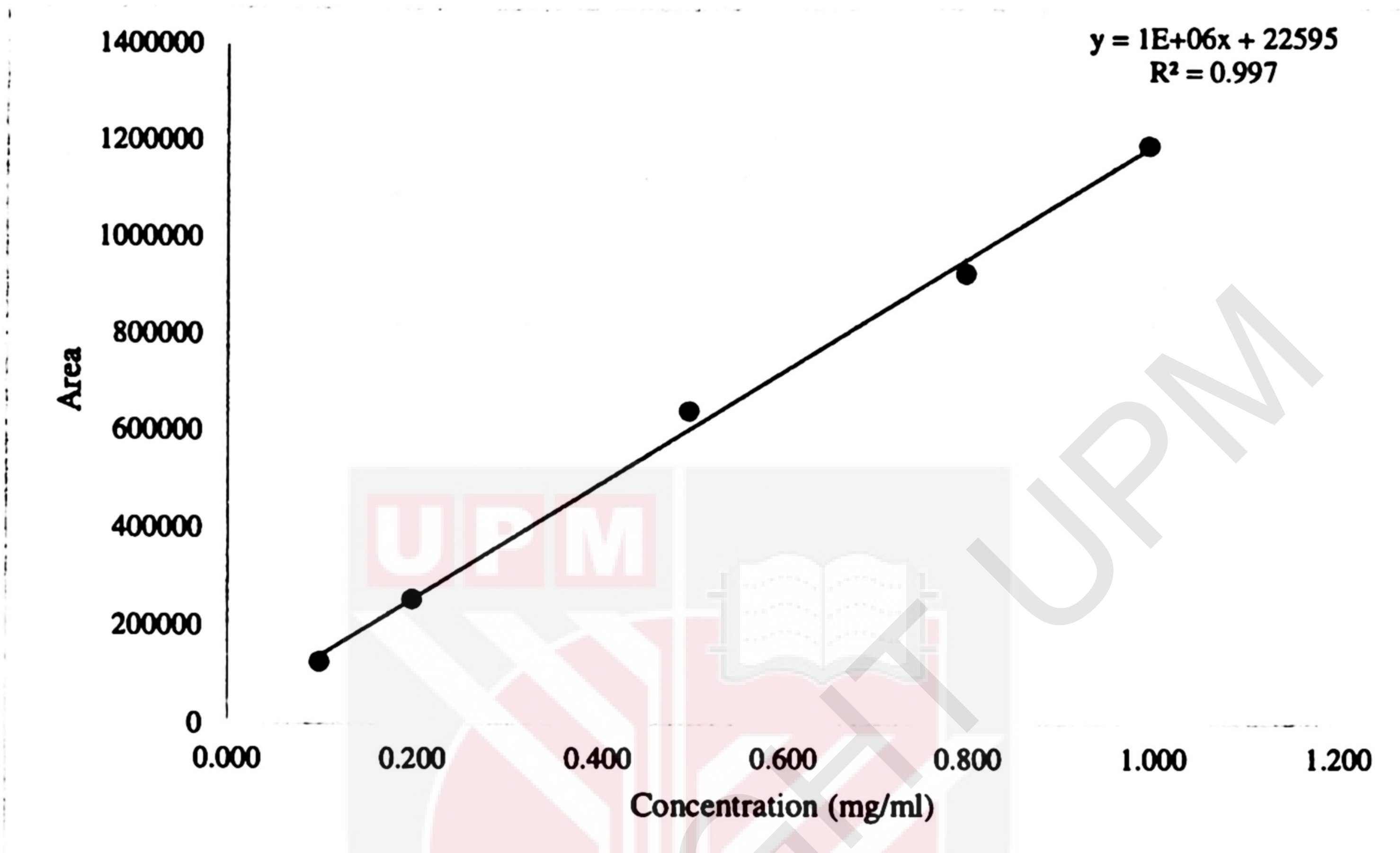
APPENDIX E

Figure 5: Lactose standard curve by HPLC-RI



Peak: Lactose

Sample Name	Peak Name	RT	Area	% Concentration	Height	Concentration
Std 1	Lactose	11.672	125417	20.45	7852	0.100
Std 2	Lactose	11.694	254212	20.31	15903	0.200
Std 3	Lactose	11.767	642659	19.87	39949	0.500
Std 4	Lactose	11.826	926704	18.24	56315	0.800
Std 5	Lactose	11.860	1192144	19.11	68045	1.000

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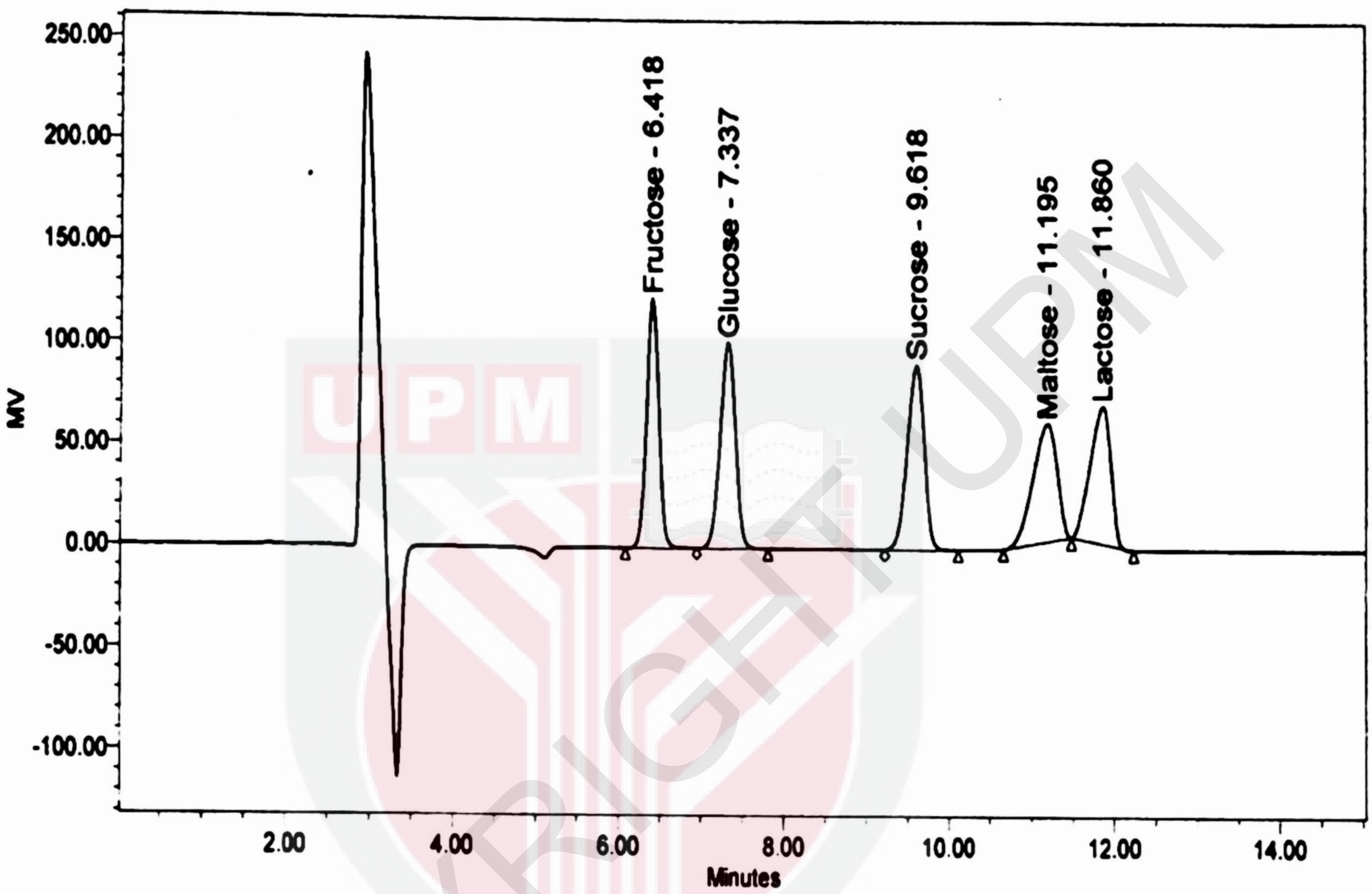
APPENDIX F

Figure 6: Chromatogram of mixed 5 sugars standard 5 (1.0 mg/ml concentration)



	Peak Name	RT	Area	% Concentration	Height	Concentration
1	Fructose	6.418	1268602	20.33	123349	1.000
2	Glucose	7.337	1305269	20.92	101834	1.000
3	Sucrose	9.618	1300273	20.84	91344	1.000
4	Maltose	11.195	1173110	18.80	58359	1.000
5	Lactose	11.860	1192144	19.11	68045	1.000
Sum						5.0

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APPENDIX G

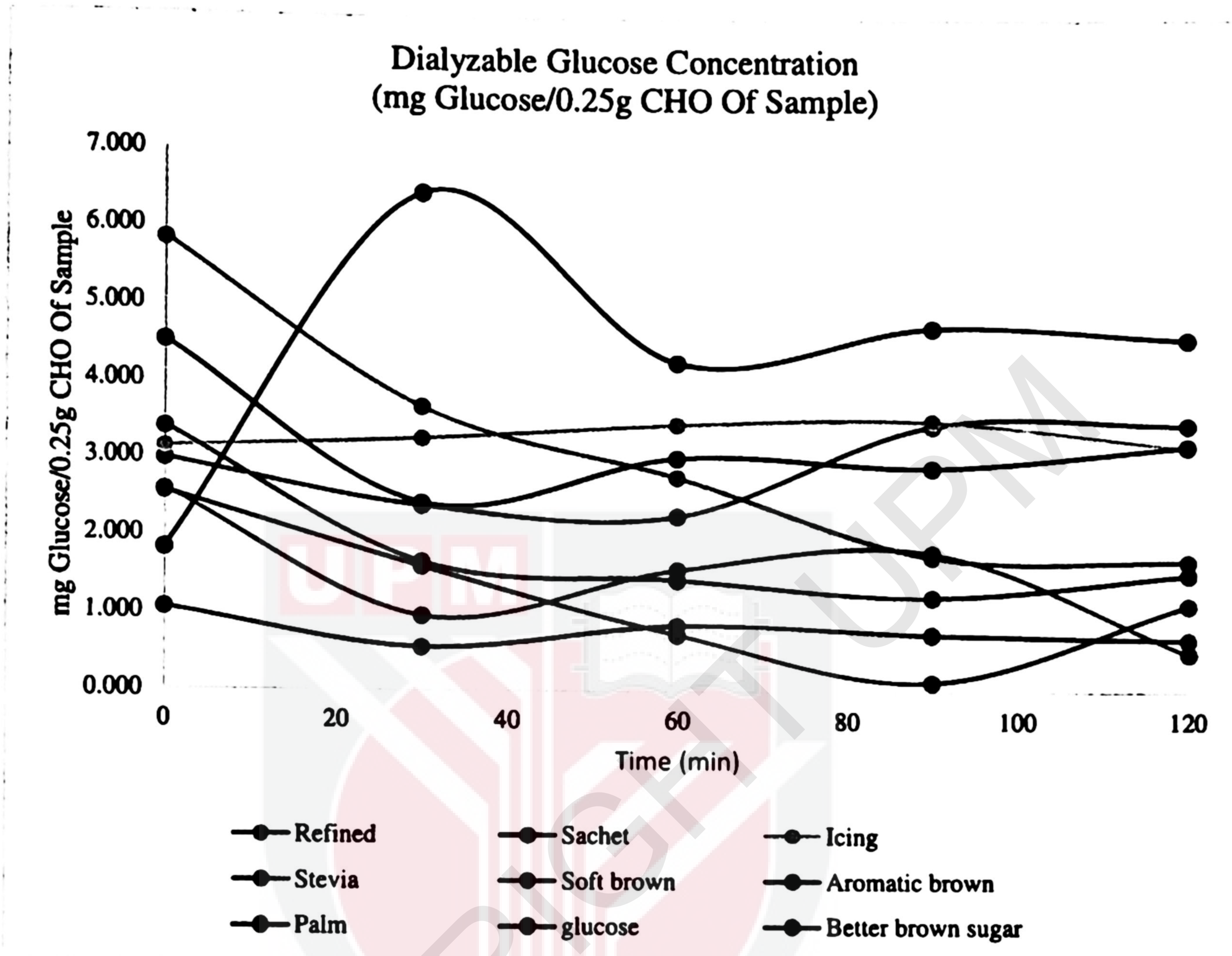

Figure 7: Dialyzable glucose concentration response (mg glucose/0.25g CHO of sample) for each types of selected sugars in Malaysia. Glucose solution as a positive control. Results are expressed as mean ± standard deviation (n=3).

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