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PREDICTING GLYCEMIC RESPONSE BASED ON IN VITRO METHOD AND DETERMINATION OF SUGAR COMPOSITION OF SELECTED RICE-BASED MEALS IN MALAYSIA

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Table of Contents

List of Tables

List of Figures

Abstract

PREDICTING GLYCEMIC RESPONSE BASED ON *IN VITRO* METHOD AND DETERMINATION OF SUGAR COMPOSITION OF SELECTED RICE-BASED MEALS IN MALAYSIA

Intan Syafinaz Binti Ahmad Fadzly

Rice is an important staple food in Malaysia and consumed in the form of mixed meals. Cooked white rice is known for its high glycemic index (GI) values (60-90). High GI foods were found to increase the risk of getting diabetes, obesity, cancers, and cardiovascular disease. Still, data of the GI values of local rice-based meals in Malaysia are still limited. The amount and type of sugars were identified to be one of the factors that affect GI values. However, information on the sugar content of Malaysians food is limited. Thus, this study aimed to predict the glycemic response using *in vitro* digestion and determine the sugar composition of four selected rice-based meals in Malaysia. The protocol of *in vitro* digestion was based on a validated method while sugar composition was determined using high-performance liquid chromatography (HPLC). Results showed that Nasi Lemak (1.306 ± 0.009) was ranked with the highest dialyzable glucose concentration followed by fried rice (1.294 ± 0.004), chicken porridge (1.255 ± 0.016) and Nasi Kerabu (1.233 ± 0.002). While the highest total sugar content was found in Nasi Kerabu (7.49%) followed by Nasi Lemak (3.21%), chicken porridge (2.48%) and fried rice (1.11%). Nonetheless, only the concentration of chicken porridge dialyzable glucose was significantly lower than plain white rice. There was a strong, negative correlation between the dialyzable glucose concentration at 120 minutes with glucose ($r= -0.888$, $p= 0.001$), fructose ($r = -0.789$, $p = 0.007$), sucrose ($r = -0.707$, $p = 0.022$), and total sugar ($r= -0.750$, $p = 0.012$) of the studied rice dishes. The correlation showed that the higher the total sugar content, the lower the predicted glycemic response. Usually, the higher total sugar content supposed to give higher predicted glycemic response. This is may be due to the presence of other components such as protein, fat, and fiber in the rice-based meals. Thus further research is needed to identify the potential factors that may influence the dialyzable glucose concentration in predicting the glycemic response of the foods.

Abstrak

ANGARAN TINDAK BALAS GLISEMIK BERDASARKAN KAEDAH *IN VITRO* DAN PENENTUAN KOMPOSISI GULA DI DALAM MAKANAN BERASASKAN NASI DI MALAYSIA

Intan Syafinaz Binti Ahmad Fadzly

Nasi merupakan makanan ruji penting di Malaysia dan dimakan dalam bentuk hidangan yang bercampur. Nasi putih mempunyai nilai indeks glisemik (GI) yang tinggi (60-90). Makanan GI tinggi meningkatkan risiko mendapat diabetes, obesiti, kanser, dan penyakit kardiovaskular. Walau bagaimanapun, data nilai GI makanan berasaskan hidangan nasi tempatan di Malaysia masih terhad. Jumlah dan jenis gula dikenal pasti sebagai salah satu faktor yang mempengaruhi nilai GI. Walau bagaimanapun, maklumat tentang kandungan gula makanan Malaysia juga terhad. Oleh itu, kajian ini bertujuan untuk menganggarkan tindak balas glisemik menggunakan kaedah pencernaan *in vitro* dan menentukan komposisi gula dalam empat jenis hidangan terpilih berasaskan nasi di Malaysia. Protokol pencernaan *in vitro* adalah berdasarkan kaedah yang disahkan manakala komposisi gula ditentukan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Keputusan menunjukkan bahawa Nasi Lemak (1.306 ± 0.009) mempunyai kepekatan glukosa *dialyzable* tertinggi diikuti nasi goreng (1.294 ± 0.004), bubur ayam (1.255 ± 0.016) dan Nasi Kerabu (1.233 ± 0.002). Kandungan gula tertinggi didapati di dalam Nasi Kerabu (7.49%) seterusnya Nasi Lemak (3.21%), bubur ayam (2.48%) dan nasi goreng (1.11%). Walaubagaimanpun, hanya kepekatan glukosa *dialyzable* bubur ayam jauh lebih rendah daripada nasi putih. Terdapat korelasi negatif yang kuat antara kepekatan glukosa *dialyzable* pada 120 minit dengan glukosa (r= -0.888, p= 0.001), fruktosa (r = -0.789, p = 0.007), sukrosa (r = -0.707, p = 0.022), and jumlah kandungan gula (r= -0.750, p = 0.012) dalam hidangan nasi yang dikaji. Korelasi menunjukkan bahawa semakin tinggi kandungan gula, semakin rendah tindak balas glisemik yang diramalkan. Kebiasaannya, kandungan gula yang lebih tinggi seharusnya memberikan tindak balas glisemik yang lebih tinggi. Ini mungkin disebabkan oleh kehadiran komponen lain seperti protein, lemak, dan serat. Oleh itu kajian selanjutnya diperlukan untuk mengenal pasti faktor-faktor yang mungkin mempengaruhi kepekatan glukosa *dialyzable* dalam meramalkan tindak balas glisemik makanan.

CHAPTER 1

INTRODUCTION

1.1 Background

Rice is the staple food for over 90% of the world's population as well as in Malaysia. Rice has been reported to be the most important food crop and the primary source of food among Malaysians (Noraida et al., 2018). Since it is the major food crop, rice is presented in a different type of rice which included fragrant white rice, parboiled rice and red rice (Atkinson et al, 2008). Cooked rice is commonly consumed during lunch and dinner where the average intake of white rice is 2.5 plates per day (Norimah et al., 2008). According to Nur Indrawaty et al. (2012), Malaysians usually consumed cooked rice with vegetables and fish or chicken. Because of the variety of multicultural races exist in Malaysia, rice is consumed in many different cooking methods and also combined with others ingredient. For example, Nasi Lemak is one of the famous local food rice-based meals in Malaysia. Rice and rice-based foods are often classified as having a high glycemic index (GI) (Hsu, Lu, Chang, & Chiang, 2015). Different cooking methods can have a significant effect on *in vitro* and *in vivo* digestibility of rice starch which in the end affect the glycemic index values. Rice-based meals in Malaysia are often added with fat and protein. Generally, the rice-based meals have a different GI as previous study show that rice-based mixed meals reduced the glycemic responses by 25% to 39% (Sun et al, 2014). Typically, sugar was added into the rice-based meals to enhance the palatability of the foods. Thus this is why Malaysia is ranked among the top countries in Asia for the availability of sugar in foods. Nonetheless, the sugar database for rice-based meals in Malaysia is still limited. (Chong et al., 2019). Sugars is known to increase the GI values; the higher the sugar content, the higher the GI values (The Glycemic Index Foundation, 2012). In general, rice-based mixed meals has shown to significantly lower the incremental peak of glucose when compared to the cooked white rice alone (Sun et al, 2014).

Glycemic index (GI) is a value that ranks the ability of carbohydrate presented in the foods to raise blood glucose levels (Jenkins et al., 1981), which focus on the effect on postprandial blood glucose levels (Wolever, 2013). The application and research in GI values are still lacking in Malaysia. Although there already have a published GI values database, yet data originated from Malaysia are still scarce.

GI has been known for its benefits and has been used in many circumstances where GI was used to plan dietary guidelines which would benefit those people who are diabetics. Also, GI is very useful in weight management intervention (Lavin et al., 1998). Next, GI can also be used for the prevention of CVDs (Xiang et al., 2012), and also cancer (George et al., 2009; Choi et al., 2012). From the benefits listed, it is clear the importance of having GI values for Malaysian local food is very crucial yet the factors that affect the GI values are still unclear.

The *in vitro* method is an alternative method to predict the glycemic response of a food where it is measures the digestibility of carbohydrates after a protocols that mimic the human digestion phase from oral to intestinal phases which is the concentration of dialyzable glucose. *In vitro* method also is a simple and inexpensive tools compared to *in vivo* measurement which requires the recruitment of volunteers. Thus, the aim of this study was to determine the glycemic response based on *in vitro* method and sugar composition values selected rice-based meals in Malaysia.

1.2 Problem Statement

The application and research about GI are still limited in Malaysia. Besides, the establishment of the GI database for local food has serve as useful information for health purpose. As limited data is available, the health practitioners or food companies only can refer to the international tables of GI and glycemic load (GL) to estimate the GI values of similar foods (Foster-Powell et al., 2002). But using the estimation calculation may not accurate to produce the GI value of similar food especially rice-based meals. Rice has been shown to have different values of GI ranging from 27 to 139 (Foster-Powell et al., 2002) which occurred due to the origin of the rice growing and the process that the rice had undergoes such as milling process as well as the gelatinization of the rice. Sugar content also is one of the factor that lead to vary GI values but data on sugar content for Malaysians local food is limited.

There are several studies that had been done to determine the GI values of rice available in Malaysia in terms of the rice alone without any added component to the rice, but only a few studies determined the GI values of rice that commonly consumed in terms of mixed meals by Malaysian. This is very inconvenient as most of the Malaysian population consumed rice mixed with other ingredients or dishes. The established GI database is not able to express the actual Malaysian local rice-based meals. Although the GI value can be estimated using formula, it may overestimate the GI values. In recent studies, only Nasi Lemak in rice-based meals categories was examined to determine the total sugar content. This data is not enough since Malaysia has variety of rice-based meals.

GI values in mixed meals have been a controversial issue since several studies have shown that GI values of rice-based mixed foods expressed different GI values compared to the rice alone, especially if the food was added with fat and protein (Wolever, 2013). Some studies showed that adding fat and protein, it will decrease the GI values of mixed foods (Sun et al., 2014). While according to Hsu et al. (2015), the mixed meal has a higher GI value compared to a single food. Contradict with the study by Sun et al. (2014) which stated that by mixing of rice with other ingredients will lower the incremental peak of glucose when compared to the rice alone. Sugar is commonly added to the rice-based meals and sugar is the source of carbohydrate where higher content of sugar can increase the GI values but the presence of other nutrients may lower the GI (The Glycemic Index Foundation, 2012). Thus a further study need to be done to confirm this.

1.3 Significance of the Study

Since the GI database for local foods in Malaysia is still limited, thus the findings of the study can be used as references for the future estimation of GI value on commonly consumed food in Malaysia. This research will be useful for the clinical purpose to plan a healthier diet specifically with dietary glycemic index and glycemic load intake of T2DM patients, reducing the risk of getting a chronic disease and also can be used for weight management and use to improve athlete's performance. Also, this study give an overview on sugars content of selected rice-based meals in Malaysia since information on sugar content is limited. This help to plan for a better diet and to avoid excessive consumption of sugar which can lead to poor health status. This study also provide *in vitro* method protocols which can be a useful tool for predicting glycemic response of test foods.

1.4 Objectives

1.4.1 General Objective

To predict the glycemic response based on *in vitro* method and determine the sugar composition values of selected rice-based meals in Malaysia.

1.4.2 Specific Objectives

1. To predict the glycemic response by using the concentration of dialyzable glucose of selected rice-based meals in Malaysia.
2. To determine the sugar composition value of selected rice-based meals in Malaysia.
3. To compare the concentration of dialyzable glucose of selected rice-based meals with plain white rice, white bread and glucose.
4. To correlate the glycemic index and sugar composition value of the selected rice-based meals in Malaysia.

1.5 Null Hypothesis

- H_o 1: There is no significant different between the concentration of dialyzable glucose of selected rice-based meals with glucose, white bread and white rice.
- H_o 2: There is no significant different in sugar composition between the selected rice-based meals.
- H_o 3: There is no significant correlation between concentration of dialyzable and sugar composition value of the selected rice-based meals.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice and rice-based meals

The scientific name of rice is *Oryza sativa L.* and it is a grain that is expressed by husk, bran, endosperm, and germ (Brown, 2008). The nutrients are mainly found in the bran which consists of fiber and mineral while, the germ consists of fat with some protein, vitamin, and mineral (Brown, 2008). The rice production comes with different types of rice which including fragrant white rice, parboiled rice and red rice (Atkinson et al, 2008). Each type of rice gives a different glycemic index value with a range of 48 to 92 (Atkinson et al, 2008).

The consumption of rice has been reported to be over one-half of the world's population staple food which contributes to 20% of total caloric intake (Bhattacharjee et al., 2002). In Malaysia, rice has been reported to be the most important food crop and the primary source of food among Malaysians (Noraida et al., 2018). Statistically, 90% of the rice production is to be consumed as food while 10% is used for others purpose. The majority of Malaysian adults consumed white rice twice a day with an average intake of 2.5 plates per day (Norimah et al., 2008). According to Noraida et al. (2018), rice typically consumed during lunch and dinner time but the rice was not consumed by its own alone but combined with other ingredients as mixed. This can be seen where the lunch and dinner food patterns in Malaysia will be a combination of rice and vegetables, with either chicken or fish (Nur Indrawaty et al., 2012). As Malaysia consists of the diversity of cultures and ethnicity, thus there were a variety of local foods including different rice mixed meals. Where the rice may come in different cooking methods and ingredients such as 'Nasi lemak' which is also a well-known local rice-based meals in Malaysia and commonly consumed during breakfast (Nur Indrawaty et al., 2012). Since rice was the main food consumed by Malaysians, it also becomes the primary ingredient in daily meals and local foods consumed by Malaysians (Nur Hafizah et al., 2013). Not only 'Nasi Lemak', but fried rice is also one of the famous dishes for breakfast among Malaysians.

The rice and rice-based foods are often classified as having a high glycemic index (GI) (Hsu et al., 2015) and different cooking methods can have a significant effect on the in vitro and in vivo digestibility of rice starch which will affect the glycemic index values. Although it has been stated that way, yet the glycemic data for these mixed meals of rice were still limited. The predicted GI values of mixed meals cannot only be calculated based on data established as in the single food GI data because the predicted GI value miscalculated the GI value of mixed meals.

Rice is a polysaccharide consisting of long chains of glucose molecules that are linked together either in the form of amylose or amylopectin (Barakatun Nisak, Ruzita & Norimah, 2005). Rice with high amylose content exhibited lower glycemic index values (Kumar et al., 2018). Rice also consist of glucose and sucrose as the main soluble sugar that gives the sweetness taste to the rice (Hu et al., 2017). Commonly, sugar was added into the mixed food to enhance sweetness and palatability. Different types and amounts of sugars are added during the cooking process which to enhances the taste of the food (Chong et al., 2019). This is why Malaysia was ranked among the top countries in Asia for the availability of sugar but sugar database for rice-based meals in Malaysia still limited (Chong et al., 2019).

2.2 Glycemic response, Glycemic Index and glycemic load

Glycemic response (GR) is defined as the post-prandial blood glucose response which can be obtained after the consumption of food that contain carbohydrates (Augustin et al., 2015). While glycemic index (GI) is defined as the incremental area under the blood glucose response curve (IAUC) elicited by a 50-g available carbohydrate portion of food, expressed as a percentage of the GR after the consumption of 50 g of anhydrous glucose or white bread (Wolever, 2006). Theoretically, GI is both the standardize GR and a relative GR (Augustin et al., 2015). In simple, GI is a value that ranks the ability of carbohydrate presented in the foods to raise blood glucose levels (Jenkins et al., 1981), which focus on the effect on postprandial blood glucose levels (Wolever, 2013).

GI values vary depends on the presence of other nutrients (Jenkins et al., 1981). The type of food grain, method, and duration of cooking, the amounts of amylose, resistant starch, fiber, and protein content, and amylopectin molecules also could affect the GI values (Kumar et al., 2018). Glycemic load (GL), is the quality and quantity of carbohydrates present in the food. Dietary GL is the sum of the GLs for all carbohydrate-containing foods consumed, usually on a daily basis, and reflects both the quantity and the quality of consumed carbohydrates (Sieri & Krogh, 2017). GL is the product of GI values multiply by the total available carbohydrates in a given amount of food (Augustin et al., 2015).

Available carbohydrates will be absorbed into the blood circulation, and it is able to raise blood glucose levels (Australian Standard, 2007). In general, consumption of carbohydrate increases the glycaemia and blood insulin level, which depend on how much of carbohydrate can affect the level including the proportion of digestion-resistant material that serves as dietary fiber, amount, processing method, and presence of other nutrients (Jenkins et al., 1981). The numerical GI value was divided into three different groups according to the range of values of GI and classified into three categories as shown in Table 2.1. This is based on in vivo GI values.

Table 2.1: Classification of Glycemic Index.

Classification of Glycemic Index	GI value
High	More than 70
Intermediate	56-69
Low	Less than 55

Source: Brand-Miller, Foster & Wolever (2003)

The international table of glycemic index and glycemic load value has been published by Foster-Powell et al. (2002) and used for reference of similar food but foods originated from Malaysia is seem very little. The GI values available in the international

GI database were used to calculate similar foods GI values to estimate the similar food. This calculation method was not supported by Dodd et al. (2014) because the calculation may overestimate the mixed food due to various factors that can altered the GI values such as cooking and processing. This mentioned that estimation calculation using formula cannot be simply applied to mixed meals because mixed food contains others macronutrients such as protein and fat where the amount may differ.

According to several studies, GI values would be affected by several factors. Combination of different ingredient in the foods is one of the factors that can affect the GI value of food. Stated by several studies, by adding fat and protein to the mixed meals reduced the glycemic responses by 25% to 39% (Sun et al, 2014). This is because the reduced GI after adding fat was due to delaying in gastric emptying (Welch et al., 1984). Where in normal subjects, the addition of fat decreased the glycemic response with a significant reduction in peak rise (Wolever, 2011). Also supported by Simpson et al. (1985) where adding fat reduced about 50% of the glucose peak rise in normal subjects.

In general, rice-based mixed meals showed a significantly lower incremental peak of glucose when consumed with proteins, fats, and vegetables compared to the rice alone ($P < 0.05$) (Sun et al, 2014). Furthermore, addition of 28-30g of fat and 20-23g of protein per 50g of carbohydrates decreased the glycemic response but addition of small amount of oil did not change the GI value of the rice (George et al., 2014).

Not only that, the availability of starch and its structure also contribute to the GI values where the presence of complex starch that retained it structure after cooking could be a reason for low GI values (Vosloo, 2005). Cooking process of rice mixed meals also affect the starch content in the rice especially amylopectin content that could cause higher

GI value but the GI value may remain low due to the present of fiber and polyphenols (Adedayo et al., 2018). Rice-based meals usually undergoes different processes such as cooking, boiling and frying which could changed the GI of the food (Ludwig, 2002).

Fiber content may also lowered the GI value due to delayed stomach emptying (Kumar et al., 2018). Types and availability of sugar also affect the glycemic index of the food which can be seen through the ripening of fruits and vegetable that cause the sugar content increase and starches decrease thus the GI of the food also change (Jones, 2012). Each type of sugar has different GI values. From the Glycemic Index Foundation 2017, maltose has the highest GI values followed by glucose, sucrose, lactose and the lowest is fructose where the GI values were 105, 100, 65, 46 and 19 respectively. Fermentation can also lower down the GI of the food by the production of organic acid that can delay the gastric emptying. There were several studies determined the GI values of commonly consumed rice in Malaysia but its applicability in the context of mixed meals is still unclear (Yusof et al, 2005).

Table 2.2: Range of Glycemic Index (GI) values of rice-based food.

Food	Range of GI value
Rice	60-90
Rice-mixed meals	41-66

Sources: Yusof et al. (2005), Robert & Ismail (2012), Ramlah (2013), Nik Shanita (2005)

There is very limited Malaysia local food being tested to estimate the GI values. Based on Table 2.2, the rice and rice-based meals GI values were ranged between 60-90 and 41-66 respectively. As shown in Table 2.2, it clearly showed that rice-based meals have lower GI values compared to rice alone. This due to several factors that have been mentioned before. Thus, more GI testing needs to be done since Malaysia consumed rice with a different type of cooking method and also combined with different ingredients comprise of multicultural races. Table 2.3 showed the studies that had been done to test the rice-based mixed meals in Malaysia. All the rice-based meals elicited low GI values.

Table 2.3: Glycemic Index of Malaysian Mixed Foods tested.

Food	**GI value**	**Reference**
Coconut milk rice (Nasi Lemak)	50 ±5	(Daniel Robert & Ismail, 2012)
	66±5	(Nik Shanita, 2005)
Fried rice (Nasi goreng kampong)	59±3.9	(Nik Shanita, 2005)
White rice served with chicken curry	41	(Ramlah, 2013)

In Table 2.4, it is shown that the GI value of rice-based meals were ranged between low to high GI with the majority has a lower GI value. According to Sun et al. (2014), rice-based mixed meals showed a significantly lower incremental peak of glucose compared to the rice alone. From Table 2.4, the rice consumed with other ingredients gives lower GI value compared to white rice alone. Although The GI value from Table 2.4 can be used as references, still this data was not based from Malaysia local foods. The GI values may slightly different because the used of different rice and food processing which could affect the GI values.

Table 2.4: Glycemic Index for rice-based meals from international food records.

Food	GI
Mushroom stroganoff with rice	26
Ground beef served with rice and an orange	31
Chicken tikka masala and rice, convenience meal	34
French style chicken with rice, reheated, lean cuisine™ frozen convenience meals	36
Lean cuisine steam indian style butter chicken with rice	43
Chicken korma and peshwari rice, prepared mea	44
Lean cuisine steam satay beef with rice	44
Chicken korma and rice, convenience meal	45
Tandoori chicken masala & rice convenience meal	45
Lean cuisine steam thai red chicken curry with rice	45
Rice (oriza sativa) and tomato soup	46
American, easy-cook rice, consumed with 10 g margarin	49
Burmese vegetable curry & rice, reheated, lean cuisine™ frozen convenience meals	49
Roasted chicken flavored, uncle ben's® ready rice (pouch)	51
Porridge (wholemeal wheat, oat, rice & wheat flour), fruit flavored, made with whole milk and water	51
Curry rice with cheese	55
White rice with fermented soybean (natto)	56
White rice, dried sea algae and milk, eaten together	57
Lentil and cauliflower cury with rice	60
White rice with pickled vinegar and cucumber (pickled food eaten with rice)	61
White rice topped with raw egg and soy sauce	72
Stir fried vegetables with chicken and boiled white rice, home made	73
White rice with dried fish strip (okaka)	79
Rice porridge	88

Source: Sydney University Glycemic Index Research Service (SUGiRS) (2017)

2.3 Low glycemic index food

Consumption of lower GI foods has been reported to help in improving cardiovascular disease risk, certain cancer, and diabetes and useful in weight management intervention. These evidences are from several studies. According to Xiang et al. (2012), diets with a high GL or GI was associated with a respectively 23% and 13% increased the risk of cardiovascular diseases (CVDs). Thus by taking a diet low in GI can reduce the risk of CVDs. Intake of low GI food also has been shown to reduce the risk of chronic diseases, in particular, type 2 diabetes mellitus (Bhupathiraju et al, 2014). Which similar with the result from the study by Salmeron et al. (1997) which involved the combination of low GL and high cereal fiber intake has positively reduced the type 2 diabetes mellitus (T2DM) risk by 2 times in both men and women. A study by Brand-Miller et al. (2003) also reported that low GI food consumption improves blood glucose control in diabetics as the lower GI food helped to delay the postprandial glycemia in diabetic patients (Miles, 2008). Consuming low GI foods was found to help increase the sleep onset latency (Afaghi et al., 2007).

Therefore, practicing low GI diet is important to preserve healthy health condition in long period because practicing high GI food intake was associated with increased risk of total cancer (George et al., 2009). This is also supported by Choi et al. (2012) where consumption of high GI food was associated with an increased risk of colorectal cancer. Breast cancer also one of the chronic disease that at increased risk when consuming high GI food (Mullie et al., 2016). Also reported by Sieri & Krogh (2017), consumption of a high GI/GL diet is associated with moderately increased risk of a few types of cancer.

Not only that, but practicing a lower GI diet also helped to improve athletic performance because lower GI foods gave a constant release of glucose which is crucial for endurance sports performance (Mondazzi & Arcelli, 2009).

In general, continuous consumption of high GI food increased high risk of obesity, cardiovascular diseases and type II diabetes (Jenkins, 2007). Since obesity has become Malaysia concern, by taking low GI food it can be used for weight management because of low GI diet play role in appetite regulation. Low GI food have a slower rate of digestion and absorption of carbohydrate which give a long time of satiety (Lavin et al., 1998).

2.4 *In vivo* and *in vitro* measurements

GI values can be obtained from two method which is *in vivo* and *in vitro* measurement where both of the measurement have its own pros and cons. *In vivo* measurement is the gold standard in determining the GI value where a human feeding method that involved the recruitment of volunteer under ethical committee approval was done. The glycemic response was obtained from the reading of the blood drawing from the volunteer (Woolnough et al.,2008). While *in vitro* measurement is a time- and cost-efficient method to analyze the glycemic properties of foods by mimicking the physiological processes occurred in the mouth, stomach, and small intestine (James et al., 2010). *In vitro* method is an alternative method to predict the glycemic response of a food where it's measured the digestibility of carbohydrates after a protocols that mimic the human digestion phase from oral to intestinal phases (Fernandez-Garcia et al., 2009).

In vivo method are complicated, expensive and time consuming while *in vitro* method are simple, inexpensive, and also applicable to larger number of samples (Ferrer-Mairal et al., 2012). *In vitro* method also a useful, method to estimate the biological response of high carbohydrate meals (Hu et al., 2004). But *in vitro* method only provide the trends of food's glycemic response which cannot give the numerical GI values. Concentration of dialyzable glucose was used to predict the glycemic response (Argyri et al., 2016).

CHAPTER 3

METHODOLOGY

3.1 Sampling and sample preparation

Rice-based meal samples (Appendix A) were selected based on purposive sampling method as data regarding specific food choices among Malaysian are quite limited (Asma A et al., 2010). Four rice-based meals which are fried rice, Nasi Kerabu, Nasi lemak and chicken porridge were selected. White rice, glucose, and white bread were used as the references. All the foods were purchased from a local cafeteria in the Faculty of Medicine and Health Sciences. Each of the samples (included the side dishes such as protein and vegetables) were ground together to homogenize followed by freeze-drying before the experiment. Non-edible part of the cooked dishes (chicken bone) was removed. The freeze-dried samples were blended into powder form and kept in an airtight container and stored in the refrigerator at 4^0C.

3.2 Chemicals

Three experiments were done in this study which are total available carbohydrate content determination, *in vitro* glycemic response (GR) determination and sugar composition determination. Chemicals used for the experiments are as follows:

Diluted sulphuric acid (sulphuric acid:water, 2.3:1.0, v/v), 52% perchloric acid (mixture of 270ml of perchloric acid (sp gr 1.70) and 100ml of water, R&M Chemical), Anthrone reagent (Friendemann Schmidt, Grade AR), glucose standard stock solution (mixture of 100mg of glucose and 100ml distilled water), glucose standard working solution (10ml of glucose standard stock solution diluted with water to 100ml volume), 0.1M HCl solution, PIPES Buffer (0.5M, pH 6.5m, 100ml, Solarbio), Pancreatin from porcine pancreas (4X cell culture, Sigma-Aldrich), Bile salts (Solarbio), 3, 5-Dinitrosalicylic acid, FS, Pepsin from porcine gastric mucosa powder (>500U/mg, Sigma Aldrich) amyloglucosidase (powder form, Solarbio), and ethanol (John Kollin Chemical).

3.3 Materials and apparatus

Materials and apparatus used were stoppered graduated cylinder, glass rod, filter paper, shaker incubator (Heidolph, Germany), 250ml volumetric flask, pipette, test tube, Bunsen burner, stand, wire, beaker and spectrophotometer (UV 1800, Shimadzu, Malaysia), six-well plate, cylindrical insert with a dialysis membrane (70µm cell strainer, Biologix), microcentrifuge, and High Performance Liquid Chromatography (HPLC) with the working condition stated specifically in the sugar composition determination method.

3.4 Determination of total available carbohydrate content

Total available carbohydrate content needed to be identified to be used in the *in-vitro* digestion method to obtain reliable GI values. In this study, the colorimetric Clegg Anthrone method was used to determine the total available carbohydrate content in each food samples.

One gram of dry sample was poured into a 100ml stoppered graduated cylinder. Subsequently, a 10ml of water was added and stirred to homogenize the sample. Next, disaccharides, trisaccharides, and higher oligomers were hydrolyzed to their reducing sugar component by adding 13ml of 52% of perchloric acid (the mixture of 270ml of perchloric acid(sp gr 1.70) and 100ml of water) into the sample mixture and stirred with a shaker for at least 20 min. The mixture was then diluted with water until it reached 100ml then stirred and filtered into a 250ml volumetric flask. The residue of the mixture in the cylinder was washed away with water and poured into the volumetric flask. Again, the mixture was diluted with water until it reached the mark of the volumetric flask.

In order to determine the total available carbohydrate content, 10ml of sample extract was diluted with water to 100ml. Then the diluted sample extract (1ml), water, and glucose standard working solution was pipetted into a different test tube. Next, anthrone reagent (5ml) was added rapidly into each test tube, mixed and placed in a boiling water bath for exact 12min then quickly cooled to room temperature. Thus, the determination was done by reading the absorbance of the reaction mixture at 630 nm against blank using the spectrophotometer.

A simplified procedure of extraction and determination of total available carbohydrates were illustrated below.

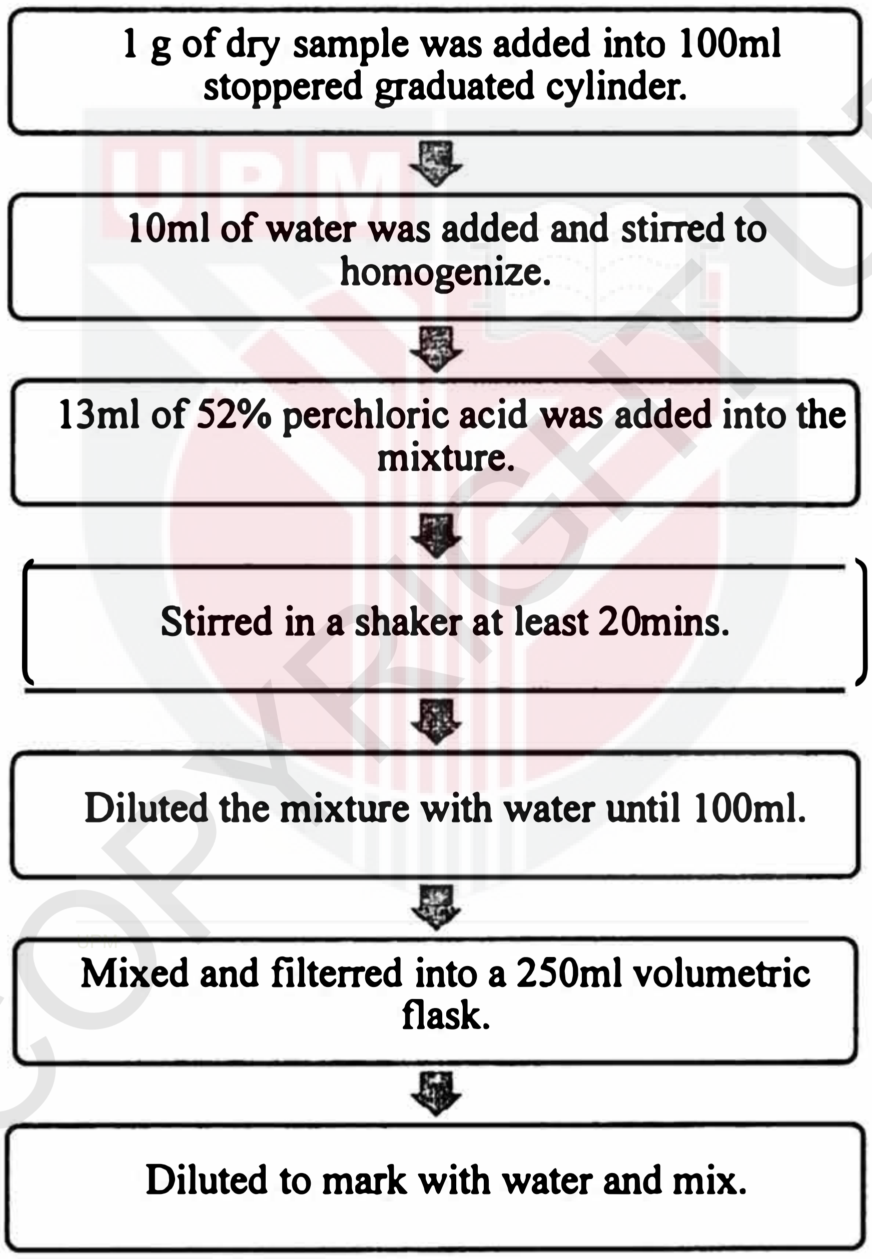

Figure 3.2: Extraction method for determination of total available carbohydrate content.

Diluted 10ml of sample extract to 100ml with water.

Pipetted 1ml of diluted sample extract/ water/ glucose standard working solution into test tube.

5ml of anthrone reagent was added rapidly to each test tube and mixed the content thoroughly.

Test tube was placed in a boiling water bath for exactly 12 mins, cool quickly to toom temperature.

Absorbance of reaction mixture was read at 630 nm against blank.

Figure 3.3: Determination of total available carbohydrate content method.

For calculation:

Total available carbohydrate content (% glucose by weight) = $\frac{25 \times b}{a \times w}$, where

a: Absorbance of glucose standard working solution.
b: Absorbance of sample extract.
w: Weight of sample taken (g).

3.5 Determination of Dialyzable Glucose Concentration to Predict Glycemic Response (GR)

The glycemic response determination was done as in vitro digestion where the procedure was based on the method developed in the past to predict glycemic responses or mineral bioavailability which consist of two phases (Argyri et al., 2016). The concentration of dialyzable glucose was used as the prediction of glycemic response.

For the first phases, samples containing 0.25 g available carbohydrate was added with water with equal weight and then homogenizesd in Ultra Turrax at a constant speed. Then the samples was incubated with α-amylase from human saliva, 185 U/g available carbohydrate at 37°C for 15 minutes in a shaking incubator at 110 rpm. This step was to imitate the oral digestion process. Afterward, the samples pH was adjusted until it reaches pH 2.5 by adding 0.1 M of HCl and water was added if the samples homogenate volume was less than 2ml and triplicates will be transferred into wells in a six-well plate. Following next, suspended 0.1 mL of pepsin in 4g/100 ml in 0.1 M HCl was added in each well and the plates was placed on a shaking incubator at 37°C for 2 hours to resemble the gastric phase of human digestion. After 2 hours, a cylindrical insert with a piece of dialysis membrane at the one end of the insert was placed in each well where the membrane is facing to the well. Each ring was filled with 2 mL 0.1 M PIPES buffer pH 6.5 to mimicking the gradual increase of pH in the human small intestine. Next, the plates was incubated for another 30 minutes, in shaking incubator at 37°C.

For the second phases, an aliquot of 0.2ml was taken from the dialysate and label as t = 0 min after the end of the incubation period from the first phases. Next, the insert on the six-well plate was removed to add 10 μL of amyloglucosidase and 0.5 mL of a pancreatin–bile salt mixture (0.2 g porcine pancreatin and 1.2 g bile extract, suspended in 100 mL 0.1 M NaHCO3) into the digested sample. Subsequently, the insert was placed back onto the well and the incubation was continued in a shaking incubator for 2 hours, simultaneously 0.2ml of aliquots was taken out every 30 min until the last 120 minutes from the dialysate which was label as 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. The aliquot was used for the determination of glucose. The aliquots was then mixed immediately with 0.8 mL ethanol in a microcentrifuge tube and 30 minutes later the tubes was centrifuged for 10 minutes at 5000 rpm at 20 °C which this process purposely to clarify the ethanol supernatant fraction before analysis of sugars. The concentration of glucose in the soluble and low molecular weight fraction of the digest which is the dialyzable glucose was tested as an index for the prediction of glycemic response. Dialyzable glucose concentration determination was using the dinitrosalicylic method against the blank at 540 nm in the spectrophotometer.

Based on Argyri et al. (2016), the highest correlation was found at 120 min of dialyzable glucose ratio. Thus, only dialyzable glucose ratio at 120 min were calculated using the following calculation:

A glucose standard curve that within the absorbance reading range was developed to obtain the y=mx +c equation. Then the x value was substituted with the absorbance reading to obtain the concentration of dialyzable glucose concentration.

3.6 Determination of sugar composition

High Performance Liquid Chromatography (HPLC) method was used to determine the sugar composition in each samples where the procedure was based on Waters (2012). First, dry sample weighted 1.0g was added into 50ml centrifuge tube. Next, acetonitrile mixture (25ml from acetonitrile and water mixture of 50 to 50 ratio) was added and homogenized for 15 minutes. Subsequently, the mixture was centrifuged at 3200 rpm for 30 minutes. After that, the supernatant was collected and filtered by 0.45μm membrane filter using syringe. Samples obtained was then kept in vial before injected into the HPLC to be analyzed.

The working condition for the HPLC used was showed in Table 3. Sugar content was determined by Refractive Index Detector against the standard solution at 1.0, 0.8, 0.5, 0.2 and 0.1 mg/mL. From the equation of standard curve of all sugar (Appendix I to Appendix M), the sugar composition (fructose, glucose, maltose, lactose, sucrose) of the rice-based meals were determined by substituting the value of peak area of chromatograph (Appendix D to Appendix H) of sugar in rice-based meals into the y-value in the equation of the standard curve. Total sugar content was the sum of the five individual sugar. Analysis for each samples was carried out in duplicate.

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

Table 3.1: The working condition of HPLC.

	Working Condition
Column	X bridge BEH Amide, 4.6mm x 100mm, 2.5μm
Detector	Refractive Index (RI) detector
Flow rate	1.0ml/min
Mobile phase	Acetonitrile : distilled water
Column temperature and RI detector temperature	40^0c

3.7 Statistical Analysis

The data obtain from this study was recorded in the IBM SPSS version 23 where the data was calculated and expressed into mean and standard deviation (SD) using one-way ANOVA and Pearson correlation calculation for the correlation of glycemic response and sugar composition. The hypothesis of this study was decided whether to be accepted or rejected where the significance different was set at value less than 0.05 (p value <0.05).

CHAPTER 4

RESULTS AND DISCUSSION

Rice was commonly consumed by Malaysians in terms of mixed food which consists of vegetables with either chicken or fish (Nur Indrawaty, Khor & Imelda, 2012). Nasi Lemak and fried rice were identified as rice-based meals chosen for breakfast among Malaysian (Nur Indrawaty, Khor & Imelda, 2012). Mixed food was reported to give higher GI values (Hsu et al., 2015). While Robert & Ismail (2012) reported that mixed food has almost similar GI values with a single food. This because there are several factors that affect the GI such as carbohydrates content, fiber content and cooking process (Allen et al., 2012). One of the factors that alter GI values is the type and amount of sugar content (Jones, 2012). Thus, the GI value and sugar composition of white rice in the form of mixed meals that commonly consumed in Malaysia was determined in this study. Four selected rice-based meals were examined which are fried rice, Nasi Lemak, Nasi Kerabu and chicken porridge. The *in vitro* glycemic response presented was yielded from 0.25g of available carbohydrates of each food where mean of the dialyzable glucose at different time were observed. While sugar composition was yielded from 1 g of sample.

Table 4.1: The percentage of total available CHO (%) in 1g sample and weight of sample for 0.25g CHO (g).

Samples	Total available CHO (%) in 1 g sample	Weight of sample for 0.25g CHO (g)
Glucose	100	0.250
White bread	58.36	0.529
White rice	49.04	0.506
Fried rice	45.22	0.542
Nasi Lemak	58.99	0.423
Nasi Kerabu	47.55	0.522
Chicken porridge	56.84	0.439

Total available carbohydrates (CHO) content was determined before the determination of glycemic response. As shown in Table 4.1, the total available CHO in 1 g of food samples was measured and ranged 47.55% to 100%. Within the rice-based meals, Nasi Lemak had the highest total available CHO (58.99%) followed by chicken porridge (56.84%), Nasi Kerabu (47.55%) and fried rice (45.22%). Glucose, white rice, and white bread were used as the reference food. A total of 0.25g of available CHO was used for the glycemic response determination.

4.1 *In vitro* dialyzable glucose concentration for prediction of glycemic response of selected rice-based meals

The prediction of glycemic response was determined by using the in vitro method where 0.25 g of available carbohydrates in each food were used to collect the concentration of dialyzable glucose readings as the index for the prediction of glycemic response (Argyri et al., 2016). The dialyzable glucose at 0, 30, 60, 90 and 120 minutes was calculated after the initiation of the second phase of the in vitro digestion to identify the trend of the selected rice-based meal's glycemic response. While, dialyzable glucose concentration at 120 minutes was used as the predictor of glycemic response (Argyri et al., 2016). The result was used to screen and estimate the glycemic potential of the foods (Kumar et al., 2018). Glucose, white bread, and white rice were used to compare the glycemic response between the selected rice-based meals.

Table 4.2: *In Vitro* dialyzable glucose concentration of selected rice-based meals.

Sample	**In-vitro dialyzable glucose concentration (mg/ml glucose)**				
	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
Glucose	1.36 ± 0.02	1.38 ± 0.08	1.36 ± 0.05	1.35 ± 0.06	1.36 ± 0.06
White bread	1.25 ± 0.01	1.27 ± 0.00	1.28 ± 0.00	1.30 ± 0.00	1.31 ± 0.01
White rice	0.13 ± 0.03	1.27 ± 0.02	1.29 ± 0.03	1.32 ± 0.00	1.32 ± 0.01
Fried Rice	0.40 ± 0.02	1.29 ± 0.01	1.30 ± 0.01	1.31 ± 0.01	1.29 ± 0.00
Nasi Lemak	1.13 ± 0.05	1.31 ±0.01	1.30 ± 0.02	1.31 ± 0.02	1.31 ± 0.01
Nasi Kerabu	1.23 ± 0.00	1.31 ± 0.03	1.28 ± 0.01	1.27 ± 0.04	1.23 ± 0.00
Chicken Porridge	1.09 ± 0.01	1.23 ± 0.00	1.25 ± 0.01	1.26 ± 0.02	1.26 ± 0.02

Values are expressed as mean ± SD, n=3.

Based on Table 4.2, all samples started low at 0 minutes and peaked at 30 minutes and glucose have the highest peak compared to other samples. The glycemic response trend of glucose and white rice where almost similar to Argyri et al. (2016) study, thus the result of this study were reliable. Unlike other trends of rice-based meals that increased moderately, Nasi Kerabu showed a trend was decreasing back after the rise at

30 minutes. This may due to the higher fiber content in Nasi Kerabu compared to other rice-based meals based on the recipes. Dietary fiber content was identified as one of the food components that reduced the glycemic response (Nayak, Berrios & Tang, 2014). Thus, this may be the reason for Nasi Kerabu to fall back the trend after the peak at 30 minutes.

The highest concentration of dialyzable glucose peak at 30 minutes was glucose (1.38 ± 0.08) followed by Nasi Lemak (1.31 ±0.01), Nasi Kerabu (1.31 ± 0.03), fried rice (1.29 ± 0.01), white bread (1.27 ± 0.00), white rice (1.27 ± 0.02) and chicken porridge (1.24 ± 0.00). Until at 120 minutes, the ranking had changed where glucose remains the highest (1.36 ± 0.06) followed by white rice (1.32 ± 0.01), Nasi Lemak (1.31 ± 0.01), white bread (1.31 ± 0.01), fried rice (1.29 ± 0.00), chicken porridge (1.26 ± 0.02) and Nasi Kerabu (1.23 ± 0.00).

From the ranking itself, it shows that mixed meals do give a lower dialyzable glucose concentration compared to white rice itself. This is consistent with Kaur, Ranawana, & Henry (2016) study where white rice was considered as high glycemic index (GI) food. This finding corresponds to several studies where the addition of fat and protein does reduce the glycemic responses (Sun et al., 2014). Also reported by Wolever (2011) where fat lowered the glycemic response with significant peak rise reduction.

Table 4.3: One-way ANOVA between *in vitro* dialyzable glucose concentration and the selected rice-based meals at 120 minutes.

Time	**Sample**	**(Mean ± SD)**
120 minutes	Glucose	1.356 ± 0.057^{a}
	White bread	1.308 ± 0.009^{c}
	White rice	1.323 ± 0.013^{b}
	Fried Rice	1.294 ± 0.004
	Nasi Lemak	1.306 ± 0.009^{d}
	Chicken porridge	1.255 ± 0.016^{ab}
	Nasi Kerabu	1.233 ± 0.002^{abcd}

Mean values are expressed in mg/ml glucose. Value in (mean ± SD) column with same superscript were significantly different. Results show significant difference when $p < 0.05$ (ANOVA).

Based on Table 4.3, there was a statistically significant difference between the dialyzable glucose concentration of selected rice-based meals at 120 minutes (F=9.355, p<0.01).The dialyzable glucose concentration of chicken porridge and Nasi Kerabu were significantly lower than glucose and white rice (p<0.01). Nasi Kerabu was significantly lower than white bread (p<0.01). From the results, this showed that Nasi Kerabu has the lowest prediction of glycemic response among the selected rice-based meals. Chicken porridge has a higher prediction of glycemic response compared to Nasi Kerabu but not significantly (p<0.05) different.

At 120 minutes, glucose (1.356 ± 0.057) was ranked with the highest dialyzable glucose concentration followed by white rice (1.323 ± 0.013), white bread (1.308 ± 0.009), Nasi Lemak (1.306 ± 0.009), fried rice (1.294 ± 0.004), chicken porridge (1.255 ± 0.016) and Nasi Kerabu (1.233 ± 0.002). Nasi Kerabu was the lowest ranked in prediction of glycemic response which may be due to the presence of protein, fat, and fiber. Based on recipes, Nasi Kerabu has more fiber content compared to other rice-based meals. The fiber component was reported to give effect to the glycemic respond by lowering it down (Kumar et al., 2018). Not only that, the content of fat and protein may also help to reduce the glycemic response. Although Nasi Lemak and fried rice contain vegetables as one of the ingredients it is not as much as in the Nasi Kerabu.

Based on the recipes (Appendix B), Nasi Lemak and fried rice may have a higher content of fat and protein compared to chicken porridge but chicken porridge have lower predicted glycemic response compared to both of the rice-based meals. Sun et al. (2014) stated that the addition of fat and protein may reduce the glycemic responses but chicken porridge showed the opposite result. This may due to the moderate amount of fat and protein content in these dishes. As reported by Robert & Ismail (2012), a moderate amount of fat and protein might give no effect to the glycemic responses. Thus, a future study needs to identify the amount of fat and protein in each rice-based meals.

The reason for chicken porridge glycemic response was lower that Nasi Lemak and fried rice were not very clear since there were many factors contributing to the alteration of glycemic responses. This may due to the gelatinization of the starch in the rice granule which causes by the swelling of the starch granules by addition of water to the rice which decreases the starch digestibility causing lower glycemic response (Parada et al., 2019).

4.2 Sugar composition of selected rice-based meals

Sugar composition of the selected rice-based meals was determined based on 1 g of the dry weight of the samples using High Performance Liquid Chromatography (HPLC) method with Refractive Index (RI) detector.. The sugars that been analyzed were fructose, glucose, sucrose, maltose, lactose. Maltose and lactose were undetectable in all the food samples. This is due to no milk and malt product that had been used as one of the ingredients of the food samples. Thus total sugar content was calculated from the sum of sugars analyzed except maltose and lactose.

Table 4.4: One-way ANOVA between sugar compositions in each selected rice-based mixed meals in Malaysia.

Sugar Composition (%)	**Sample**	**Mean**	**SD**
Fructose	White rice	0.32^{a}	0.00
	Fried Rice	0.39^{a}	0.02
	Nasi Lemak	0.71^{b}	0.05
	Nasi Kerabu	1.33	0.01
	Chicken porridge	0.68^{b}	0.05
Glucose	White rice	0.28	0.01
	Fried Rice	0.39	0.02
	Nasi Lemak	0.55	0.01
	Nasi Kerabu	1.36	0.02
	Chicken porridge	0.73	0.06
Sucrose	White rice	0.08^{a}	0.01
	Fried Rice	0.33^{a}	0.01
	Nasi Lemak	1.96	0.12
	Nasi Kerabu	4.80	0.20
	Chicken porridge	1.07	0.04
Total sugar	White rice	0.68	0.01
	Fried Rice	1.11	0.00
	Nasi Lemak	3.21	0.06
	Nasi Kerabu	7.49	0.17
	Chicken porridge	2.48	0.06

Values are expressed as mean ± SD, n=2. Valued in the same column with same superscript letters were not significantly different ($p > 0.05$, ANOVA, Tukey-HSD)

Based on Table 4.4, the mean difference of fructose content was significantly different (F 3486.36, p=<0.01) among all the rice-based meals. Nasi Lemak and chicken porridge plus white rice and fried rice were not significantly different (p= <0.05). Within the rice-based meals, Nasi Kerabu has the highest fructose content (1.33%) followed by Nasi Lemak (0.71%), Chicken porridge (0.68%), and fried rice (0.39%).

The presence of a variety of vegetables in Nasi Kerabu was higher compared to other meals may be the reasons for Nasi Kerabu to have the highest fructose content as fructose is commonly found in fruits and vegetables (Barclay et al., 2012). Also reported by White (2013), vegetables contain comparable proportions of fructose and glucose. In Nasi Kerabu, torch flower, cabbage, bean sprouts, cucumber, long beans, and lemongrass were used as the ingredients in the finished meals. This shown that Nasi Kerabu has a higher amount of vegetables compared to Nasi Lemak which only have cucumber while chicken porridge only has parsley and fried rice only has mixed vegetables (carrots, peas, and corns). This is based on the recipes of the rice-based meals used in this study.

Based on Table 4.4, the mean difference of glucose content was significantly different (F−3017.87, p<0.01) among all the rice-based meals. Within the rice-based meals, Nasi Kerabu has the highest glucose content (1.36%) followed by Chicken porridge (0.73%), Nasi Lemak (0.55%), and fried rice (0.39%). Rice basically contain glucose and obtain from the starch gelatinization during cooking process.

The mean difference of sucrose content was significantly different (F=705.36, p<0.01) among all the rice-based meals. Within the rice-based meals, Nasi Kerabu has the highest sucrose content (4.8%) followed by Nasi Lemak (1.96%), chicken porridge (1.07%) and fried rice (0.33%). Sucrose is obtained from the table sugar and sauce added during the cooking process (Chong et al., 2019). Rice itself also contains sucrose (Hu et al., 2017). Based on the recipes, all the rice-based meals were added with table sugar and sauces. The table sugar and sauces were added into the side dishes of the rice-based meals such as the 'sambal' for the Nasi Lemak, Sucrose content in Nasi Kerabu was the highest also may be caused by the way of the food was cooked since all the food samples were purchased from local restaurant, thus no accurate data on how to rice-based meals were prepared.

Within the rice-based meals, Nasi Kerabu has the highest total sugar content (7.49%) followed by Nasi Lemak (3.21%), chicken porridge (2.48%), fried rice (1.11%). All the rice-based meals total sugar content was significantly different (F: 3239.76, $p<0.05$). The main contributor for the total sugar was sucrose content where among the three types of sugar, sucrose has a higher percentage in most of the selected rice-based meals compared to other sugars. This is because sucrose is commonly available in the form of granulated sugar, loaf sugar, castor sugar and powdered sugar (MOH, 2012). This table sugar was also known as one of the ingredients used in most local food (Amarra et al., 2016). Based on the recipe, all selected rice-based meals were added with table sugar.

When compared with Chong et al. (2019) study, the total sugar content of Nasi Lemak was 1.5%, while in this study total sugar was higher at 3.21%. This showed that the cooking process does affect the sugar content. Its also may due to the sugar added to the Nasi Lemak since everyone have their own sweetness preferences.

4.3 Correlation between predicted of glycemic response and sugar composition in selected rice-based meals

A Pearson product-moment correlation was run to determine the correlation between dialyzable glucose concentration and sugar composition in selected rice-based meals. The correlation between sugars and dialyzable glucose concentration at 120 minutes was determined.

Table 4.5: Pearson Correlation between the sugar compositions with *in vitro* dialyzable glucose concentration at 120 minutes

Sugar composition	*In-vitro* dialyzable glucose concentration at 120 minutes	
	r	*p-value*
Fructose	-0.789**	0.007
Glucose	-0.866**	0.001
Sucrose	-0.707*	0.022
Total sugar	-0.750*	0.012

**. Correlation is significant at the 0.01 level (2-tailed)

*. Correlation is significant at the 0.05 level (2-tailed)

Based on the Table 4.5, there was a strong, inverse correlation between dialyzable glucose concentration at 120 minutes and sugar composition. The strongest correlation was between dialyzable glucose concentration at 120 minutes and glucose (r= -0.888, p= 0.001). While fructose (r = -0.789, p = 0.007), sucrose (r = -0.707, p = 0.022), and total sugar (r= -0.750, p = 0.012) also show statistically significant strong, negative correlation with dialyzable glucose concentration at 120 minutes. Although there was a strong, negative correlation between the sugars and predicted glycemic response at 120 minutes, only Nasi Lemak was significant ($p < 0.05$) between the samples. Based on Table 4.6, Nasi Lemak was positively, strong correlated with glucose ($r = 0.998$, $p = 0.041$) and fructose ($r = 0.997$, $p = 0.046$). While negatively, strong correlated with sucrose ($r = -0.998$, $p = 0.041$) and total sugar ($r = -0.998$, $p = 0.037$).

The results reflected that the higher the total sugar content (fructose, glucose, sucrose), the lower the prediction of glycemic response. According to The Glycemic Index Foundation (2017), glucose (GI=100) and sucrose (GI=65) were giving high GI values while fructose were categorised as low GI sugar (GI=19). Thus, a high amount of sucrose and glucose supposed to increase the glycemic response. In this study, sucrose was the main contributor to the sugar content where it is may be obtained from the table sugar added or the hydrolyzed starch of the rice itself. Although sucrose is the main sugar found in the rice-based meals, yet it is in a small percentage which is below 5%. Thus, the GI values of the sugar itself may not contributing to the glycemic response. This is due to the rice-based meals is a mixture of protein, fat, fiber, carbohydrates, sugar and others nutrients. This is why sugar is concluded not as the main factor affecting the glycemic response. Other components in the rice-based meals may contributed to the results such as cooking process, fiber content and the addition of fat and protein. These factors are also postulated to contribute to the negative correlation observed.

Table 4.6: Pearson Correlation between the sugar compositions with *in vitro* dialyzable glucose concentration in each selected rice-based meals.

Sample	Sugar Composition (%)							
	Fructose		Glucose		Sucrose		Total sugar	
	r	p-value	r	p-value	r	p-value	r	p-value
White rice	-0.715	0.493	0.715	0.493	0.715	0.493	0.715	0.493
Nasi Lemak	0.997*	0.046	0.998*	0.041	-0.998*	0.041	-0.998*	0.037
Fried rice	-0.394	0.742	-0.392	0.743	0.371	0.758	-0.929	0.242
Chicken porridge	0.578	0.608	0.578	0.608	-0.569	0.614	0.583	0.604
Nasi Kerabu	0.038	0.976	0.023	0.985	0.002	0.999	0.07	0.995

*.Correlation is significant at the 0.05 level (2-tailed)

CHAPTER 5

CONCLUSION, LIMITATION AND FUTURE RECOMMENDATION

5.1 Conclusion

Rice is known as the staple food for Malaysian. Cooked rice is commonly eaten in mixed meals with combination of vegetables and either chicken or fish. Glycemic index (GI) values were known to help people with diabetes, obesity problem, cancers and athletes to plan for their diet for better health status. Yet, the availability of known GI values for rice-based meals food are still limited. While estimating the GI value from published GI value of similar rice-based meals are debatable.

This is due to several factors that could affect the GI values and one of it is sugar content. Sugar included glucose, fructose and sucrose which have its own GI values. Rice itself also contain glucose and sucrose which give the taste to rice. Rice-based meals are commonly added with table sugar and sauces which also contributed to the increase of sugar content. Yet, database for sugar content of rice-based meals also limited. Thus, this study determined the glycemic response based on concentration of dialyzable glucose and sugar composition of selected rice-based in Malaysia.

The predicted glycemic response showed that Nasi Kerabu have the lowest GI values and was significantly different among the studied rice-based meals. However, Nasi Kerabu elicited the highest sugar content compared to others selected rice-based meals. Although The Glycemic Index Foundation (2017) stated that the higher sugar content supposed to give higher glycemic response, it cannot be generalized for the studied rice-based meals because the meals is a mixture of several components such as protein, fat, fiber and others which affect the glycemic response. Thus, a further study need to be done for further explanation of the result. Although Nasi Kerabu have the lowest glycemic response among other selected rice-based meals, it is important to take into account the amount of fat and protein in the meals as the low GI food may contain high energy or high amount of fat that also could reduce the glycemic response (Mann et al., 2007).

5.2 Strengths and Limitations

This study is one of the few study that determined the sugar composition and estimated the glycemic response of selected rice-based meals in Malaysia. Similar study need to be done using *in vivo* methods to identify the actual values of GI and more selection of food need to be included since Malaysia have varieties of rice-based meals.

Furthermore, this study provided an overview of the trend of glycemic response of the selected rice-based meals to be used in future clinical study to estimate the actual GI values. Next, this study also help the health professional for dietary management by exposing that although rice have been categories as high GI food but it is better to consume with fibers, protein and fat because it can lower down the glycemic response.

This study does not measured the content of fiber, fat and protein in each rice-based meals. Thus, a direct conclusion of the main cause of the reduction in the glycemic responses be made. Also, the selected rice-based meals used in this study were purchased from local cafeteria. Thus, the results was reflecting what is available for purchase rather than the controlled amount of nutrient content.

5.3 Recommendations

Future research is needed to determine the actual GI values by using *in vivo* method and sugar composition for future use since this study only give overview about the glycemic response of selected rice-based meals and sugar composition database are still lacking in Malaysia. The nutrient content such as fat, protein and vegetables should also be measured and control to identify the potential factors on the glycemic response and better understanding which factors could affect the GI value of the rice-based meals. Similar study need to be done as there are more variety of rice-based meals in Malaysia and can be add to Malaysian Food database.

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APPENDICES

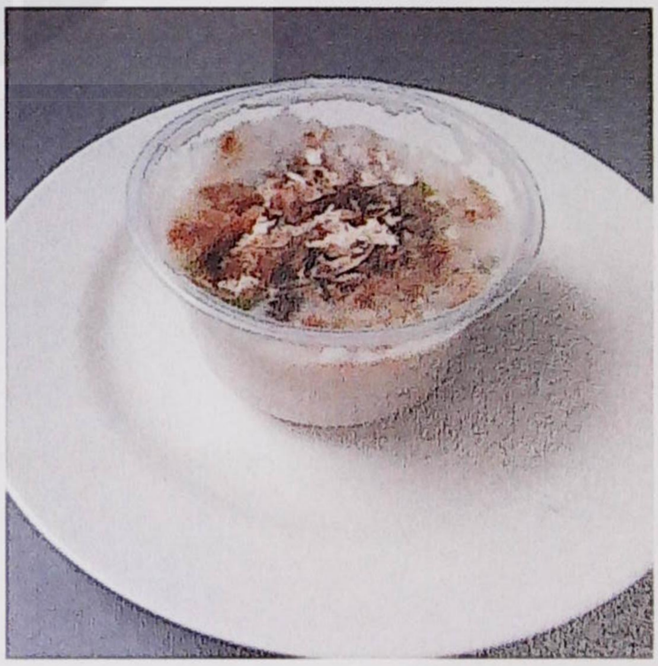
APPENDIX A

Photos of selected rice-based meals

Nasi Kerabu

Nasi Lemak

Fried rice



Chicken porridge

APPENDIX B

General recipes of each selected rice-based meals

Nasi Lemak

Coconut milk steamed rice, hardboiled egg, dried anchovies sambal, small cucumber slice, salt and sugar

Nasi Kerabu

White rice, flower cloth, fried chicken, coconut fish flakes, spiced sauce, salt, sugar, gula melaka, and salted egg.

The Kerabu (Herb Salad) is a mix of finely shredded local herbs and raw vegetables like the following.

Long beans, bean sprouts, cabbage, cucumber, Vietnamese mint, kaffir lime leaves, lemongrass and torch ginger.

Fried rice

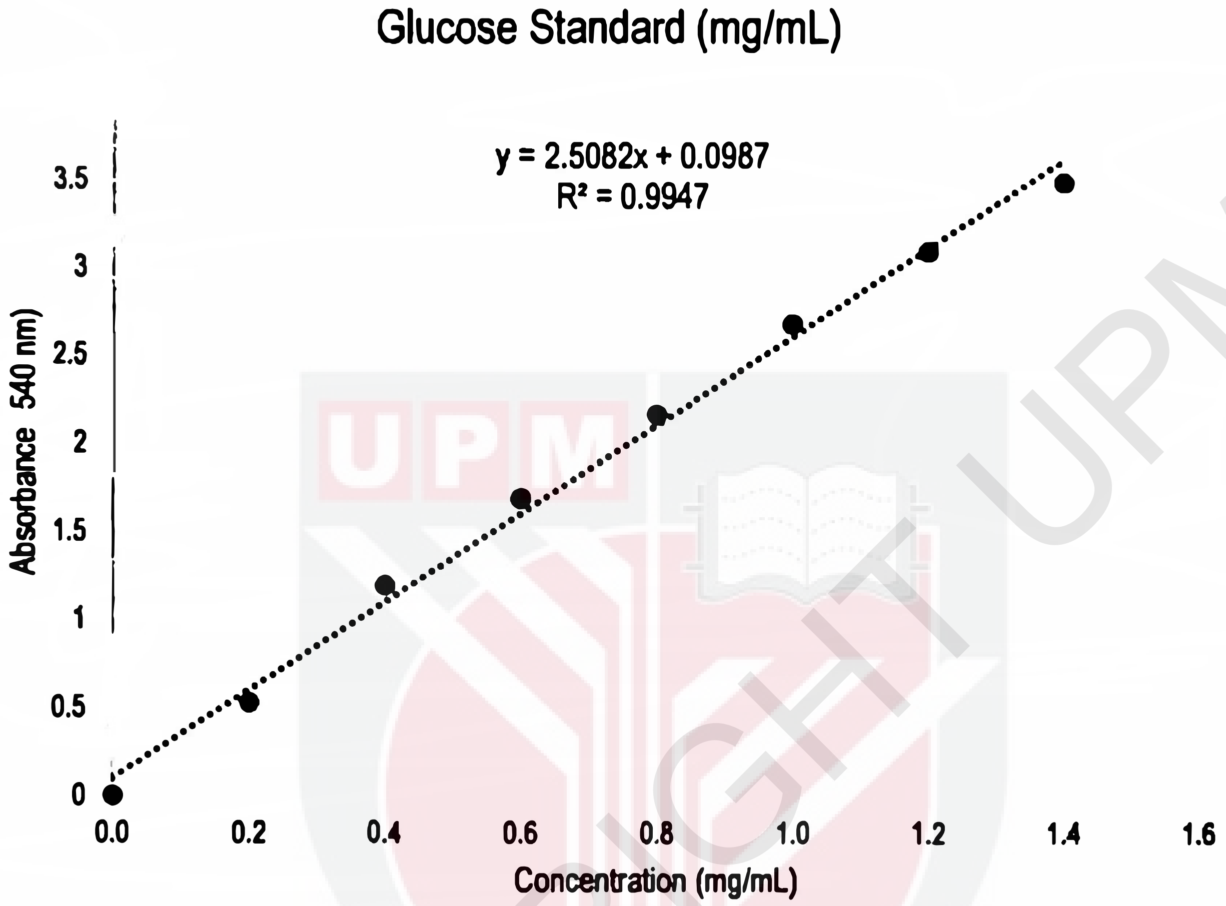
White rice, egg, chicken cube, mixed vegetables (carrots, peas, and corn), oil, garlic, peppers, salt, sugar and sambal shrimp paste.

Chicken porridge

Porridge, shredded chicken breast, fried onions, salt, sugar and parsley.

APPENDIX C

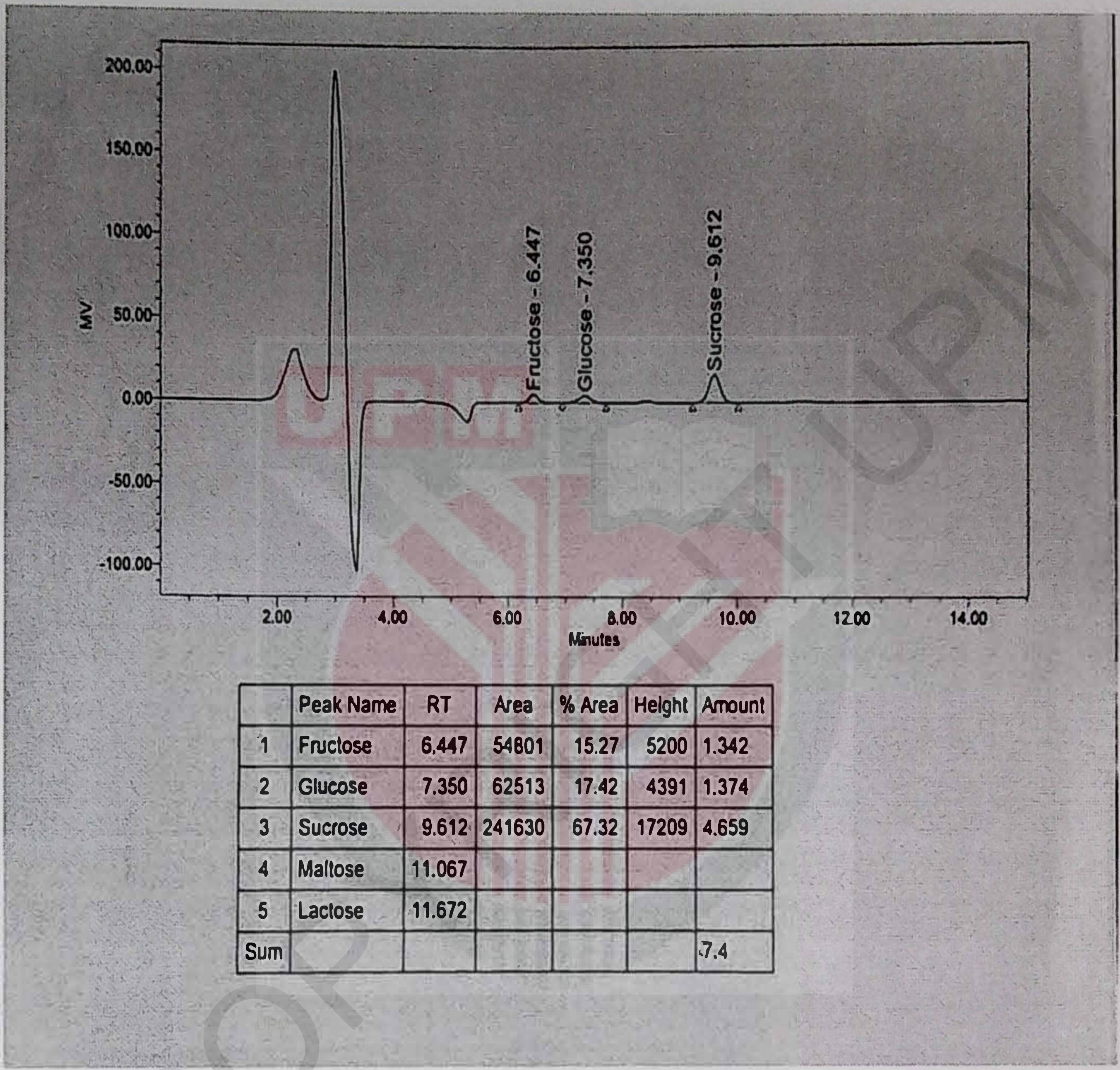
The glucose standard curve of dialyzable glucose concentration



Concentration of glucose (mg/mL)	Absorbance reading (540nm)
0.0	0.000
0.2	0.529
0.4	1.195
0.6	1.689
0.8	2.165
1.0	2.681
1.2	3.092
1.4	3.484

APPENDIX D

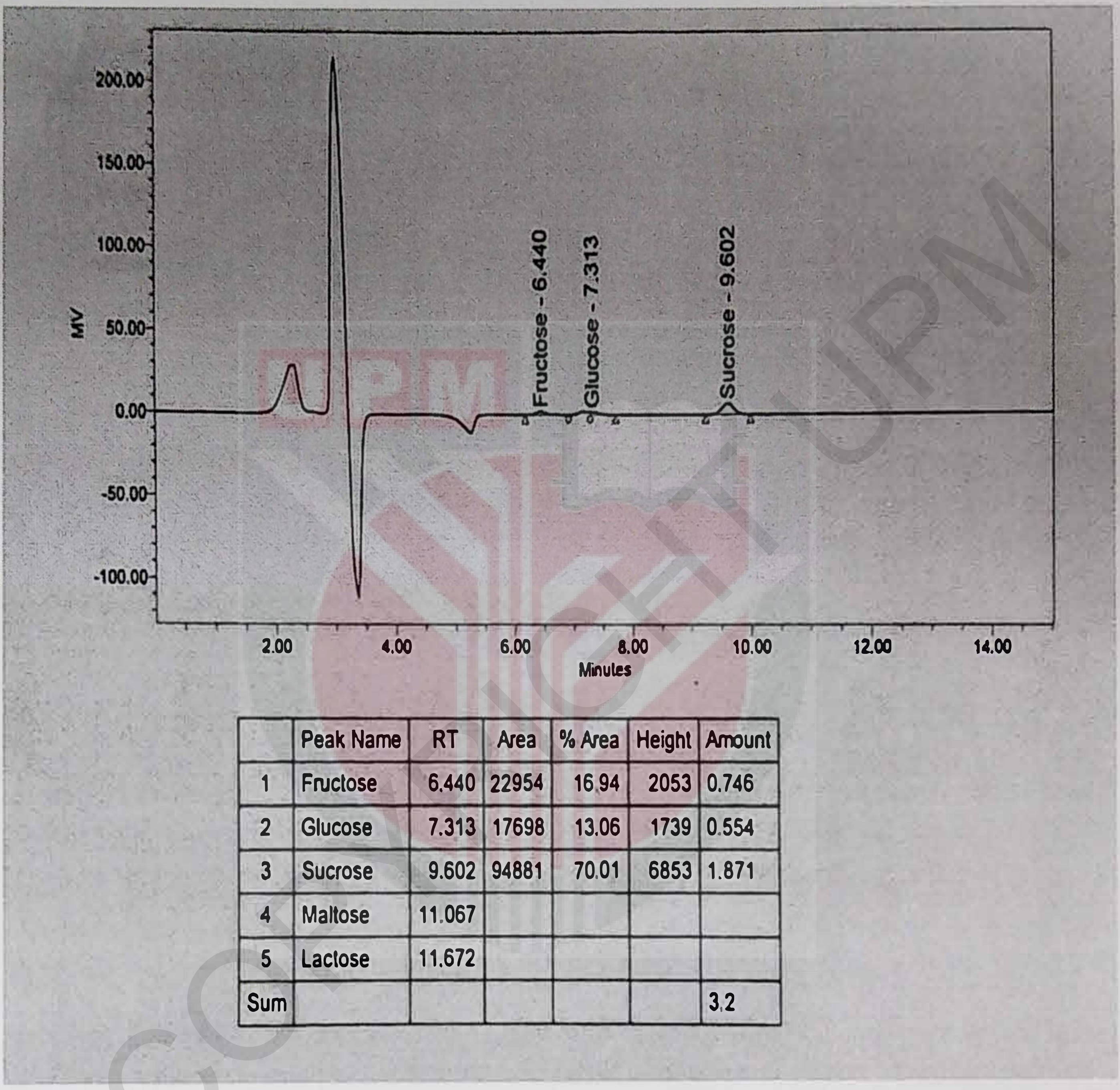
Chromatograph of Nasi Kerabu.



	Peak Name	RT	Area	% Area	Height	Amount
1	Fructose	6.447	54801	15.27	5200	1.342
2	Glucose	7.350	62513	17.42	4391	1.374
3	Sucrose	9.612	241630	67.32	17209	4.659
4	Maltose	11.067				
5	Lactose	11.672				
Sum						7.4

APPENDIX E

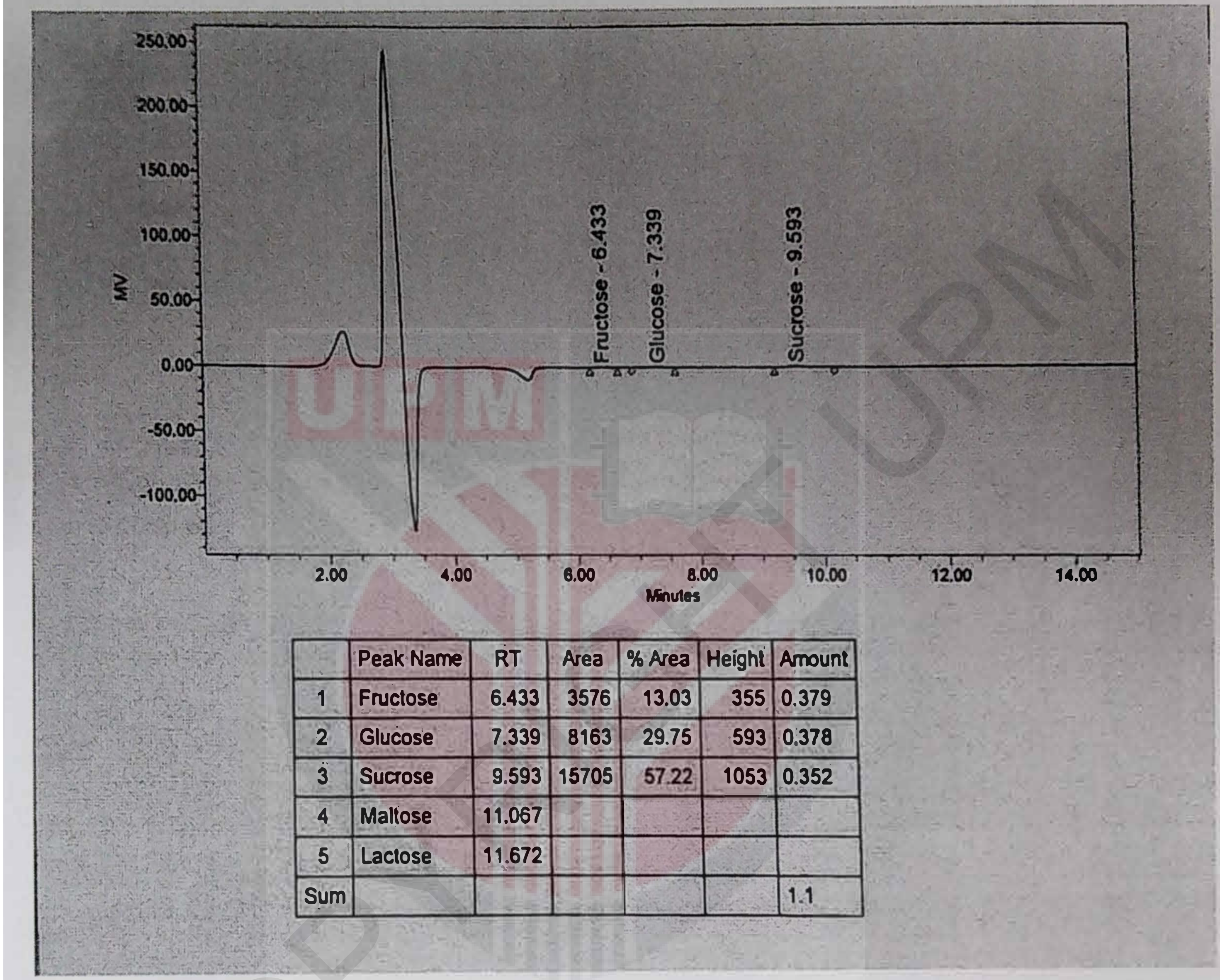
Chromatograph of Nasi Lemak.



	Peak Name	RT	Area	% Area	Height	Amount
1	Fructose	6.440	22954	16.94	2053	0.746
2	Glucose	7.313	17698	13.06	1739	0.554
3	Sucrose	9.602	94881	70.01	6853	1.871
4	Maltose	11.067				
5	Lactose	11.672				
Sum						3.2

APPENDIX F

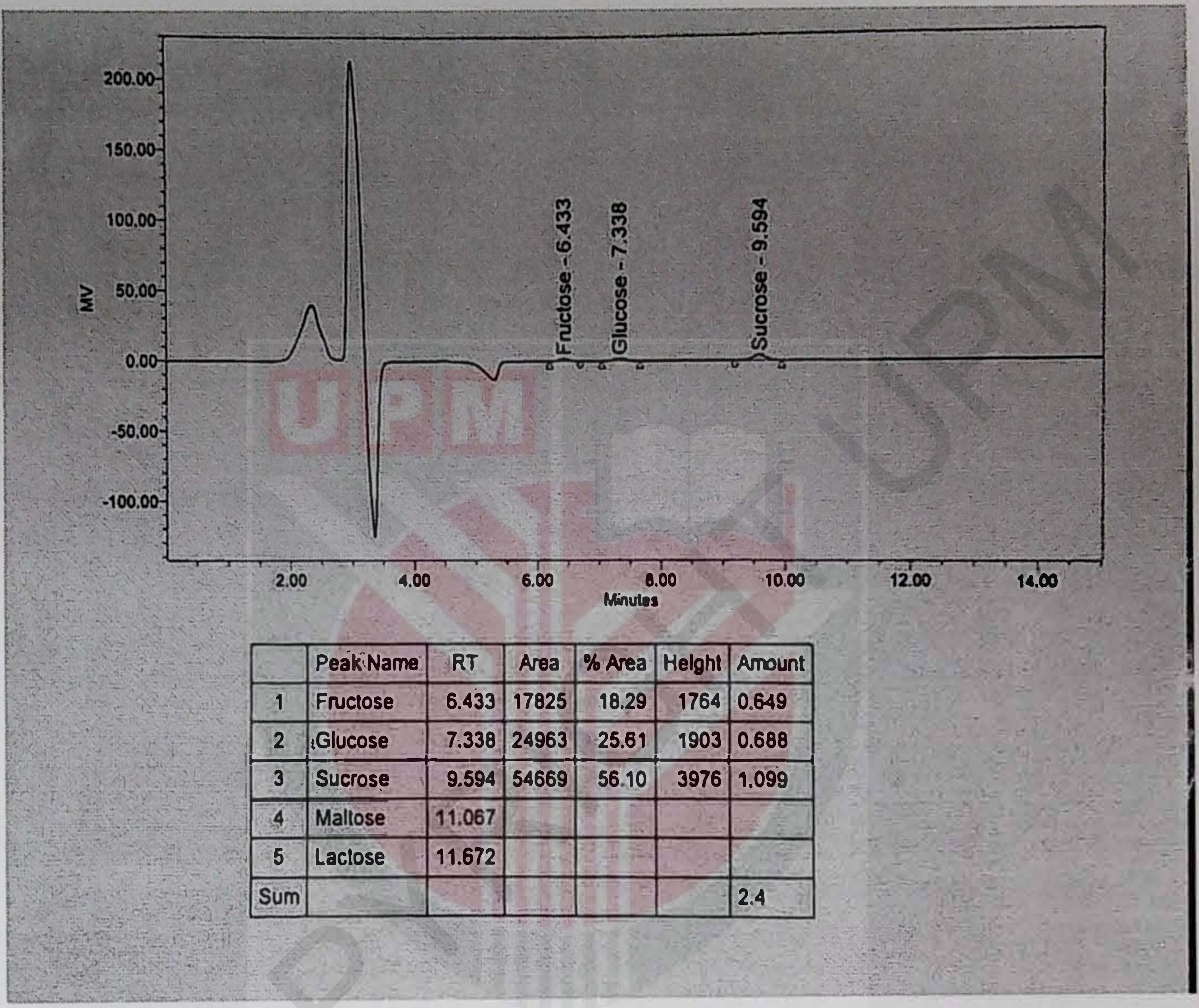
Chromatograph of Fried rice.



	Peak Name	RT	Area	% Area	Height	Amount
1	Fructose	6.433	3576	13.03	355	0.379
2	Glucose	7.339	8163	29.75	593	0.378
3	Sucrose	9.593	15705	57.22	1053	0.352
4	Maltose	11.067				
5	Lactose	11.672				
Sum						1.1

APPENDIX G

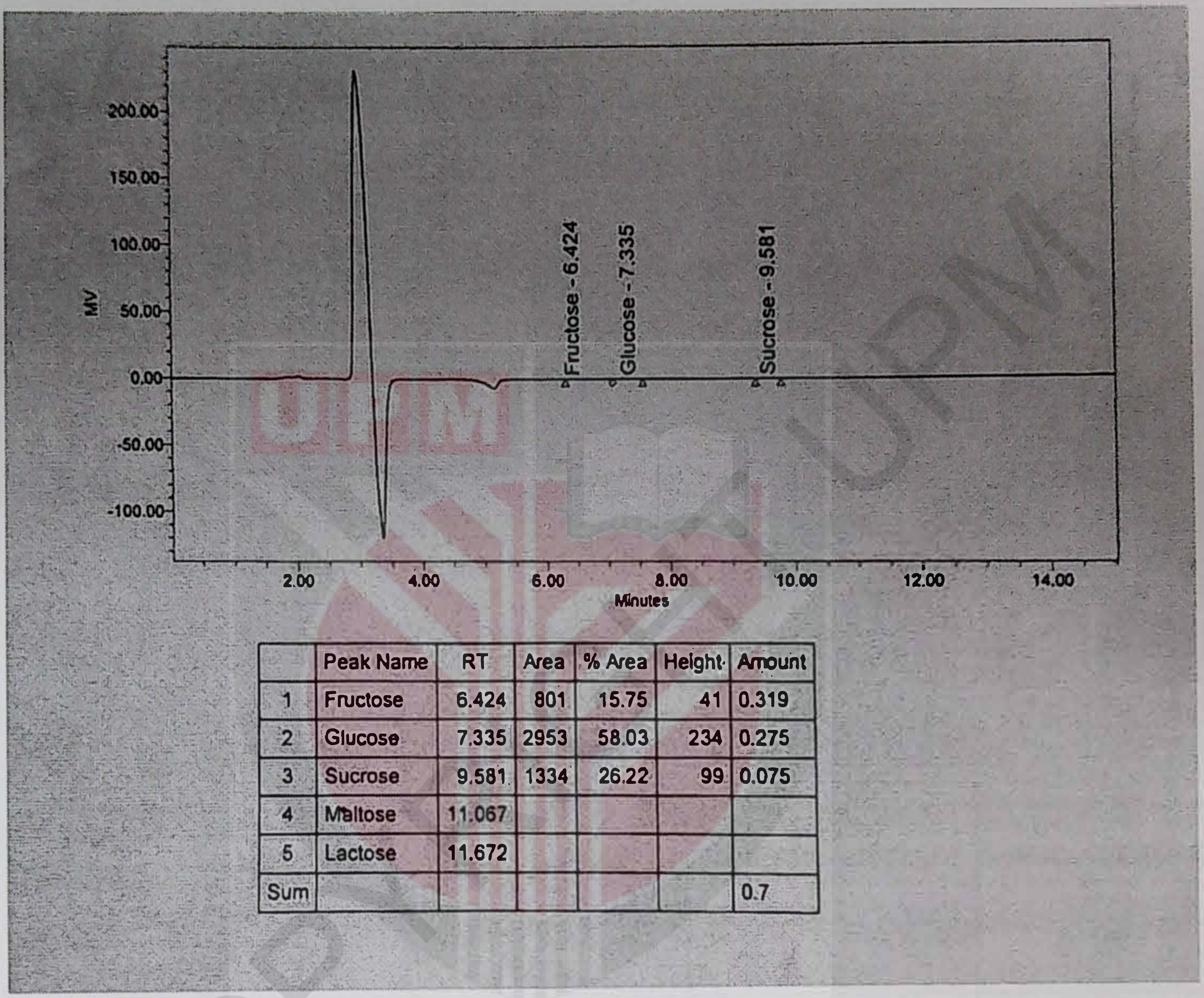
Chromatograph of Chicken porridge.



	Peak Name	RT	Area	% Area	Height	Amount
1	Fructose	6.433	17825	18.29	1764	0.649
2	Glucose	7.338	24963	25.61	1903	0.688
3	Sucrose	9.594	54669	56.10	3976	1.099
4	Maltose	11.067				
5	Lactose	11.672				
Sum						2.4

APPENDIX H

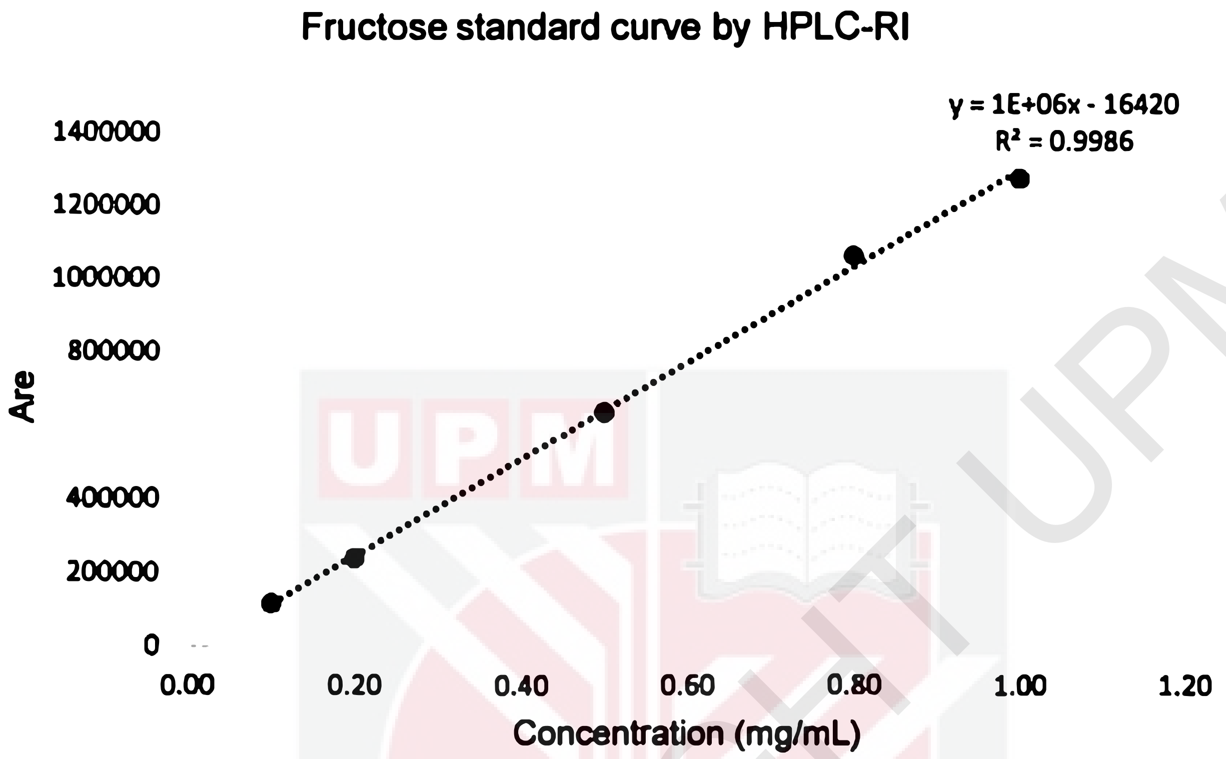
Chromatograph of white rice.



	Peak Name	RT	Area	% Area	Height	Amount
1	Fructose	6.424	801	15.75	41	0.319
2	Glucose	7.335	2953	58.03	234	0.275
3	Sucrose	9.581	1334	26.22	99	0.075
4	Maltose	11.067				
5	Lactose	11.672				
Sum						0.7

APPENDIX I

Fructose standard curve by HPLC-RI.

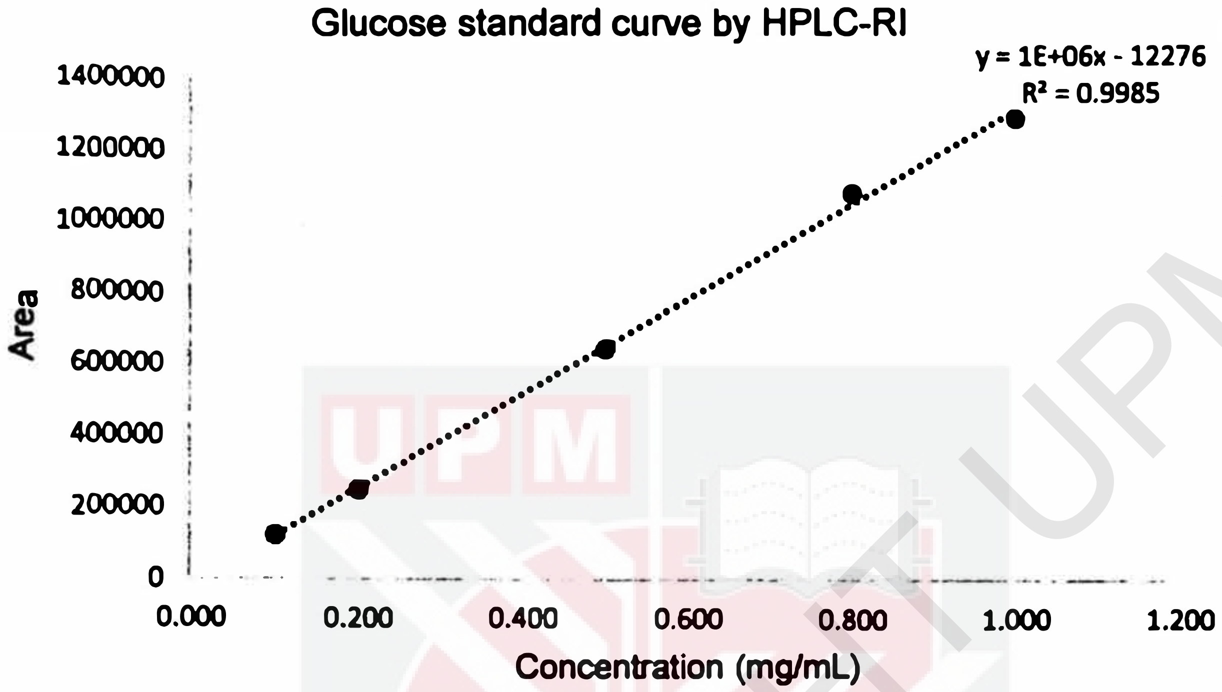


Sample Name	Peak Name	RT	Area	% Area	Height	Concentration
Std 1	Fructose	6.387	115369	18.81	11872	0.100
Std 2	Fructose	6.391	238620	19.07	24417	0.200
Std 3	Fructose	6.405	632222	19.55	63888	0.500
Std 4	Fructose	6.415	1059135	20.85	104193	0.800
Std 5	Fructose	6.418	1268602	20.33	123349	1.000

Std = Standard

APPENDIX J

Glucose standard curve by HPLC-RI.

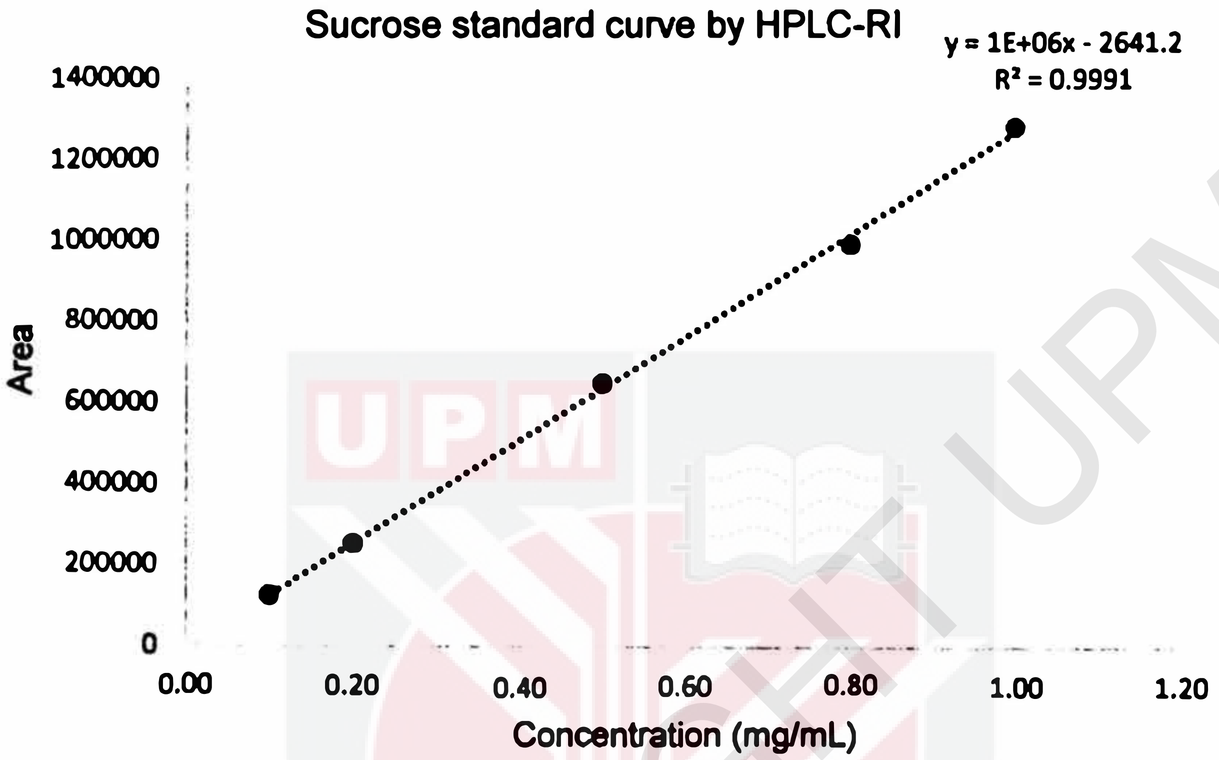


Sample Name	Peak Name	RT	Area	% Area	Height	Concentration
Std 1	Glucose	7.298	122891	20.03	10123	0.100
Std 2	Glucose	7.302	250163	19.99	20454	0.200
Std 3	Glucose	7.318	650809	20.12	52595	0.500
Std 4	Glucose	7.331	1091580	21.49	86197	0.800
Std 5	Glucose	7.337	1305269	20.92	101834	1.000

Std = Standard

APPENDIX K

Sucrose standard curve by HPLC-RI.

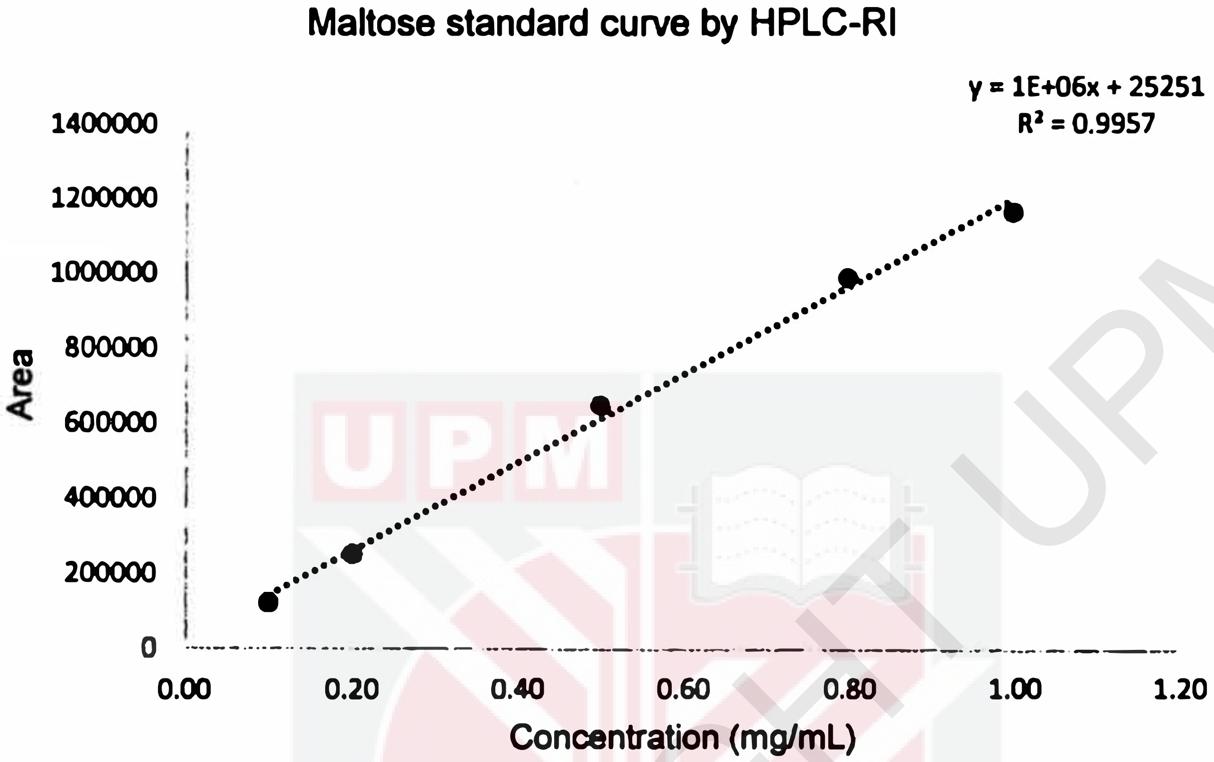


Sample Name	Peak Name	RT	Area	% Area	Height	Concentration
Std 1	Sucrose	9.562	125152	20.40	9620	0.100
Std 2	Sucrose	9.568	254360	20.32	19316	0.200
Std 3	Sucrose	9.591	655817	20.28	49093	0.500
Std 4	Sucrose	9.606	1005388	19.79	72177	0.800
Std 5	Sucrose	9.618	1300273	20.84	91344	1.000

Std = Standard

APPENDIX L

Maltose standard curve by HPLC-RI.

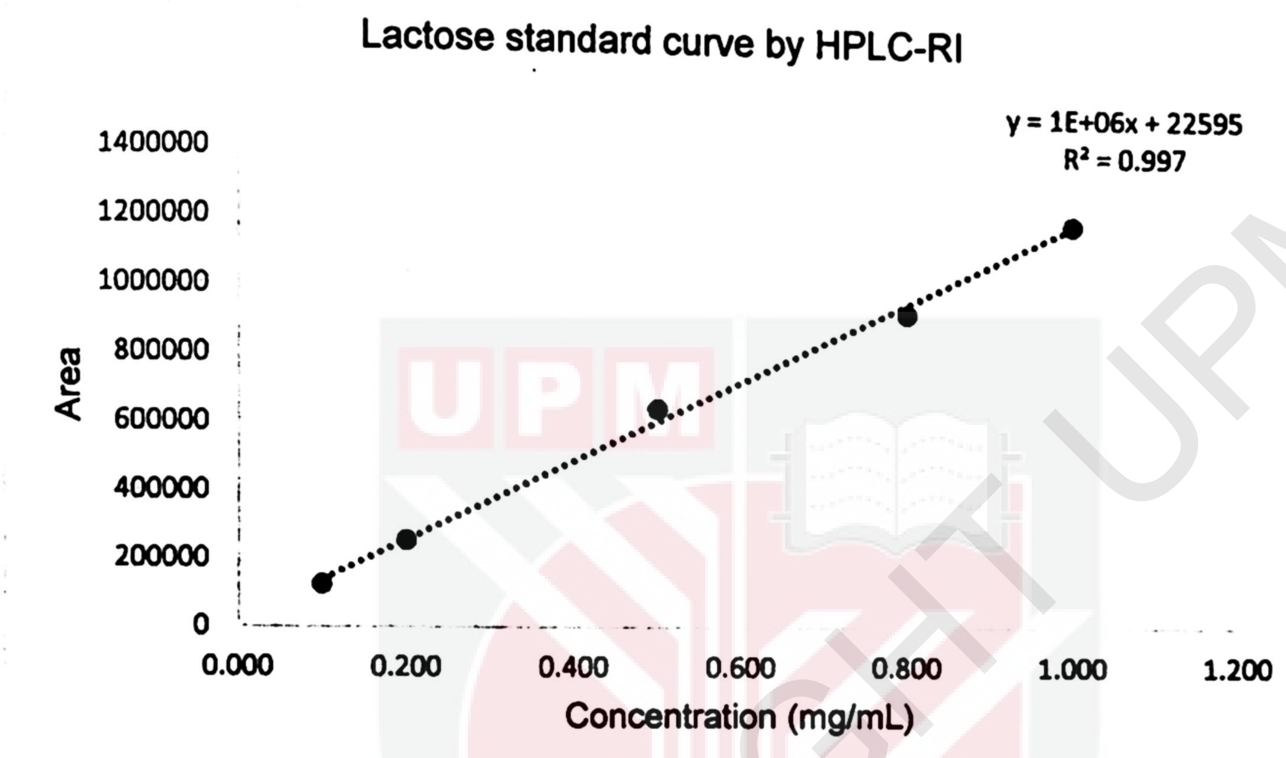


Sample Name	Peak Name	RT	Area	% Area	Height	Concentration
Std 1	Maltose	11.067	124592	20.31	6694	0.100
Std 2	Maltose	11.082	254168	20.31	13433	0.200
Std 3	Maltose	11.132	652767	20.18	33506	0.500
Std 4	Maltose	11.172	996524	19.62	50011	0.800
Std 5	Maltose	11.195	1173110	18.80	58359	1.000

Std = Standard

APPENDIX M

Lactose standard curve by HPLC-RI.



Sample Name	Peak Name	RT	Area	% Area	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

Std = Standard