



UNIVERSITI PUTRA MALAYSIA

***IN VITRO GLYCAEMIC INDEX OF SELECTED PACKAGED BAKED
PRODUCTS IN THE LOCAL MARKET***

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PRODUCTS IN THE LOCAL MARKET**

BY

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

This project entitled “*In vitro* glycaemic index of selected packaged baked products in the local market” was prepared by Nur ‘Aqilah Salehah Mohd Aziss and submitted to the Faculty of Medicine and Health Sciences as a partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously or concurrently for any other degree at UPM or other institutions.



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LIST OF ABBREVIATIONS AND ANNOTATIONS

%	Percentage
µL	Microlitre
AMG	Amyloglucosidase
AOAC	Association of Official Analytical Chemists
AUC	Area Under Curve
°C	Degree Celsius
eGI	Estimated Glycaemic Index
eGL	Estimated Glycaemic Load
FAO	Food and Agriculture Organization
g	Gram
GI	Glycaemic Index
GL	Glycaemic Load
GOPOD	Glucose oxidase/oxidase
HI	Hydrolysis Index
M	Molar
MANS	Malaysian Adult Nutrition Survey
mg	Milligram
min	Minutes
mL	Millimetre
NHMS	National Health Morbidity Survey
NIP	Nutrition Information Panel
Nm	Nanometer
rpm	Rotations per minutes
RSH	Rate of Starch Hydrolysis
U	Unit
WHO	World Health Organization

ABSTRACT

***IN VITRO* GLYCAEMIC INDEX OF SELECTED PACKAGED BAKED PRODUCTS IN THE LOCAL MARKET**

Nur 'Aqilah Salehah Mohd Aziss

Foods with a high glycaemic index have been linked to a higher risk of diabetes, obesity, cancer, and cardiovascular disease. Packaged baked products are commonly consumed in Malaysia. However, there is a lack of information about the glycaemic index value of packaged baked products in Malaysia. Therefore, this study aims to determine the *in vitro* glycaemic index of selected packaged baked products available in the local market. *In vitro* method was applied as it is an inexpensive and alternative method to *in vivo*. A total of 6 samples of packaged baked products (cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filler) were sampled from the local market in Serdang, Selangor. The samples were assayed for moisture, fat, total available carbohydrate and total starch content. A validated *in vitro* digestion method was used to determine the estimated glycaemic index and glycaemic load. Among the studied samples, moisture content was the highest in fine wholemeal bread (35.73 ± 0.81). Fat content was the highest in butter cake (32.10 ± 1.02). Cookies with cream have the highest total available carbohydrate (18.11 ± 0.47). Cream crackers had the highest total starch content (58.63 ± 0.65) while the lowest in chiffon cake (28.72 ± 0.71). The highest eGI value was observed in fine wholemeal bread (83.79 ± 0.92) and the lowest was in butter cake (50.79 ± 0.60). eGL value was highest in fine wholemeal (25.30 ± 0.28) and butter cake has the lowest eGL value (7.31 ± 0.09). Significant correlations exist between eGI and eGL ($p < 0.01$). eGI and fat content was significantly correlated too. However, no correlation exists between eGI and total available carbohydrate content among the selected packaged baked products. Nutrients such as starch and fat influence the *in vitro* glycaemic response of the samples studied.

Abstrak

IN VITRO GLISEMIK INDEKS DALAM PRODUK BAKERI BERBUNGKUS TERPILIH YANG TERDAPAT DI PASARAN TEMPATAN

Nur 'Aqilah Salehah Mohd Aziss

Makanan dengan indeks glisemik tinggi dikaitkan dengan risiko diabetes, obesiti, barah, dan penyakit kardiovaskular yang lebih tinggi. Pengambilan produk bakeri berbungkus adalah satu kebiasaan di Malaysia. Walau bagaimanapun, maklumat tentang indeks glisemik dalam produk bakeri berbungkus masih kurang. Oleh itu, kajian ini bertujuan untuk menentukan indeks glisemik secara *in vitro* dalam produk bakeri berbungkus terpilih yang terdapat di pasaran tempatan. Kajian *in vitro* digunakan kerana ia adalah kaedah yang murah dan merupakan kaedah alternatif bagi *in vivo*. Sebanyak 6 sampel produk bakeri berbungkus dipilih (krim kraker, biskut berkrim, kek mentega, kek chifon, roti bijirin penuh halus dan roti berinti coklat) dibeli dari pasaran tempatan di Serdang, Selangor. Sampel diuji untuk kelembapan, lemak, jumlah karbohidrat yang ada dan jumlah kanji. Kaedah penghadaman *in vitro* yang telah disahkan digunakan untuk menentukan indeks glisemik dianggarkan dan beban glisemik. Di antara sampel yang dikaji, kandungan lembapan tertinggi adalah roti bijirin penuh halus (35.73 ± 0.81). Kandungan lemak tertinggi didapati di dalam kek mentega (32.10 ± 1.02). Biskut berkrim mempunyai jumlah karbohidrat tertinggi (18.11 ± 0.47). Krim kraker mengandungi kanji tertinggi (58.63 ± 0.65) manakala yang paling rendah adalah kek chifon (28.72 ± 0.71). Nilai eGI tertinggi didapati pada roti bijirin penuh halus (83.79 ± 0.92) dan yang terendah adalah pada kek mentega (50.79 ± 0.60). Nilai eGL adalah tertinggi dalam roti bijirin penuh halus (25.30 ± 0.28) dan kek mentega mempunyai nilai eGL terendah (7.31 ± 0.09). Hubungan yang signifikan wujud antara eGI dan eGL ($p < 0.01$). Kandungan eGI dan lemak juga berhubung secara signifikan. Walau bagaimanapun, tidak ada hubungan antara eGI dan jumlah kandungan karbohidrat yang ada di antara produk bakeri berbungkus. Nutrien seperti kanji dan lemak didapati mempengaruhi tindak balas glisemik *in vitro* daripada sampel bakeri yang dikaji.

CHAPTER 1

INTRODUCTION

1.1 Background

Packaged baked products are food made from a dough or batter that is baked such as bread, cake or cookie that is packaged. Baked products are commonly consumed in between meals time (Louie & Rangan, 2018) or during breakfast (di Giuseppe et al., 2012). According to the Malaysian Adults Nutrition Survey (MANS) in 2014, cream crackers were among the top ten daily food items consumed while white bread was the top ten weekly consumed foods by Malaysian adults. Packaged baked products included in the national survey were bread, cream crackers, buns, cakes, flavoured/cream/filled cookies, wholemeal bread and pastries that had a prevalence of consumption ranging from 10% up to 78% (Institute for Public Health, 2014). Packaged baked products are often high in added sugar and among the major contributors to energy (Bernstein et al., 2016; Johnson et al., 2017; Luo et al., 2019; Russo et al., 2020).

The glycaemic index (GI) is defined as the area under the blood glucose response curve of 50g carbohydrate portion of a test food expressed as a percentage of the area after taking the same amount of carbohydrate from a standard or reference food (Jenkins et al., 1981). GI can be classified into three categories where GI value below 55 is defined as low, 56-69 as moderate and 70 and above as high (International Standard ISO 26642:2010, 2010). Both high and low GI values meals are argued to provide a useful effect in enhancing athletic performance based on the time consumed (Chua et al., 2019; Goto, 2016; Wolever, 2006; Wong et al., 2017). Low GI diets are agreed to be relevant in the prevention and management of chronic

diseases such as diabetes mellitus (Augustin et al., 2015; Gomes et al., 2017; Hasbullah et al., 2020; Zafar et al., 2019) and coronary heart disease (Livesey & Livesey, 2019; Shahdadian et al., 2019; Yu et al., 2016).

Determining GI can be done by using *in vivo* as well as *in vitro* studies. *In vivo* method is the gold standard in determining GI and as a prerequisite in applying the GI symbol (FAO/WHO, 1998; Glycemic Index Foundation, 2018; International Standard ISO 26642:2010, 2010). Meanwhile, *in vitro* method is usually being used in food product development to reformulate ingredients for better GI values (Doménech-Asensi et al., 2016; Luo et al., 2019; Wee & Henry, 2020). *In vitro* studies mimic the physicochemical processes involved in carbohydrate digestion that occur in the upper gastrointestinal tract of humans (Ferrer-Mairal et al., 2012). This study design helped in reducing the demands of humans and preventing the complexities related to human management involved *in vivo* experimental design (Barine & Yorte, 2016). *In vitro* study is also inexpensive, non-invasive and can apply to a large number of samples. However, there is no standardized methodology for *in vitro* study of GI and various *in vitro* studies can be found with many differences.

1.2 Problem Statement

According to the Household Expenditure Survey Report 2019, Malaysians spent 13.8% of food and non-alcoholic beverages expenses to purchase bread and other cereals (Department of Statistics Malaysia, 2020). Russo et al. (2020) discovered that baked goods and breakfast foods were among the top ten sources of added sugar. In the Malaysian Adults Nutrition Survey (MANS) 2014, sugar was among the top five foods that Malaysian consumed daily (55.9%). Besides, the National Health and Morbidity Survey (NHMS) 2017 revealed that the increment of sugar intake in adolescents in Malaysia was observed from 29.5g in 2012 to 40.7g in 2017 (Institute for Public Health, 2017). Excessive intake of sugar is associated with obesity and non-communicable diseases such as diabetes and heart disease. Based on NHMS 2019 data, an alarming increase in diabetes trends can be observed in Malaysia as the prevalence of diabetes in 2011 (11,2%) increased to 13.4% in 2015 and up to 18.3% in 2019. Similarly, the trend of obesity in Malaysia continues to rise from 15.1% in 2011 to 17.7% in 2015 and 19.7% in 2019 (Institute for Public Health, 2020).

In Malaysia, the interest in GI has been increasing from identifying GI of frequent Malaysian foods to the association of GI diet with disease prevention and management. Robert et al. (2006) managed to determine selected individual Malaysian foods where rice, kuih teow and sweet potato were identified as high GI foods and bananas as low GI food. GI for four common mixed meals from three ethnic groups in Malaysia which are coconut milk rice (50 ± 5), lacy pancake with chicken curry (51 ± 5), flatbread with dhal curry (64 ± 2) and fried noodles (61 ± 6) was also discovered by Robert & Ismail (2012).

In addition, Recommended Nutrient Intakes (RNI) for Malaysia 2017 has compiled the glycaemic index value of selected Malaysian foods from various studies done on frequently consumed food in Malaysia. Moreover, a study that highlights the role of low GI and GL diet in the prevention of gestational diabetes mellitus was also done recently by Hasbullah et al. (2020). Most of the studies related to GI in Malaysia used *in vivo* studies either involving normal healthy subjects (Bakar et al., 2019; Chapagai et al., 2016; Ghani et al., 2018; Hasbullah et al., 2020; Mohd Yusof et al., 2005, 2009; Robert et al., 2006, 2008, 2016) or patients (Mohd Yusof et al., 2010; Robert & Ismail, 2012; Shyam et al., 2013).

Despite this, the GI value of packaged baked products available in the local market is still lacking. While being the gold standard in determining GI, *in vivo* study may be regarded as resource-demanding and expensive when a large food sample is tested. Thus, there is a need for the alternative method with simpler and economical procedures to determine GI using *in vitro* study. According to Augustin et al. (2015), a list of glycaemic index values of foods is important to be developed for both health professionals and the consumer to use as a guide. Therefore, this study aims to determine *in vitro* glycaemic index and glycaemic load of selected packaged baked products (cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filling) from the local market in Serdang, Selangor.

1.3 Significance of Study

Most recently, there was a study done locally to determine the GI using *in vitro* study of selected sugars, rice-based meals and traditional kuih (Mohamad Nawawi et al., 2020). However, no study has been published on the determination of the glycaemic index of packaged baked products from the local market using an *in vitro* study method. This study would give benefits in several aspects. Firstly, this study will add the body of knowledge regarding *in vitro* study of GI. This study would also provide the estimated GI value of packaged baked products in the local market.

The findings of this study could be used as baseline data for future research in understanding the glycaemic index in the packaged baked products in the local market. This study may also give information for consumer education in choosing healthy food choices available in the local market. Furthermore, the information from this study can be used by nutritionists and dieticians during the dietary advice to their patients and clients.

1.4 Objectives

1.4.1 General Objective

To determine *in vitro* glycaemic index and glycaemic load of selected packaged baked products (cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filling) in the local market.

1.4.2 Specific Objectives

- i. To identify and compare the information available on the Nutrition Information Panel (NIP) of the selected packaged baked products in the local market obtained from the market survey.
- ii. To determine the moisture, total starch, total available carbohydrate and fat content of selected packaged baked products using the standard methodology.
- iii. To determine the estimated glycaemic index of selected packaged baked products in the local market based on *in vitro* method.
- iv. To determine the glycaemic load of selected packaged baked products in the local market based on the estimated glycaemic index.
- v. To determine the correlation between the estimated glycaemic index with analyzed macro nutrients content (total available carbohydrate, fat) of selected packaged baked products.
- vi. To determine the correlation between the estimated glycaemic index with the glycaemic load.

1.4.3 Hypotheses

1. Wholemeal bread has the highest means of estimated glycaemic index and glycaemic load value among the selected packaged baked products (cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filling).
2. There is a significant correlation between the estimated glycaemic index with analysed macro nutrients content (total available carbohydrate, fat) of selected packaged baked products.
3. There is a significant correlation between the estimated glycaemic index with the glycaemic load.

CHAPTER 2

LITERATURE REVIEW

2.1 Packaged Baked Products

2.1.1 Consumption of Packaged Baked Products

Baked products are foods made from dough or batter that is baked. The most common type of baked product is bread. There are also other types of baked products such as biscuits, cakes and pastries. Usually, baked products are consumed during snack time (Louie & Rangan, 2018) or breakfast (di Giuseppe et al., 2012). By packaging, the baked product can extend the shelf life, minimize the texture changes (Galić et al., 2009) decrease food waste and increase food safety (Sand, 2019). At present, packaged baked products can easily be found in the local supermarket as one of the convenience foods. According to the Household Expenditure Survey Report 2019, Malaysian households spent RM 783 monthly on food and non-alcoholic beverages including RM108 for bread and other cereals (Department of Statistics Malaysia, 2020). In addition, the consumption of packaged baked products in Malaysia has a varied range of prevalence. In the Malaysian Adults Nutrition Survey (MANS) 2014, bread had the highest consumption with a prevalence of 78.2% followed by cream crackers (65.3%), bun (46.3%), cakes (38.5%), cookies (31.1%), wholemeal bread (21.8%) and pastries (10.7%). To highlight, cream crackers were among the top ten daily food products eaten, while white bread was the Malaysian adults' top ten weekly foods consumed (Institute for Public Health, 2014).

Packaged baked products are known as food high in added sugar and among the main contributors to energy. Sucrose is a commonly used type of sugar in baked products to enhance sweetness. Luo et al. (2019) stated that sucrose also helps in

bulking, retaining moisture and extending the shelf life of the baked products. A cross-sectional study done by Russo et al., (2020) to identify the top 10 sources of added sugar and types of high GI foods in the diet of US children by race and ethnicity discovered that baked goods and breakfast foods were most frequent in the top 10 sources of added sugar. Similarly, Bernstein et al. (2016) revealed that bakery products were the third-highest food group containing free sugar ingredients with a mean of 17g total sugar when determining the amount of free sugar in Canadian pre-packaged foods using a food composition database. A secondary analysis among 2182 Australian children and adolescents in determining main discretionary choice subgroups that contribute to energy and key nutrient intake identified that cakes, muffins and slices were among major contributors to energy intake (4%), saturated fat (5%) and added sugars (11%) (Johnson et al., 2017).

2.1.2 Ingredients in Packaged Baked Products

Flour, fats and sugar are the main ingredients in making baked products. According to Mamat and Hill (2018), flour determines the consistency whereas fat exhibits a lubricating effect and prevents the formation of gluten by coating the starch granules. Meanwhile, sugar influences the various rheological properties of dough and biscuits. The study also mentioned that wheat flour is the most common type of flour being used in baked products with lard, beef fats and hydrogenated vegetable oils as common fats used. Other than sucrose, 10-20% by weight of the total sugars is usually added with reducing sugars. Apart from these main ingredients, dried fruits and nuts are widely used to create a variety of flavours, appearances and textures in baked products. As mentioned by Manley (2011), when including dried fruits and nuts, extra attention should be given to the flavour needed, size and cleanliness of the added substances by the baker. Commonly, dried fruits used are dried grapes, dates, cherries while tree nuts, peanuts, Arachis and ground nuts are examples of nuts used in baked products. Also, fruit pastes and syrups are normally added to baked products as a colouring agent or as fillings (Manley, 2011).

The healthier option of baked products is currently a popular choice among consumers. Substitution of less healthy ingredients with healthier ingredients will provide a healthier baked product. Soong et al. (2015) mentioned that oat and barley flours can be used as a substitution of wheat flour in muffins as they blunted the postprandial glycaemic response. By the same token, replacing 10% of refined wheat flour with fenugreek seed powder significantly reduced the glycaemic response in buns and flatbreads (Robert et al., 2016). Moreover, Srikaeo and Thongta (2015) discovered that bread using palm and coconut sugars instead of sugarcane provided a better starch digestion rate and lower estimated GI values. Doménech-Asensi et al.

(2016) did a study on reformulating four different bakery products (cookies, croissants, Spanish muffins and Spanish sponge cake) with healthier properties. The modified bakery products with supplemented inulin, replacement of margarine and sunflower oil with high oleic sunflower oil and reduced total fats and simple sugar added had better nutritional properties than the original recipe. Despite various successful studies done in replacing less healthy ingredients with a nutritionally better ingredient, the challenge lies in combining these better components in given products (Peris et al., 2019).



2.1.3 Moisture, Carbohydrate and Fat Content of Baked Products

Moisture content is important to baked products. According to Ibrahim et al. (2020), moisture content can influence the freshness of the food and the stability of the food storage duration. Based on the Malaysian Food Composition Database 1997, bread has up to 38 g moisture per 100g bread. Whereas, the cake has a lower moisture content which is 14.8g to 28.1g per 100g depending on the type of cake. Meanwhile, crackers and cookies only have 1.4g to 5.3g moisture per 100g depending on the types (Institute for Medical Research, 1997). Mamat & Hill (2018) stated that food that has less than 4% moisture, tend to have a longer shelf life, up to six months or longer.

Sugar and flour used in making baked products could contribute to the carbohydrate content of the final product. As determined by the Malaysian Food Database, crackers, biscuits and cookies contained the most carbohydrate for almost 76g per 100g, higher than bread (around 50g per 100g) and cakes (around 57g per 100g) (Institute for Medical Research, 1997). When calculating the carbohydrate, carbohydrate by differences was used in the Malaysian Food Database 1997 where the 100 minus the sum of ash, moisture, fat, protein and total dietary fibre.

As mentioned above, fat is one of the main ingredients in baked product. Fat content in baked products varies across the type of baked products. In the Malaysian Food Composition Database 1997, among bread, crackers and cakes, the highest fat content can be seen in cakes with up to 20g fat per 100g. Following cakes are crackers, biscuits and cookies with slightly lower fat at up to 17g per 100g. Meanwhile, bread only contained up to 1 g per 100g (Institute for Medical Research, 1997). Fats play a crucial role in bread production, providing desired characteristics such as better gas retention, a moist mouthfeel, and flavour (Lau et al., 2016).

2.1.4 Glycaemic Index of Baked Products

Based on the International Tables of Glycemic Index and Glycemic Load Values: 2008 by Atkinson et al. (2008), baked products such as bread, breakfast cereals, rice and snack products, including whole-grain version categorised in low, moderate and high GI value. There is a wide range of bread included in the table with various GI values such as wheat bread (52), wholemeal bread (68 ± 9), white wheat bread (69 ± 5) and fruit loaf (57 ± 6). Next, a cake such as banana cake with sugar has a GI value of 47 ± 8 and a pound cake has a GI value of 38 ± 5 . Both filled and flavoured biscuits had different GI values such as wheat biscuits (61) and cocoa flavour biscuits (46 ± 4). Lastly, crackers showed a mixed GI value where cream cracker is 65 ± 11 and grain cracker is 49 ± 7 .

Various factors can influence the GI in baked products mainly the ingredient. Flour is the main ingredient in baked products. If different flour was used, a different GI is produced. Soong et al. (2015) also did a study in GI in muffins with different types of flours. The study discovered that rice muffin has the highest GI (79.1) followed by wheat and corn muffin (both 74.4), barley muffin (55.4) and oat muffin (53.6). Similarly, if a different type of grains was incorporated in the flour, the GI value produced also varied. A study was done by Mohd Yusof et al. (2009) in four types of commercialized bread observed that wholemeal bread has the highest GI (85 ± 5.9) followed by white bread (83 ± 6.5), wholemeal with oatmeal bread (67 ± 6.9) and multigrain bread (56 ± 6.2). However, the authors mentioned that the reference food, glucose with an assigned GI value of 100 was not significantly different from wholemeal bread and white bread. Likewise, when a different type of fat was used, the GI value could change. Lau et al. (2016) found that bread baked with coconut oil

has a lower glycaemic response compared to bread baked with olive oil, grapeseed oil and butter.



2.2 Glycaemic Index

2.2.1 Concept of Glycaemic Index and Glycaemic Load

The glycaemic index concept (GI) was introduced by Jenkins et al. (1981). Glycaemic index is defined as the area under the blood glucose response curve of 50g carbohydrate portion of a test food expressed as a percentage of the area after taking the same amount of carbohydrate from a standard or reference food. The reference food used usually is glucose or white bread. GI can be used as a method to classify foods based on their blood glucose-raising potential (FAO/WHO, 1998). According to International Standard ISO 26642:2010, (2010), GI can be categorized into three categories. A GI value below 55 is defined as low, 56-69 as moderate and 70 and above as high. A compilation of the average GI of 62 common foods from 205 studies by Atkinson et al. (2008) revealed that dairy products, legumes and fruits have a low GI whereas bread, breakfast cereals and rice including whole grain have both high and low GI.

The glycaemic index (GI) serves as a tool used to systematically rank carbohydrates in different foods according to their impacts on postprandial glucose response (Brand-Miller et al., 2009). GI measures the quality of the carbohydrate present in the food but not the quantity. Glycaemic load (GL) was first introduced by Salmerón et al. (1997) which serves both quality and quantity of carbohydrate. GL incorporates the product of GI and the total available carbohydrate content in a given amount of food which can be expressed in corresponding units of g per serving, g per 100g food, and g per 1000 kJ or 1000 kcal (Augustin et al., 2015). GL also has a classification system where low (≤ 10), medium (11-19) or high GL (≥ 20) is used (Venn & Green, 2007). Venn and Green (2007) mentioned that the relationship between GI and GL is not straightforward. For instance, if consumed in small amounts,

a high GI food may have a low GL. In contrast, depending on the portion size consumed, a low GI food may have a high GL. In short, although a fixed GI value is assigned to food, any food could have a low, medium or high GL because GL depends on the quantity of food consumed.



2.2.2 Glycaemic Index Value and Health

High GI and low GI are useful in enhancing athletic performance depending on the food consumption time. A low-GI food before prolonged vigorous exercise tends to increase endurance but not work output. Meanwhile, high-GI foods enhance muscle glycogen content in the recovery period of prolonged vigorous activity (Wolever, 2006). Goto (2016) observed a faster-repeated sprint performance among trained adult female soccer players after consumption of pre-exercise low GI high carbohydrate meals. However, Wong et al. (2017) did a meta-analysis to determine whether a pre-exercise low GI carbohydrate meal leads to greater endurance performance than a high GI meal. The meta-analysis discovered only 1 study from 15 randomized controlled or crossover trials showed greater exercise performance after consumption of low GI meals compared to high GI meals. Similarly, Chua et al. (2019) also did not find a significant influence of ingesting either high GI or low GI meals in the intermittent sprint and endurance performance.

The glycaemic index can be a useful tool in managing and preventing diabetes mellitus. A study among 20 patients with type 2 diabetes done by Gomes et al. (2017) found that a low GI diet reduced body fat and prevented the negative metabolic and inflammatory responses induced by the high GI diet. The study discovered an increased concentration in fructosamine, NEFA and TNF- α after consuming a high GI diet for 30 consecutive days whereas consuming a low GI diet for 30 days straight led to 1.8% of body fat reduction. Similarly, Zafar et al. (2019) did a systematic review and meta-analysis from 54 randomized controlled trials in adults or children with impaired tolerance, type 1 or type 2 diabetes. The study concluded that a low GI diet can be useful for glycaemic control and blood lipids, especially for type 2 diabetes patients. A low GI diet was effective in reducing glycated haemoglobin (HbA1c),

fasting blood glucose, body mass index (BMI), total cholesterol and low-density lipoprotein (LDL). Furthermore, Augustin et al. (2015) recognized a strong association between lower dietary glycaemic index or glycaemic load (GL) in reducing the risk of developing type 2 diabetes mellitus in both genders. In contrast, Hasbullah et al. (2020) argued that no significant association was found between dietary GI and GL with gestational diabetes mellitus. Nevertheless, a significant association was found between high dietary GI and GL intakes with established risk factors of gestational diabetes mellitus.

The glycaemic index has influences on the risk of developing coronary heart disease, especially in women. A prospective cohort study in urban Chinese women done by Yu et al. (2016) revealed that GI, GL and intakes of refined and total carbohydrates were significantly associated with increased risk of developing stroke. The study also found that dietary GI was significantly associated with total stroke risk with a hazard ratio (HR) of 95% CIs was 1.19 (1.04, 1.36) at 90th percentile compared to 10th percentile. Dietary GI was also significantly associated with both risks of ischemic and haemorrhagic stroke (both $p < 0.05$). The finding is also supported by a meta-analysis done by Livesey and Livesey (2019) among 12 studies. The study determined the risk relation (RR) and dietary GI and remarked a strong relationship between coronary heart disease and GI with a mean RR of 1.24 per 10 U GI. Higher relation was noted in women-only studies with RR 1.35 as compared to men-only studies with RR of 1.04. Nonetheless, Shahdadian et al. (2019) observed no significant association between dietary GI and all-cause mortality (RR: 1.07; 95% CI: 0.96, 1.19) and CVD mortality (RR: 1.02; 95% CI: 0.87, 1.20) in 18 cohort studies. Whereas, there is a positive association was observed between GI and all-cause mortality in women.

2.2.3 Factors that Influence Glycaemic Index

2.2.3.1 Carbohydrate

The glycaemic index of a food can be influenced by different components of carbohydrates present in foods such as monosaccharides, disaccharides and polysaccharides (Eleazu, 2016). Sucrose, for example, which is made up of glucose and fructose, has a lower GI than glucose because half of it is fructose, which causes a low blood sugar response. Furthermore, while sucrose has a GI of 68, glucose has a GI of 100. Polysaccharides can reduce starch digestibility. Wee and Henry (2020) summarized that polysaccharide can form a network on the surface and around the starch digestibility which restrict the enzymatic access. The authors also mentioned that polysaccharides can rise food viscosity, limiting the release of glucose in the intestinal phase.

2.2.3.2 Protein and Fat

Other food components present in the food such as fat and protein influenced the glycaemic response which will impact the GI. Ye et al. (2018) discovered a significant increase in starch digestibility after removal of proteins, lipids or both in rice flour. The lowest estimated GI is observed in the original rice flour containing both proteins and lipids (88.9) followed by rice flour without lipid (91.5), without proteins (92.3) and absence of both (94.5).

Lau et al. (2016) stated that the incorporation of fats during bread baking reduces the glycaemic response. The study found that controlled bread without any added fats had the highest mean of incremental area under the curve (iAUC) which was 246 as compared to bread baked with butter (203), grapeseed oil (203), olive oil

(191) and coconut oil (180). Fats slow down the rate of emptying food in the gastric into the intestine (Eleazu, 2016). This caused the rate of carbohydrates digested in the intestine to become slower which will lower the glycaemic response. The addition of fat in carbohydrate meals can form amylose-lipid complexes which act as a barrier to starch digestibility (Wee & Henry, 2020). This can reduce the GI of the food by limiting the available carbohydrate for digestion.

Another finding by Menon et al. (2016) also revealed that protein fortification in sweet potato starch noodles can reduce the estimated glycaemic index (eGI). Fortification of whey protein concentration at 10, 20 and 30% levels reduced the eGI of sweet potato starch noodles to 74.55, 70.83 and 68.73 respectively as compared to control (78.89). Food with high protein stimulates insulin production, which lowers postprandial blood glucose levels (Eleazu, 2016). Wee and Henry, (2020) mentioned that protein-starch interaction would form a network surrounding the starch granules which can limit the digestibility of starch and further the GI. Therefore, a mixed meal of carbohydrates with protein such as chicken noodles can contribute to the lowered glycaemic response.

2.3 Starch Hydrolysis

Starch hydrolysis can affect the glycaemic response which will further affect the GI. Starch is a polysaccharide that is commonly present in food such as bread, pasta and rice. Starch can be classified as rapidly digestible starch, slowly digestible starch and resistance starch depending on the amount of starch converted to glucose (Wee & Henry, 2020). Rapidly digestible starch converted into glucose in the first 20 minutes whereas slowly digestible starch in the first 100 minutes of digestion. Meanwhile, resistant starch refers to the type of starch that does not convert into glucose after 120 minutes of digestion. Therefore, different types of starch can lead to different raise in blood glucose levels after ingestion.

Amylose-amylopectin ratio and amylose-lipid complex in food can also influence starch hydrolysis. High amylose food would produce a lower glycaemic response. Belobrajdic et al. (2019) did a comparison study of high-amylose wheat bread with conventional low-amylose wheat bread in postprandial glycaemia. The author found that high-amylose wheat bread produced 39% less glycaemic response than conventional low-amylose wheat bread. Similarly, a high amylose-lipid complex can reduce the glycaemic response which will also reduce the GI. Lau et al. (2016) discovered that bread baked with coconut oil and olive oil has higher amylose-lipid complexes than bread baked with butter and grapeseed oil. The glycaemic response exerted by bread baked with coconut oil and olive oil were also lower than the other two breads.

CHAPTER 3

METHODOLOGY

3.1 Study Design

This is an experimental study aimed to determine the *in vitro* glycaemic index of selected packaged baked products in the local market. The simplified study design for the experiments can be referred in Figure 1.

3.2 Market Survey on Available Baked Products in the Local Market

A general market survey was done according to Ringuette et al. (2019) with slight modifications. Five stores located in Serdang, Selangor were selected using convenient sampling. The categories of packaged baked products from the stores were surveyed and identified. Four categories of packaged baked products available from the market were surveyed and selected via purposive sampling which includes crackers, cookies, cakes and bread. In each category, all brands and different types available such as plain, flavoured, filled, topped, cream-containing and ingredients used were noted (Appendix I).

3.3 Sample Selection and Collection

A total of six samples of packaged baked products from the four categories were selected and purchased from the local market around Serdang, Selangor which were cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filling. The samples were then taken to the Nutrition laboratory in Universiti Putra Malaysia. The information of the samples purchased such as the front of the products, nutrition information panel and ingredients used were recorded (Appendix II). The samples were then stored according to the recommended storage condition stated in the packaging of the products. Wholemeal bread is an

example of a whole grain product. Nutrition Society of Malaysia (2012) had differentiated whole grain and whole meal. Whole grain contains the bran, germ and endosperm of the grain while wholemeal also contains the bran, germ and endosperm of the grain, but is finely milled, giving a finer texture.



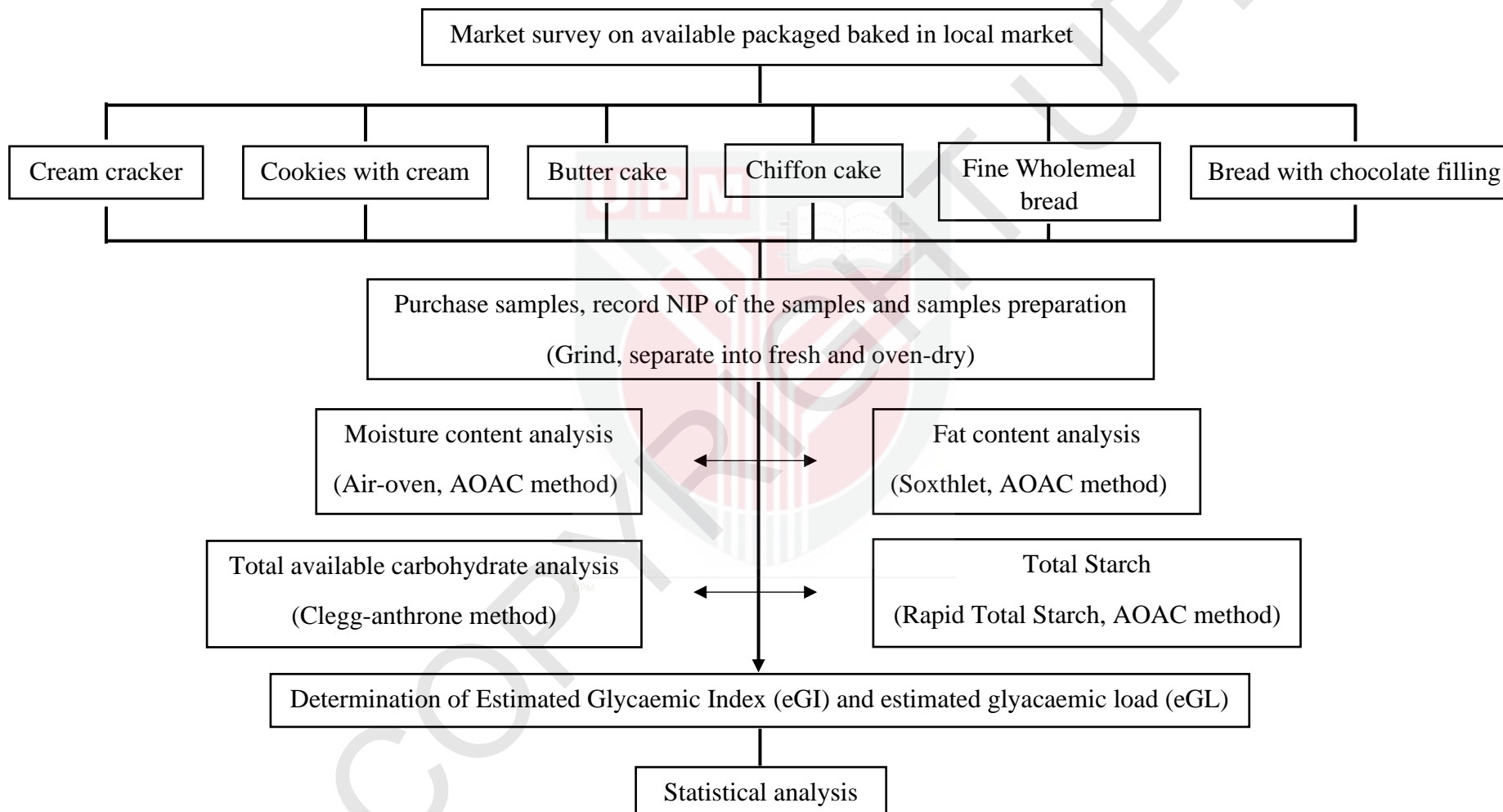


Figure 1. Overview of research activity

3.4 Material, Chemical Reagents, Enzymes and Equipment

3.4.1 Materials and Reference Foods

Materials needed were samples purchased from the local market which are cream crackers, cookies with cream, butter cake, chiffon cake, fine wholemeal bread and bread with chocolate filling.

The reference foods used were white bread (Gardenia (KL) Sdn. Bhd.) and glucose (Glucolin™; Reckitt Beckinsler, Malaysia). Both reference foods were applicable in the *in vitro* GI since most studies used white bread (Ferrer-Mairal et al., 2012; Germaine et al., 2008; Goni et al., 1997; Hettiaratchi et al., 2012; Jun et al., 2014; Ren et al., 2016; Zhu et al., 2020) and glucose (Barine & Yorte, 2016; Kumar et al., 2020) as the reference food.

3.4.2 Chemical Reagents and Enzymes

Chemical reagents being used in this study were 52% perchloric acid (HClO₄), (R&M Chemical), petroleum ether 40 -60°C (System, Malaysia), Anthrone reagent (ACROS ORGANIC, Belgium), GOPOD D-glucose Assay kit (Megazyme, Ireland), sodium acetate and Trizma maleate salt (Sigma Aldrich, USA), diluted sulphuric acid (H₂SO₄) (sulphuric acid: water, 2.3:1.0, v/v), calcium chloride, hydrochloric acid (HCl), and potassium chloride (Merck, Germany), glucose standard (food grade) stock solution (mixture of 100 mg of glucose and 100 mL distilled water), glucose standard working solution (10 mL of glucose standard stock solution diluted with water to 100mL volume).

The enzymes used in this study pepsin from porcine gastric mucosa 250 U/mg (Sigma Aldrich, United Kingdom), amyloglucosidase 100 000U/mg (Solarbio,

Beijing, China), α -amylase from human saliva 87.5 U/mg (Sigma Aldrich, USA) and thermostable α -amylase (Sigma Aldrich, USA).

3.4.3 Equipment

The equipment was food processor (National MK-5070N, Osaka, Japan), weighing scale GF-300 (AND Weighing, Japan), oven (Mettler, Germany), water bath (Mettler, Germany), spectrophotometer UV-1800 (Shimadzu Corporation, Japan), Soxhlet system (Electrothermal, United Kingdom), rotary evaporator R200 (Buchi, Switzerland), magnetic stirrer MR Hei – Tec (Heidolph Instruments, Germany), centrifuge Rotafix 32 (Hettich, Germany) and pH meter PB-10 (Sartorius Group, Germany).

3.5 Preparation of Samples

Each of the samples was ground to homogenize using a food processor (National MK-5070N, Osaka, Japan). The grounded samples were separated into two according to the assays; fresh samples for moisture and total carbohydrate content and oven drying at 40°C for total starch, fat content and *in vitro* glycaemic index determination before the experiment. The samples were then kept in an airtight container with oxygen absorbent to prevent insect damage and maintain sample quality. The samples were stored in the refrigerator at 4°C before analysis.

3.6 Determination of Moisture Content

The moisture content of packaged baked products was determined by the oven-drying method by AOAC (2008). The moisture content was done in triplicate. Firstly, the aluminium dish with cover was washed and dried in the oven for 3 hours at 105°C. The dish was then cooled in a desiccator and weighed as soon as it attained room temperature. After that, 10g of ground homogenized sample was placed in the aluminium dish and was dried in air-oven at 105°C overnight, uncovered. On the next day, the dish was cooled in a desiccator and weighed immediately after reaching room temperature. The procedure was repeated until a constant weight was obtained. The moisture content in the food sample was calculated using the formula:

$$\text{Moisture content (\% by weight)} = \frac{\text{loss of weight of the sample (g)}}{\text{weight of sample taken (g)}} \times 100$$

3.7 Determination of Fat Content

The fat content was determined using the semi-continuous Soxhlet extraction method by AOAC (2000). Firstly, 10g of sample was put inside an extraction thimble and cotton wool was placed as a lid. Meanwhile, an empty round bottom flask was weighed and 150 ml petroleum ether at 40-60°C was added to it. The thimble was placed in an extraction chamber below a condenser which is suspended above the flask containing petroleum ether. The fat was extracted using an automated fat extraction system for 3-6 hours until the solvent is clear and colourless which indicated all fat has been extracted.

After fat extraction had been completed, petroleum ether was removed by evaporation using a rotary evaporator. Then, the flask was dried in an air oven at 100°C for one hour and cooled in a desiccator. The flask was weighed afterwards. The fat content in the food sample was calculated using the below formula:

$$\text{Fat content (\% by weight)} = \frac{W_2 - W_1}{W_{\text{sample}}} \times 100, \text{ where}$$

W₁: weight of the flask without fat (g)

W₂: weight of the flask with fat (g)

W_{sample}: weight of the sample taken (g)

3.8 Determination of Total Available Carbohydrate Content

The total available carbohydrate content of the samples was determined using the colorimetric Clegg-anthrone method (AOAC, 2000). Firstly, 2.5g of wet sample was placed into a 100 mL stoppered graduated cylinder. Then, 10 mL of water were added and the mixture was stirred using a glass rod to disperse the sample thoroughly. The mixture was added with 13 mL of 52% perchloric acid and stirred frequently for 20 minutes. The mixture was diluted to 100 mL with water and filtered into a 250 mL graduated flask. The cylinder was washed with water and the washing was collected into the same flask. The content was diluted to the mark and mixed thoroughly.

The extracted sample then undergoes the determination process. 10 mL of sample extract was taken and diluted to 100 mL of water. A dilution of 10 factors was done where 1 mL of diluted sample extract was further diluted to 10 mL. Next, 1 mL of the diluted sample extract was transferred into a test tube using a pipette. Then, 5 mL of anthrone reagent was rapidly added into the test tubes and was mixed thoroughly. All test tube was placed in a boiling water bath for 12 minutes and cooled down to room temperature. Lastly, the solutions were transferred into cuvettes and the absorbances were read at 630 nm against the blank. The total available carbohydrate was calculated using the following formula:

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

a: absorbance of glucose standard working solution

b: absorbance of sample extract

w: weight of sample taken (g)

3.9 Determination of Total Starch

The total starch was determined using the rapid total starch method by McCleary et al. (2019). Firstly, four test tubes containing approximately 100 mg of dried sample each was prepared with three of the tubes were triplicate of the sample and the other one was the sample background. In all test tubes, 10 mL of 0.1 M pH 5.0 of sodium acetate buffer containing 5 mM calcium chloride (CaCl_2) was added and the mixture was shaken well. Then, the three sample tubes were added with 0.1 mL undiluted thermostable α -amylase while the sample background tube was added with 0.1 mL of sodium acetate buffer. The tubes were vortexed to mix the content and capped with aluminium foil. The tubes were then incubated at 100°C for 15 minutes where the tubes were taken out and shaken every 5 minutes. After incubating, the tubes were cooled at 60°C for 5 minutes.

Next, the three sample tubes were added with 0.1 mL of amyloglucosidase (3300 U/mL) while the sample background was added with 0.1 mL of sodium acetate buffer. The tubes were then capped and incubated at 60°C for 30 minutes where the tubes were shaken thoroughly every 15 minutes to homogenize the enzyme with the mixture. After that, the content of the tubes was transferred into 15 mL centrifuge tubes to be centrifuged at 4000 rpm for 5 minutes. A dilution of 11-fold was done to all food samples and backgrounds where 1 mL of supernatant was added with 10 mL of sodium acetate buffer.

Finally, the procedure was continued to GOPOD procedure to determine the d-glucose using the GOPOD assay kit. From diluted samples and sample backgrounds, 0.1 mL was pipetted out and added with 3.0 mL GOPOD reagent in new test tubes. Meanwhile, GOPOD standard and GOPOD reagent blank were prepared by mixing 0.1 mL D-glucose standard with 3.0 mL GOPOD reagent and 0.1 mL sodium acetate

buffer with 3.0 mL GOPOD reagent in new test tubes respectively. All tubes were then incubated at 50°C for 20 minutes. The contents were transferred into cuvettes and the absorbance readings were read at 510 nm against the GOPOD reagent blank. The GOPOD reagent blank absorbance reading should be less than 0.05 when read against distilled water. The following formula was used to calculate the total starch content:

Total starch, % = $\Delta A \times F \times 102 \times D \times 1/1000 \times 100/W \times 162/180$, where

ΔA : Absorbance of sample solution read against reagent blank minus the absorbance of the sample background read against the reagent blank;

F : Factor of conversion absorbance values to μg glucose (100 μg glucose divided by the absorbance value obtained for 100 μg of glucose);

102 : volume correction, (0.1mL taken from 10.2mL of sample solution);

D : further dilution of sample solution

1/1000 : conversion from μg to mg;

100/W : conversion to 100mg sample;

162/180 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

3.10 Determination of Estimated Glycaemic Index

3.10.1 *In Vitro* Kinetic of Starch Digestion

The determination of the estimated glycaemic index (eGI) was a modified method of Goni et al. (1997) by Graça et al. (2020). Before analysis, all samples with more than five per cent of fat, determined based on the samples' nutrition information panel provided by the manufacturer were defatted using Soxhlet extraction with petroleum ether. The analysis was done in triplicates.

First and foremost, a portion of the sample containing approximately 50mg of total starch was dissolved in 0.1 M HCl-KCl buffer (pH 1.5) in a 50 mL centrifuge tube. Then, 200 μ L of pepsin from porcine gastric mucosa solution (220 U/mL) was added and the tube was incubated at 40°C for 1 hour to mimic the gastric phase, preventing the protein-starch interaction.

The mixture was added with 25 mL of 0.1 M pH 6.9 Tris-maleate buffer solution containing 0.4mM calcium chloride (CaCl_2) to stop the pepsin reaction and create an optimum condition for the small intestinal phase. The phase was imitated by adding 5 mL of α -amylase solution from human saliva (3 U/mL) and mixing it thoroughly. At this time, a sample solution of 5 mL was taken out and placed in a 15 mL centrifuge tube for $t = 0$ minute. Then, the remaining sample solution was incubated at 37°C water bath for 3 hours. For different time points which were $t = 30, 60, 90, 120$ and 180 minutes, 5 mL of sample solution were pipetted out.

Following every 5mL aliquot of sample solution, the 15 mL tube was placed in a boiling water bath for 5 minutes to stop the enzymatic reaction and brought cool to room temperature. In a new 15 mL tube, 1 mL of sample aliquot was added with 3.0 mL of 0.4 M sodium acetate buffer (pH 4.75) and 60 μ L of amyloglucosidase

(3300 U/mL). The aliquots were then incubated at 60°C for 45 minutes and centrifuged afterwards at 4000 rpm for 5 minutes at room temperature. All sample aliquot was diluted 11- fold by pipetting 100 μ L of the supernatant with 1 mL of sodium acetate buffer in a 2 mL microcentrifuge tube.

Lastly, the process was continued to GOPOD procedure using the GOPOD d-glucose assay kit. In a test tube, 0.1 mL of diluted sample aliquot was mixed with 3 mL of GOPOD reagent and incubated at 50 °C for 20 minutes. Then, the content was transferred into cuvettes and the absorbance readings were read at 510 nm against the GOPOD reagent blank.

3.10.2 Estimation of Rate of Starch Hydrolysis (RSH)

The rate of starch hydrolysis was calculated based on Goni et al. (1997) using the equations below according to the weight of sample taken during *in vitro* kinetics of starch digestion:

For a sample with a weight of 100 mg, RSH was calculated by equation **1 (a)**, for a sample with weight more than 100 mg, RSH was calculated by equations **1 (a)** and **1 (b)**:

$$\text{Rate of starch hydrolysis} = \left(\frac{a}{b} \times 100\right) \times 11 \times 0.9 \dots\dots\dots \mathbf{1 (a)}$$

$$\text{Rate of starch hydrolysis per 100 mg} = \left(\frac{RSH}{c} \times 100\right) \times 11 \times 0.9 \dots\dots\dots \mathbf{1 (b)}$$

Where,

a: average absorbance reading of sample at different t = 30, 60, 90, 120 and 180 min

b: average absorbance reading of GOPOD standard solution

c: weight of sample taken (g)

3.10.3 Estimation of Area Under Curve (AUC), Hydrolysis Index (HI), and Estimated Glycaemic Index (eGI)

Determination of estimated glycaemic index (eGI) was calculated based on a first-order rate equation established by Goni et al. (1997) using hydrolysis index that can provide better GI prediction. Goni et al. (1997) found a good correlation ($r = 0.894$) between *in vitro* GI to referenced GI. Similarly, Germaine et al. (2008) showed a good linear correlation between eGI and GI values of 14 tested grain foods ($r = 0.93$). Likewise, Ren et al. (2016) also stated a significant positive correlation between GI of pure foxtail millet with eGI ($r = 0.988, p = 0.002$).

The area under the curve (AUC) was calculated using the software of Graphpad Prism version 8.0.1 (GraphPad Software., San Diego, California).

The hydrolysis index (HI) and estimated GI (eGI) were calculated based on two reference foods which were white bread and glucose since most studies used white bread (Ferrer-Mairal et al., 2012; Germaine et al., 2008; Goni et al., 1997; Hettiaratchi et al., 2012; Jun et al., 2014; Ren et al., 2016; Zhu et al., 2020) and glucose (Barine & Yorte, 2016; Kumar et al., 2020) as the reference food. Both reference foods were applied to *in vitro* GI study. The HI values were expressed as a percentage using the below equation:

$$HI = \left(\frac{AUC \text{ of sample}}{AUC \text{ of reference food}} \times 100 \right)$$

Lastly, eGI was estimated according to the formula below:

$$eGI = 39.71 + 0.549 HI$$

3.10.4 Estimation of Glycaemic Load (eGL)

The glycaemic load was estimated based on Kumar et al. (2020) with the following formula:

$$eGL = \frac{eGI \times \text{carbohydrate per serving size (g)}}{100}, \text{ where}$$

carbohydrate per serving size (g) was obtained from the nutrition information panel (NIP) provided by the manufacturer on the packaging. Similarly, eGL was also estimated based on both reference foods which were white bread and glucose.

3.11 Statistical Analysis

Statistical analysis was performed by using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA). All analyses were carried out in triplicate and the results were expressed as mean and standard deviation. The data were subjected to one-way ANOVA followed by Tukey's post hoc test to see which sample was significantly different. As for the correlation between eGI with total available carbohydrate, fat content analysed and eGL, the data was subjected to Pearson Correlation. The level of significance was set at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter provides information on results and inferential analysis of the studied variables. The chapter begins with nutritional facts based on the nutrition information panel (NIP) provided by the manufacturer. Then, the moisture content, fat content, total available carbohydrate content and total starch in the packaged baked products are further described in this chapter. Apart from these, the hydrolysis index, estimated glycaemic index and glycaemic load are also detailed. Next, the correlation of estimated glycaemic index with estimated glycaemic load and nutrient facts from the manufacturer were presented as bivariate analysis and discussed. The total number of packaged baked products analyzed were six samples.

4.2 Nutritional Data in NIP of Packaged Baked Products

The nutritional facts of the packaged baked products are described in Table 1. Generally, carbohydrate was the major macro nutrient in baked products ranging from 41.4 to 71.3 g/100g, followed by fat and protein ranging from 5.4 to 30.5 g/100g and 4.7 to 9.7 g/100g respectively. The serving size varied among packaged baked products ranging from 31g to 67g per serving. Overall, the baked products mainly consist of carbohydrates, fat and protein except for fine wholemeal bread which contains more protein than fat.

Table 1: Nutritional facts per 100g of packaged baked product based on nutrition information panel (NIP) by manufacturer

Sample	Pack Serving size (g)	Carbohydrate (g)	Sugar (g)	Fibre (g)	Fat (g)	Protein (g)
Cream cracker	31	64.5	3.2	-	25.8	6.5
Cookies with Cream	28.5	71.3	38.2	2	20.6	4.7
Butter Cake	35	41.1	21.6	3.5	30.5	5.9
Chiffon Cake	40	52	-	-	23	8.5
Fine Wholemeal Bread	67	45	-	5.1	5.4	9.7
Bread with Chocolate Filling	50	51	-	9.3	23	7.4

Among these baked products, the highest carbohydrate was found in cookies with cream (71.3 g/100g) while the lowest was found in butter cake (41.1 g/100g). Butter cake contains the most fat (30.5 g/100g) and the least fat belongs to fine wholemeal bread (5.4 g/100g). Meanwhile, the protein was the highest in fine wholemeal bread (9.7 g/100g) whereas cookies with cream had the lowest protein (4.7 g/100g). Among the six baked products analyzed, only three products provided the amount of sugar and only four samples provided the fibre content in the nutritional information panel. The highest sugar content was observed in cookies with cream (38.2 g/100g) while the lowest was in cream crackers (3.2 g/100g). On the contrary, cookies with cream have the least fibre (2 g/100g) while the most fibre was seen in bread with chocolate filling (9.3 g/100g).

4.3 Moisture, Fat, Total Available Carbohydrate and Total Starch Contents of Packaged Baked Products

A one-way ANOVA with a Post hoc comparison using the Tukey test was conducted to compare the mean of moisture, fat, total available carbohydrate and total starch contents among the studied baked products. The results of the analysis are shown in Table 2.

Table 2: Moisture content, total available carbohydrate, fat content and total starch of packaged baked products

Sample	Moisture Content (%)	Fat content (%)	Total available carbohydrate content (%)	Total starch (g/100g dry weight basis)
Cream Cracker	2.69 ± 0.16 ^e	23.46 ± 0.80 ^{bc}	13.83 ± 1.19 ^b	58.63 ± 0.65 ^a
Cookies with Cream	2.75 ± 0.06 ^e	22.09 ± 1.16 ^{bc}	18.11 ± 0.47 ^a	32.39 ± 2.06 ^c
Butter Cake	18.58 ± 0.12 ^d	32.10 ± 1.02 ^a	13.39 ± 1.14 ^b	32.22 ± 0.44 ^c
Chiffon Cake	21.48 ± 0.51 ^c	24.81 ± 3.21 ^b	10.90 ± 0.27 ^c	28.72 ± 0.71 ^c
Fine Wholemeal Bread	35.73 ± 0.81 ^a	6.21 ± 0.40 ^d	12.65 ± 0.60 ^{bc}	54.95 ± 7.28 ^a
Bread with Chocolate Filling	26.57 ± 0.18 ^b	19.58 ± 0.22 ^c	12.18 ± 0.08 ^{bc}	44.15 ± 0.95 ^b

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate a significant difference ($p < 0.05$, ANOVA, Tukey)

4.3.1 Moisture Content

There was a significant difference in moisture content at the $p < 0.05$ level among the baked products [$F(5,12) = 3184.49, p < 0.05$]. Moisture content in fine wholemeal bread was significantly greater (35.73 ± 0.81) than bread with chocolate filling (26.57 ± 0.18), chiffon cake (21.48 ± 0.51) and butter bake (18.58 ± 0.12). Meanwhile, cream crackers and cookies with cream did not significantly differ between each other ($2.69 \pm 0.16, 2.75 \pm 0.06$ respectively) but were significantly lowest among the baked products.

Moisture content for fine wholemeal bread was similar to data reported in the Malaysian Food Composition Database which is 38.7g moisture per 100g (Institute for Medical Research, 1997). Similarly, Belobrajdic et al. (2019) showed a range of 37 to 44% of moisture content while Ibrahim et al. (2020) reported 37.06 % moisture in the bread. Ibrahim et al. (2020) also stated that moisture assists in moistening and lubricating the bread which may slow down the crumb firming process.

The moisture content for cream crackers and cookies with cream obtained were comparable with the Malaysian Food Composition Database which is 4g moisture per 100g (Institute for Medical Research, 1997). Likewise, a study was done by Norhayati et al. (2015) also reported 2.83 %, 3.89 % and 3.02 % moisture for crackers with vegetable flavour, crackers with sugar and biscuits with cream-filled respectively. Moisture is an important parameter related to the quality and shelf life of food. Foods with moisture content less than 4% usually have a long shelf life of six months or longer (Mamat & Hill, 2018).

4.3.2 Fat Content

Similarly, fat content was reported to vary significantly at $p < 0.05$ level [$F(5,12) = 96.96, p < 0.05$]. Butter cake had significantly more fat (32.10 ± 1.02) than chiffon cake (24.81 ± 3.21), bread with chocolate filling (19.58 ± 0.22) and fine wholemeal bread (6.21 ± 0.40). No significant difference was found between chiffon cake, cream cracker (23.46 ± 0.80) and cookies with cream (22.09 ± 1.16). In the same way, cookies with cream crackers and cookies with cream were no different than bread with chocolate filling.

This result was comparable with the nutritional information table stated in Table 1 where butter cake contained the most fat (30.5g /100g) and fine wholemeal bread as the least fat content (5.4 g/100g). The result was slightly different to the Malaysian Food Composition Database, where fat in plain cake is 19.8%, and fine wholemeal bread is 0.6% fat. The major ingredient in butter cake is butter which consists of high saturated fat. According to Brassard et al. (2017), consumption of butter greatly increases low-density lipoprotein cholesterol (LDL-C) concentration compared to cheese. High LDL-C may lead to heart disease.

4.3.3 Total Available Carbohydrate

Significant differences were also observed in total available carbohydrate content at $p < 0.05$ level [$F(5,12) = 32.70, p < 0.05$] among the baked products. Cookies with cream had a significantly higher total available carbohydrate (18.11 ± 0.47) compared to cream crackers (13.83 ± 1.19), butter cake (13.39 ± 1.14) and chiffon cake (10.90 ± 0.27). However, fine wholemeal bread (12.65 ± 0.60), bread with chocolate filling (12.18 ± 0.08) were not significantly different from cream crackers, butter cake and chiffon cake. The possible justification was a higher amount of carbohydrate and sugar in cookies with cream. As tabulated in Table 1, cookies with cream had the highest carbohydrate and sugar content among the baked products.

4.3.4 Total Starch

Likewise, the total starch contents were significantly different [$F(5,12) = 49.36, p < 0.05$] among the studied baked products. Cream crackers had significantly greater total starch (58.63 ± 0.65) than bread with chocolate filling (44.15 ± 0.95), cookies with cream (32.39 ± 2.06), butter cake (32.22 ± 0.44) and chiffon cake (28.72 ± 0.71). However, no significant difference was observed between cream crackers and fine wholemeal bread (54.95 ± 7.28). Similarly, cookies with cream, butter cake and chiffon cake were not significantly different.

A previous study done by Whitney (2013) reported a total starch of 58.48 % for blended whole wheat bread which was comparable to the total starch gained for fine wholemeal bread in this study. Barine and Yorte (2016) also reported a total starch of 63.53% for cookies which is similar to the total starch obtained by cream crackers. Lower total starch resulted may be contributed by granules properties. Wee and Henry (2020) mentioned that larger and smoother starch granules were digested more slowly, due to the smaller specific surface area available for enzyme binding.

4.4 Determination of Estimated Glycaemic Index (eGI) of Packaged Baked Products

The rate of starch hydrolysis of studied packaged baked products based on different time points was calculated and presented in Figure 2. In addition, one-way ANOVA was conducted to compare the mean hydrolysis index (HI), estimated glycaemic index (eGI) and estimated glycaemic load (eGL) of the packaged baked products. Table 3 describes the results of the Post hoc comparison using the Tukey test. The determination of HI, eGI and eGL were done based on two reference foods, white bread and glucose.

4.4.1 Rate of Starch Hydrolysis

The estimation of GIs based on the rate of starch hydrolysis depending on the sampling times was also analyzed and presented in Figure 2.

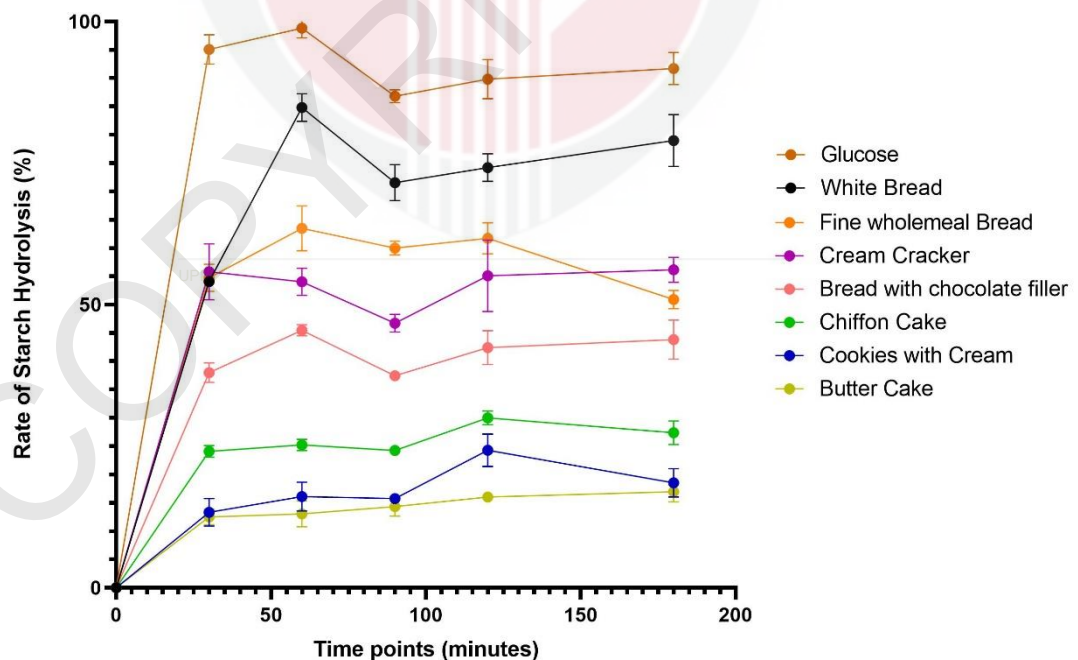


Figure 2. Rate of starch hydrolysis based on sampling time for packaged baked products

Table 3: Hydrolysis index, estimated glycaemic index and estimated glycaemic load of packaged baked products

Sample	Hydrolysis Index (HI)		Estimated Glycaemic Index (eGI)*		Estimated Glycaemic Load (eGL)**	
	HI ₁	HI ₂	eGI ₁	eGI ₂	eGL ₁	eGL ₂
White Bread (Refence food)	100.00 ± 0.99 ^b	79.01 ± 0.79 ^b	94.61 ± 0.55 ^b	83.09 ± 0.43 ^b	28.57 ± 0.16 ^b	25.09 ± 0.13 ^b
Glucose (Reference food)	126.57 ± 2.11 ^a	100.00 ± 1.67 ^a	109.20 ± 1.16 ^a	94.61 ± 0.92 ^a	43.57 ± 0.46 ^a	37.75 ± 0.37 ^a
Cream Cracker	73.72 ± 3.14 ^d	58.24 ± 2.48 ^d	80.18 ± 1.72 ^d	71.69 ± 1.36 ^d	16.04 ± 0.34 ^e	14.34 ± 0.27 ^e
Cookies with Cream	25.00 ± 1.18 ^g	19.75 ± 0.94 ^g	53.44 ± 0.65 ^g	50.56 ± 0.51 ^g	10.85 ± 0.13 ^g	10.26 ± 0.10 ^g
Butter Cake	20.19 ± 1.10 ^h	15.95 ± 0.87 ^h	50.79 ± 0.60 ^h	48.47 ± 0.48 ^h	7.31 ± 0.09 ^h	6.98 ± 0.07 ^h
Chiffon Cake	36.43 ± 0.97 ^f	28.79 ± 0.77 ^f	59.71 ± 0.53 ^f	55.51 ± 0.42 ^f	12.42 ± 0.11 ^f	11.55 ± 0.09 ^f
Fine Wholemeal Bread	80.29 ± 1.67 ^c	63.44 ± 1.32 ^c	83.79 ± 0.92 ^c	74.54 ± 0.72 ^c	25.30 ± 0.28 ^c	22.51 ± 0.22 ^c
Bread with Chocolate Filling	56.98 ± 0.18 ^e	45.02 ± 0.14 ^e	70.99 ± 0.10 ^e	64.42 ± 0.08 ^e	18.10 ± 0.03 ^d	16.43 ± 0.02 ^d

Values are expressed as mean ± standard deviation. Different superscript letters within the same column indicate a significant difference ($p < 0.05$, ANOVA, Tukey) Subscript 1 indicates fresh white bread is used as the reference food; Subscript 2 indicates glucose is used as the reference food. *eGI was estimated based on $eGI = (0.549 \times HI) + 39.71$ (Goni et al., 1997). **eGL are calculated based on $eGL = eGI \times \text{carbohydrate per serving} / 100$

Based on Figure 2, the rate of starch hydrolysis of most samples showed an irregular increase and decrease. It should be noted that different baked products achieved the highest peak of starch hydrolysis at different time points. At this 30 minutes, cream crackers had the highest peak of starch hydrolysis (55.81 % starch/min). Following that, glucose (98.86 % starch/min), white bread (84.82 % starch/min), bread with chocolate filling (45.48 % starch/min) and fine wholemeal bread (63.49 % starch/min) reached the highest peak at 60 minutes of incubation. Subsequently, at 120 minutes, cookies with cream (24.29 % starch/min) and chiffon cake (30.03 % starch/min) attained the highest peak. Lastly, butter cake achieved its peak the latest at 180 minutes (16.97 % starch/min). Amongst the analyzed baked products, fine wholemeal bread had the greatest rate of starch hydrolysed during the highest peak with 63.49 % starch/min.

The glycaemic response given by food can be seen through its starch hydrolysis properties. The rate of starch hydrolysis can be influenced by the type of starch present in the food samples. Different types of starch can lead to different raises in blood glucose levels after ingestion (Wee & Henry, 2020). Generally, there are three types of starch. First, the rapidly digestible starch is converted into glucose in the first 20 minutes. Second, the slowly digestible starch is converted into glucose in the first 100 minutes of digestion. Third, resistant starch refers to the type of starch that is not converted into glucose after 120 minutes of digestion.

Moreover, starch hydrolysis or digestibility can also be influenced by the amylopectin/amylose ratio and amylose-lipid complex (Eleazu, 2016). Other than that, Wee & Henry (2020) concluded that starch digestibility of flour is affected by the degree of milling, the particle size, flour composition and protein-lipid interaction. The

author also mentioned that starch hydrolysis depends on the starch properties such as granule morphology, amylose-amylopectin content and degree of gelatinization.



4.4.2 Hydrolysis Index (HI) of the Analyzed Packaged Baked Products

When using white bread as the reference food, the HI of the analyzed baked products varied significantly. Glucose had a significantly higher value (126.57 ± 2.11) than white bread (100.00 ± 0.99). Among the analyzed packaged baked products, the HI value of fine wholemeal bread was significantly greater (80.29 ± 1.67) compared to cream crackers (73.72 ± 3.14) and bread with chocolate filling (56.98 ± 0.18). Meanwhile, the lowest HI value was seen in butter cake (20.19 ± 1.10).

Similarly, when glucose is used as the reference food, significant differences were observed in HI values of the studied baked products. Glucose showed a significantly greater HI value (100.00 ± 1.67) than white bread (79.01 ± 0.79). The same trend was observed in HI values of studied baked products where fine wholemeal bread had a significantly higher value (63.44 ± 1.32) than cream cracker (58.24 ± 2.48) and bread with chocolate filling (45.02 ± 0.14). Whereas, butter cake had the lowest HI value (15.95 ± 0.87) among samples.

4.4.3 Estimated Glycaemic Index (eGI) of the Analyzed Packaged Baked Products

Next, eGI values of the analyzed baked products also showed significant differences when attributed to white bread as reference food. Glucose recorded a significantly higher eGI value (109.20 ± 1.16) compared to white bread (94.61 ± 0.55). Among the six baked products analyzed, fine wholemeal bread had the highest eGI value (83.79 ± 0.79) while the lowest eGI value was observed in butter cake (50.79 ± 0.60).

Likewise, significant differences were seen in eGI values of the studied baked products when glucose was the reference food. A greater eGI value was shown in glucose (94.61 ± 0.92) compared to white bread (83.09 ± 0.43). The highest eGI value among the studied baked products was fine wholemeal bread (74.54 ± 0.72) while the lowest value was in butter cake (48.47 ± 0.48).

Butter cake has the lowest eGI values amongst the packaged baked products studied in both reference foods. The addition of fat forms amylose-lipid interaction which increased the resistant starch and reduced the glycaemic response (Wee & Henry, 2020). Butter is the main ingredient in butter cake made from dairy products which mainly consist of fat, specifically saturated fat. The formation of amylose-lipid interaction is varied with the saturation and chain length of the fat (Lau et al., 2016). Meanwhile, fine wholemeal bread had the highest eGI values based on both reference foods among the studied packaged baked products. Fine wholemeal bread is made from the whole grain that has been milled into a fine texture. Milling exposed starch to digestion and further milling increased the starch surface area together with its digestibility (Wee & Henry, 2020).

4.4.4 Glycaemic Load (eGL) of the Analyzed Packaged Baked Products

Apart from this, eGL values of the analyzed baked products varied significantly when white bread was used as the referenced food. Glucose showed a significantly greater eGL value (43.57 ± 0.46) compared to white bread (28.57 ± 0.16). Among the baked products analyzed, fine wholemeal bread produced the greatest eGL value (25.30 ± 0.28) while butter cake had the least eGL value (7.31 ± 0.09) when white bread was used as the reference food.

Correspondingly, a significant difference was found in eGL values of studied baked products with glucose as reference food. A higher eGL value was seen in glucose (37.75 ± 0.37) than white bread (25.09 ± 0.13). Among the baked products studied, the highest eGL value was seen in fine wholemeal bread (22.51 ± 0.22) while the lowest value was in butter cake (6.98 ± 0.07).

Overall, all packaged baked products studied were significantly lower in eGI values than both white bread and glucose. To conclude, a similar pattern was observed in HI, eGI and eGL values of the studied packaged baked products when glucose and white bread were used as the reference foods. When white bread was used as the reference food, higher HI, eGI and eGL values of the packaged baked products were observed compared to glucose as the reference food. Therefore, to compare which values are better, the International Table of GI and GL Values 2008 by Atkinson et al. (2008) was used as guidance.

4.4.5 Glucose as the Reference Food

Atkinson et al. (2008) had tabulated the GI values on both reference foods in the international table. However, only two baked products analyzed were reported in the international table which were cream cracker and fine wholemeal bread. When comparison was done, the eGI values obtained were 12-13 units lower than the international table when using white bread as the reference food. Meanwhile, when glucose was used as the reference food, the eGI values obtained were 6-7 units lower than the international table. Some previous studies also used glucose as the reference food for baked products samples (Barine & Yorte, 2016; Soong et al., 2015). Plus, the eGI values for glucose (94.61 ± 0.92) aligned well with Atkinson et al. (2008) (96) while the eGI of white bread (83.09 ± 0.43) was comparable with Henry et al. (2021) (83 ± 6.50). Therefore, the eGI values of studied baked products with glucose as the reference foods are used to classify and compare with previously published reported GI and GL values.

GI can be classified into three categories where GI value below 55 is defined as low, 56-69 as moderate and 70 and above as high (International Standard ISO 26642:2010, 2010). In this study, among the analyzed packaged baked products, fine wholemeal bread and cream crackers were classified as high-GI foods while chiffon cake and bread with chocolate filling as moderate-GI foods. The low-GI foods were cookies with cream and butter cake. Next, the classification of GL by Venn & Green (2007) was used in this study where value ≤ 10 indicates a low GL, the value of 11-19 as medium GL and value of ≥ 20 indicates high GL foods. Among the packaged baked products studied, fine wholemeal bread was the only high-GL food. Cream cracker, chiffon cake and bread with chocolate filling were medium-GL foods and cookies with

cream and butter cake were low-GL foods. Overall, the studied baked products were successfully categorized in all categories for GI and GL.



4.5 Comparison of Estimated Glycaemic Index (eGI) and Estimated Glycaemic Load (eGL) of Analyzed Packaged Baked Products with Previously Reported Values

In Table 4, the eGI values obtained with glucose as the reference food were compared with the values reported in the International Table by Atkinson et al. (2008) and Henry et al. (2021). In this study, eGI value for cream crackers was 71.69 ± 1.36 which is higher than the international table value at 65 ± 11 (Atkinson et al., 2008). Similarly, eGI value for fine wholemeal bread (74.54 ± 0.72) was higher than the value in the international table by Atkinson et al. (2008) (68 ± 9) but lower than the value reported by Henry et al. (2021) (85 ± 5.9). Meanwhile, the eGI values of 48.47 ± 0.48 and 55.51 ± 0.42 for butter cake and chiffon cake, respectively were lower than GI values provided by Henry et al. (2021) with values of 64.72 and 60 ± 6 respectively. However, there were no reported GI values for cookies with cream and bread with chocolate filling. In short, the eGI values obtained for the studied baked products were lower than the previously reported values except for cream crackers and fine wholemeal bread.

There is a slight difference in the classification of the previously reported GI values of packaged baked products with this study. In the previously reported GI values, cream crackers, butter cake, chiffon cake were categorized as moderate-GI foods. In this study, the cream cracker was categorised as high-GI food, butter cake as low-GI food and chiffon cake as moderate-GI food. Whereas, wholemeal bread was categorised as moderate-GI food based on values reported in the international table (Atkinson et al., 2008) or high-GI food as reported by Henry et al. (2021).

Table 4: Comparison of estimated glycaemic index (eGI) and estimated glycaemic load (eGL) with previously reported values

Sample	Pack Serving size (g)	eGI ₂	eGL ₂ (based on pack serving size)	eGL ₂ (based on equivalent serving size)	Based on previously reported values ^{ab}			
					GI ₂	Serving size (g) ^a	Available Carbohydrate (g/serve) ^a	GL ^a
Cream Cracker	31	71.69 ± 1.36	14.34 ± 0.27	11.56	65±11 ^a	25	17	11
Cookies with Cream	28.5	50.56 ± 0.51	10.26 ± 0.10	na	na	na	na	na
Butter Cake	35	48.47 ± 0.48	6.98 ± 0.07	na	64.72 ^b	na	na	na
Chiffon Cake	40	55.51 ± 0.42	11.55 ± 0.09	na	60 ± 6 ^b	na	na	na
Fine Wholemeal Bread	67	74.54 ± 0.72	22.51 ± 0.22	10.08	68±9 ^a 85 ± 5.9 ^b	30	11	7
Bread with Chocolate Filling	50	64.42 ± 0.08	16.43 ± 0.02	na	na	na	na	na

eGI and eGL values used with glucose as the reference food

na: not available.

eGL are calculated based on carbohydrate per serving in the nutrition information panel by the manufacturer.

Superscript a indicates (Mean ± SEM) from the International Table of GI and GL Values 2008 (Atkinson et al., 2008)

superscript b indicates (Mean ± SEM) from Henry et al. (2021)

The possible explanation for the differences is due to different types of flour used or different processing methods (Foster-Powell et al., 2002). The glycaemic response is reported to be lower in bread when high amylose wheat flour was used (Belobrajdic et al., 2019). Zhu et al. (2020) also discovered a strong inverse correlation between GI values and amylose. Elevated amylose content produced a higher resistance starch which can lower the postprandial glucose (Hallström et al., 2011).

The eGL values acquired with glucose as the reference food was compared with the reported values in the International Table (Atkinson et al., 2008) (Table 4). Only two of the studied baked products can be compared, which include the cream cracker and wholemeal bread. The eGL values for cream cracker (14.34 ± 0.27) and fine wholemeal bread (22.51 ± 0.22) based on the pack serving size were greater than the reported GL values (11 and 7, respectively). It should be noted that the pack serving size of the studied baked products were bigger than the serving size used in the International table (Atkinson et al., 2008). Indeed, different amounts of serving sizes give different eGL values. When the serving size was fixed and equivalent according to the International Table (Atkinson et al., 2008), the eGL values were similar to the reported GL values. Cream crackers had eGL value of 11.56 while fine wholemeal bread had eGL value of 10.08 compared to reported GL values of 11 and 7, respectively. The majority of the foods used in the International Table by (Atkinson et al., 2008) are of Australian, British or Canadian origin, and some of Danish, Indian and Japanese origin (Sun et al., 2021). Currently, there is only a small number of literature about GI and GL values of Southeast Asian foods, especially for Malaysia.

4.6 Correlation of Estimated Glycaemic Index (eGI) with Estimated Glycaemic Load (eGL) and Analysed Nutrient Content

Pearson correlation coefficients were computed to assess the relationship between eGI with eGL and analysed nutrient content where $p < 0.01$ was treated as significant. The results analysis is presented in Table 5.

Table 5: Correlation of estimated glycaemic index (eGI) with estimated glycaemic load (eGL) and analyzed nutrient content

Measure	eGI ₁	
	<i>r</i>	<i>p</i>
eGL ₁	.905*	0.000
Total available carbohydrate content	-.318	0.198
Fat Content	-.737*	0.000

The correlation was determined by conducted Pearson's Correlation analysis. *Correlation is at $p < 0.01$. Subscript 1 indicates fresh white bread is used as the reference food

A significant, very strong positive correlation was observed between eGI and eGL (glucose as the reference food, $r(16) = .905$, $p < 0.01$). A similar finding was reported by Mohd Yusof et al. 2010) where dietary GI was positively correlated with dietary GL ($r = 0.49$, $p < 0.001$). However, Venn & Green (2007) mentioned that the relationship of GI and GL is not straightforward because GL is dependent upon the amount of carbohydrate consumed. The authors mentioned that a high GI food can have a low GL if it is eaten in small quantity. GI is a relative glycaemic response and represents the quality of the carbohydrate in food (Augustin et al., 2015). Meanwhile, GL represents the quantity of carbohydrates that gives an impact on glucose response

which is dependent on GI and the total amount of carbohydrate ingested (Eleazu, 2016).

In this study, no significant correlation was found between total available carbohydrates and eGI ($r(16) = -.318, p = 0.198$). The finding contradicts previous papers that showed a positive correlation between GI and carbohydrates. Hakimah et al., (2020) found a weak correlation while a moderate correlation was recorded by Raczkowska et al., (2019). The contradictory finding might be due to different methods of carbohydrates determination were used. The previous study used carbohydrate by difference when performing the correlation while this study used the Clegg-anthrone method.

In addition, this study found that fat content ($r(16) = -.737, p < 0.01$) showed strong, significant but negatively correlated with eGI. The result is aligned with previous studies (Hakimah et al., 2020; Hettiaratchi et al., 2009; Jenkins et al., 1981; Zhu et al., 2020). However, some papers remarked no relationship exists between GI value and fat (Henry et al., 2005; Lok et al., 2010; Sun et al., 2021). Furthermore, the previous correlation was not specifically done in baked products. Therefore, future studies can be done to identify the correlation of GI with macro nutrients in baked products. Amylose - lipid complexes are formed when fat is present in a carbohydrate meal. The amylose-lipid complexes limit starch digestion which resulting in lower GI (Wee & Henry, 2020). Fat also slows down the rate of gastric emptying which will lower the rate of carbohydrates digested in the intestine (Eleazu, 2016). This can also lower the glycaemic response.

Unfortunately, the correlation for protein was not done due to limited time. In short, protein is insulinotropic which can stimulate insulin production that can reduce

the glycaemic response (Eleazu, 2016). Protein-starch interaction can also limit the digestibility of starch (Wee & Henry, 2020).



CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study aimed to determine *in vitro* glycaemic index among selected packaged baked products available in the local market. Above all, based on the findings, the studied packaged baked products can be categorized into all three classifications for both GI and GL. In terms of GI classification, fine wholemeal bread and cream cracker were high-GI foods while bread with chocolate filling and chiffon cake was moderate-GI foods. Meanwhile, low-GI foods were cookies with cream and butter cake. When GL classification was applied, fine wholemeal bread was the only high-GL food. Medium-GL foods were chiffon cake, bread with chocolate filling and cream cracker. Whereas, butter cake and cookies with cream were low-GL foods.

Secondly, the highest eGI value was found in fine wholemeal bread while the lowest eGI value was observed in butter cake. Similarly, fine wholemeal bread also obtained the highest eGL value while butter cake has the lowest eGL value. Next, this study managed to determine *in vitro* GI and GL in bread with chocolate filling and cookies with cream which was not reported in the previous studies, especially in Malaysia. The values obtained can be used as a guide for future research.

Furthermore, this study found that eGI was significantly and strongly correlated with eGL. Whereas, no significant correlation was observed between eGI and total available carbohydrates. Lastly, eGI exhibited significantly strong but negatively correlated with fat content. In the previous studies, the mixed finding was recorded for the correlation of GI with macro nutrient content.

5.2 Limitations and Recommendations

Several recommendations to the public and diabetic patients can be proposed based on the findings of this study. Firstly, low-GI foods are regarded as a good GI and suitable for the public especially for diabetic patients, to avoid a surge of blood glucose level. The GI claims available on the package of certain baked products can be used as a guideline by the public. However, the public is recommended to not only focus on the GI value of the foods alone. The low GI can be due to the higher content of fat in the baked products as found in butter cake in this study. Thus, the public should identify the fat content in the baked products and choose a lower fat content product within the low-GI foods. Next, the public should consider the GL of the baked product, and not the GI alone. GL can also be used as a guide as it is based on the serving size per consumed. Therefore, it is suggested for the manufacturer to include the GL label together with the GI claims to provide clearer guidance to the public for healthier choices.

In the Malaysian Dietary Guidelines, Malaysians are recommended to choose at least half of the grain products from whole grains (National Coordinating Committee on Food and Nutrition, 2010). This can be done by substituting the refined grain product such as white bread with a whole grain product such as wholemeal bread. This study also found that fine wholemeal bread had lower eGI values than white bread. However, the fine wholemeal bread was categorized as high-GI food in this study which can increase the blood glucose immediately after being consumed. Hence, the public is recommended to choose a better choice of whole grain products with lower GI values such as wholegrain oat bread and wholegrain mixed rye bread (Lanzerstorfer et al., 2018).

There are several limitations and recommendations identified from this study. First and foremost, the eGI obtained in this study was through *in vitro* study that can be served as a predictive GI for *in vivo* and can be used in food development studies. Therefore, to obtain a more reliable eGI value, the incorporation of *in vivo* GI should be done in the future. Secondly, due to limited time, protein and dietary fibre analysis cannot be done. Thus, we are unable to see the trends of protein and dietary fibre in our samples. We are also incapable of observing the correlation between eGI with protein and dietary fibre content of all the samples. Future research should perform the protein and dietary fibre analysis to acquire a comprehensive correlation.

Next, this study only determined total starch content. Due to this, the composition of the starch in the samples remain unknown. In the future, estimation of resistant starch should be included to have more information on starch in the samples. Then, the study only sampled packaged baked products each of category available in the local market. Future studies are suggested to focus on one category to have an overall representation. Lastly, the studied baked product had undergone over-drying before *in vitro* analysis. Oven-drying is a processing step that could influence the starch hydrolysis in baked products. As a recommendation, before analysis, baked products should be freshly ground or freeze-dried.

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APPENDICES

APPENDIX I

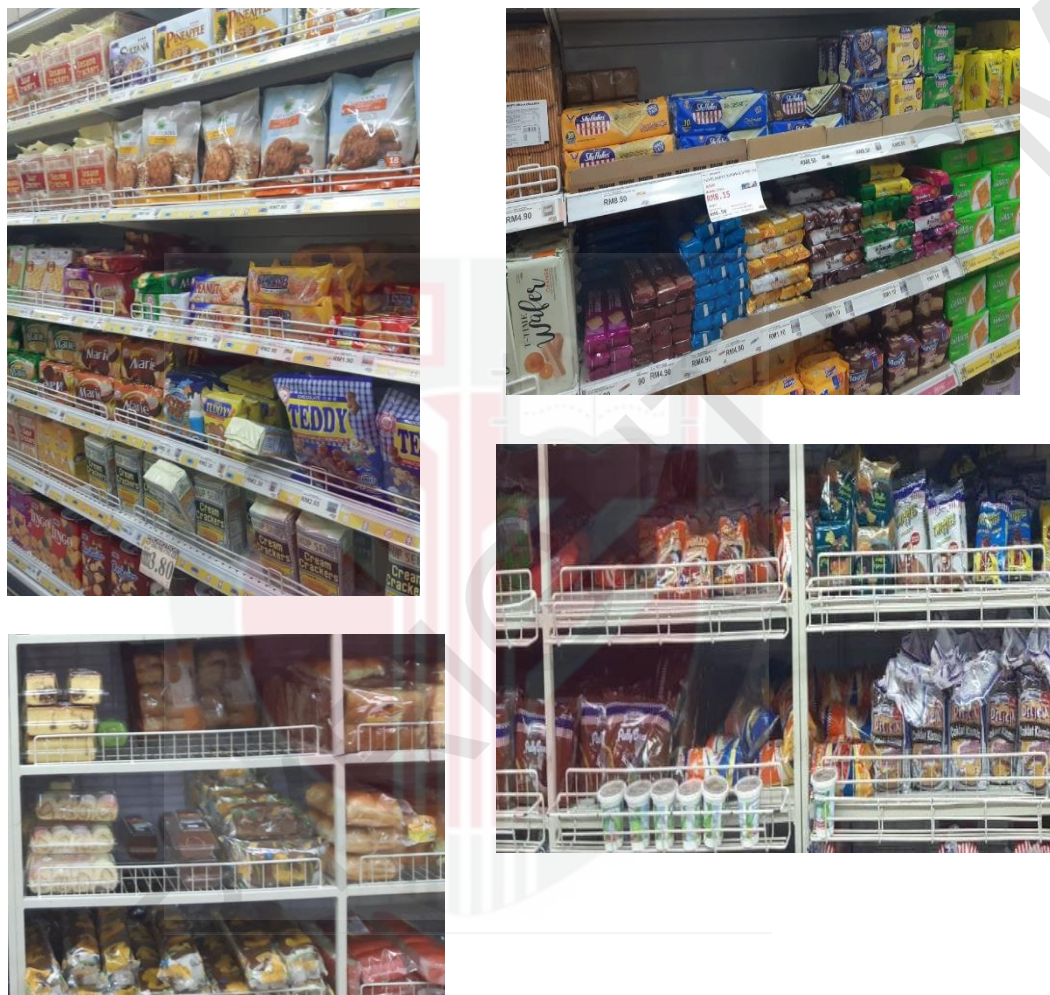


Figure 1: Various categories, brands and types of packaged baked products observed in the local market during the survey



Figure 2. Cream crackers



NUTRITION INFORMATION			INFORMASI NILAI GIZI	
Serving Size: 28.5 g (3 Sandwiches) Servings Per Package: about 4			Takaran Saji : 28.5g (3 Sandwich) Jumlah Sajian per Kemasan : sekitar 4.5	
	Per Serving	Per 100g	JUMLAH PER SAJIAN	
Energy	139 kcal	489 kcal	Energi Total	140 kkal
Protein	1.3 g	4.7 g	Energi dan Lemak	50 kkal
Fat	5.9 g	20.6 g		
Saturated Fat	2.8 g	9.9 g	Lemak Total	6g 9%
Trans Fat	0 g	0 g	Lemak Jenuh	3g 14%
Monounsaturated Fat	2.0 g	7.1 g	Lemak Tidak Jenuh Tunggal	2g
Polyunsaturated Fat	1.0 g	3.6 g	Lemak Tidak Jenuh Ganda	1g
Cholesterol	0 mg	0 mg	Lemak Trans	0g 0%
Total Carbohydrates	20.3 g	71.3 g	Kolesterol	0 mg 0%
Sugars	10.9 g	38.2 g	Protein	1g 2%
Dietary Fibre	0.6 g	2.0 g	Karbohidrat Total	20g 6%
Sodium	148 mg	520 mg	Serat Pangan	1g 2%
			Gula	11g
			Natrium	150 mg 10%

*Persen ANG berdasarkan pada kebutuhan energi 2150 kkal. Kebutuhan energi anda mungkin lebih tinggi atau lebih rendah.

KOMPOSISI: TEPUNG TERIGU, GULA, MINYAK NABATI (MENGANDUNG ANTIOKSIDAN TBHQ), BUBUK COKLAT, SIRUP FRUKTOSA, PATI JAGUNG, PENGEMBAK (NATRIUM & AMONIUM BIKARBONAT), GARAM, PENGEMULSI (LESITIN KEDELAI), PERISA SINTETIK VANILA.

MENGANDUNG ALERGEN, LIHAT DAFTAR BAHAN YANG DICETAK TEBAL.

DIPRODUKSI PADA PERALATAN YANG JUGA MEMROSES PRODUK YANG MENGANDUNG SUSU DAN KACANG.

Figure 3. Cookies with cream



Figure 4: Butter cake



Ramuan:
 Telur ayam, gula, tepung gandum "unbleached", lemak sayuran (berasaskan kelapa sawit tanpa penghidrogenan), gliserin, sorbitol, tepung susu, agen penaik, garam, tepung kanji yang diubahsuai, asid sitrik, kalium sorbat. Mengandungi penstabil and pengemulsi sebagai kondisioner makanan yang dibenarkan. Mengandungi perisa makanan yang dibenarkan. Semua aditif adalah daripada tumbuhan dan sintetik.

Maklumat Pemakanan	
Hidangan Setiap Bungkus:	1
Saiz Hidangan:	40g
Per Hidangan	
Tenaga	179kcal
Karbohidrat	20.8g
Protein	3.4g
Lemak	9.2g
Asid Lemak Monotidaktepu	4.0g
Asid Lemak Politidaktepu	1.2g
Asid Lemak Tepu	4.0g
Asid Lemak Trans	0g

Figure 5: Chiffon cake

APPENDIX II



RAMUAN:
Tepung gandum penuh berprotein tinggi "unbleached", air, tepung gandum berprotein tinggi "unbleached", lemak sayuran (berasaskan kelapa sawit tanpa penghidrogenan), gula, protein gandum, garam, vitamin dan mineral, yis, kondisioner doh (mengandungi gandum), kalsium propionat.
Mengandungi pengemulsi dan enzim (mengandungi gandum dan soya) sebagai kondisioner makanan yang dibenarkan. Produk ini dikilangkan dengan menggunakan mesin yang memproses produk yang mengandungi susu.

INGREDIENT LIST:
Unbleached high protein whole wheat flour, water, unbleached high protein wheat flour, vegetable fat (non-hydrogenated palm based), sugar, wheat protein, salt, vitamins and minerals, yeast, dough conditioner (contains wheat), calcium propionate. Contains emulsifiers and enzymes (contains wheat and soy) as permitted food conditioners.
This product is manufactured on equipment that processes products containing milk.

NUTRITION INFORMATION/ MAKLUMAT PEMAKANAN		
Serving Per Package/ Hidangan Setiap Bungkus	6	
Serving Size/ Saiz Hidangan	67g (2 slices)	
	Per Serving/ Per Hidangan (67g)	Per 100g
Energy/ Tenaga	179kcal	267kcal
Carbohydrate/ Karbohidrat	30.2g	45.0g
Protein/ Protein	6.5g	9.7g
Fat/ Lemak	3.6g	5.4g
Monounsaturated Fatty Acid/ Asid Lemak Monotidaktepu	1.0g	1.5g
Polysaturated Fatty Acid/ Asid Lemak Politidaktepu	0.6g	0.9g
Saturated Fatty Acid/ Asid Lemak Tepu	2.0g	3.0g
Trans Fatty Acid/ Asid Lemak Trans	0g	0g
Cholesterol/ Kolesterol	0mg	0mg
Sodium/ Natrium	289mg	431mg
Dietary Fibre/ Serabut Diet	3.4g	5.1g
Thiamin/ Tiamina (Vitamin B1)	0.6mg	0.8mg
Riboflavin/ Riboflavin (Vitamin B2)	0.2mg	0.3mg
Niacin/ Niasin (Vitamin B3)	2.7mg	4.0mg
Calcium/ Kalsium	101mg	150mg
Iron/ Zat Besi	1.9mg	2.8mg
Folic Acid/ Asid Folik	55µg	82µg

Figure 6: Fine wholemeal bread

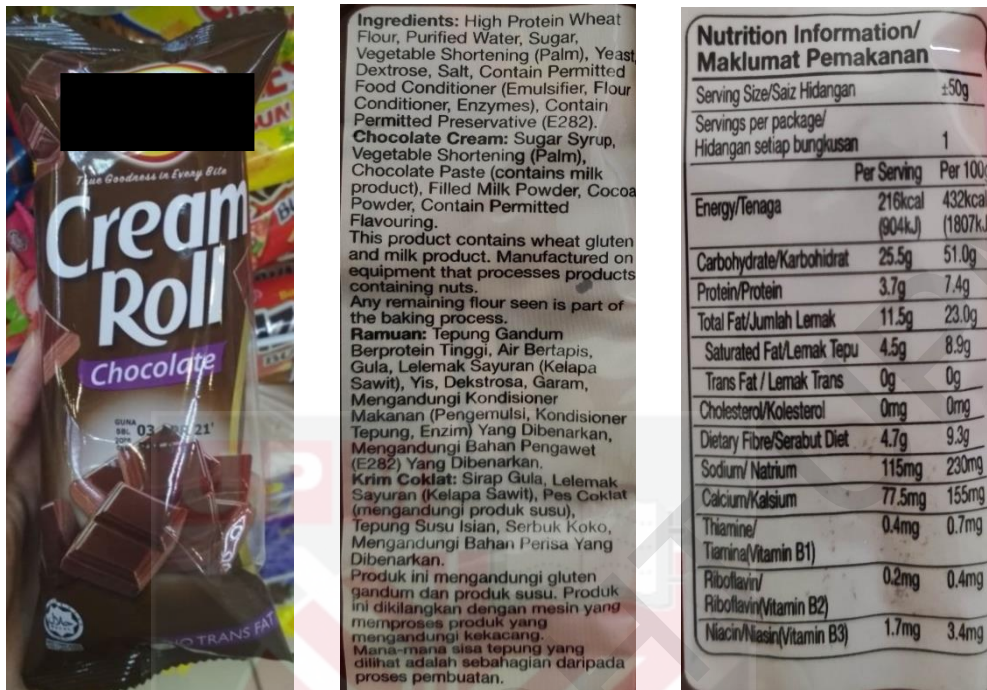


Figure 7: Bread with chocolate filling