EFFECT OF SWEETENER AND RESIDUE OF OVERRIPE BANANA INCORPORATION ON PHYSICOCHEMICAL PROPERTIES, SENSORY ACCEPTABILITY AND GLYCAEMIC INDEX OF CHOCOLATE COOKIES

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EFFECT OF SWEETENER AND RESIDUE OF OVERRIPE BANANA INCORPORATION ON PHYSICOCHEMICAL PROPERTIES, SENSORY ACCEPTABILITY AND GLYCAEMIC INDEX OF CHOCOLATE COOKIES

by

NG YEE VERN

Thesis submitted in fulfilment of the requirements for the degree of Master of Science

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<tr>
<td>cm</td>
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</tr>
<tr>
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<td>Micrometre</td>
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<tr>
<td>nm</td>
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<td>Greater than</td>
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</tr>
<tr>
<td>≥</td>
<td>Greater than or equal to</td>
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<tr>
<td>≤</td>
<td>Lower than or equal to</td>
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<td>Kilogram</td>
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<tr>
<td>p</td>
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<td>mmol/l</td>
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<td>N</td>
<td>Normality</td>
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<tr>
<td>°Brix</td>
<td>Degree brix</td>
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<tr>
<td>w/v</td>
<td>Weight per volume</td>
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<tr>
<td>M</td>
<td>Molar</td>
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<td>s</td>
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<tr>
<td>AACC</td>
<td>American Association of Cereal Chemist</td>
</tr>
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<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>et al.</td>
<td>And others</td>
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<tr>
<td>FAO</td>
<td>Food and Agricultural Organization</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GI</td>
<td>Glycaemic index</td>
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<td>GL</td>
<td>Glycaemic load</td>
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<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
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<tr>
<td>HCL</td>
<td>Hydrochloric acid</td>
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<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental area under curve</td>
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<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
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<tr>
<td>IOM</td>
<td>International Organization for Migration</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<td>MANS</td>
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<td>Malaysian Dietary Guidelines</td>
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<tr>
<td>Min</td>
<td>Minute(s)</td>
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<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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<td>QC</td>
<td>Quality control</td>
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<tr>
<td>RDA</td>
<td>Recommended Daily Allowance</td>
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<td>RNI</td>
<td>Recommended Nutrients Intake</td>
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<td>SBP</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>Statistical Package for Social Science</td>
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<td>TDF</td>
<td>Total dietary fibre</td>
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<td>Texture profile analysis</td>
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<tr>
<td>TSS</td>
<td>Total soluble solid</td>
</tr>
<tr>
<td>UHT</td>
<td>Ultra-heat treatment</td>
</tr>
<tr>
<td>USDA</td>
<td>United State Department of Agricultural</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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KESAN PENAMBAHAN PEMANIS DAN SISA PISANG RANUM KE ATAS CIRI-CIRI FIZIKOKIMIA, PENERIMAAN SENSORI DAN INDEKS GLISEMIK BISKUT COKLAT

ABSTRAK

Permintaan untuk produk bakeri yang diperkaya serat dan rendah gula semakin meningkat kerana prevalens diabetis yang tinggi. Pisang adalah salah satu buah yang disukai ramai dan memberikan faedah kesihatan pemakanan yang sangat baik. Sementara itu, banyak pisang ranum telah dibuang kerana kualiti dan penampilannya yang rendah. Namun begitu, pisang ranum menunjukkan sumber yang kaya dengan pemanis semulajadi dan serat dietari yang berpotensi digunakan sebagai ramuan makanan baru untuk menggantikan gula dan tepung gandum dalam produk bakeri. Oleh itu, kajian ini bertujuan untuk menentukan komposisi pemakanan, profil tekstur, penerimaan sensori dan nilai indeks glisemik (GI) biskut coklat yang ditambah dengan pemanis pisang ranum (OBS) sebagai pengganti separa (0, 5, 10, 15 dan 20%) untuk gula dan penggunaan residu pisang ranum (OBR) sebagai pengganti separa (8%) untuk tepung gandum. Keputusan telah menunjukkan bahawa penambahan OBR dan OBS meningkatkan nilai pemakanan biskut coklat. Biskut coklat yang ditambah dengan 8% OBR + 20% OBS mencatat kandungan serat dietari (7.80%) dan abu (1.47%) tertinggi. Kedua-duanya kandungan karbohidrat dan sukrosa biskut coklat dikurangkan dengan peningkatan tahap OBS. Analisis profil tekstur menunjukkan peningkatan bagi nisbah sebaran dan menurun bagi kekerasan dengan peningkatan tahap OBS dalam biskut coklat. Skor sensori untuk 0% dan 8% OBR biskut coklat tidak menunjukkan perbezaan yang signifikan. Selain itu, penambahan OBS dalam biskut coklat sehingga 15% menghasilkan skor tertinggi...
dari segi aroma, rasa dan penerimaan keseluruhan. Berdasarkan keputusan yang didapati dari ujian penerimaan sensori, tiga formulasi biskut coklat (0%, 8% OBR dan 8% OBR + 15% OBS) dipilih untuk ujian GI. Keputusan didapati bahawa nilai GI untuk 0%-biskut coklat, 8% OBR-biskut coklat dan 8%OBR + 15%OBS-biskut coklat masing-masing adalah 63, 56 dan 50. Hasil kajian ini menunjukkan bahawa pisang ranum boleh digunakan sebagai ramuan makanan dalam penghasilan biskut coklat yang berkhasiat dan rendah GI.
EFFECT OF SWEETENER AND RESIDUE OF OVERRIPE BANANA INCORPORATION ON PHYSICOCHEMICAL PROPERTIES, SENSORY ACCEPTABILITY AND GLYCAEMIC INDEX OF CHOCOLATE COOKIES

ABSTRACT

Demand for dietary fibre-enriched and low sugar bakery products is increasing rapidly due to current high incidence of Type 2 diabetes mellitus. Banana is one of the most consumed fruit which provide excellent nutritional health benefits. Meanwhile, overripe banana has been discarded due to its low quality and appearance. Despite its appearance, overripe banana exhibits rich sources of natural sweetener and dietary fibre which could potentially be used as a novel food ingredient to replace added sugar and wheat flour in bakery product. Thus, the study aims to determine the nutritional values, physical properties, sensory acceptability and glycaemic index (GI) value of chocolate cookies formulated with overripe banana sweetener (OBS) as partial replacement (0, 5, 10, 15 and 20%) for table sugar and utilization of overripe banana residue (OBR) as partial replacement (8%) for wheat flour. Results have shown that incorporation of OBR and OBS significantly (p<0.05) increased nutritional values of chocolate cookies. Chocolate cookies formulated with 8% OBR+20% OBS recorded the highest TDF (7.80%) and ash (1.47%) content. Both carbohydrate and sucrose content of chocolate cookies were reduced significantly with increasing level of OBS. In texture profile analyses, an increment in spread ratio as well as a decrease in firmness were shown with increasing levels of OBS in chocolate cookies. Sensory scores for control and 8% OBR-incorporated cookie were not significant difference for all the sensory attributes. Moreover, incorporation of OBS up to 15% produced the highest scores in
term of aroma, flavour and overall acceptance. Based on the sensory acceptability results, Three formulations of chocolate cookies (0%, 8% OBR and 8% OBR+15% OBS) were selected for GI testing. It was found that the GI values for 0%-cookie, 8% OBR-cookie and 8%OBR+15%OBS-cookie were 63, 56 and 50, respectively. The results of this study showed that overripe banana can be used as a food ingredient in developing low-GI chocolate cookie.
CHAPTER 1
INTRODUCTION

1.1 Background and problem statements

In recent decades, the incidence of chronic diseases is increasing at an alarming rate. Chronic diseases include cardiovascular diseases (CVD), cancers, chronic respiratory diseases and diabetes mellitus (DM) are the major cause of mortality in the world (Ramli and Taher, 2008). The prevalence of chronic diseases is rising rapidly and is forecasted to exceed as the common causes of death by 2030 (WHO, 2011). Among the chronic diseases, DM is currently a global public health concern and reported to have led to 1.6 million death globally in 2016 (WHO, 2016). The prevalence of DM has doubled in the past three decades, ranging from 4.7% in 1980 to 8.8% in 2017 and it is expected to increase to almost 10% by the year 2045, equalling to 9.9% of the population (Cho et al., 2018). Furthermore, Malaysia was reported to probably have the distinction of having the highest prevalence of DM among all the countries in ASEAN region (Chan, 2015). The Ministry of Health (MOH, 2015) reported that DM in Malaysia increased in line with age which starting from 0.7% within the age group of 20 – 24 years old, arriving a peak of 27.9% at age group 70 – 74 years. The incidence of DM is due to many factors, for example rapid urbanization, eating habit and increasing rates of obesity and sedentary lifestyle (Hu, 2011). Even there are plenty of efforts and anti-diabetic agents provided, DM is still a major cause of morbidity and mortality worldwide (Erejuwa et al., 2012).

The increasing trend of DM has led to high demand of diabetes-related functional food with the purpose of improving their blood glucose control. One of the approaches is by assessing the physiological effects of food using the concept of glycaemic index (GI) which is a value given to carbohydrate-rich foods based on
their effect on post-meal glycaemia (Esfahani et al., 2009). The GI of a food depends on the rate of digestion and absorption of carbohydrates in the small intestine (Arvidsson-Lenner et al., 2004). As a result, low-GI foods are able to produce a gradual rise in blood glucose level and are therefore, more favourable in terms of health, especially for the management of diabetes. There are several food components that can be used to reduce the GI of a food. These include the presence of dietary fibre and types of sugar added in the food.

Dietary Fibre (DF) is the edible part of plant which is resistant to enzymatic digestion and absorption in human small intestines with complete or partial fermentation in the large intestine (Dhingra et al., 2012; AACC 2001). DF is an important component in our daily diet and naturally present in cereals, vegetables, fruits and nuts (Dhingra et al., 2012). DF plays an important role in human health and body function (Dreher, 2001). High consumption of DF has been proven to reduce the risk of certain types of diseases such as obesity, diabetes, cancer and cardiovascular diseases (Cho et al., 2013; Lattimer et al., 2010). DF particularly soluble fibre is mostly found in fruits and vegetables and has been shown to have a direct effect on the GI of a food (Weickert and Pfeiffer, 2018). The viscous, gel-forming and more readily fermented properties of these fibres increase viscosity of food which leads to prolonged carbohydrate digestion and absorption as well as improved satiety. Thus, lowering postprandial blood glucose level (Post et al., 2012). The recommended DF intake for adults is 20-35 g/day (ADA, 2001). Nevertheless, the intake of DF among populations is low, ranging from only 3 – 16 g/day (Lee and Wan Muda, 2019).
Sugars are one of the major components in our diet and are normally added into foods and beverages to enhance the colour, texture and flavour development (Goldfein and Slavin, 2015). The most common of which is sucrose or table sugar which is widely used in the food industry for the production of commercialised food products such as bakery products, ice cream and carbonated beverages (Zargaraan et al., 2016). However, over consumption of sucrose-rich product may lead to weight gain, cardiovascular diseases and type 2 diabetes (Goldfein and Slavin, 2015). As a consequence, artificial sweeteners (non-nutritive sweetener) such as aspartame, sucralose, neotame and saccharin have received great attention to replace added sugar as these sugars generally have little to no calories and provide low glycaemic response (Mattes and Popkin, 2008). Nevertheless, the effect of artificial sweeteners on human health is still a controversial topic. There are previous studies that found the links between artificial sweeteners and adverse health effects, such as weight gain, cancer, nausea, diarrhea and metabolic disorders (Harpaz et al., 2018; Hampton, 2008; Whitehouse et al., 2008). Moreover, there was a study which reported that artificial sweeteners induce glucose intolerance by altering the gut microbiota (Suez et al., 2014). Hence, there is an increasing interest in searching for a more natural and nutrient-rich sweetener. Alternative sweeteners that are believed to be healthier are natural sweeteners from high sugar tropical fruits such as banana, pineapple, mangoes and pomegranate. Interestingly, these are safer to be consumed due to no toxicity claimed (Song et al., 2006).

Banana (Musa sp.) is one of the most widely consumed fruit and main international trade fruit in the world (Castelo-Branco et al., 2017). Today, banana is the world second largest fruit crop with an estimated gross production exceeds 117 million tonnes (FAO, 2017). Banana has long been recognised as a great source of
nutrients and beneficial effects on human health, for instance lower risk of high blood pressure and stroke, improve bowel movement, maintain blood sugar level and help in weight loss (Sidhu and Zafar, 2018; Kumar et al., 2012). However, banana is a perishable fruit that has a short lifespan from harvest until the onset of deterioration (Karim et al., 2018). Previous studies have claimed that the purchase intention for overripe banana was significantly low due to low quality, appearance of brown spots as well as decrease in firmness of the pulp (Rohm et al., 2017; Symmank et al., 2018). As a result, banana has been shown to be one of the most-wasted products as most retailers ask for fruit in yellow colour which associated with ripe banana (Mattsson et al., 2017; Shahir and Visvanathan, 2014). Despite that, overripe banana provides an excellent source of vitamins (A, B₆, C and D), minerals (potassium and magnesium), DF and natural sweetener (Kumar et al., 2012). The sugar content, which is mainly composed of sucrose, fructose and glucose is increased tenfold from unripe banana to overripe banana, rising from 1.26 to 12.28% (Yap et al., 2017). A previous study by Chaipai et al. (2018) revealed that the DF in banana pulp does not vary with maturity although most of the starch will be converted into sugar in overripe banana. There have been a few studies that utilized overripe banana or second grade banana to produce banana puree films (Martelli et al., 2012) and biodegradable starch laminates (Alanís-López et al., 2011). However, overripe banana is still underutilized and very little effort has been made to identify its functionality in terms of application to food (Padam et al., 2014). Hence, the utilization of overripe banana will not only help in increasing the value of food products but also indirectly reduce food waste.

Presently, bakery products such as breads, cakes, cookies, and pastries have a great demand as one of integral part in our daily life. Most of people eat baked goods
to reduce the consumption of staple food such as rice (Sheng et al., 2008). Cookies are small, flat and sweet bakery product that are widely consumed due to their ready-to-eat, convenient, affordable cost, high availability and longer shelf life (Adeyeye and Akingbala, 2015). According to Malaysian Adults Nutrition Survey (MANS, 2014), cookies is in the top 10 lists of daily consumed food among Malaysians (Kasim et al., 2018). Hence, cookies could be an alternative carrier of nutrients and utilised as a source for addition of various nutritionally rich ingredients for their diversification (Nandeesh et al., 2011). Nevertheless, cookies in the market are mostly high in fat and sugar content as well as lack of DF content. Currently, research has been conducted on the use of banana pulp powder as a source of DF in noodle (Ritthiruangdej et al., 2011), ice cream (Yangılar, 2015) and wheat-based product (Loong and Wong, 2018; Adubofuor et al., 2016). However, there is a lack of overripe banana application in bakery product such as cookie. In this context, the objective of this study was to determine the nutritional properties, sensory acceptability and glycaemic index (GI) value of chocolate cookies formulated with overripe banana sweetener (OBS) as partial replacement for table sugar and utilization of overripe banana residue (OBR) as partial replacement for wheat flour. OBS is the sugar extracted from overripe banana pulp whereas OBR is the remaining residue of the banana pulp after the removal of sugar.
1.2 Rational and significance of the study

Eating chocolate cookies can be associated with a range of emotions. Aside from the emotional comfort that chocolate cookies may provide, there are scientific explanations for why people salivate for them. Some research suggests that the ingredients in chocolate cookies may have addictive properties with sugar has the most profound effect. Evidence has shown that sugars can induce rewards and cravings in magnitude to those induced by addictive drugs (Ahmed et al., 2013). Besides, chocolate contains small amounts of a compound known as anandamide which is also a brain chemical that is responsible for mood-altering effects (Benton and Nehlig, 2004). According to Wenk (2019), chocolate which in addition to sugar and fat may raise further the level of anandamide in our brains making chocolate cookies so universally craved that most people find them irresistible.

However, most of commercialized chocolate cookies are often high-fat and sugar-rich which resulted in low nutrient content and high level of GI. Hence, chocolate cookies are often been identified as unhealthy food or snack. There is a continuous worrying trend of developing food products which are focusing on the taste but neglecting the health issue. As a consequence, greater numbers of people are diagnosed with chronic illness. By incorporating natural ingredients into chocolate cookie, consumers have greater chance to have healthy food items in their daily food choice as well as making chocolate cookie part of a healthy diet.

Due to the fact that overripe banana are often treated as waste, its immense nutritional content is believed to have the potential to develop DF-rich and low-GI chocolate cookie. Furthermore, by utilizing locally available natural ingredients can
avoid increment in the production cost as well as selling price of the chocolate cookies, at the same time could reduce the wastage of food.

1.3 Objectives of the study

1.3.1 General objective

To investigate the physicochemical properties, sensory acceptability and glycaemic index (GI) value of chocolate cookies incorporated with overripe banana sweetener (OBS) and overripe banana residue (OBR).

1.3.2 Specific objectives

1. To determine the nutritional compositions of chocolate cookies incorporated with different percentage of OBS and OBR.

2. To examine the texture profiles and microstructure characteristics of chocolate cookies incorporated with different percentage of OBS and OBR.

3. To evaluate the sensory acceptibility of chocolate cookies incorporated with different percentages of OBS and OBR

4. To determine the GI of chocolate cookies incorporated with OBS and OBR.
CHAPTER 2
LITERATURE REVIEW

2.1 Banana (Musa sp.)

Figure 2.1 shows a taxonomy background of banana. The taxonomy group of banana was first identified and described by Carl Linnaeus in 1753 (Hyam and Pankhurst, 1995).

Scientific classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Liliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Zingiberales</td>
</tr>
<tr>
<td>Family</td>
<td>Musaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Musa</td>
</tr>
<tr>
<td>Species</td>
<td>Musa accuminata</td>
</tr>
<tr>
<td></td>
<td>Musa balbisiana</td>
</tr>
</tbody>
</table>

Common names

Dessert banana, forbidden fruit, banana, nana

Figure 2.1 Taxonomy background of banana (The Plant List, 2013)

Banana (Musa sp.) is an edible fruit produced by a plant belonging to the genus of Musa from the family of Musaceae, which is a large monocotyledonous herb (Marikkar et al., 2016). It can grow from 2 to 9 meters in height at maturity (Marikkar et al., 2016; Nelson et al., 2006). As banana plants (Figure 2.2(a)) are normally tall and sturdy, they are often mistaken for trees, but the trunk is actually a “false stem” or pseudostem. A pseudostem composed of leaf sheaths and a terminal crown of leaves through which an inflorescence (flower spike) emerges carrying a
bunch of banana (consists of 50 – 150 bananas) as shown in Figure 2.2(b) (Stevens and Ware, 2018).

Figure 2.2(a)  Banana plant (Petruzello et al., 2019)

Figure 2.2(b)  Bananas growing in a bunch (Petruzello et al., 2019)
Bananas are plants of the tropical humid lowlands and are mostly grown between 40° north and south of the equator. According to Sulaiman et al. (2011), there are more than 20 edible banana cultivar types and most of them are derived from two wild species, known as *Musa acuminate* (A-genome) and *Musa balbisiana* (B-genome). *Musa acuminate* is the most widespread of the species and have their centre of diversity in the Malaysian (Simmonds, 1962) and Indonesia (Horry et al., 1997). As a result, this species is focused in this study. Meanwhile, the distribution of *Musa balbisiana* is reported from East India to South China, Philippines, Moluccas and New Guinea (Smartt, 1976; Tucker, 1993). Some examples of cultivated varieties of *Musa* are listed in Table 2.1.

Banana fruit is a very popular fruit in terms of its importance as a food crop in the world market (Singh et al., 2016). The fruit is variable in size, colour but is usually elongated-cylindrical and curved (Nelson et al., 2006). Furthermore, the fruit can vary in taste from starchy to sweet, and texture from firm to mushy depends on the ripeness of the banana (Stevens and Ware, 2018). Ripen banana is mainly eaten raw as a dessert or sweet fruit, whereas unripe banana is utilised in cooking (fried banana, banana chips etc) (Singh et al., 2018; Singh et al., 2016). Moreover, banana also can be processed into a number of food products, for example ripe banana can be made into puree form, which can further used in variety of products such as yogurt, ice cream, bakery products and baby food (Singh et al., 2016; Aurore et al., 2009); unripe green banana can be dried and ground into flour so that it can be stored for longer periods and utilized for other purposes (Cheok et al., 2018).
Table 2.1  Example of some cultivated varieties of *Musa*

<table>
<thead>
<tr>
<th>Crossed species</th>
<th>Main distribution</th>
<th>Genome group</th>
<th>Subgroup (Cultivar)</th>
<th>Common cultivar names</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. acuminate  × M. acuminate</td>
<td>Malaysia, India, Indonesia, Philippines, Sri Lanka, Thailand, Vietnam, Australia</td>
<td>AA</td>
<td>Inarnibal</td>
<td>Pisang lemak</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lakatan</td>
<td>Pisang berangan, Phayan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pisang Lilin</td>
<td>Lidi, Pisang Lidi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sucier</td>
<td>Lady’s Finger, Amas, Caramelo</td>
</tr>
<tr>
<td>M. acuminate  × M. balbisana</td>
<td>Philippines, Bhutan, China, India, Vietnam, Papua New Guinea, Sri Lanka</td>
<td>AB</td>
<td>Kamarangasenge</td>
<td>Sukali Ndizi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ney Poovan</td>
<td>Lady’s Finger</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maoli-Popoulu</td>
<td>Pacific Plantain, Comino, Pompo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pisang Raja</td>
<td>Pisang Raja, Larip, Houdir</td>
</tr>
</tbody>
</table>

Adopted from Ploetz *et al.*, 2007

2.1.1 The production of banana

Bananas are the fourth most important food crop after wheat, rice and maize in terms of production and are the world’s favourite fruit in terms of consumption quantity. It is an important staple food and contributes to the food security of millions of people in the developing world (Tripathi *et al.*, 2014). With the global population surpassing 7 billion people, the main driver of fast production expansion has been the increasing consumption requirements of rising population in developing countries. The bulk of the global production increase has come from top producers who are also top consumers such as Brazil, Philippine, India and China. Furthermore,
increasing health awareness in Western markets has contributed to the rising demand, with banana consumption having substantially gained in popularity among European and North American consumers.

As shown in Figure 2.3(a) and Figure 2.3(b), bananas are predominantly produced in Asia with Asia-Pacific leads the banana market with 61% share of global consumption. Within Asia-Pacific, India is the largest producer of bananas in the world, with a production of 29.7 million metric tons per years on average between 2010 and 2017, from an area of 0.84 million hectares (FAO, 2019). Other major banana-producing countries are China (11.58 million tons), Indonesia (7.26 million tons), Philippine (6.14 million tons) and Brazil (6.75 million tons) (FAO, 2020a; Shahbandeh, 2020).

The global banana exports were estimated at 19.2 million metric ton in 2018 and set a new record high of 20.2 million tonnes in 2019, an estimated increase of 5% as compared to 2018. Two leading exporters, Philippine and Ecuador is accountable for the rise (FAO, 2020b). Moreover, most of the bananas are consumed in the countries where they are grown and only 20% are exported to other countries (Singh et al., 2016). India’s exports of banana represent only 0.3% of the world exports since most of the banana grown in India are for the domestic market. Due to the structure of landholdings in India, there are certain limits on land usage. Thus, the contract-farming model is used, which allows the agribusiness producers to produce bananas in larger areas than the legal constraints. In India, production and productivity have increased significantly with the expansion of area under cultivation.
Figure 2.3(a)  World production of bananas in 2018 (Shahbandeh, 2020)

Figure 2.3(b)  Bananas production in 2018 (FAO, 2020a)
In Malaysia, banana is also one of the important fruit crops cultivated with ranked second in terms of production area and fourth in export revenue (Kayat et al., 2016). In Figure 2.4, the production trend shows that banana has increased continually from 2.8 million tons in 2013 to 3.5 million tons in 2017, an increase about 21.4% in 5 years (Abu Dardak, 2019). Most of the bananas grown commercially are found in Johor, Perak, Kelantan, Pahang, Kedah and Selangor (MOA, 2019). About 50% of the bananas cultivated are Pisang Berangan and the Cavendish type, and the remaining popular cultivars are Pisang Mas, Pisang Rastali, Pisang Raja, Pisang Awak, Pisang Nangka and Pisang Tanduk (Kayat et al., 2016). Bananas are mostly cultivated for local consumption as banana is the most-consumed fruits (10 kg/year) as compared with other fruits as reported by Department of Statistic Malaysia (2018) presented in Table 2.2. The consumption trend will determine the supply and demand of fruits in Malaysia as higher demand from domestic market will lead to higher income. Meanwhile about 12% of the total production (mostly the Cavendish variety) is exported, mainly to Singapore, Brunei, Hong Kong and the Middle East (MOA, 2015). Considering the importance of banana in domestic and export markets, the government has banana as one of the fruits to be prioritised for development under the National Agricultural Policy (Mohamad Roff et al., 2012).
Figure 2.4  Bananas production in Malaysia (Abu Dardak, 2019)

Table 2.2  Tropical fruit consumption per capita in Malaysia, 2018

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of fruits</th>
<th>Consumption (kg/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Banana</td>
<td>10.0</td>
</tr>
<tr>
<td>2.</td>
<td>Pineapple</td>
<td>7.6</td>
</tr>
<tr>
<td>3.</td>
<td>Durian</td>
<td>6.4</td>
</tr>
<tr>
<td>4.</td>
<td>Watermelon</td>
<td>3.3</td>
</tr>
<tr>
<td>5.</td>
<td>Guava</td>
<td>2.5</td>
</tr>
<tr>
<td>6.</td>
<td>Mango</td>
<td>2.0</td>
</tr>
<tr>
<td>7.</td>
<td>Jackfruit</td>
<td>1.9</td>
</tr>
<tr>
<td>8.</td>
<td>Papaya</td>
<td>1.7</td>
</tr>
<tr>
<td>9.</td>
<td>Rambutan</td>
<td>1.1</td>
</tr>
<tr>
<td>10.</td>
<td>Mangosteen</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Source: Department of Statistic Malaysia (2018)
2.1.2 Ripening of banana

Fruit ripening is a genetically programmed, highly coordinated process of organ transformation from unripe to ripe stage, in order to yield an attractive edible fruit with an optimum blend of colour, taste, aroma and texture (Maduwanthi and Marapana, 2017; Perotti et al., 2014). Bananas are climacteric fruits which are artificially ripened regularly. Ripening process of banana can be divided into three stages: pre-climacteric stage, climacteric/ripening stage and lastly ripe and senescence stage (Maduwanthi and Marapana, 2017; Robinson and Saúco, 2010). During ripening process of banana, bananas undergo a set of biochemical and physical changes that lead to a soft and edible ripe fruit.

2.1.2(a) Changes in physical properties of banana

Textural change is very important and a major event in fruit ripening. According to few studies, there are dramatic changes occur in banana peel colour and pulp texture during the rise in respiration during storage of climacteric fruit as shown in Table 2.3 and Figure 2.5. Skin colour changes from green to yellow, firmness is decreased and fruit gets softened (Adi et al., 2019; Karim et al., 2018; Mba et al., 2013; Tapre and Jain, 2012). Banana normally exhibit fruit softening mainly due to depolymerisation and solubilisation of cell wall components and loss of cell structure. Change in turgor pressure and degradation of cell wall polysaccharides and enzymatic degradation of starch are determinant mechanisms of fruit ripening (Adane et al., 2015; Li et al., 2010). According to Maduwanthi and Marapana (2017), the compounds responsible for the changes in peel colour are chlorophyll and carotenoids. As banana ripens, chlorophyll content decreases and become absent in ripe fruit (Moser et al., 2012). At the same time, the level of total carotenoids decrease to half the level at the colour break and subsequently again reached a level
similar to that in green banana (Aquino et al., 2018). Mainly colour changes in banana during ripening are based on the peel colour rather than the pulp colour. Hence, colour of banana peel has been used in the assessment of the stages of ripeness of banana (Karim et al., 2018; Sogo-Temi et al., 2014).

The physical and mechanical properties of banana fruit and changes in these parameters during different ripening stages is important attributes to design handling, sorting, peeling, processing and packaging system. Knowing these properties of agricultural products would help engineers to prepare the machine’s unit properly to protect fruits from bruises, injuries and other defects that emanate as results of post-harvest processing treatments.

Table 2.3 Description of banana ripening stages

<table>
<thead>
<tr>
<th>Maturity index</th>
<th>Storage day</th>
<th>Ripening stage</th>
<th>Peel colour</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>Mature</td>
<td>All peels are green</td>
<td>Hard, rigid</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>Early ripening</td>
<td>Green peel with a trace of yellow</td>
<td>Slightly bend, ripening started</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Intermediate ripening</td>
<td>More green area than yellow</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>Ripe</td>
<td>Yellow with green hint</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>9</td>
<td>Ripe</td>
<td>All yellow colour</td>
<td>Peel readily, pulp firm, aromatic</td>
</tr>
<tr>
<td>VI</td>
<td>11</td>
<td>Fully ripe</td>
<td>All yellow with brown speckles</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>13</td>
<td>Overripe</td>
<td>Yellow with increasing brown areas</td>
<td>Pulp soft and darkening, highly aromatic</td>
</tr>
</tbody>
</table>

Modified from Mba et al. (2013) and Karim et al. (2018)
2.1.2(b) Changes in chemical compositions of banana

The effects ripening on the chemical composition of banana at different levels of ripening stages have been studied by various researchers. The results showed that the chemical composition of banana pulp was diversely affected by ripening as shown in Table 2.4. Moisture content is an important characteristic as it affects the consumers’ acceptability of the fruit. The moisture content of banana was reported to increase significantly during ripening (Yap et al., 2017; Tapre and Jain, 2012). The increment of moisture content is mainly due to breakdown of carbohydrate within the fruit and moisture migrated from peel to pulp which also explains the softening of banana pulp as ripening proceeds (Mohapatra et al., 2010; Sakyi-Dawson et al., 2008).

A few studies reported a significant decrease in ash content with increase in banana maturity (Yap et al., 2017; Khawas et al., 2014; Adeyemi and Oladiji, 2009).
The variation in ash content may be due to differential absorption capacity of minerals at different stages of development (Adeyemi and Oladiji, 2009). Changes in mineral composition varied and were not consistent with the stages of ripeness. Study by Adeyemi and Oladiji (2009) reported an increase in zinc and manganese content until they reached a peak at the ripe stage and decreased afterward meanwhile a constant decline was shown in magnesium content as banana ripen. The decrease in magnesium level could be due to the conversion of chlorophyll (green pigment) in unripe banana to carotenoids (yellow colour characteristic) in ripe banana. It forms a non-enzymatic covalent bonding with chlorophyll thus its conversion to carotenoids (Toma et al., 2018). Magnesium is an important component of chlorophyll thus is higher in unripe banana (Adeyemi and Oladiji, 2009). In contrast, the concentration of magnesium and potassium was shown to increase as ripening occurred by Yap et al., (2017). Such variations could be because of different varieties of banana used and its growing condition (Yap et al., 2017).

Fat content was very low in unripe banana but appeared subsequently in ripe banana. Meanwhile, protein content of banana was also quite low with only 3% detected and did not markedly change during ripening (Chaipai et al., 2018; Robinson and Sauco, 2011).

The most prominent chemical change that occurs during ripening of banana is the hydrolysis of starch and the accumulation of sugars (glucose, fructose and sucrose) which are responsible for the sweetening of the fruit as the ripening progress has been reported by various studies. As banana ripen, starch content was shown to reduce gradually while total sugar content was observed to increase tenfold from stage 1 to stage 7 (Chaipai et al., 2018; Toma et al., 2018; Maduwanthi et al., 2017;
Yap et al., 2017; Zulkifli et al., 2016). Fructose content was found to be the lowest in unripe banana but was the dominant sugar in overripe banana followed by glucose and lastly sucrose (Yap et al., 2017). According to Xiao et al (2018), the conversion of starch-to-sugars during banana ripening is associated with the transformation of inedible-to-edible. The decline of starch content is explained by the degradation of starch and the formation of free sugars through enzymatic breakdown process. A study by Chaipai et al., (2018) found that the starch in unripe stage is resistant to enzymes. This is because the starch granule in unripe banana is generally tightly packed in a radial pattern and is dehydrated thus limits the accessibility of digestive enzymes. As ripening proceed, the starch granule is rehydrated by the increment of moisture content which loosens the compact structure of starch and therefore became less resistant.

Increase in solubility of pectin polysaccharides is also one of the major changes happens during banana ripening. Several studies have claimed that the total dietary fibre (TDF) was shown to increase significantly with the progress of maturity (Chaipai et al., 2018; Maduwanthi and Marapana, 2017; Khawas et al., 2014). The increase in DF content at ripe stage over unripe stage is most possibly due to increase in soluble fibre pectin which responsible for the softening of banana pulp. The increase of soluble pectin along with softening of banana pulp is related with pectin degradation of insoluble protopectin to soluble pectin by pectic enzymes (Maduwanthi and Marapana, 2017; Emaga et al., 2008). According to Chaipai et al. (2018), the cell wall of the unripe banana is more compact due to the pectin molecules being tightly bound in the cell wall, which contribute to the firmness of the fruit.
There are variations in pH and total soluble solids (TSS) at the different ripening stages of banana. Most of the studies indicated pH value of banana is inversely proportional to the banana ripeness (Adi et al., 2019; Toma et al., 2018; Zulkifli et al., 2016). The high pH at unripe stage which then reduced as banana ripens was a result of high organic acid contents in the fruit (Adi et al., 2018). The changes in banana acidity are mainly caused by the changes in malic acid, citric acid and oxalic acid during ripening process (Etienne et al., 2014). Besides that, a significant different of banana pH was observed by Mohapatra et al., (2016) and result showed that pH value could also be affected due to the conversion of starch to sugar in banana. Meanwhile, TSS reflects the sugar concentration in banana fruit, the changes in TSS content in ripe banana were found to be significant (Adi et al., 2019; Toma et al., 2018; Yap et al., 2017; Zulkifli et al., 2016). Moreover, the increment of TSS can be used as an indicator to relate the conversion of starch into sugar for banana (Tapre and Jain, 2012).

The stages of ripening of banana play an important role in physical, chemical and functional properties of the fruit. By understanding the physicochemical changes of banana ripening can forms the basis for expanding the utilization of bananas.
Table 2.4  Chemical compositions of banana at different ripening stages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ripeness stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td><strong>Energy (g/100g)</strong></td>
<td>388.64</td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
</tr>
<tr>
<td><strong>Moisture (%)</strong></td>
<td>76.13</td>
</tr>
<tr>
<td></td>
<td>±0.06</td>
</tr>
<tr>
<td><strong>Protein (g/100g)</strong></td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>±0.11</td>
</tr>
<tr>
<td><strong>TDF (g/100g)</strong></td>
<td>6.47</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
</tr>
<tr>
<td><strong>Starch (%)</strong></td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>±0.20</td>
</tr>
<tr>
<td><strong>Total sugar (%)</strong></td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>±0.31</td>
</tr>
<tr>
<td><strong>Glucose (%)</strong></td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>±0.19</td>
</tr>
<tr>
<td><strong>Fructose (%)</strong></td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>±0.13</td>
</tr>
<tr>
<td><strong>Sucrose (%)</strong></td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
</tr>
<tr>
<td><strong>Ash (mg/100g)</strong></td>
<td>4.198</td>
</tr>
<tr>
<td></td>
<td>±0.24</td>
</tr>
<tr>
<td><strong>Potassium (mg/100g)</strong></td>
<td>421.81</td>
</tr>
<tr>
<td></td>
<td>±75.1</td>
</tr>
<tr>
<td><strong>Magnesium (mg/100g)</strong></td>
<td>36.26</td>
</tr>
<tr>
<td></td>
<td>±2.94</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.18</td>
</tr>
<tr>
<td></td>
<td>±0.06</td>
</tr>
<tr>
<td><strong>TSS</strong></td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>±0.5</td>
</tr>
</tbody>
</table>

Titratable acidity

<table>
<thead>
<tr>
<th>Parameter</th>
<th><strong>Citric acid (mg/100g)</strong></th>
<th><strong>Malic acid (mg/100g)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citric acid (mg/100g)</strong></td>
<td>2.34</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.13</td>
</tr>
<tr>
<td><strong>Malic acid (mg/100g)</strong></td>
<td>2.24</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.13</td>
</tr>
</tbody>
</table>

Adopted from Adi et al. (2019), Chaipai et al. (2018) and Yap et al. (2017)
2.1.3 The potential health effects of banana

Banana is a ready-to-eat and affordable fruit for human consumption, which works to build good health due to its immense nutritional and medicinal value. Table 2.5 shows the list of potential health effects of banana.

<table>
<thead>
<tr>
<th>Health effect</th>
<th>Explanation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduce risk of high blood pressure</td>
<td>Banana contains high amount of potassium which is an essential mineral for maintaining normal blood pressure and heart function. Furthermore, potassium also helps to maintain normal fluid and electrolyte balances in the cell.</td>
<td>Olvera-Hernández et al. (2018); Dayanand et al. (2015); D'Elia et al. (2014);</td>
</tr>
<tr>
<td>Cardiovascular protection</td>
<td>Potassium-rich fruit like banana was found to able to lower the risk of stroke. A number of studies have demonstrated the ability of banana to maintain normal blood pressure and heart function.</td>
<td>Hjartåker et al. (2015); Cressey et al. (2014)</td>
</tr>
<tr>
<td>Restore normal bowel activity</td>
<td>Banana is rich in DF (insoluble and soluble fibre) which can help to restore normal bowel activity and help to prevent both constipation and diarrhea. The soluble fibre especially pectin can normalize the colon’s function by absorbing large amount of water and provide bulk producing ability. Banana is also a rich source of prebiotic (fructooligosaccharide). The beneficial bacteria do not just nourish probiotic bacteria in the colon but also produce vitamins and digestive enzymes that improve body’s ability to absorb nutrients.</td>
<td>Cassettari et al. (2019); Bae (2014); Wang et al. (2014)</td>
</tr>
<tr>
<td>Protection from ulcers and heartburn</td>
<td>The antacid effects of bananas have been recognized to protect against stomach ulcers damage. Leucocyanidin, a flavonoid in banana, has been reported to increase the thickness of the mucous membrane layer of the stomach. Banana can help to neutralize acidity thus able to prevent heartburn. A simple mixture of banana and milk was found to significantly supressed acid secretion in an animal study.</td>
<td>Ali et al. (2018); Rafa Zubair et al. (2018);</td>
</tr>
</tbody>
</table>
| Protection against neurodegenerative diseases (Alzheimer’s disease) | The effect of banana extracts on neuron cells was investigated and found that the phenolic phytochemicals prevented neurotoxicity on the cells.  

The results indicated banana in our daily diet may protect neuron cells against oxidative stress-induced neurotoxicity and have the potential to reduce the risk of neurodegenerative disorders such as Alzheimer’s disease.  

Moreover, ripe banana contains carotenoid which has been shown to have beneficial effects on neurodegenerative diseases. Several results obtained from animal and cell culture model studies have linked the consumption of carotenoid-rich food with decreased risk of neurodegenerative diseases. |
|---|
| Lower cholesterol | The effect of DF and polyphenol properties of banana on serum cholesterol in rats fed with cholesterol enriched diet was studied. The results reported a decline in body weight and serum total cholesterol.  

Banana is also rich in unsaturated fatty acid, vitamin E, total saponin and flavonoids which may regulate the lipid metabolism. |
| Regulate blood glucose level | Banana contains high amount of DF and amylase-resistant starch which slow down carbohydrate absorption in the body and prolong feeling of fullness thus prevent sharp increase of blood glucose level.  

A few studies compared few varieties of banana and found that most banana exhibit low glycaemic index value and suggested that banana may be a snack for both healthy or diabetic patient who are under dietary management. |
| Kidney health | The high potassium content in banana can improve overall functional efficiency of kidneys. Potassium intake can suppress excretion of calcium in the urine and reduce the risk of kidney stones.  

A prospective study on Swedish population showed that the population consume more than 75 servings of fruits and vegetables per month minimize their risk of kidney cancer by 40%. Among the fruits, banana was shown to be the most protective. Furthermore, eating bananas four |

Honarvar et al. (2017); Kesse-Guyot et al. (2014); Heo et al. (2008)

Dikshit et al. (2016); Liyanage et al. (2016); Liyanage et al. (2015)

Olvera-Hernández et al. (2018); Adedayo et al. (2016); Cressey et al. (2014)

Silva et al. (2016); Mosa and Khalil (2015); Rashidkhani et al. (2005)
to six times a week could halve the risk of developing kidney disease as compared to those who did not consume banana.

As energy booster
Bananas consist of three natural sugars – sucrose, fructose and glucose combined with fibre which provides an instant and substantial boost of energy.

Potassium found in banana also plays an important role in helping muscles to contract properly during exercise and minimizes muscle cramping.

Research has shown that just two bananas are enough to provide energy for a strenuous 90-minutes workout.

Nieman et al. (2018);
Nieman et al. (2015);
Nieman et al. (2012)

As immunity booster
Banana has been found to contain 25% of the recommended daily allowance (RDA) for vitamin B6 which is necessary for producing antibodies and red blood cells as well as aiding in fat metabolism. Vitamin B6 serves as an immunity booster by strengthen your immune system against infectious diseases.

In addition, an average sized banana can provide about 15% of RDA for vitamin C which known for its antioxidant properties.

Abhishek et al. (2019);
Parra et al. (2018);
Sidhu et al. (2018)

Depression
A research was done among people who are suffering from depression and most of them claimed to be feeling better after consume a banana. This is because banana contains a type of protein known as tryptophan which will be converted into serotonin in the body. Serotonin is known for its contribution for wellbeing and happiness.

Gouda (2017);
Samad et al. (2017)
2.1.4 Overripe banana pulp as food ingredient in food product

Presently the method of preparation or preservation of banana only focus on certain processing techniques such as pulped into puree which further used in various food products (ice cream, jam, yogurt, bakery products, baby food and etc) (Yap et al., 2017; Phuapaiboon, 2016; Bana and Gupta, 2015; Chanbisana and Banik, 2014); sliced and canned with syrup or fried as chips (Aurore et al., 2009); fermented into vinegar or alcoholic beverages (Pauline et al., 2017; Kaur and Kaur, 2015); and dried into flour or powder (Batista et al., 2017; Salvak et al., 2016). The stage of banana ripeness and their nutritional qualities are important factors in the processing of banana and its uses in various food products. As most ripen banana fruit is mainly consumed as raw, thus unripen banana fruit is usually being further processed into food product or raw food ingredient as unripe fruit has high starch content and low moisture content which make it more shelf-stable and best suited for processing (Singh et al., 2018). As a result, mostly overripe bananas are screen out from bulk. However, overripe banana still exhibits health benefits such as source of dietary fibre, natural sweetener and minerals (Table 2.4). The effort of utilizing overripe banana as functional food ingredient in food products had never been properly explored or studied previously. To the best of our knowledge, there is no report has been published or documented on the utilization of overripe banana as raw ingredient, especially in bakery-based food which can potentially enhance the nutritional quality of the food products.
### 2.2 Dietary fibre

Dietary fibre (DF) has a long history, its term was originated from Hispley (1953) who suggested that DF is a nondigestible components making up the plant cell wall. After several revisions, DF was then defined as ‘a constituent consisting of remnants of plant cells which resists to digestion by the alimentary enzymes of man’ (Trowell et al., 1985). Until last decade, the most consistent and widely accepted definition come from AACC (2001) and Westernbrink et al. (2013) who described DF as ‘the edible parts of plant that are resistant to digestion and absorption in the human small intestines which complete or partial fermentation in the large intestine’. DF includes polysaccharides, oligosaccharides, lignin and associated plant substances.

According to Mudgil and Barak (2013), DF is categorized into two categories, soluble and insoluble fibre based on chemical, physical and functional properties. The classification of DF components is presented in Table 2.6. Soluble fibre dissolves in water forming viscous gels that bypass the digestion of the small intestine and is easily fermented by the microflora of the large intestine. They consist of pectin, gums and mucilage. Foods rich in soluble fibre include fruits, oats, barley and beans. Meanwhile, insoluble fibre is not water soluble by which they do not form gels due to their water insolubility and fermentation is severally limited. Examples of insoluble fibre are cellulose, lignin and some hemicelluloses (Ötles abd Ozgoz, 2014). Most fibre containing foods have approximately one-third soluble and two-third insoluble fibre (Claesson et al., 2012).
Table 2.6  Classification of DF components, their description, main food sources and major physiological effect

<table>
<thead>
<tr>
<th>Fibre component</th>
<th>Description</th>
<th>Main food sources</th>
<th>Major physiological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insoluble DF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>Main structural component of plant cell wall. Soluble in concentrated acid and insoluble in concentrated alkali.</td>
<td>Vegetables, fruits, brans and grains</td>
<td>Delay gastric emptying, regulate blood glucose levels, lower serum cholesterol levels, due to its effects of increasing viscosity of gut content and colonic fermentation</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Cell wall polysaccharides containing backbone of β-1,4 glucosidic linkages. Soluble in dilute alkali.</td>
<td>Cereal grains</td>
<td></td>
</tr>
<tr>
<td>Lignin</td>
<td>Non-carbohydrate cell wall component. Complex cross-linked phenyl propane polymer. Resists bacterial degradation.</td>
<td>Woody plants</td>
<td></td>
</tr>
<tr>
<td><strong>Soluble DF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td>Components of primary cell wall with D-galacturonic acid as principal components. Water soluble and gel forming.</td>
<td>Fruits, vegetables, legumes, sugar beet, potato</td>
<td>Shorten bowel transit time, improve laxation due to its bulking capacity, support growth of intestinal microflora due to its fermentation in the large intestine</td>
</tr>
<tr>
<td>Gums</td>
<td>Secreted at site of plant injury by specialized secretary cells. Food and pharmaceutical use.</td>
<td>Leguminous seed plants, seaweed extracts, microbial gums</td>
<td></td>
</tr>
<tr>
<td>Mucilage</td>
<td>Synthesized by plant, prevent desiccation of seed endosperm. Food industry use, hydrophilic, stabilizer.</td>
<td>Plant extracts (gum acacia, gum karaya, gum tragacanth)</td>
<td></td>
</tr>
</tbody>
</table>

Adopted from Dai and Chau (2017), Li and Komarek (2017) and Dhingra *et al.*, (2012)
2.2.1 Trend of dietary fibre intake

Dietary fibre (DF) has been long recognized for its health benefits since decades ago. As a treasured protector of our health, DF is an underrated nutrient that often perhaps doesn’t receive the consumers’ recognition it deserves. Consumption of DF has been observed globally and was reported that most of the populations consume less DF than WHO recommendation of at least 25 g of DF per day (WHO, 2011). In fact, the average person in US was reported to consume less than 50% of the DF levels recommended for good health (Hoy and Goldman, 2014). Similarly, only 9% of adults in UK consume sufficient DF and similar trends are reported in most of the developing world (Hooper et al., 2015; Harland et al., 2012). It is estimated 3.9 million deaths worldwide were caused by the inadequate consumption of fruits and vegetable in 2017 (WHO, 2019). Nevertheless, consuming over 25 g of DF every day has been well documented to provide great health benefits and reduce the risk of cardiovascular diseases, type 2 diabetes, colorectal cancer, stomach cancer and a swarm of other health issues (Weickert and Pfeiffer, 2018; Aune et al., 2017; Yu et al., 2015).

The Malaysia Dietary Guidelines (MDG) recommends at least 3 servings of vegetables and 2 servings of fruits per day as part of a balanced diet (Tee, 2011). According to Nurul Izzah et al. (2012), 50 – 85% of Malaysian did not meet the fruits and vegetables intake recommendations. An assessment was done by Lee et al. (2019) on the DF intake among adults in Penang and Kota Bharu showed that the consumption of fruits (< 1 serving) and vegetables (< 2 servings) was significantly lower than the suggested levels by the MDG indicated low DF intake among the participants. Additionally, low DF consumption pattern were also observed among local Malaysian communities in other studies (Pei et al., 2018; Ismail et al., 2016) as
well as in Indonesia who shares similar socio-cultural practices with Malaysia (Lee and Ryu, 2018).

Table 2.7  Recommended nutrient intake of DF for adults in different countries

<table>
<thead>
<tr>
<th>Country/Association</th>
<th>Recommended Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOM, USA (2002)</td>
<td>14 g DF / 1000 kcal</td>
</tr>
<tr>
<td>ADA (2008)</td>
<td>Men = 38 g / day</td>
</tr>
<tr>
<td></td>
<td>Women = 25 g / day</td>
</tr>
<tr>
<td>Dietitians Association of Australia (2008)</td>
<td>30g DF / day</td>
</tr>
<tr>
<td>WHO (2003)</td>
<td>&gt;25 g DF / day</td>
</tr>
<tr>
<td>United Kingdom (UK)</td>
<td>30 g DF / day</td>
</tr>
<tr>
<td>SACN (2008)</td>
<td>Minimum = 12 – 24 g DF / day</td>
</tr>
<tr>
<td>Malaysia RNI (2005)</td>
<td>20 – 30 g DF / day</td>
</tr>
</tbody>
</table>

Different countries have slight different in recommended intake of DF for adults as shown in Table 2.7. Despite the country guidelines showing different sources of DF according to the characteristics of the local diet, other factors such as taste, price, convenience or trends can directly affect food choice (García-Meseguer et al., 2017). A study done by Bielemann et al. (2015) stated that different population group particularly young adults demonstrate a significant challenge due to the emotional, physiological and environmental changes. Young adults are generally sensitive to the influence of trends including the consumption of fast food, soft drinks, snacks and ready-to-eat products which are rich in sugars and fats but lack of DF. Thus, learning healthy food habit is important at early age for improving future health. Correspondingly, several studies have highlighted the lack of knowledge or awareness as one of the major factors in relation to low consumption of DF. It was
reported that consumption of fruits and vegetables was higher among those with higher education as compared to those with low education (Markland et al., 2013; Lin et al., 2011). Generally speaking, consumption of DF is associated with better health status and the health consciousness is more pronounced among educated population (Ismail et al., 2016). Furthermore, the trend of fruits and vegetables intake is strongly influenced by socioeconomic background. Inadequate consumption of DF was found to be higher among people with lower income than those with higher income, mainly due to affordability issue (Msambichaka et al., 2018).

In the food pyramid, fruits and vegetables are located at the second level indicates that they are important in our daily diet as a primary source of DF. More efforts and strategies have to be implemented globally in order to encourage consumption of DF to help maintain health status and lowering risk of non-communicable diseases.

2.2.2 Application of dietary fibre in food product

Dietary fibre (DF) can be used to provide various functional properties when it is incorporated in food systems, for example it may be used as a tool for improving texture, as a bulking agent in reduced-sugar applications, as a colouring and natural antioxidant as well as can be used to manage moisture in the replacement of fat (Ramirez-Santiago et al., 2010). Furthermore, addition of fibre contributes to the modification and improvement of the texture, sensory acceptability and shelf-life of the food product due to their water binding capacity, gel forming ability, fat mimetic, anti-sticking, anti-clumping and thickening effects (Yangilar, 2013).

Most commonly, DF are added into bakery products to prolong freshness attributed to their capacity to retain water, hence reduce economic losses (Kurek and
Wyrwisz, 2015). Different sources of DF have been used to replace wheat flour in bakery products. Ballester-Sánchez et al. (2019) used white, red and black quinoa seed flour to enhance DF and other nutritional content in bread making. A Chinese cabbage outer-leaf, which is a main by-product of kimchi was made into powder to replace wheat flour in the preparation of muffins and it was reported the DF content increased with increasing level of kimchi by-product powder (Heo et al., 2019). A study on utilizing DF from black carrot and xanthan gum in development of gluten free rice muffins was done by Singh et al. (2016) and the finding showed that incorporation of DF significantly improved appearance and specific volume of the rice muffins. DF has also been applied into other flour products such as noodles which is an integral part of the diet in Asian countries. The health effect of insoluble DF from wheat bran in dry Chinese noodle was investigated by Zhang et al. (2019) and shown smaller particle size of insoluble DF improved the texture, cooking loss and sensory score of the noodle. Crizel et al. (2015) did a research on the potential of orange by-product as source of DF in pasta. Interestingly, orange by-product increased both DF content and antioxidant capacity without affecting the quality properties of the pasta. Banana peel is also one of the popular sources of DF that has attracted a great deal of attention due to the production of banana in large volumes annually which lead to the disposal of banana peel. According to Agama-Acevedo et al. (2016) and Pathak et al. (2016), banana peel is rich in both DF and bioactive compounds which also has shown to improve nutritional quality and sensory score of noodle (Castelo-Branco et al., 2017) and chapatti (Kurhade et al., 2016).

DF also has been utilized to enhance nutritional quality of drinks and beverages with soluble fibre being the most employed. Bosch-Sierra et al. (2019) suggested that consumption of fibre-enriched drink could be an appropriate way to
supplement daily fibre intake and achieve beneficial effects on metabolic health. Recent findings have claimed that beverages enriched with fibre significantly decreased postprandial serum glucose and circulating insulin as well as feelings of satiety and fullness was observed as compared to beverages without added fibre (Bosch-Sierra *et al.*, 2019; Anunciação *et al.*, 2018; Paquet *et al.*, 2014). Other than that, incorporation of DF in drinks and beverages can improve the viscosity and stability of the beverages. A research was done by Alqahtani *et al.* (2014) who incorporated four types of fibres (orange fibre, kibbled wheat, oat flour and oat fibre) into UHT beverages. The result demonstrated a positive outcome in terms of consistency, stability and sensory acceptability of the UHT beverages with the inclusion of fibre.

Meat is often an important part of daily meals to provide source of high biological value protein, fatty acids, vitamins and many essential micronutrients. However, most meat is deficient in DF. Therefore, attempts have been made to incorporate different sources of DF into meat product to enhance the nutritional values, improve yield and shelf life as well as the processing characteristics of meat products (Talukder, 2015). The effect of DF as a fat substitute in sausage was determined by Ham *et al.* (2016) and it was shown that replacing fat with DF contributed to reduction of lipid oxidation and sensory properties were maintained during storage. Similar study was done by Fang *et al.* (2019) by incorporating sugarcane fibre to improve eating quality and health benefits of chicken sausage. The effect of DF on rheological properties of meat emulsions was also investigated. Addition of DF into meat was shown to be able to lower cooking loss and improve emulsion stability and viscosity (Agar *et al.*, 2016).
Recently, there is an increasing demand for food products with low calories, low fat and high DF functional food as nowadays more and more people are concerned about their health and nutritional status with the increasing environmental pollution and stress in their life. Food product enriched with DF is an effective way to enhance functionality as well as to promote healthy eating habit.

2.3 Sugars and sweeteners

Sugar is a type of naturally occurring sweetener that is nontoxic and sweet tasting soluble crystalline carbohydrates. Sugars can be absorbed quickly into our body and are less filling as compared to other types of carbohydrates. Therefore, sugars are mainly used as a source of energy in our daily diet (Zaitoun et al., 2018). Furthermore, sugars play an important role in food and drink industry as they are often used to enhance flavour, colour and texture and also as a preservative in food products. The main source of sugar is cane sugar or beet sugar and there are also other several sources such as honey, corn syrup, fruits and etc (Zaitoun et al., 2018). There are two types of sugars described as either intrinsic or extrinsic as presented in Figure 2.6. Intrinsic sugars are sugars that occur naturally within the cellular structure of food whereas extrinsic sugars do not. Extrinsic sugars are sugars that can be added by individual, caterer and food manufacturer which also known as free sugar. Free sugars are commonly found in sugared carbonated drinks, bakery products, sugared breakfast cereals and jam. Unexpectedly, free sugars can also be found in foods and drinks that claimed to be ‘healthy’ including fruit juices, smoothies and dried fruit or can be hidden in other foods such as ready-made sauces and yoghurts (Yeung et al., 2015). Generally, intrinsic sugars are not shown to have an adverse effect on general health. On the other hand, extrinsic sugars especially
free sugar can contribute to several diseases particularly obesity, cardiovascular
diseases and type 2 diabetes (Kaartinen et al., 2017).

Artificial sweeteners, also known as non-nutritive sweeteners has gained
popular due to their ability to provide low or zero calories and do not raise blood
sugar level. Moreover, artificial sweeteners has been used widely because of their
high sweetness intensity, thus only small amount of artificial sweetener is needed to
achieve the same sweetness as sugar (Table 2.8). The United States Food and Drug
Administration (FDA) authority has approved few artificial sweeteners (saccharine,
aspartame, sucralose, neotame, acesulfame-K and advantame) for human use and
classified them as safe to consume category (Sharma et al., 2016). Artificial
sweeteners are increasingly used in manufacturing processed food products such as
baked goods, cereals, and granola bars, dairy products including sugar-free yogurts,
no-sugar added ice cream and flavoured milk. Similarly, artificial sweeteners are also
added to a wide range of condiments including sugar-free jam, sugar-free syrup and
reduced-sugar ketchup (Sylvetsky and Rother, 2016).

Table 2.8 Relative sweetness of sugars and artificial sweeteners

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Relative sweetness</th>
<th>Artificial sweetener</th>
<th>Relative sweetness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>Saccharine</td>
<td>200 – 700</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.6 – 0.75</td>
<td>Aspartame</td>
<td>120 – 200</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.8 - 2.0</td>
<td>Sucralose</td>
<td>400 – 800</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.4 - 0.5</td>
<td>Acesulfame-K</td>
<td>130 - 200</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.2 – 0.5</td>
<td>Neotame</td>
<td>7000 - 13000</td>
</tr>
</tbody>
</table>

Adopted from Karl (2017) and Jagan Mohan Rao and Ramalakshmi (2011)
2.3.1 Trend of sweetener intake

The World Health Organization (WHO) recommended that free sugar should be taken less than 10% of total energy intake per day. However, the new guideline from WHO suggests that reducing the amount of sugar intake to below 5% of total energy intake per day would have additional benefits (WHO, 2015). In addition, the Scientific Advisory Committee on Nutrition (SACN) recently also has recommended that free sugars account for no more than 5% daily dietary energy intake (SACN, 2015). For an adult woman of normal BMI with an intake of around 2000 calories
per day, 5% would equate to 100 calories. Sugar provides 4 calories per gram so this would mean that 5% was approximately 25 g of sugar (6 teaspoons). For the average man with around 2500 calories per day, 5% would be nearer to 8 teaspoons of sugar per day. The Malaysian Dietary Guidelines (2010) is also in line with those recommendations through key message 10 with the statement ‘Consume foods and beverages low in sugar’ (Amarra et al., 2016). Given the standard 330 ml can of cola contains 35 g of sugar (8 – 9 teaspoons), the challenge in reducing free sugars to this level can be highlighted.

Malaysian is one of the highest sugar consumers in the Asia Pacific region (Hamiruddin et al., 2018). The total teaspoons of sugar consumed by a Malaysian in a day increased from 17 in 1970 to 26 in 2009 (Cheah et al., 2019). Furthermore, the demand for sugar in Malaysia was estimated to increase from 1.4 million tonnes in 2011 to 1.9 million tonnes in 2020 (Cheah et al., 2019). According to Amarra et al. (2016), the escalating availability of sugar coupled with sedentary lifestyles are the main factors attributed to the country’s rising problem of obesity and other non-communicable diseases. As a result, Malaysia has been declared as the most obese country in Southeast Asia with diabetes rate increased from 11.5% in 2006 to 17.7% in 2015, or an estimated 3.5 million Malaysian adults (Chan et al., 2017). This epidemic of overweight and obesity are among the consequences of the consumption of sugar intake especially sugar-sweetened beverages as these kinds of beverages have partial compensation for total energy, added sugar content and low satiety (Hamiruddin et al., 2018). Smith et al. (2015) showed that sugar-sweetened beverages topped all other food and beverages options as the primary source of sugar intake in most children and adolescents. Other studies also have shown that high sugar intake in overweight and obese adolescents were strongly correlated with sugar
consumed via sugar-sweetened beverages (Smith et al., 2015; Vanderlee et al., 2014). The Malaysian Adult Nutrition Surveys (MANS) reported that 59% of Malaysians consumed sugar and sweetened condensed milk on a daily basis which would directly affect their child’s sugar intake (Norimah et al., 2008). Furthermore, the average intake of sugar among Malaysian was about 4 teaspoons (21 g) per day, and this only represents the sugar added into beverages. In other words, if sugars from food like snacks and desserts were taken into consideration, the dietary proportion from sugar would be exceeded (Hamiruddin et al., 2018).

The growing concerns about health have increased people awareness to avoid consumption of food and drink rich in sugar, salt or fat. There is an increasing demand in alternative sweeteners that can be used in food product instead of sugar such as artificial sweeteners. Aspartame and sucralose are the common sweeteners used to sweeten a range of products in food and beverage industry. The food and beverage industries are increasingly replacing sugar or corn syrup with artificial sweetener (Chattopadhyay et al., 2014). High demand from soft drink and confectionery industries is expected to act as a growth driver for artificial sweeteners market. The reduced cost of production and the better economy of scale are also boosting the growth of the artificial sweetener market. It is expected to reach 2.2 billion dollars by 2020, with its annual growth at approximately 5.1% per year from 2008 to 2015, and with the greatest growth expected in Latin America and in China (Sylvetsky et al., 2016). However, the toxicological evidence and the concerns regarding the safety of artificial sweeteners are the major restraint of the market as their role in weight management and health remains a topic of continued controversy. Although artificial sweeteners have claimed to provide health benefits primarily by reducing sugar intake in daily diet and thus reducing the risk of overweight and
obesity (Harpaz et al., 2018). There are studies who reported the use of artificial sweeteners could increase the risk of overweight, diabetes, cancer and some other adverse health effects presented in Table 2.9.

Limiting consumption of any sweeteners may well be the best health advice. Nevertheless this recommendation may be impractical and difficult to implement when sugar craving is a genetically predetermined behaviour at least in a substantial sub-group of people (Hwang et al., 2015). For this reason, the demand for novel sweeteners, especially natural sweeteners with low calories, acceptable taste and healthy qualities is pressing. Most of the commercial sweeteners present on the market are usually made of a combination of natural and artificial sweet substances (Mooradian et al., 2017). However, it is advisory to eliminate artificial sweeteners from the diet particularly those pregnant and during lactation (Al-Qudsi et al., 2019).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernardo et al., 2016</td>
<td>Adults and children</td>
<td>Artificial sweetener use</td>
<td>Adverse clinical effects</td>
</tr>
<tr>
<td>Greenwood et al., 2014</td>
<td>Generally healthy population</td>
<td>Artificially-sweetened beverage consumption</td>
<td>Incidence of type 2 diabetes</td>
</tr>
<tr>
<td>Rebholz et al., 2017</td>
<td>Middle-age adults (45 – 64 years old)</td>
<td>Diet soda consumption</td>
<td>Risk of renal disease</td>
</tr>
<tr>
<td>Imamura et al., 2015</td>
<td>Healthy adults</td>
<td>Artificially sweetened beverages</td>
<td>Incidence of type 2 diabetes</td>
</tr>
<tr>
<td>Miller and Perez, 2014</td>
<td>Generally healthy population</td>
<td>Low-calorie sweeteners from foods and beverages</td>
<td>Body weight or body composition</td>
</tr>
<tr>
<td>Romo-Romo et al., 2016</td>
<td>Adults</td>
<td>Artificial sweeteners consumption</td>
<td>Glucose metabolism and appetite regulating hormones, development of metabolic chronic diseases</td>
</tr>
<tr>
<td>Spencer et al., 2016</td>
<td>Humans and animals</td>
<td>Aspartame, saccharin or sucralose consumption</td>
<td>Fermentation, absorption, gastrointestinal symptoms</td>
</tr>
<tr>
<td>Schernhammer et al., 2012</td>
<td>Adults</td>
<td>Artificial sweetener-soda consumption</td>
<td>Carcinogenic potential</td>
</tr>
<tr>
<td>Reid et al., 2016</td>
<td>Pregnant women, infants or children</td>
<td>Early life exposure of artificial sweeteners</td>
<td>Long-term metabolic health effect (BMI, birth weight, growth velocity, incidence of overweight/obesity, change in adiposity, incidence of impaired glucose tolerance, metabolic syndrome, insulin resistance or type 2 diabetes)</td>
</tr>
</tbody>
</table>
2.3.2 Utilization of natural sweetener in food product

As consumers are looking for something healthier, the search of sugar substitutes of natural sources has led to the discovery of a number of substances that possess an intensely sweet taste or taste-modifying properties. Many plants and fruits materials have been found to taste sweet because they contain large amounts of sugars and polyols or other sweet constituents, for example honey, maple syrup, agave nectar and date syrup. Although these sweeteners may contain some caloric value, their contribution to energy intake is negligible in the amounts used (Carniel Beltrami et al., 2018). The application of natural sweeteners in the food and beverage industry has been accelerating and diverse in usage from alcoholic and non-alcoholic drinks, sauces, dairies to confectionaries.

Honey is the oldest natural sweetener and the health benefits of honey such as anti-inflammatory, antioxidant, antibacterial, antidiabetic, antimutagenic, antifungal and antitumoural effects are well documented (Ahmed et al., 2018). The effect of honey as partial sugar substitute on microbial shelf stability of cassava-wheat composite bread was studied by Adeboye et al. (2015). The growth of bacteria and moulds were inhibited in bread incorporated with honey attributed to the antimicrobial and antibacterial properties of honey (Adeboye et al., 2015). There is also study reported food products incorporated with honey have lower glycaemic index value, thus may be suitable for consumption particularly for people with impaired glucose tolerance and other health problems (Rana and Katere, 2013).

Date fruits are also considered as one of the good sources of natural sweetener, DF and some important minerals. Date syrup as sucrose replacement in cookie has been studied by Sengev and Oguche, (2017) and Alsenaien et al. (2015). Both of the studies revealed the nutritional content of the cookies prepared using date
syrup showed a significant improvement in fibre, ash and protein content meanwhile decreased in fat and carbohydrate content without adversely affecting the physical and sensory qualities of cookies.

Monkfruit or *LuoHanGuo* has recently emerged as a potential natural sweetener and has gained popularity due to its low calorie and several beneficial effects such as anti-carcinogenic, weight control and regulation of blood sugar (Pandey and Chauhan, 2019). Lee *et al.* (2016) used monkfruit as sugar substitute to developed monkfruit-Omija extract tea as a functional health food compared to sugar-Omija extract tea. The results obtained showed that monkfruit-Omija extract tea displayed anti-hyperglycaemic effect as well as improved liver function and lipid metabolism in diabetic-induced mice.

Other than that, a study done by Kulkarni *et al.* (2018) who developed glazed tamarind candy using natural sweetener from sweet sorghum syrup and it was found that the carbohydrate content and caloric value of the candy was significantly decreased with increasing levels of sweet sorghum syrup, with notable increased in total ash content. Furthermore, concentrated neera sap was utilized by Tai *et al.* (2019) as a source of natural sweetener to improve physicochemical properties and antioxidant capacity of carrot cake.

Natural sweeteners are derived through extraction processes from fruits, roots, leaves and other parts of plants. Unlike artificial sweeteners, these sugars are obtained naturally with no synthesis or artificial production processes involved. Global adoption of natural sweeteners as sugar substitute are still very low, but has slowly gained more popularity than artificial sweetener due to health benefits.
Consumers’ health awareness has creating a shift from artificial sweeteners to natural substitutes.

2.4 Bakery product

Bakery products is defined as ‘food manufactured from recipes largely based on or containing significant quantities of wheat or other cereal flours which are blended with other ingredients. They are formed into distinctive shapes and undergo a heat-processing step which involves the removal of moisture in an oven located in a bakery’, according to Cauvain and Young (2007). The term ‘baked products’ is applied to a wide range of food products, including breads, rolls, cookies, pies, pastries and muffins which are usually prepared from flour or meal derived from some form of grain. Bread is already a common staple in prehistoric times, provides many nutrients in the human diet (Arranz-Otaegui et al., 2018).

Flour, water and leavening agents are the ingredients primarily responsible for the characteristic appearance, texture and flavour of most bakery products. Eggs, milk, salt, shortening and sugar are effective in modifying these qualities, and various minor ingredients may also be used (Costantina et al., 2019). Bakery products prepared with wheat flour is often referred as standard against bakery products made with non-wheat flour. Wheat flour contains protein composite known as gluten which is essential for texture development especially in bread-making. Whole wheat flours are produced using the entire kernel while white flours are made only from the endosperm, without bran and germ. Bran and germ contain substantial amount of dietary fibre, fatty acids, iron and vitamin B (Kumar et al., 2011). But still, white flour or refined flours are used extensively for preparation of many products despite its low nutritional quality (Oghbaei and Prakash, 2018).
Bakery products are routinely consumed and appreciated around the globe. They are enjoyed by consumers of various age groups during snack times or special occasions other than as part of their meals. According to Malaysian Adult Nutrition Survey (MANS) which involved 6,742 subjects all over Malaysia, bread and cookies appeared in the list of top 10 most commonly consumed foods (Kasim et al., 2018). Due to their high popularity and availability, bakery products can be chosen as suitable carriers to deliver some essential nutrients. There are ongoing studies being done to identify nutritive ingredients which can incorporate as partial replacement for refined flour or sugar in order to enhance the nutritional quality of bakery products without jeopardizing the physical and sensory qualities.

### 2.4.1 Cookie

Cookies are sometimes confused with biscuit. The term cookie actually refers to hard sweet or semi-sweet types of biscuit and is adopted in North America where biscuits can be confused with small soda raised bread or muffins. In other countries, cookies refer to wire cut products that contain large pieces of various ingredients like nut and chocolate (Manley, 2000). Cookie has a lot of similarities with other bakery products such as bread or cakes. According to Smith (1972), cookies can be defined as a type of bread that is crispy, hard, have many varieties of flour and made into small and thin shape. Meanwhile, Baking Industry Research Trust (2010) described cookie as ‘baked product that has a cereal base (wheat, oat or barley) of at least 60% and low moisture content of 1 – 5%, excluding moisture content from fillings and icings’ (Cauvain and Young, 2007). The difference between bread and cookie is in terms of the levels of fat and sugar used and moisture content of the final product (Manley, 2000).
Cookies are popular and well-accepted snack food by all age groups throughout the world (Zouari et al., 2016). It is very established in industrialized countries and is rapidly expanding in developing countries. The global market for cookies has been growing rapidly with market size valued at 124 billion dollar in 2019 (Statista Research Department, 2019). In Malaysia, the sales value of manufactured cookies and biscuits was approximately MRY 2.2 billion in 2019 as shown in Figure 2.7 (Hirschmann, 2020). According to Sindhuja et al. (2015), the main four key factors for the success of cookie are:

I. Their relatively long shelf-life
II. Their great convenience as food products
III. The human liking and weakness for sugar and chocolate
IV. Their relatively good value for money

Figure 2.7  Sales values of cookies and biscuits in Malaysia, 2012 – 2019 (Hirschmann, 2020)
However, cookies are regarded as unhealthy and are rejected by weight conscious consumers because majority of cookies have high levels of fat and sugars but low in fibre, protein, vitamin and minerals (Li et al., 2016). Typical cookies formulation has a fat content of 20 – 60% and sugar of 25 – 55% based on the weight of flour (Baltsavias, 1996). Thus, cookies are usually been classified as a snack food instead of as a nutrient contributor. Cookies are eaten mainly for its organoleptic attributes, but not for nutritional factor. For all these reasons, cookies have the potential to be a delivery system of essential nutrient in the human diet due to its popularity and well accepted by consumers including children Furthermore, cookies have longer shelf-life, low manufacturing cost and can be produced on a large scale for wider distribution (Amin et al., 2016).

The development of healthier cookies products has been on the rise in recent years. The market for cookies is expected to grow further with new products innovation that emphasize on product fortification and the continued emergence of organic food. The growth demand of cookies in the market will also benefit the manufacturer investment in new products, particularly in low calorie product such as fat and sugar free cookies.
2.4.2 Bakery product as functional food

In recent years, society has become more aware of the existing relationship between food consumption and personal health. As a response, researchers have been working on the design and development of not only more nutritious food, but also food that provide an extra health benefit to its consumer which known as functional food. Studies have been conducted for the development of different kind of functional products, for example ice-cream (Ahmade et al., 2014), fruit juices (Fontes et al., 2015), chocolate (Erdem et al., 2014), cheese (Makelainen et al., 2010) as well as bakery products (Collar and Angiolobi, 2014; Marpalle et al., 2014).

Bakery products are considered to be the better vehicles for fortification. Thus, the addition of functional ingredients to bakery products has risen in popularity due to the ability to reduce risk of chronic diseases beyond basic nutritional functions. Most of the food industries utilized industrial-by-products as sources of functional ingredients, for example fruits, vegetables, nut, legume, cereal, seed, winery and brewery are rich sources of dietary fibre, proteins, vitamins, minerals and other bioactive compounds that can be recovered and used as value added functional ingredients (Jeddou et al., 2017; Belghith-Fendri et al., 2016; Helkar et al., 2016; Wani et al., 2015). The incorporation of functional ingredients obtained has impact on technological, nutritional and health promoting properties of bakery products, as described in previous studies listed in Table 2.9.
Table 2.10  Literature review of bakery products as functional food

<table>
<thead>
<tr>
<th>Product</th>
<th>Intervention</th>
<th>Percentage used</th>
<th>Functionality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat cookie</td>
<td>Breakfruit flour substituted with margarine</td>
<td>10, 20, 30</td>
<td>Decreased fat and increased protein content</td>
<td>Li et al. (2016)</td>
</tr>
<tr>
<td>Cookie</td>
<td>Cashew-apple residue substituted with wheat flour</td>
<td>5, 10, 15, 20</td>
<td>Enhanced dietary fibre content</td>
<td>Ebere et al. (2015)</td>
</tr>
<tr>
<td>Cookie</td>
<td>Moringa leave to replace egg and milk</td>
<td>5</td>
<td>As source of dietary fibre and protein content particularly for vegetarians</td>
<td>Emelike et al. (2015)</td>
</tr>
<tr>
<td>Non-wheat cookie</td>
<td>Plantain and Bambara groundnut protein concentrate to replace wheat flour</td>
<td>10, 20, 30, 40, 50</td>
<td>Improved protein, ash, fibre and energy content</td>
<td>Kiin-Kabari and Giami (2015)</td>
</tr>
<tr>
<td>Novel cookie</td>
<td>Jering seed flour substituted with wheat flour</td>
<td>5, 10, 15, 20, 100</td>
<td>Improved protein, fibre and ash content</td>
<td>Cheng and Bhat (2016)</td>
</tr>
<tr>
<td>cookie</td>
<td>Fortified with pea flour, soya bean flour and oat flake</td>
<td>5, 10, 15</td>
<td>Protein rich and low in sugar</td>
<td>Amin et al. (2016)</td>
</tr>
<tr>
<td>Biscuit</td>
<td>Incorporation of pomegranate peel powder</td>
<td>2.5, 5, 7.5, 10</td>
<td>Increased protein, fibre, minerals, antioxidant activity and β-carotene contents</td>
<td>Srivastava et al. (2014)</td>
</tr>
<tr>
<td>Cookie</td>
<td>Incorporation of watermelon seed protein concentrate</td>
<td>2.5, 5, 7.5, 10</td>
<td>Increased protein content</td>
<td>Wani et al. (2015)</td>
</tr>
<tr>
<td>Cookie</td>
<td>Pitaya peel flour substituted with wheat flour</td>
<td>5, 10, 15</td>
<td>Improved ash and fibre content</td>
<td>Ho et al. (2016)</td>
</tr>
<tr>
<td>Biscuit</td>
<td>Utilization of defatted mango kernel</td>
<td>5, 10, 15, 20, 25, 30</td>
<td>Improved mineral and fibre content</td>
<td>Shabeer et al. (2016)</td>
</tr>
<tr>
<td>Product</td>
<td>Description</td>
<td>Added Amount</td>
<td>Benefits</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
<td>--------------</td>
<td>--------------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Cookie and Muffin</td>
<td>Incorporation of goji-by product powder</td>
<td>10, 20, 30, 40</td>
<td>Increased protein, free phenolic, insoluble and soluble dietary fibre</td>
<td>Bora et al. (2019)</td>
</tr>
<tr>
<td>Muffin</td>
<td>Grape pomace skin flour replaced with wheat flour</td>
<td>5, 7.5, 10</td>
<td>Enhanced protein and fibre content</td>
<td>Bender et al. (2017)</td>
</tr>
<tr>
<td>Muffin</td>
<td>Pumpkin powder substituted with wheat flour</td>
<td>10, 20, 40, 60, 80</td>
<td>Enhanced β-carotene and minerals content</td>
<td>Mala et al. (2018)</td>
</tr>
<tr>
<td>Balady flat bread</td>
<td>Incorporation of banana peel powder</td>
<td>5, 10</td>
<td>Enhanced fibre, protein and minerals content</td>
<td>Eshak (2016)</td>
</tr>
<tr>
<td>Cake</td>
<td>Incorporation of passion fruit and orange residue flour</td>
<td>5, 10</td>
<td>Enhanced fibre, protein and minerals content</td>
<td>Oliverira et al. (2016)</td>
</tr>
<tr>
<td>Cupcake</td>
<td>Incorporation of guava pomace and seed</td>
<td>5, 10, 15, 20</td>
<td>Higher fibre, total phenolics and antioxidant capacity</td>
<td>Khalifa et al. (2016)</td>
</tr>
<tr>
<td>Cracker</td>
<td>Addition of tomato pomace</td>
<td>4, 8, 12</td>
<td>Increase in protein, ash, dietary fibre, minerals, total phenolics and antioxidant capacity</td>
<td>Isik and Topkaya (2016)</td>
</tr>
<tr>
<td>Cake</td>
<td>Utilization of fruit pulp waste powder</td>
<td>5, 10, 15, 20</td>
<td>Enrichment of dietary fibre and minerals</td>
<td>Singh (2016)</td>
</tr>
<tr>
<td>Sponge cake</td>
<td>Supplemented with button mushroom powder</td>
<td>5, 10, 15</td>
<td>Enhanced protein and ash content</td>
<td>Salehi et al. (2016)</td>
</tr>
<tr>
<td>Bread</td>
<td>Incorporation of jackfruit rind powder</td>
<td>5, 10, 15</td>
<td>Higher fibre and minerals content</td>
<td>Felli et al. (2018)</td>
</tr>
</tbody>
</table>
2.5 **Nutritional composition analysis**

The determination of food composition is fundamental to theoretical and applied investigations in food and technology and is often the basis of establishing the nutritional value and overall acceptance from the consumer standpoint. Proximate analysis is carried out to determine the major components of food such as moisture, ash, fat, protein and carbohydrate. To obtain accurate results of homogenous and representative sample, sample preparation and collection should therefore be done and considered carefully.

2.5.1 **Moisture**

Moisture determination can be one of the most vital analysis performed on a food product and yet one of the most challenging from which to obtain accurate and precise data. In addition, moisture content of food varies greatly. Among the common fruits, watermelon and honeydew melon contain the most water (93%) followed by citrus, grape, peach, orange, mango, apple (82 - 89%), green banana and avocado contain the least water content (76%), according to Khan *et al.* (2017). Fruits and vegetables are often dried to enhance their shelf life with the water content is preferably below 15% (FAO, 2007).

The dry matter that remains after removal of moisture is commonly referred to as total solids (Bradley, 2003). Moisture content has direct economic importance to the processor and the consumer because the amount of dry matter in a food is inversely related to the amount of moisture. Water is an important constituent of all foods we eat. However, at the same time, the moisture in foods can be considered as necessary evil. In another words, the spoilage and the rate of spoilage are directly proportional to the water content of food. Excessive water content in food is vulnerable to quick deterioration caused by mould growth (Grumezescu and Holban,
2017). Therefore, moisture is a quality factor in products such as dehydrated fruits and vegetables, dried milks, dehydrated potatoes, spices and herbs. Moisture content is important to the food processor as a quality factor for jams and jellies to prevent sugar crystallization. A reduction in moisture is useful for convenience in packaging or shipping of concentrated fruit juices and dehydrated food products which are difficult to package if the moisture content is too high. In addition, another importance of moisture assay is that the moisture content is often specified in the compositional standard of a food product, for instance, the moisture content for enriched flour must be \( \leq 15\% \) and cheddar cheese must be \( \leq 39\% \) (Nielsen, 2010).

The procedures for determination of moisture content stated in the food standards generally involved thermal drying methods. The sample is heated under described conditions and the weight loss is taken as a measure of the moisture content of the sample. The moisture determination from the reduction of weight due to heating necessarily involves an empirical choice of the type of oven, temperature and length of drying (Pomeranz and Meloan, 2000). Thus, the values obtained for moisture content depend on the randomly selected conditions where some of the methods provide approximate rather than accurate moisture values. With that, drying methods are simple, relatively rapid and allow the simultaneous analyses of many samples and continued to be the preferred procedure for moisture determination.

### 2.5.2 Ash

According to Harbers and Nielsen (2003), ash is described as “the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff”. The nature of the food ignited and the method of ashing will determine the amount and composition of ash in a food product. There are various minerals that compose the ash in different foods which occur in different proportions as well.
Determination of ash in foods can be performed by weighing the dry mineral residue of organic materials after heating at elevated temperatures (550°C). The usual procedure generally used to determine total ash is dry-ashing technique. The sample is weighed into a porcelain crucible and the organic matter is burned off without flaming and heated to constant weight. The residues of ashing are carbon-free. There are considerable difference in term of mineral constituent in ash and original food.

The ash content determination is essential for several reasons. Other than it is a part of the proximate analysis for nutritional evaluation, total ash content is a useful parameter to measure the index of refinement of foods such as wheat flour or sugar. The ash assay can fundamentally shows the separation of bran and germ from the rest of the kernel because the mineral content of the bran is about 20 times that of the endosperm. Ash assay is carried out prior to specific elemental analysis for individual mineral. Ash content becomes important because certain foods contain high amount of particular minerals. The ash content of most fresh food is less than 5% (USDA, 2011).

2.5.3 Fat

The terms lipid, fat and oil are often used interchangeably. Lipids are defined as “a group of substances that, in general, are soluble in ether, chloroform or other organic solvents but are sparingly soluble in water” (Min and Ellefson, 2010). The term lipid commonly refers to the broad, total collection of food molecule that meet the definition. Meanwhile, fats generally refer to those lipids that are solid at room temperature whereas oils refer to those lipids that are liquid at room temperature.

Lipids consist of a broad group of substances that have some common properties and compositional similarities. They were differentiated by general
classification such as simple lipids, compound lipids and derived lipids. Simple lipids include ester of fatty acids with alcohol, namely triacylglycerol and waxes. Compound lipids are phospholipids, cerebrosides and sphingolipids, for instance fatty acids, long chain alcohols, sterols, fat soluble vitamins and hydrocarbons. Triacylglycerol was the most prevalent class of lipids in the diet. They comprise of three fatty acids esterified to a glycerol molecule backbone (Ratnayake and Galli, 2009).

The measurement of total lipid content in a food is usually carried out by organic solvent extraction method. Solvents which have a high solvent power for lipids and low or no solvent power for proteins, amino acids and carbohydrates are ideal for extraction because they have low-boiling point, evaporate easily, non-flammable and non-toxic in both liquid and vapour states. Petroleum ether which composes of pentane and hexane is the low boiling point fraction of petroleum. It has a boiling point of 35 - 38°C and has more hydrophobic lipids, cheaper, less hygroscopic and less flammable compared to ethyl ether (Nielsen, 2010).

An accurate and precise quantitative analysis of lipids in foods is critical for accurate nutritional labelling, determination if the foods meet the standard of identity and manufacturing specifications. Inaccuracies in analysis may result in a product of undesirable quality and functionality, thus increase manufacturing cost. In bakery product, fat is important to provide tenderness, moist mouth feel, structure, lubricate, incorporate air and transfer heat (Rios et al., 2014).
2.5.4 Protein

Protein is an abundant component in all cells. Almost all except storage proteins are essentially required for biological functions and cell structure. Apart from their nutritional significance, proteins also affect the organoleptic properties of foods (Pomeranz and Meloan, 2000). The protein content of food has been determined on the basis of total nitrogen content since years ago because nitrogen is the most distinguish element present in protein. The Kjeldahl method has been widely employed to determine the nitrogen content in food. Nitrogen content is then multiplied by a conversion factor to obtain the protein content. By using this method, it was assumed that dietary carbohydrates and fats do not contain nitrogen and that nearly all of the nitrogen in the food present as amino acids in proteins (FAO, 2003). Based on early determination, the average nitrogen (N) content of proteins was found to be about 16%, which lead to the use of the calculation N × 6.25, whereby 1 / 0.16 = 6.25, in order to convert nitrogen into protein content.

In the Kjeldahl procedure, digestion of protein and other organic compounds in a sample using sulfuric acid with the presence of catalyst convert the total organic nitrogen to ammonium sulphate. Neutralization of the digested mixture with alkali then takes place followed by distillation into a boric acid solution that forms the borate anions. Lastly, the borate anions which are proportional to the amount of nitrogen are titrated with standardized acid. The result of the analysis shows the crude protein content of the food because the nitrogen also comes from non-protein components. However, the content of non-protein compounds is generally smaller than the protein content of most foods (Pomeranz and Meloan, 2000). Kjeldahl method is applicable to all types of food and it is inexpensive and accurate. However, there are some disadvantages such as the method does not just measure protein
nitrogen but total organic nitrogen, is time consuming which requires at least 2 hours to complete, has poorer precision than biuret method and uses corrosive reagent (sulfuric acid).

Although protein consists of a complex mixture, but still most food analysts usually want to find out the total protein content of a food. Total protein content of food is determined empirically. An absolute method can be done by isolation and direct weighing of the protein. Such method is completely impractical for food analysis but it is occasionally used in biochemical investigation. In addition, a “true protein” can be measured by summing up amino acids which represents the protein content of the food (FAO, 2003). It is because proteins are made up of chains of amino acids joined by peptide bonds which can be hydrolysed to their component amino acids. Analysis of amino acids is performed by ion-exchange, gas-liquid or high-performance liquid chromatography (HPLC). However, this approach requires more sophisticated equipment than the Kjeldahl method and thus may be beyond the capacity of many laboratories. Furthermore, experience with the method is important as some amino acids (the sulphur-containing amino acids and tryptophan) are more difficult to determine than others. The advantage of this method is that it overcomes the problem with the use of total N × conversion factor. Therefore, no assumptions require regarding either the non-protein nitrogen content or the relative proportions of specific amino acid of the food. In spite of the complexities of amino acid analysis, there has been reasonably good agreement among laboratories and methods.

2.5.5 Carbohydrate

Total carbohydrate can be measured by two principles, either by difference or by direct measurement of the individual components which are combined to give a total. Carbohydrate determined by calculating the differences has been widely used
where the content of protein, fat, ash and moisture of a food are determined analytically, then being subtracted from the total weight of the food. The remaining value is considered to be the carbohydrate content. Yet there are some drawbacks with this method, in that the by difference figure includes a number of non-carbohydrate components such as lignin, organic acids, waxes and some Maillard product. In addition to this error, it includes cumulative analytical errors from the other analyses. As stated by FAO/WHO (1998), a single figure of total carbohydrate in food is uninformative because it does not reflect many types of carbohydrates that have different potential physiological properties.

Dietary carbohydrate can be divided into two categories which are unavailable and available carbohydrate (FAO/WHO, 1998). Available carbohydrate refers to that fraction of carbohydrate that can be digested by human enzymes, absorbed and entered into intermediary metabolism (FAO, 2003). Dietary fibre which can be a source of energy only after fermentation is an example of unavailable carbohydrate.

Available carbohydrate can be obtained by two different ways; firstly, it can be estimated by calculating the difference or secondly, by direct analysis. By using calculation, the amount of dietary fibre is determined and deducted from total carbohydrate which yields the estimated weight of available carbohydrate (FAO/WHO, 1998). Yet, the composition of the various saccharides comprising available carbohydrate is not indicated from the calculation. In this situation, available carbohydrate can be obtained by adding up the analysed weights of individual available carbohydrates.
An optimum diet has at least 55% of total energy from carbohydrate which is provided in various food sources (FAO/WHO, 1998). Globally, more than 70% of the caloric value of human diet comes from carbohydrates (BeMiller, 2003). Carbohydrate-containing foods provide available carbohydrate for oxidative metabolism as well as vehicle for important micronutrients and phytochemicals. Another function of dietary carbohydrate is to maintain glycaemic homeostasis and gastrointestinal integrity and function.

### 2.5.6 Dietary fiber

There are two basic approaches can be followed to estimate dietary fibre (DF) content, namely gravimetric and chemical. The gravimetric approach involves the usage of chemicals or enzymes where digestible carbohydrates, lipids and proteins are selectively solubilized or removed by hydrolysis. The materials that are not digested are collected by filtration and the fibre residue can be determined gravimetrically. Meanwhile in the chemical approach, digestible carbohydrates are removed by enzymatic digestion and fibre components are hydrolysed by acid. The sum of the released monosaccharides in the acid hydrolysate is the value for DF (BeMiller, 2003).

Determination of DF is important in terms of making food label claims as labelling of food products for DF content is now required, thus an official method for its determination is needed. Currently, it is clear that two types of DF methodology are required: enzymatic gravimetric for food labelling and control purpose; enzymatic chemical for research purposes. These methods must yield similar DF results. Undoubtedly, the combination of Association of Official Analytical Chemists (2000) methods 985.29 and 991.43 (TDF) and the Uppsala enzymatic-chemical method form the basis of filling these requirements. This basic solution is achieved.
on the condition that the enzymes used in the methods (α-amylase, protease and amyloglucosidase) are of the required activity and purity (McCleary, 2003).

2.6 Evaluation of food quality

Food quality is at utmost importance when consumer choosing their food. Consumer preference is important to the food manufacturer who wants to dominate the market for the product. Quality is difficult to define precisely but it generally refers to the degree of excellence of a food includes the entire characteristics of a food that are significant and that make the food acceptable, according to Vaclavik and Christian (2008). Food quality goes hand in hand with both food acceptability and food safety. Therefore, it is important that food quality is monitored to ensure the foods being produced are acceptable to the consumer. For this reason, human senses and instruments are used in research to evaluate the quality of food. The importance of human senses in food evaluation is to perceive the sensory properties whereas instruments are crucial to quantify the physical properties (Singham et al., 2015). More complete picture of the product can be obtained with the combination of both methods.

2.6.1 Sensory evaluation

Sensory plays a major role in defining food quality. According to Sensory Evaluation Division of the Institute of Food Technologists (1981), sensory evaluation is defined as a method used to evoke, measure, analyse and interpret those responses to products perceived through senses of sight, smell, touch, taste and hearing. Sensory analysis can be considered to be an interdisciplinary science that uses human panellists’ sensory perception related to thresholds of determination of attributes, the variance in individual sensory response experimental design to measure the sensory
characteristics and acceptability of food products. Since there is no instrument that can replicate the human psychological and emotional response, the sensory evaluation of any food study is essential (Singh-Ackbarali and Maharaj, 2014). In food product development, sensory evaluation is important particularly to assess the acceptability of new product or formulation. Besides, it is also useful in product quality control, quality assurance, product sensory specification, product optimization and support for advertising claims (Kemp and IFST PFSG committee, 2008). Sensory analysis is performed to reveal regarding product quality by asking questions related to discrimination, description or preference.

Sensory test can be divided into two general categories: affective and analytical. Affective test is also known as acceptance or consumer testing. This method allows the researcher to learn whether the panellists prefer a product or to learn its potential for its acceptability by the consuming public. Meanwhile, analytical test includes difference or discrimination testing. This method is used to observe whether there is a detectable difference between samples or to monitor the nature of any such differences (Sharif et al., 2017). The other analytical test is Descriptive tests are commonly used in the product development context which aims to develop a product that matches a known target quality, to reformulate an existing product using different formulations or to study the differences among several commercially available or experimental products. Sensory properties and their intensity can be described by descriptive tests (Carpenter et al., 2012). In addition, this method can also be used as a research guidance tool to support or interpret instrumental methods and other sensory tests. Descriptive tests can be grouped into two categories which are attribute rating and descriptive analysis. There are different types of rating test in attribute rating; one of it is category scaling. Category scaling
is where the panellist is assigned to check the code samples against a scale or against descriptive terms such as none, slight, moderate, strong and extreme which could apply to a number of attributes for foods. Alternatively, panellist can also be asked to mark an unstructured line with verbal anchors at each end that mark the limits of the attribute instead of using structured words. The line is digitized so each point can have a numerical assignment for analysis (Charley and Weaver, 1998).

2.6.2 Instrumental evaluation

Food quality can also be tested instrumentally without relying on human senses. Some methods may apply combination of both sensory and instrumental evaluation. There are a great number of methods and instruments have been designed to assess the three principal physical attributes of quality such as appearance, flavour components and texture of food. Most of these methods and instruments are unique to a specific food or application rather than having general application. Hence, the best testing method and instrument can be chosen by referring to the objective.

In the instrumental method, texture profiling generally involves compressing the test sample at least twice and quantifying the mechanical parameters from the recorded force-deformation curves (Szczesniak, 2002). Texture profile analysis (TPA) is a versatile instrument for assessing the texture of food with its numerous prove attachments. The analyser is driven by a mechanism that applies force toward the food during a compression or away from the food sample during a tension. The instrument then records the effect of the applied force while the test is being performed. There are five primary textural parameters defined by Pons and Fiszman (1996) as listed in table 2.11.
A study by Bland et al. (2018) on the comparison of sensory and instrumental methods found that there were linear correlations between both methods for hardness, cohesiveness and springiness. Similar pattern is shown by Di Monaco (2008) who worked with fifteen solid food samples and found satisfactory correlations between instrumental and sensory attributes, mainly hardness and springiness. Furthermore, Pascua et al. (2013) commented that the mechanical properties for firmness between sensory and TPA are similar. Researchers find correlations between sensory and instrumental measurements due to a number of reasons:

I. The need for quality control instruments

II. The desire to predict consumer response

III. The desire to understand what is being perceived in sensory texture assessment

IV. The need to develop optimized instrumental tests

<table>
<thead>
<tr>
<th>Textural parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fracturability / brittleness</td>
<td>The force at the first significant break in the curve.</td>
</tr>
<tr>
<td>Hardness</td>
<td>The peak force during the first compression cycle.</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>The ratio of the positive force area during the second compression portion to that during the first compression (Area 2/ Area 1), excluding the areas under the decompression portion in each cycle.</td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>The negative force area for the first bite, representing the work necessary to pull the plunger away from the food sample.</td>
</tr>
<tr>
<td>Springiness / elasticity</td>
<td>The height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite.</td>
</tr>
</tbody>
</table>
2.7 Microstructure characteristics

Microscopy is being increasingly employed to study the influence of food ingredients and processing conditions on food structure and only in the last few decades the full potential of electron microscopy has been recognised (Wilson, 1991). Recent developments in these fields have further improved our understanding on food structures and also the types of information which can now be expected to obtain regarding structure (Aguilera and Stanley, 1999). Scanning electron microscopy (SEM) which combines the best features of both light and transmission electron microscopy with a magnification of 500 000 times is a very useful tool to visualize food structure (Barbosa-Canovas, 2009).

The study of the microstructure of food has taken on increasing significance because the structure of foods can have a profound influence on its nutritional value, rheology and texture attributes (Fazaeli et al., 2012). In SEM, the image is formed step-by-step with scanning a focused electron beam across the specimen. The primary electrons penetrate the solid specimen and are deflected by a large number of elastic scattering processes. Various signals are generated as results of the impact of the incidents electrons which then collected to form an image or to analyse the sample surface. There are many advantages of SEM in examining food microstructure, for example wide range of magnification, simple preparation of sample, high depth of field and the image is a representation of electron data allowing for image analysis and quantification (Aguilera and Stanley, 1999). However, the disadvantages of SEM include the difficulties associated with examining insulating specimens and hydrated samples without altering their state in some way (either drying or freezing) (Harker et al., 2006).
Several articles have reviewed the application of SEM to provide a valuable introduction to the aspects of food structure that can be revealed by this technique (Fazaeli et al., 2012; Xiao and Gao, 2012). A few researches revealed the internal structure of bakery products are likely to be affected by the presence of fibre component. Incorporation of fibre was showed to disturb the starch granule structure causing them a more compact structure as well as the protein matrix leading to product with low volume due to poor gas retention properties of the dough (Ng, 2017; Indrani et al., 2015).

Sucrose replacer such as inulin was also found to affect the structural integrity of muffin by Jingrong et al. (2018). The muffin matrix was found to become more irregular and the starch granules were not fully embedded due to the presence of inulin which would limit gluten and starch hydration during mixing and baking causing less developed structure. Moreover, a similar research done on the effect of sugar replacer by Sahin et al. (2018) who demonstrated burger bun formulated with xylitol resulted in incomplete protein-network formation. The hygroscopic ability of xylitol causes it to compete with gluten for water, resulting in delayed and weakened the gluten network development.
2.8 Glycaemic index and glycaemic load

The concept of glycaemic index (GI) was proposed by Jenkins et al. (1981) as an alternative system for classifying carbohydrate-based foods. GI values do not define carbohydrate by their chemical structure but rather by their physiological glycaemic response. GI is defined by FAO/WHO (1998) as the “incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food expressed as a present of the response to the same amount of carbohydrate from a standard food taken by the same subject”. The carbohydrate in the definition excludes dietary fibre and refers to carbohydrate that will affect the postprandial glycaemia which is also known as available carbohydrate.

Based on the definition of GI, the concept is practical only foods that provide a considerable amount of available carbohydrate. Determination of GI values for low carbohydrate-containing foods such as vegetables or foods that consist of mainly fat and protein can be quite challenging and may be incorrect when used in practise. Hence, GI concept is recommended to use on foods that provide at least 15 g and preferably 20 g of glycaemic carbohydrate per portion, such foods are bread, cereals, rice, pasta and potatoes (Asp et al., 2004).

The GI ranks carbohydrate foods on a scale of 0 to 100 based on their impact on postprandial glucose. For example, glucose drink has the highest GI value of 100 while food with GI value of 70 or more is considered high-GI, between 56 – 69 is intermediate-GI and 55 or less is low-GI (Brand-Millet et al., 2003). Generally, high GI foods are food with the most refined starchy whereas unrefined foods (DF-rich and resistant starch) tend to have low GI. According to Mitchell (2008), the GI concept has been applied worldwide as an indicator in selection of food products.
The longest exposure to GI phenomenon is in Australia where the awareness was the highest at 82% of all respondents, followed by Europe and United States.

There is another concept known as glycaemic load (GL) which was introduced to quantify the overall glycaemic effect of a standard portion of food. It is calculated as the product of the amount of available carbohydrate in that serving and the GI of the food divided by 100. When a food contains a relatively small amount of carbohydrate, it is usually essential to consider the GL together with the GI values. A GL value of 10 or less is considered as low, between 11 – 19 is medium and 20 or more is high (Braclay et al., 2008). Examples of comparison of GI and GL of some common foods as listed in Table 2.12.

The hydrolysis rate of food and the gastric emptying rate in the gastrointestinal tract determine the absorption rate of glucose after a meal. High GI foods such as white rice, mashed potato and white bread are broken down and absorbed rapidly, resulting in a sharp increase in blood glucose level. On the contrary, low GI foods are absorbed more slowly, resulting in a slower and more sustained rise in blood glucose level. This is particularly relevant for patients with impaired postprandial glucose regulation. Interpretation of GI values should consider about other relevant properties of food such as energy content and amount of other macronutrients, whereby it should not be interpreted in isolation.
Table 2.1  Examples of comparison of GI and GL of some common foods. Reference food: glucose

<table>
<thead>
<tr>
<th>Glycaemic load</th>
<th>Glycaemic index</th>
<th>Glycaemic index</th>
<th>Glycaemic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (≤ 10)</td>
<td>Low (≤ 55)</td>
<td>Medium (56 - 69)</td>
<td>High (≥ 70)</td>
</tr>
<tr>
<td></td>
<td>Apple (6, 38)</td>
<td>Beets (5, 84)</td>
<td>Popcorn (8, 72)</td>
</tr>
<tr>
<td></td>
<td>Orange (5, 42)</td>
<td>Cantaloupe (4, 65)</td>
<td>Watermelon (4, 72)</td>
</tr>
<tr>
<td></td>
<td>Skim milk (4, 33)</td>
<td>Pineapple (7, 65)</td>
<td>Whole wheat flour bread (9, 71)</td>
</tr>
<tr>
<td></td>
<td>All bran cereal (8, 42)</td>
<td>Table sugar (7, 68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carrots (3, 47)</td>
<td>Pure honey (10, 58)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peanuts (1, 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium (11 – 19)</td>
<td>Apple juice (11, 40)</td>
<td>Life cereal (16, 66)</td>
<td>Cherries (15, 74)</td>
</tr>
<tr>
<td></td>
<td>Banana (12, 52)</td>
<td>Wild rice (18, 57)</td>
<td>Shredded wheat (15, 75)</td>
</tr>
<tr>
<td></td>
<td>Sourdough wheat brand (15, 50)</td>
<td></td>
<td>Doughnut (17, 76)</td>
</tr>
<tr>
<td>High (≥ 20)</td>
<td>Macaroni (23, 47)</td>
<td>White rice (23, 64)</td>
<td>Baked russet potatoes (26, 85)</td>
</tr>
<tr>
<td></td>
<td>Spaghetti (20, 42)</td>
<td>Couscous (23, 65)</td>
<td>Cornflakes (21, 81)</td>
</tr>
<tr>
<td></td>
<td>Brown rice (20, 50)</td>
<td></td>
<td>Pizza (22, 80)</td>
</tr>
</tbody>
</table>

Adopted from Nayak et al. (2014) and Atkinson et al. (2008)
2.8.1 Factors affecting glycaemic index value of food

There are several factors that may contribute to the differences in glucose and insulin responses. One of the factors is the cooking and processing of food, which could alter the food structure, thus affect the glucose response. Processing conditions alter postprandial glucose responses of starch by disturbing the cell wall and structure of the granule and gelatinization increases the GI. Starch is presented as the large granules in carbohydrate food and if the granules are disrupted, both of the amylose and amylopectin starch macromolecules will become available for hydrolysis. Food processing such as rolling, pressing, grinding and flaking of carbohydrate foods can disturb the germ layer and starch granules integrity, causing the carbohydrate portion more accessible to digestible enzyme and resulted in an increase in GI value. Besides, cooking with heat and a moist environment promotes gelatinization in which starch granules swell and break, allowing the accessibility of α-amylase to starch.

Ratio of amylose to amylopectin could also affect the glucose responses and the GI values. Foods containing greater proportion of amylopectin are likely to have higher GI value. This is because amylopectin consists of branched-starch molecules which are more easily hydrolysed in the small intestine than the single-strand amylose. Additionally, if the starch goes through processing such as by application of heat or moisture, amylose tends to recrystallize or retrograde. The retrograded amylose is minimally digested by amylase enzyme in comparison to intact amylose. Moreover, amylose tends to interact with other food components such as lipids and protein. Ahmadi-Abhari et al. (2013) reported that the rate of amylolysis has diminished as a result of complex formation of phospholipids and amylose. Hence,
the reduced rate of amylolysis leads to slower absorption of glucose into the blood stream and lower GI value.

Several studies suggested that different GI values are obtained from food when eaten alone or included in a mixed meal. The effect of protein and fat affect the glycaemic response as they are particularly efficacious in promoting gut peptide release, thereby stimulate insulin secretions. For instance, Kim et al. (2019) reported a meal containing three macronutrients showed significant lower postprandial glucose response as compared to meal with just one or two macronutrients. As shown in Figure 2.8, DF especially soluble DF or viscous fibre has significant influence on glycaemia. It improves carbohydrates tolerance through increasing viscosity and volume of the alimentary bolus, slowing down the rate of gastric emptying as well as the rate of intestinal absorption by forming an intermediate film between the intestinal lumen and brush border enzymes (Brownlee, 2011).

The existence of certain food components such as organic acids or antinutrients is one of the factors affecting GI value. Polyphenols and phytic acids are antinutrients that delay the digestion of available carbohydrate by binding to α-amylase (Singh et al., 2010). An increment in the acidity of a meal could also slow down the rate of gastric emptying, and thus lower the glucose response. It was documented that the postprandial blood glucose and insulin responses were decreased with the addition of sourdough lactobacilli in wheat bread (De Angelis et al., 2007).

The nature of monosaccharide compounds is another aspect influencing glycaemic responses. For example, fructose exhibited lower blood glucose response than glucose. This is due to the absorption of fructose is through a process of
saturable facilitated diffusion and must be converted to glucose by the liver before entering the blood circulation. On the contrary, glucose is readily absorbed into small intestine (Bornet et al., 1997).

Figure 2.8  Factors influencing the rate of digestible carbohydrate availability in gastrointestinal tract (Nayak et al. 2014)

2.8.2 Health benefit of glycaemic index (GI) concept

Lot of efforts have been carried out and demonstrated that selecting carbohydrate foods according to their GI values provide significant impact on many aspects of human physiology and metabolism. Hence, GI concept may contribute to the prevention and management of several chronic diseases. Previous literature reported that low GI or GL diets offered comparable or better protection than whole grains or DF on the risk of chronic diseases include type 2 diabetes, coronary heart disease and colorectal cancer (Livesey et al. 2019).
The link between high GI diet and certain chronic diseases can be explained through several plausible mechanisms. High GI foods produce higher blood glucose concentrations and demand for more insulin compared to low GI foods with similar carbohydrate content. The high demand for insulin may lead to the failure of pancreatic β-cell and impaired glucose tolerance which then result in diabetes, especially in genetically susceptible individuals. Evidence also suggested that increase insulin resistance caused by high GI is mediated by the effect on glycaemia, free fatty acids and counter-regulatory hormones (Ludwig, 2002).

There are studies claimed that low-GI diets can significantly improve diabetic control in diabetic individuals. The regulation of glucose metabolism is impaired in diabetic patients, thus they are more susceptible to the influence of diet on plasma glucose, particularly in the postprandial period. A study by Gomes et al. (2017) demonstrated a great improvement in glycated haemoglobin and fasting blood glucose with low-GI diet compared to higher-GI diet in type 2 diabetes patients. Similar trend was also reported by Yusof et al. (2009) and Jenkins et al. (2008). Furthermore, an intervention study by Turner-McGriey et al. (2011) revealed that low GI-diet appears to be one of the determinants of success of the 2003 American Diabetes Association (ADA) recommended diet in improving HbA1c and body weight among type 2 diabetes patients. These findings also suggested that overweight individuals with type 2 diabetes may benefit not only from increased fibre intake but particularly from a reduction in the GI of their diets. The beneficial effects of low GI foods are also noticeable in type 1 diabetic individuals. Although insulin is the mainstay of treatment for type 1 diabetes, but few studies conducted in children with type 1 diabetes suggested that a low GI-diet can be a useful adjunctive
Gast et al. (2012) reported elevated glucose and insulin concentrations are direct consequences of insulin resistance which is known as risk factors for cardiovascular diseases (CVD). A randomized controlled trial by Jenkins et al. (2012), it was found that diet with low GI improved both glycaemic control and reduced the risk of CVD in type 2 diabetes patients by reducing blood pressure. According to Fleming (2013) and Goff (2013), low-GI diets can significantly lower total and low density lipoprotein (LDL) cholesterol levels resulted in reduced rate of CVD events. There has also been emerging evidence on the positive effects of low-GI diets on CVD risk factors such as oxidative damage (Botero et al., 2009) and inflammation (Wolever et al., 2008) in type 2 diabetic patients. As obesity is also a risk factor for CVD, low-GI diets have been reported as causing greater weight loss in overweight or obese people compared with control diets, as well as improving lipid profile. Thomas et al. (2007) reported low-GI diet was shown to result in significant decrease in body mass, total fat mass, BMI, total cholesterol and LDL-cholesterol compared with control diets.

Several studies have investigated the associations between dietary GI and GL on the risk of colorectal cancer. Evidence suggests that high postprandial glucose and insulin are involved in the etiology of several cancers, including colorectal and endometrium cancer (Sieri et al., 2015). The increase risk of colon cancer appears to cause by high levels of insulin and insulin-like growth factor 1 (IGF-1) in particularly, and a carbohydrate-rich diet which results in hyperglycaemia and
consequently hyperinsulinemia plays a role in the development of this cancer (Giovannucci and Michaud, 2007).

A study by Sieri et al. (2015) stated that high dietary GI with high consumption of carbohydurate from high GI foods were associated with significantly increased risk of colon cancer whereas the relation between dietary GL and colorectal cancer yielded inconsistent results. The finding is supported by Sieri et al. (2017) who also found that high consumption of low-GI carbohydrate foods was positively correlated with lowered colon cancer risk. Therefore, it is suggests that colon cancer risk depends more on the ability of the carbohydurate foods consumed to increase postprandial blood glucose rather than the overall quantity of carbohydurate consumed. Based on a meta-analysis by Galeone et al. (2013, it was shown that there were significant associations between dietary GL with endometrial cancer risks. High levels of GI and GL may increase the level of systemic markers inflammation such as reactive protein, interleukin-6 and tumour necrosis factor alpha which involved in pathophysiology of many types of cancer (Galland, 2010).

Lowering GI of the diet appears to be an effective way to improve glycaemic control in diabetes as well as reduce the risk of chronic diseases, thus the use of low-GI diet as a long-term maintenance diet should be considered as part of overall strategy to improve quality of life.
CHAPTER 3
MATERIALS AND METHODS

3.1 Collection of raw material

As shown in Figure 3.1(a), fully ripened *Musa acuminate cv. Berangan* banana (stage 6) was purchased from a local fruit store in Kota Bharu, Kelantan and kept at room temperature (25°C) and relative humidity of 80% - 85% until the fruit reached the desired ripeness (stage 7) without any ripening agent (Figure 3.1(b)). The stage of ripening was determined according to Karim *et al.* (2018) using a colour chart and physical observation. In order to standardize the sample, software imageJ 1.38 (National Institutes of Health, USA) was used to calculate the number of black spot (>200) on the banana skin of every single bananas as shown in Figure 3.2. The overripe banana was then processed to obtain overripe banana sweetener (OBS) and overripe banana residue powder (OBR). Three batches of bananas were processed in the study with each batch consisted of three bunches of banana.

Figure 3.1 (a) Fully ripened *Berangan* banana (b) Overripe *Berangan* banana
Figure 3.2 Calculation of black spot on banana skin using software ImageJ.
(a) Image editing and analysis (b) Black spot counting
3.2  Processing of raw material

Extraction of banana was done by using the method establish by Albuquerque et al. (2005) with modification. The banana pulps were homogenised with water in the ratio of 1:3 and centrifuged at 15000× g for 25 mins at 4°C. Filtration was done with Whatman No. 4 filter paper to extract the non-fibrous portions which mainly consist of minerals, organic acid, soluble polysaccharides and sugars from the liquafied banana pulps. The extracted supernatant and remaining residue were then further processed into OBS and OBR, respectively.

3.2.1 Overripe banana sweetener

The extracted clear banana liquid was dehydrated using a technique described by Tadakittisarn et al (2007) with slight modifications. 50g of banana liquid was dehydrated in a thermal dehydrator (Anywin FD770, China) at 60°C overnight (16 hours) to remove the moisture content. The concentrated syrup (Figure 3.3) was then kept in a screw cap bottle at 4°C prior to analysis and further use. Both pH and total soluble solids (TSS) °Brix of the syrup were measured using a HANNA pH 211 microprocessor pH meter (USA) and a hand refractometer (Atago 3851 PAL-BX/RI, Japan), respectively.

Figure 3.3  Overripe banana sweetener
3.2.2 Overripe banana residue

The residue (w/v) obtained after filtration was dried in a conventional oven (Memmert, Germany) at 55°C for 24 hours, followed by milling into powder using an electrical blender and then sieved into fine powder (125 µm diameter) using a sieve. The residue powder (Figure 3.4) obtained was kept in a screw cap bottle at 4°C until further use.

Figure 3.4 Overripe banana residue powder
3.3 Development of chocolate cookie

The recipe of the chocolate cookie was adopted from Mohan et al. (2018) with slight modification. The chocolate cookies were formulated by using commercially available raw ingredients such as butter, margarine, castor sugar, egg, baking powder, cocoa powder, wheat flour and corn flour. Incorporation of 8% overripe banana pulp powder to replace wheat flour was identified as the best formulation in a previous study by Ng et al. (2020). Hence, chocolate cookies formulated with 8% OBR were used in this study. OBS was used to partially substitute castor sugar at the percentage of 10, 15 and 20% as listed in Table 3.1.

Table 3.1 Incorporation levels of OBS and OBR in chocolate cookie

<table>
<thead>
<tr>
<th>Items</th>
<th>Ingredients</th>
<th>Items</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*Control (0% OBS and OBS)</td>
<td>Quantities (g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0% OBS</td>
<td>10% OBS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8% OBS</td>
<td>8% OBS</td>
</tr>
<tr>
<td>1</td>
<td>Wheat flour</td>
<td>76</td>
<td>69.9</td>
</tr>
<tr>
<td>2</td>
<td>OBR</td>
<td>0</td>
<td>6.1</td>
</tr>
<tr>
<td>3</td>
<td>Castor sugar</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>OBS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Butter</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Margarine</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Egg</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>Baking powder</td>
<td>$\frac{1}{2}$</td>
<td>$\frac{1}{2}$</td>
</tr>
<tr>
<td>9</td>
<td>Cocoa powder</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>Corn flour</td>
<td>5</td>
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</tbody>
</table>
All ingredients were carefully weighed on a portable digital scale (OHAUS Scout, USA). In a mixing bowl, butter and castor sugar were beaten together by using an electric hand mixer (Khind HM200, Malaysia) followed by adding eggs into the mixture slowly until a creamy texture was achieved. After addition of all dry ingredients, the mixture was beaten again for 5 mins and kept in the fridge for 2 hours. The refrigerated dough was manually shaped into 3 mm thick cookies using a 5 cm diameter mould. After that, they were placed on a baking sheet and baked in an oven (Zanussi ZCG841W, England) at 170°C for 12 mins. The cookies (Figure 3.5) were then cooled at room temperature for 1 hour before being grinded into powder form and kept in a screw cap bottle at 4°C until further analyses. The cost calculation for one piece of cookie is shown in Appendix B.

Figure 3.5 Chocolate cookie after baking in oven
3.4 Nutritional analyses

The nutritional analyses were conducted on both OBR and chocolate cookies to determine their proximate composition and TDF content. Proximate analyses for moisture, total ash, protein and fat content were conducted following the AOAC (1996) method.

3.4.1 Moisture determination

In determination of the moisture content, the air-oven method 925.10 was followed. Firstly, aluminium dish was put in an oven (Memmert GmbH & Co. KG, Germany) for drying at 105°C for three hours, followed by cooling down in desiccator (NORMAX, Portugal) to reach room temperature and weighted (W1) using an analytical balance (Mettler-Toledo Dragon 204, Switzerland).

Samples were ground in a laboratory blender (Waring Commercial 8010S, USA) until fine. The homogenized sample was weighed 5.0000 ± 0.001 g into the aluminium dish and then dried together in an oven at 105°C overnight. After drying, the aluminium dish with sample was cooled in desiccator and weighted (W2) until constant weight was attained. The difference between the constant final weight and initial weight after drying process was expressed as the moisture lost. Hence, this difference was reported to be the moisture content of sample. The following formula was used to calculate moisture content of the sample.

\[
\text{Moisture content (\%)} = \frac{\text{Loss of weight of the sample (g)}}{\text{Weight of the sample (g)}} \times 100
\]

\[
= \frac{(\text{Weight of sample} + W1) - W2}{\text{Weight of the sample (g)}} \times 100
\]
3.4.2 Total ash determination

Total ash in foods refers to the inorganic residue which remains after the organic matter has been burnt away at elevated temperature (around 550°C). It represents the total mineral contents in foods.

The determination of ash was done following the method 942.05. In the beginning, crucibles were oven-dried at 105°C for three hours. After drying, they were immediately cooled in desiccator and weighted (W1). Ground sample was weighed 0.500 ± 0.01 g into crucible and charred on hotplate (ERLA EM2-V7070, Malaysia) until smoke was ceased. They were then transferred into a muffle furnace (Barnstead Thermolyne F6020C-33, USA) and incinerated at temperature of 550°C for three hours until the sample turned to whitish or greyish. They were removed from muffle furnace, cooled in desiccator and weighted (W2) until constant weight was obtained. Total ash content of the sample was calculated using the formula as below.

\[
\text{Total ash content (\%) } = \frac{\text{Weight of ash (g)}}{\text{Weight of the sample (g)}} \times 100
\]

\[
= \frac{W2 - W1}{\text{Weight of the sample (g)}} \times 100
\]

3.4.3 Fat determination

Crude fat of the sample was determined by Soxhlet method 960.39. Initially, oven-dried extraction beaker (VELP Scientifica, Italy) was cooled down in desiccator to room temperature and weighted (W1). Cellulose thimble (Whatman, USA) with previously filled sample (3.000 ± 0.01 g) was attached to the Soxhlet
extractor unit (VELP Scientifica SER148/6, Italy). Next, 80 ml of petroleum ether solvent (b.p.t. 40 – 60°C) was added into each of the extraction beaker which was then attached to the extraction unit.

After cellulose thimbles with sample and extraction beakers with petroleum ether solvent were introduced to the extraction unit, the unit was closed, and then the cooling water flow and heating were started. In order to immerse the thimbles, the extractor slider was pushed to ‘Immersion’ position. After immersion for 30 min, the slider was changed to ‘ashing’ position to remove thimbles from solvent. This step took 25 min to allow reflux washing. Then, the slider was switched to ‘Recovery’ position and the stopcock placed below the water cooled condenser was switched off. This last step of extraction was lasted for 30 min.

All of the extraction beakers were taken out from the unit and moved into an oven to dry the remaining petroleum ether at 100°C for a period of 30 min. After the solvent was entirely removed, the residue remaining in the extraction flask was considered to be the fat content of the sample. Next, they were cooled in a desiccator before the beakers containing fat of the samples were weighed (W2). The residue in the extraction thimble turned into the defatted sample. Fat content of the sample was determined using the following formula.

\[
\text{Fat content (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of the sample (g)}} \times 100
\]

\[
= \frac{W2 - W1}{\text{Weight of the sample (g)}} \times 100
\]
3.4.4 Protein determination

The Kjeldahl method 991.20 was principally used to investigate the protein content of sample. The method can be divided into three steps: digestion, distillation and titration.

3.4.4(a) Reagents preparation

Sodium hydroxide, NaOH (40% w/v)

Naoh (400 g) was dissolved in 700 ml of distilled water in a volumetric flask with 1000 ml volume. Then, the content was further diluted to 1000 ml with distilled water.

Boric acid solution (4% w/v)

Boric acid (40 g) was dissolved in 700 ml of distilled water in a volumetric flask with 1000 ml volume. Then, the content was further diluted to 1000 ml with distilled water.

Hydrochloric acid, HCL (0.1 N)

Thirty seven percent of HCL (8.88 ml) was added into a 1000 ml volumetric flask having 700 ml of distilled water. Then, the content was further diluted to 1000 ml with distilled water.

3.4.4(b) Procedure

In the digestion step, sample (1000 ± 10 mg) was put into a 250 ml of long neck digester flask. Next, two tablets of selenium catalyst (Gerhardt 1000 Kjeltabs ST, Germany) and concentrated sulphuric acid (20 ml) were added into the flask including the blank flask without sample. Then, the tubes were brought to Kjeldatherm block digestion unit (Gerhardt GmbH & Co. KG, Germany) and placed in inclined position on the electric coil heating rack. The Turbosog scrubber unit
(Gerhardt GmbH & Co. KG, Germany) was turned on to remove and neutralize acid fumes. The digestion unit was programmed to achieve 400°C by gradual heating to digest the sample until clear solution was observed.

After the digestion step was completed and the flasks were cooled to room temperature, they were transferred to Vapodest distillation unit (Gerhardt GmbH & Co. KG, Germany). Next, a conical flask containing two drops of methyl red indicator (0.1% w/v) was put on the receiver platform. Previously prepared 60 ml of distilled water, 40 ml of NaOH solution (40% w/v) and 60 ml of boric acid solution (4% w/v) were released automatically into the digester flask.

After the distillation process was ended, the conical flask was taken out and the content was titrated to become light purplish colour, by adding 0.1 N HCl transported from a burette. The amount of HCl needed for the titration step was obtained. The quantity of nitrogen in the sample reveals its protein content after the quantity of the nitrogen content was multiplied by a conversion factor of 6.25 because most of the proteins contain 16% nitrogen. Thereby, protein content was calculated as follows:

\[
\text{Protein} (\%) = \frac{(\text{ml HCl} - \text{ml HCl blank}) \times 14.008 \times 0.1 \text{ N HCL} \times 6.25}{\text{Weight of the sample (mg)}} \times 100
\]

3.4.5 Total carbohydrate determination

Total carbohydrate content was calculated by difference (Charrodiere et al., 2004). The calculation was as follow:

Total carbohydrate, CHO (%) = 100 – % (moisture + fat + ash + protein)
3.4.6 Total dietary fibre determination

The total dietary fibre of the sample was carried out according to enzymatic-gravimetric method 991.43 (AOAC, 1996). In this study, analysis was performed by using Fibertec E system (FOSS Analytical, Sweden) which implements the enzymatic-gravimetric principle. The system comprises of Filtration Module (Fibertec E 1023) and Thermostatic Shaking Water Bath (FOSS 1024).

3.4.6(a) Reagents preparation

Protease solution

Protease (100 mg) was dissolved in 2 ml of deionised water.

Hydrochloric acid, HCl (0.325 M)

Thirty seven percent HCl (26.90 ml) was added into a volumetric flask containing 700 ml distilled water. Then, the content was further diluted to 1000 ml using distilled water.

Sodium hydroxide, NaOH (0.275 N)

NaOH (11 g) was dissolved in distilled water (700 ml) in a volumetric flask. Then, the content was further diluted to 1000 ml using distilled water.

Phosphate buffer (0.08 M, pH 6)

Sodium phosphate dibasic anhydrous, Na₂HPO₄ (1.4 g) and Sodium phosphate monobasic monohydrate, NaH₂PO₄.H₂O (9.68 g) were dissolved in distilled water (700 ml) in a volumetric flask. Next, the content was further diluted to 1000 ml using distilled water and the pH was checked with pH meter (Mettler Toledo S20, USA).
Ethanol solutions (95% and 78%)

For 95% ethanol, absolute ethanol (950 ml) was diluted with distilled water (50 ml) in a 1000 ml volumetric flask.

For 78% ethanol, absolute ethanol (780 ml) was diluted with distilled water (220 ml) in a 1000 ml volumetric flask.

3.4.6(b) Procedure

Prior to the experiment, celite (0.5 g) was weighed in fritted crucibles which were then put into an oven for drying at 105°C overnight. After they were taken out and cooled in desiccator, they were weighted. For sample preparation, samples were defatted with petroleum ether by Soxhlet method (explained in Section 3.4.3) and homogenized by grinder prior to analysis. The defatted sample (1000 ± 5 mg) was put on lid of incubation flask which was then attached to the flask.

Phosphate buffer solution (50 ml) was added into each flask to start the step one incubation. They were stirred on hot plate with magnetic stirrer (ERLA, Malaysia) to ensure sample was completely dispersed. pH (6.0 ± 0.2) was checked using HANNA pH 211 microprocessor pH meter (USA). If necessary, either 0.275 N NaOH or 0.325 M HCL was added for pH adjustment. Alpha-amylase (50 μl) was added into each flask and the content was stirred at low speed. Flasks were then covered by aluminium foil and incubated in boiling (at 95 to 100°C) water bath (Protech Model 830, Malaysia) for 30 min. After that, the flasks were taken out from water bath and cooled to room temperature.

Prior to step two incubation, the pH of the content was initially adjusted to 7.5 ± 0.1 by adding 0.275 N NaOH. Each flask was filled with protease solution (100 μl) when the target pH was achieved. The flasks were then covered and incubated in
a shaking water bath at 60°C for 30 min with continuous agitation (speed 3.0). The flasks were cooled to room temperature after removed from shaking water bath.

Third step incubation was carried out by adjusting pH ranging from 4.0 to 4.6 by adding 0.325 M HCl. After pH was adjusted, amyloglucosidase (200 μl) was added into each flask. The flasks were covered and incubated again in shaking water bath at 60°C for 30 min with continuous agitation (speed 3.0). These three incubation steps were aimed to digest starch and protein. The flasks were transferred out and immediately added with 280 ml or 4 volumes of 95% ethanol. They were then let to precipitate for 1 hour at room temperature.

The crucibles were brought to the filtration unit. The beds of celite in the crucibles were wet and redistributed using a stream of 78% ethanol. Suction was applied to draw the celite onto the fritted glass in order to get an even mat.

The crucibles were removed from the unit and mounted upside down on top of the incubation flask containing the sample. The flasks were attached to the bayonet fittings and they were folded up. The bottom lids of each flask were then removed. Residues on the lid and seal were washed off into the flask with a small portion of 78% ethanol.

In each flask, washing procedure was carried out using ethanol and acetone. The produce was repeated 3 times using 20 ml 78% ethanol, 2 times using 10 ml 95% ethanol and 2 times using 10 ml acetone. Water aspiration pump was started for filtration. The control valves were turned to ‘V’ position for suction and ‘P’ position for breaking up clogged residue, whichever necessary.
After filtration, residues in crucibles were dried at 105°C overnight and weighed. The dried residues were divided into two groups of replicates. The first replicated were continued with determination of ash in furnace (Carbolite CWF1100, UK). Weigh of residue ash was taken. The second replicates were taken for determination of protein. As to continue with protein analysis, the residue in each crucible was scrapped into digestion flask and protein analysis was run as in Section 3.4.4. Protein content of residual was calculated.

Calculation

Residue weight = Weight of (Residue + Celite + Crucibles) – Weight of (Celite + Crucibles)

\[
\text{Blank value} = \left( \frac{B1 + B2}{2} \right) - \text{mg protein} - \text{mg ash}
\]

\[
\text{Dietary fibre (%) } = \left\{ \left( \frac{(R1 + R2)}{2} \right) - \text{mg protein} - \text{mg ash} - \text{Blank} \right\} \times \frac{M1 + M2}{2} \times 100
\]

References for the formulas are as follow:

B1/B2 = Residue weight (mg) of blank duplicates

R1/R2 = Residue weight (mg) of sample duplicates

M1/M2 = Weight (mg) of sample duplicates
3.4.7 Sugar analysis

The sugar content of OBS and chocolate cookies were analysed with the Boehringer Mannheim/ R Biopharm, Germany 10 716 260 035 sugar analysis kit for the enzymatic analysis of sucrose, glucose and fructose according to the manufacturer's instructions.

3.5 Physical analyses

3.5.1 Physical evaluation of chocolate cookie

Measurements of physical characteristics (Saha et al., 2011; Tiwari et al., 2011) were carried out using a 15 cm scale. One hour after baking, weight (W) of ten pieces of cookies from each formulation was taken by using analytical balance (Mettler-Toledo Dragon 204, Switzerland) and the mean value (g) was obtained. The cookie diameter (D) was taken by arranging ten pieces of cookies edge-to-edge. The cookie thickness (T) was measured by stacking ten pieces of cookies. The spread ratio (D/T) was calculated as the diameter to thickness ratio.

3.5.2 Texture profile analysis

Three-point break technique (Gains, 1991) was used to measure firmness and crispiness of the cookies by using a Texture Analyser TA.XTplus (Stable Micro Systems, Surrey, UK) (Figure 3.6). The analyser was equipped with Texture Exponent Software package and the accessories for the test were heavy duty platform (HDP/90) and 3-point bend rig (HDP/3PB). In order to support the cookie, the two adjustable supports of the rig base were separated to be 20 mm apart and the distance was kept constant for comparison purpose. The base plate was fixed onto the heavy duty platform which was then adjusted and locked in a position that allowed equal distance between the upper blade and the two lower supports. Next, cookie sample
(approximately 50 mm in diameter and 4 mm in thickness) was put centrally on the supports.

The operating settings are listed in Table 3.2. Once the trigger force obtained, the force increased until the cookie was broken into two pieces. The maximum or peak force detected to break the cookie was regarded as ‘firmness’. At the same time, the mean distance compressed prior to breaking value was reported as ‘crispiness’. A curve and the values of interest were produced from the software. The peak force (kg) and the mean distance at point break (mm) were indicated as firmness and crispiness of the cookie, respectively.

Figure 3.6 Texture Analyser TA.XTplus
Table 3.2 Operating settings for TPA

<table>
<thead>
<tr>
<th>Test mode</th>
<th>Compression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-test speed</td>
<td>1.0 mm/s</td>
</tr>
<tr>
<td>Test speed</td>
<td>3.0 mm/s</td>
</tr>
<tr>
<td>Post-test speed</td>
<td>10.0 mm/s</td>
</tr>
<tr>
<td>Trigger force</td>
<td>50g</td>
</tr>
<tr>
<td>Distance between probe and cookie</td>
<td>10 mm</td>
</tr>
<tr>
<td>Compression distance</td>
<td>3 mm</td>
</tr>
<tr>
<td>Option</td>
<td>Return to start</td>
</tr>
</tbody>
</table>

3.5.3 Scanning electron microscopy

In order to observe the morphological characteristics of the cookie, they were cut into small fine pieces with a blade to create a clean fracture surface and then viewed by using a SEM (Brand: Fei, Model: Quanta FEG 450, Netherland). Samples were mounted on an aluminium sample holder (12 mm in diameter) with double sided conductive carbon tape to improve conductivity. Then, the samples were sputter coated with a thin layer of gold in a vacuum evaporator (Baltex SCD005 Sputter Coater, Hi-Tech Germany) and placed in the SEM chamber for investigation. The microstructure of the samples with magnification of 500 and 1000× were photographed using a 5kV of electron beam-accelerating voltage.

3.6 Sensory evaluation

Sensory evaluation session was carried out based on seven-point hedonic scale (Aminah, 2000) where high score indicates higher preference (1= dislike very much and 7= like very much). Sensory attributes such as colour, appearance, aroma,
crispiness, flavour and overall acceptability were evaluated. Sensory evaluation form was attached as Appendix C.

After the cookie samples were cooled to room temperature after baking, they were placed into a plastic pouch and heat-sealed. All of the samples were coded with three digits number and randomly permutated. The code was created from a table of random numbers where three digits were chosen to be used as a code. Set of numbers that could possibly have meaning to the volunteers such as 911, 999, 007 were avoided. The random numbers were then listed on a master sheet, with one code for each sample and for each panel.

After preparing three-digit coded cookie samples, sensory evaluation of cookies was carried out in School of Health Sciences, USM. Samples were judged by randomly-selected 60 panellists consisting of students and staffs. Initially, five cookie samples were served to every panellists and drinking water was also provided to rinse their mouth before testing the next sample in order to minimize bias from previous sample. Short briefing was given prior to the evaluation session.

3.7 **In vivo glycaemic index determination**

3.7.1 **Selection of participants**

Informative poster and social networking were used to act as a medium to introduce the study and recruit participants. Based on the methodology described by FAO/WHO (1998), 13 healthy human participants (nine females and four males) were randomly selected from the School of Health Sciences, USM. The inclusion criteria were: age between 18 to 60 years old, BMI of 18.5 to 24.9 kg/m2, non-smoker, non-pregnant, non-lactating, carrying no history of acute or chronic diseases
and did not experience any surgical procedures during the past six months. After collecting a written informed consent form (Appendix E) from participants, a clinical examination was performed by a physician. The clinical examination form was attached as Appendix D. Ethical approval for this study was acquired from the Human Research Ethics Committee of USM (Appendix A).

Measurement of body weight (kg) was done on an electronic scale (Seca 767, UK), with participant wearing light clothing and their shoes were taken off. Height measurement (m) was conducted by utilising an electronic stadiometer (Seca 242, UK), with participant standing straight and without shoes. Fasting blood glucose was measured using dry chemistry analyser (Reflotron Plus, Roche, Switzerland). Blood pressure was determined using a mercurial sphygmomanometer (Fazzini, Italy). BMI was calculated using the standard formula: weight (kg)/ height² (m²). Characteristics of participants were summarized in table 3.3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.70</td>
<td>2.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.50</td>
<td>6.82</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.60</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.06</td>
<td>2.00</td>
</tr>
<tr>
<td>Systolic BP (mm/Hg)</td>
<td>107.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Diastolic BP (mm/Hg)</td>
<td>72.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>5.24</td>
<td>0.32</td>
</tr>
</tbody>
</table>
3.7.2 Preparation of participants

Participants were instructed to fast overnight for about 10 – 14 hours prior to attending the test session in the morning. They were also informed to avoid any unusual vigorous activity on the day before the test was carried out (Brouns et al., 2005). In order to minimise carry-out effects, two test sessions were separated for at least 72 hours as a washout period.

3.7.3 Preparation of reference food

The reference food used in this study was glucose. It was prepared by dissolving 25 g of original flavoured Glucolin (Reckitt Benckiser, Malaysia) in 250 ml drinking water. 25 g of Glucolin contains 25 g of available carbohydrate (dextrose monohydrate).

3.7.4 Preparation of test foods

Three highly favourable cookie formulated with 0 (control), 0% OBS + 8% OBR and 15% OBS + 8% OBR were chosen for GI determination based on the sensory evaluation score. Participants were served with 25 g available CHO portions of the three test foods to avoid unrealistically oversized serving. This formula was used to calculate available CHO:

\[
\text{Available carbohydrate, } CHO \ (g) = \text{Total carbohydrate (g)} - \text{Total dietary fibre (g)}
\]

The nutritional compositions of the test foods containing 25 g of available CHO are shown in Table 3.4.
Table 3.4  Serving size and nutritional composition of test foods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (0% OBR and OBS)</th>
<th>0% OBS + 8% OBR</th>
<th>15% OBS + 8% OBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portion size (g)</td>
<td>38.48</td>
<td>41.54</td>
<td>41.85</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>189.45</td>
<td>204.35</td>
<td>205.18</td>
</tr>
<tr>
<td>Total CHO (g)</td>
<td>26.22</td>
<td>28.22</td>
<td>28.24</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.85</td>
<td>3.00</td>
<td>3.03</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>8.13</td>
<td>8.83</td>
<td>8.90</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>1.22</td>
<td>3.22</td>
<td>3.24</td>
</tr>
<tr>
<td>Available CHO (g)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

3.7.5  Study protocol for glycaemic index determination

The procedure used was according to FAO/WHO (1998) international-recognised GI methodology. This clinical trial involved six experimental sessions in which participants were randomly assigned to consume three repetitions of glucose as standard food and three different test foods on separate occasions. Consumption of glucose drink for three times at different occasions was for the sake of improving the precision of measurement, hence could reduce the variation of mean GI values due to glucose tolerance (Brouns et al., 2005). Meanwhile, every formulation of test food was tested once by each participant. All test meals were consumed in the dietetic laboratory, USM in the morning after an overnight fast. Drinking water (250 ml) was provided along with each test food. Participants were instructed to finish the test foods within 15 minutes at comfortable pace and remain sedentary during the experiment session. During each session, fingertip capillary blood samples were collected at fasting level (0 min), then repeatedly at 15, 30, 45, 60, 90 and 120 min after consuming the test food.
3.7.6 Capillary blood sampling

Capillary blood sampling has several advantages over venous blood sampling because of easier to access, higher sensitivity and large variations in measured glucose concentration can be avoided (FAO/WHO, 1998; Brouns et al., 2005). This technique has become popular, especially with the widespread of point-of-care testing nowadays. The guidelines of capillary blood sampling by WHO (2010) was followed. Participants were directed to warm their hands prior to finger prick to increase their blood flow. Alcohol pad containing 70% v/v isoprophyl alcohol (BD Alcohol Swabs, USA) was gently applied to the entry site on finger. Skin was then deliberately punctured utilising a lancet device (Accu-Chek Safety T-Uno, Roche, Switzerland). The first drop of blood was wiped away as possible contamination with debris or tissue fluid may occur. Whole blood of approximately 4 µl was drawn from fingertip capillary into cavity of disposable plastic microcuvette (HemoCue Glucose 201 RT Microcuvette, Sweden) by capillary action. During finger blood extraction, hard finger squeezing was avoided to minimize dilution of plasma.

3.7.7 Glucose measurement

The filled microcuvette was inserted without delay in the cuvette holder of glucometer (HemoCue Glucose 201 RT, Sweden) (Figure 3.7). After 40 – 240 seconds, the glucose vale of the blood sample is displayed in mmol/L. HemoCue Glucose 201 RT System applies the glucose dehydrogenase method and photometric detection. The glucose reaction is an enzymatic method, in which tetrazolium salt (MTT) is used to obtain a quantification of glucose in visible light. The HemoCue analyser is factory calibrated and does not need any further calibration by the user. It has an internal quality control, “SELFTEST” is performed each time it is turned on.
External quality control test was performed using the liquid control (GlucoTrol-NG Level 1, Eurotrol, Netherlands) before each test session.

Figure 3.7  HemoCue Glucometer and the devices used for GI study

3.7.8  Calculation of glycaemic index

Calculation of incremental area under curve (iAUC) was performed using Microsoft Excel (Version 365 ProPlus, USA), in which the trapezoid rule was applied. If the blood glucose response falls below the baseline, only the area above the fasting level was included. The calculation of GI was done by applying the equation below (FAO/WHO, 1998).

\[
GI = \left( \frac{\text{Area under the glucose curve of test food}}{\text{Area under the glucose curve of reference food}} \right) \times 100
\]

The GI of each test food is expressed as the percentage of iAUC for the test food over the mean iAUC for glucose consumed by the same participant. The resulting values for all participants were averaged to calculate the GI for each test food.
3.8 Statistical analysis

The data was analysed by using Version 24 IBM SPSS Statistics (SPSS, USA). Data obtained were subjected to one-way analysis of variance (ANOVA) followed by post-hoc Turkey HSD for multiple comparisons of means. Three batched of chocolate cookies formulated with OBS and OBR were produced in this study for all measurements. Results were expressed as mean values of three replicates + standard deviation (SD) except for dietary fibre determination (n=2), sensory evaluation (n=60) and GI determination (n=13). Significance level was established at p<0.05.

Incremental area under curve (iAUC) was calculated geometrically and ignoring the area under the baseline fasting level (Wolever et al., 1991). The iAUC of three times repeated were calculated for mean and SEM of each subject. The iAUC of each test food was expressed as percentage of the mean iAUC of glucose taken by the same subject. All subjects’ values were averaged and taken as the GI of each test food. The results of individual’s iAUC and GI > 2SD from the mean were considered as an outlier. The outlier was excluded. Results without outlier were expressed in mean ± SEM. Glucose response curves were constructed using GraphPad Prism Software (Version 6, San Diego California USA). The data obtained were subjected to repeated measures ANOVA followed by Bonferroni’s multiple comparisons test to compare the mean differences among the individual means.
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Chemical characteristics of overripe banana sweetener

The chemical properties of OBS after processing are presented in Table 4.1. The moisture content of OBS was 16.54%, indicating that most of the moisture content in OBS had been removed through dehydration at 60°C. Benhura et al. (2016) recommended a drying temperature between 40°C to 80°C for syrup to obtain the highest concentration of reducing sugars.

The concentrated OBS obtained was found to have a pH value of 4.74. Similar results were found in banana syrup (4.9) (Tadakittisarn et al., 2007) and dates syrup (4.6 – 5.3) (Al-Mutairi and Al-Jasser, 2012). According to Kumar et al. (2009), the reduction in pH value upon dehydration was a concentration effect due to large amounts of water being removed from the tissues. Besides, Zulkifli et al. (2016) found that pH of bananas decrease as they ripens. The changes in acidity are most probably caused by the biochemical changes (malic acid, citric acid, oxalic acid and potassium) during ripening of banana (Etienne et al., 2013). From the acidity value of OBS, it can be suggested that OBS is not purely a sugar solution but also contains minerals and organic acids which is also supported by the high ash content (3.05%) of OBS obtained in this study. This result is slightly lower than plum syrup which has an ash content of 3.8% (Abu, 2002). This is mainly because mineral content varies in different types of fruit.

The high TSS of OBS (81.54°Brix) obtained was attributed to the high amount of sugar and low moisture content. The major carbohydrates found in OBS were sucrose (50.39%) followed by fructose (16.33%) and glucose (13.65%) which
is in contrast with a study by Tadakittisarn et al. (2007) where the content of sucrose (35.99%) was followed by glucose (16.94%) and fructose (14.75%) which was observed in banana syrup. The differences were probably due to the different ripening stages and types of banana used and also growing conditions of the plant. According to Yap et al. (2017), total sugar content increased drastically from unripe to overripe banana. Furthermore, fructose content was reported to be the lowest in unripe banana but appeared to be the dominant sugar in overripe banana. This is consistent with the higher sucrose and fructose content in OBS. The high sugar content (above 68% sugar level) can prevent the growth of microorganisms (Rawat, 2015). Based on the lower moisture content and shelf stable potentials, OBS could be used as a nutritive sweetener in bakery products.

<table>
<thead>
<tr>
<th>Table 4.1 Chemical properties of OBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong>*</td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>TSS (°Brix)</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
</tbody>
</table>

*The analysis was replicated thrice (n=3) and expressed in mean ± SD.
4.2 Nutritional composition of overripe banana residue

The nutritional compositions of OBR, by-product of OBS are presented in Table 4.2. Proximate analyses indicated OBR contains low level of moisture (4.19%) which meets the flour specifications which often limit the level of flour moisture to not more than 14% (Carter et al., 2015). OBR also contains low level of fat (0.17%) but high amount of ash (2.76%), protein (5.21%) and carbohydrate (87.74%). The data were close to the previous study conducted by Zakpaa et al. (2010) who investigated that ripe plantain flour possessed fat (0.34%), ash (2.68%) and carbohydrate (91.16%).

Table 4.2 Nutritional composition of OBR

<table>
<thead>
<tr>
<th>Nutritional composition*</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>4.19 ± 0.03</td>
</tr>
<tr>
<td>Fat</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>5.21 ± 0.09</td>
</tr>
<tr>
<td>Ash</td>
<td>2.76 ± 0.06</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>87.74 ± 0.04</td>
</tr>
<tr>
<td>TDF</td>
<td>33.61 ± 0.23</td>
</tr>
</tbody>
</table>

*The analysis was replicated thrice (n=3) and expressed in mean ± SD.

From the result obtained, OBR contains an appreciable amount of TDF (33.61%). According to Xiao et al. (2018), most of the starch including insoluble fibre in banana will turn into sugar as it ripens. Surprisingly, the TDF content in the OBR was still in a great quantity. A previous study by Ramli et al. (2009) found that the TDF in ripe banana flour was higher as compared to unripe banana flour because of the increment of water-soluble pectin. Pectin is a fibre that is also responsible for
the pulp softening when banana ripen (Duan et al., 2008). Thus, OBR could be used for enhancing the DF content of the chocolate cookies.

4.3 Nutritional composition of chocolate cookies

The nutritional values of chocolate cookies formulated with OBS and OBR at different levels are shown in Table 4.3. In general, incorporation of OBS and OBR in chocolate cookie resulted in significantly differences in all nutritional compositions except fat content. There were no significant differences (p>0.05) in fat content in OBS and OBR formulated chocolate cookies as compared to control chocolate cookie. This is because both OBS and OBR originally contained negligible amount of fat.

Incorporation of 8% OBR showed a significantly (p<0.05) increment in TDF content and ash content as compared to control chocolate cookie, raising from 3.18 to 7.77% and 0.72 to 1.47%, respectively. Similar trend was observed in bakery product formulated with different fruits (orange, passion fruit and watermelon) and vegetables residue (Ferreira et al., 2015) and pineapple residue powder (Singh, 2016). There is a solid health claim that increase of DF intake is associated with better diet quality, decrease incidence of chronic diseases and improvement of overall body function (Walia et al., 2009). A study was done by Brauchla et al. (2013) regarding the effect of high fibre snack (cereal, cracker and bread) on diet quality in children. The result revealed that the children accepted the high fibre snacks easily. The addition of high fibre snack significantly increased their daily fibre intake and physical well-being. Therefore, high fibre snack could be an alternative source of fibre. Indeed, food product which contains high DF is able to increase DF intake of individual who does not favour of taking fruits and vegetables.
in their daily diet. With that, OBR could be used as a potential source of DF in food products. In contrast, both protein and moisture content of 8% OBR-chocolate cookies decreased significantly (p<0.05) compared to control chocolate cookies, from 7.41 to 7.20% and 2.58 to 2.44%, respectively. The results were in line with Ng et al. (2017) who replaced wheat flour with oyster mushroom powder in cinnamon biscuits. Reduction in protein content is due to the lower protein content in OBR compared to wheat flour. Cookies are dry products with very low moisture content as thermal processing reduces the final moisture content in the product. Moreover, the high fibre content in OBR might absorb large amounts of water which results in further decline of moisture content during baking. A reduction in water activity with high levels of fibre content could lead to a microbial-free and shelf-stable cookie.

While OBR was being utilized as a fibre-enriched ingredient in the chocolate cookies, OBS was partially substituted for table sugar at 10, 15 and 20% with the purpose of reducing the sucrose content and to improve the nutritional quality in the chocolate cookies. There were no significant differences (p>0.05) observed in protein and TDF content when levels of OBS increased compared to the 0% OBS-chocolate cookies. There was a significant increase (p<0.05) in moisture content in the 10 and 15% OBS-chocolate cookies compared to the 0% OBS-chocolate cookies (2.44 to 2.63%) but no significant difference was observed when compared with the control chocolate cookie. Meanwhile, when the levels of OBS increased up to 20%, a significant increment (p<0.05) in moisture content was observed where is increased to 2.75%. A similar result was reported by Tai et al. (2019) who partially replaced sugar with concentrated Nypa fruticans Sap in carrot cake. The increment in moisture content is influenced by the different solubility rate of sugar during mixing. The crystalline form of sucrose has fewer interactions with water, causing it to evaporated
more easily which makes drier cookies during baking. Meanwhile, high moisture absorption of glucose and fructose in OBS has more interactions with water by hydrogen bonding which prevents it from evaporation during baking, thus producing chocolate cookie with higher moisture content. Due to the high amount of ash content in OBS (3.05%), increasing levels of OBS from 10 to 20% in chocolate cookies significantly increased (p<0.05) the ash content, from 1.12 to 1.47% when compared with 0% OBS-chocolate cookies. Majzoobi et al. (2016) reported a similar result in biscuits formulated by replacing sucrose with date syrup. In contrast, there was a significant reduction (p<0.05) in carbohydrate (67.95 to 67.19%) and sucrose content (24.95 to 17.67%) with increasing percentage of OBS incorporated into the chocolate cookies compared to 0% OBS chocolate cookies. This finding is in agreement with Mehrabi et al. (2017) who used grape syrup as a sucrose replacement in sponge cake. The trend is attributed to the proportion of sucrose, fructose and glucose in OBS. According to Amarra et al. (2016), Malaysia is one of the countries with the highest consumption of sugar, approximately 96 - 118 g/day which is significantly above the recommended intake (roughly 50 g/day). One of the major sources of contributing sugar comes from commercial biscuits in Malaysia (Norhayati et al., 2015). Hence, it is important to reduce sugar content in bakery products to improve carbohydrate metabolism in the body.
Table 4.3  Nutritional composition of chocolate cookies incorporated with OBS and OBR

<table>
<thead>
<tr>
<th>Nutritional Compositions</th>
<th>Concentration (%)</th>
<th>0% OBS</th>
<th>10% OBS</th>
<th>15% OBS</th>
<th>20% OBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Control (0% OBR and OBS)</td>
<td>*Control (0% OBR and OBS)</td>
<td>2.58 ± 0.07\textsuperscript{b}</td>
<td>2.44 ± 0.04\textsuperscript{c}</td>
<td>2.55 ± 0.01\textsuperscript{b}</td>
<td>2.63 ± 0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>Moisture</td>
<td>Ash</td>
<td>0.72 ± 0.02\textsuperscript{e}</td>
<td>1.12 ± 0.02\textsuperscript{d}</td>
<td>1.30 ± 0.01\textsuperscript{c}</td>
<td>1.39 ± 0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>Protein</td>
<td>Fat</td>
<td>7.41 ± 0.04\textsuperscript{a}</td>
<td>7.20 ± 0.02\textsuperscript{b}</td>
<td>7.21 ± 0.03\textsuperscript{b}</td>
<td>7.25 ± 0.04\textsuperscript{b}</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>TDF</td>
<td>21.15 ± 0.03\textsuperscript{a}</td>
<td>21.28 ± 0.07\textsuperscript{a}</td>
<td>21.28 ± 0.01\textsuperscript{a}</td>
<td>21.24 ± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>68.14 ± 0.11\textsuperscript{a}</td>
<td>67.95 ± 0.06\textsuperscript{a}</td>
<td>67.64 ± 0.03\textsuperscript{b}</td>
<td>67.47 ± 0.07\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.18 ± 0.53\textsuperscript{b}</td>
<td>7.77 ± 0.13\textsuperscript{a}</td>
<td>7.74 ± 0.18\textsuperscript{a}</td>
<td>7.74 ± 0.32\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.12 ± 0.10\textsuperscript{a}</td>
<td>24.95 ± 0.04\textsuperscript{a}</td>
<td>21.65 ± 0.06\textsuperscript{c}</td>
<td>19.42 ± 0.11\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Mean ± SD values bearing different subscript letter within the same row indicate significant differences (p<0.05).
4.4 Physical properties of chocolate cookies

The effect of OBS and OBR incorporation on the physical properties of chocolate cookies is shown in Table 4.4. In terms of physical characteristics, addition of 8% OBR demonstrated a slight decrease in weight (6.40 to 6.34 g), diameter (50.43 to 50.28 mm) and spread ratio (16.81 to 16.76), but there were no significant (p>0.05) differences as compared to control chocolate cookies. However, both control and 8% OBR-chocolate cookies showed no changes in terms of thickness (3 mm). The observation was in line with the biscuits incorporated with increasing level of mango peel powder (Ajila et al., 2008), citrus peel powder (Nassar et al., 2008) and apple powder (Kohajdová et al., 2014).

Incorporation of OBS increased the weight (6.36 – 6.42 g) and diameter (50.35 – 50.46 mm) of the chocolate cookies at 10, 15 and 20%, but there were no significant (p>0.05) differences as compared to 0% OBS-chocolate cookies. There was a significant (p<0.05) increment in spread ratio of chocolate cookies with increasing levels of OBS (17.01 to 17.40) as compared to chocolate cookies with 0% OBS but no significant difference was observed in 10 and 15% OBS-chocolate cookies. In contrast, addition of OBS slightly decreased the thickness of the chocolate cookie (2.96 to 2.90 mm), but there were no significant (p>0.05) differences as compared to chocolate cookies without OBS. In line with other study, Handa et al. (2012) observed an increment in diameter and spread ratio as well as a slight decreased in thickness of the biscuit enriched with fructooligosaccharide sweetener. The crystallized sucrose is not completely dissolve prior to baking, so the undissolved sugars will dissolve during baking which lead to cookie spread to occur (Handa et al., 2012). OBS containing cookies were found to be larger in spread ratio.
than cookies without OBS. This is because the high solubility of OBS as compared to sucrose is likely to maintain its dissolved nature longer during baking, which would also facilitate flow of the dough. Thus, increasing the OBS concentration tends to increase the spread ratio of the chocolate cookies. Furthermore, OBS has more affinity for water than sucrose; thereby limit the water for gluten development caused poor gas retention which results in decreased thickness of cookies.

Firmness and crispiness are textural properties which attract attention in the evaluation of baked goods due to their connection with human perception of freshness (Pereira et al., 2013). Addition of OBR in chocolate cookies did not produce significant (p>0.05) differences in crispiness which ranged from 0.35 to 0.38 mm. A previous study by Kuchtová et al. (2018) showed a similar trend of result when grape skin and seed flours were added into biscuit formulation. Meanwhile, the firmness of the chocolate cookies was shown to increase when OBR is added, rising from 1.05 to 1.23 kg. It was significantly (p<0.05) increased in 8% OBR-chocolate cookies compared with the control chocolate cookies. This result is in agreement with a study done by Varastegani et al. (2015) which claimed that the addition of papaya pulp flour as DF source increased the firmness of the cookie. The increase in firmness of chocolate cookie could be attributed to the decreasing moisture content (2.58% to 2.44%) when the OBR is added. The DF component has high capacity for water absorption, causing the matrix to be hardened by interacting with the gelatinized starch thus making it less available for dough inflation during baking. Consequently, more compact structure and higher degree of firmness cookies are produced (Leiva-Valenzuela et al., 2018).
Both firmness and crispiness of the chocolate cookies were found to decrease with increasing levels of OBS. The firmness of chocolate cookies decreased from 1.19 to 1.14 kg, significantly at 20% OBS as compared to 0% OBS-chocolate cookies but there were no significant (p>0.05) differences between 0 and 10% OBS-chocolate cookies as well as 15 and 20% OBS-chocolate cookies. It was also noted that the crispiness of chocolate cookies decreased from 0.34 to 0.31 but no significant difference (p>0.05) among all the formulations. The results indicate a lower snapping characteristic, making the texture of the cookie soft which is in agreement with a previous study by Ayyappan et al. (2016) who used oxyloooligosaccharides for replacing sugar (5 – 15%) in cookies, which showed a decline in both hardness and fracturability as compared to control cookies. Similar result for the effect of sucrose replacers also has been reported by Majzoobi et al. (2016) who utilized date syrup in biscuit. As OBS is substituted for sucrose, the peak force required to penetrate the cookie tends to decline which can be attributed to the higher spread ratio of the cookies. Furthermore, cookie firming involves the recrystallization of sucrose leading to a firmer and drier texture (Handa et al., 2012). This observation correlated well with the finding of several studies on high fructose corn syrup (Zargaraan et al., 2016), raffinose (Belcourt and Labuza, 2007) and trehalose (Kawai et al., 2014) have been used successfully to prevent sucrose recrystallization. In the present study, OBS being highly soluble doesn’t recrystallize; it binds more water and therefore gives softer cookies.
Table 4.4  Physical properties of chocolate cookies incorporated with OBS and OBR

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control (0% OBR and OBS)</th>
<th>0% OBS + 8% OBR</th>
<th>10% OBS + 8% OBR</th>
<th>15% OBS + 8% OBR</th>
<th>20% OBS + 8% OBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (W, g)</td>
<td>6.40 ± 0.04</td>
<td>6.36 ± 0.03</td>
<td>6.38 ± 0.02</td>
<td>6.42 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Diameter (D, mm)</td>
<td>50.43 ± 0.20</td>
<td>50.35 ± 0.05</td>
<td>50.41 ± 0.03</td>
<td>50.46 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Thickness (T, mm)</td>
<td>3.00 ± 0.00</td>
<td>2.96 ± 0.01</td>
<td>2.94 ± 0.01</td>
<td>2.90 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Spread ratio (D/T)</td>
<td>16.81 ± 0.07</td>
<td>17.01 ± 0.02</td>
<td>17.15 ± 0.01</td>
<td>17.40 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Firmness (kg)</td>
<td>1.05 ± 0.03</td>
<td>1.19 ± 0.05</td>
<td>1.17 ± 0.02</td>
<td>1.14 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Crispiness</td>
<td>0.38 ± 0.04</td>
<td>0.35 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0.33 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD values bearing different subscript letter within the same row indicate significant differences (p<0.05).
4.4.1 Microstructure characteristics

The microstructure of the chocolate cookies formulated with OBS and OBR was viewed by using SEM to observe the structural characteristics. The visual magnification of 500× and 1000× of four different formulations of chocolate cookies are shown in Figure 4.1 and Figure 4.2, respectively.

By observation, for control cookie (Figure 4.1A), gluten proteins formed a smooth and continuous network in which the starch granules were embedded in the protein matrix and almost completely gelatinized. The control cookie was featured by an open structure with larger size of starch granule, corresponding to the entrapment of gas bubbles that expand during baking which might increases the surface area exposed to enzyme activity. For cookies incorporated with 8% OBR (Figure 4.1B), a large number of partially gelatinized starch granules were clearly visible. The gluten matrix was almost aggregated and partially interrupted by starch granules and the presence of DF, suggesting a poorly developed gluten network due to the lack of water in the formulation. Furthermore, it could be noticed that the DF component from OBR embedding in the cookies disturbed the starch granule structure, causing them irregular shape and less smooth granule surface. A similar observation was done in research evaluating the effect of DF on the internal structure of biscuit (Ng, 2017) and bread (Indrani et al., 2015). The incorporation of DF-rich OBR generates a more compact structure, with starch granules trapped within the fibre matrix. Therefore, DF reduced the integrity retention of the starch granule by disturbing the structure of starch granule and reducing the size of starch granule, both of which led to the restriction of starch susceptibility to enzymatic hydrolysis (Wu et al., 2014). Furthermore, DF has higher water absorption capacity which preferentially absorbs water under conditions of water shortage making more
surfaces of the starch granules is protected against penetration by water and thereby limit starch gelatinization (Schuchardt et al., 2016). Similarly, a previous study by Sivam et al. (2013) demonstrated pectin-fortified breads formulated with 20% extra water resulted in a greater degree of starch gelatinization and a smooth microstructure compared to control pectin-fortified bread.

The size of starch granule decreased when OBS is incorporated into the cookies (Figure 4.2 C and D) as compared to control cookies and OBR-cookies. The increased in continuous phase in the cookie also can be observed as the levels of OBS increased. Similar result was revealed in research done on the effect of sugar replacer in muffin (Jingrong et al., 2018) and burger bun (Sahin et al., 2018). It has been shown that different sugars can delay starch gelatinization. This is based on the ability of sugar to mask starch granules and slow down water penetration by interacting with the water molecules, thus making the water less available for starch (Alamri et al., 2016). A study by Sun et al. (2014) reported that the increasing concentration of fructose syrup in starch caused the onset of gelatinization is shifted to higher temperature causing a greater level of thermal energy required before the starch granule can swell and begin to gelatinize. The effect of syrups on starch physicochemical properties is due to the penetration of small sugars into the amorphous regions of starch instigates complex formation between sugars and starch components which lead to the increase of gelatinization temperature. This will cause delay in starch swelling, amylose leaching, and limit the start of starch gelatinization (Mohamed and Babucurr, 2015). This is supported by Mohamed et al. (2019) who investigated the dynamic rheological properties of date syrups in corn starch. This could be attributed to the possible interaction of OBS with amylose causing weaker network and extended phase separation.
The addition of OBR and OBS into the chocolate cookies was shown to disturb the structure and reduce the size of starch granules as well as limit starch gelatinization. All of these resulted in reducing the surface area of starch granule exposed to enzyme activity. Hence, reduce starch digestion and lead to slower release of glucose from the starch, in turn improve glycaemic response. Based on the results, it could be concluded that the chocolate cookies formulated with OBR and OBS were more resistant to digestion compared to control chocolate cookie which can be correlated with the reduction of postprandial glycaemic response.
Figure 4.1  Scanning electron micrograph (500×) of chocolate cookies. (A) Control (B) 0% OBS + 8% OBR (C) 10% OBS + 8% OBR (D) 20% OBS + 8% OBR
Figure 4.2  Scanning electron micrograph (1000×) of chocolate cookies. (A) Control (B) 0% OBS + 8% OBR (C) 10% OBS + 8% OBR (D) 20% OBS + 8% OBR cookie
4.5 Sensory acceptability of chocolate cookies

The development of low sugar and fibre-enriched products with acceptable sensory characteristics is one of the challenges facing by food industry in order to fulfil consumers’ expectations. In this study, cookies formulated with OBS and OBR were evaluated by 60 panellists for the selected sensory attributes as compared to the control chocolate cookie. The sensory scores for the chocolate cookies formulated with OBS and OBR is shown in Figure 4.3. The result showed that the scores for all the attributes in all formulations ranged from 4.52 to 5.42 which can still be considered as acceptable values. A similar result was shown in a previous study by Ng et al. (2017) who replaced wheat flour with Pleurotus sajor-caju mushroom in cinnamon biscuit with the scores from 4.33 to 5.48.

Chocolate cookies formulated with 8% OBR has improved the sensory score for colour (5.22), appearance (4.87), aroma (4.70), flavour (4.97) and overall acceptability (5.07) but no significant (p>0.05) difference compared with control chocolate cookies. A previous study revealed that the addition of banana flour to 7% – 10% in cookie was found to have the optimum sensory scores (Dhar et al., 2013). A similar trend was also found by Kuchtová et al. (2018) which replaced different levels of grape skin and seed flours in cookie development. Notably, the scores for all the attributes increased with increasing levels of OBS added into the chocolate cookies. Among all the formulations, 15% OBS-chocolate cookies recorded the highest score for all attributes, but no significant difference (p>0.05) was observed in comparison with other formulations (control, 0 and 10% OBS). However, incorporation of OBS up to 20% showed a decline in scores for all attributes, with significant decreases (p<0.05) in flavour (4.52) and overall acceptance (4.62). The
addition of higher amounts of OBS led to lower scores in sensory attributes possibly due to the strong flavour of banana. This may be due to consumers’ previous expectations and perception of chocolate cookies as well as individual preferences on banana flavour intensity. A study by Piqueras-Fiszman and Spence (2015) suggested that taste and flavour is mostly determined by the expectations generated prior to tasting. Hence, enhancing banana flavour in the chocolate cookie may have negatively influenced consumer ratings. The data obtained suggests that consumers generally prefer chocolate cookies with 8% OBR + 15% OBS as it has a more balanced flavour.
Figure 4.3  Sensory acceptability of chocolate cookies formulated with OBS and OBR.
4.6 Glycaemic index of chocolate cookies

In order to provide a comprehensive evaluation of the effect of OBS and OBR incorporation in chocolate cookies, the blood glucose response after consuming the OBS and OBR formulated chocolate cookies was investigated and presented in Appendix F and illustrated in Figure 4.4. At 15 mins and 30 mins, the blood glucose levels for three test foods (ranging from 5.87 to 6.27 mmol/L at 15 mins) and (6.73 to 7.50 mmol/L at 30 mins) were significantly lower (p<0.05) compared to the reference food (6.99 and 8.63 mmol/L). The peak time of all test foods was at 45 mins. The highest blood glucose response at 45 mins was recorded for reference food (8.84 mmol/L), followed by control chocolate cookies (7.83 mmol/L), 0% OBS chocolate cookies (7.59 mmol/L) and lastly 15% OBS chocolate cookies (7.06 mmol/L). The blood glucose responses for all test foods at 45 mins were significantly lower (p<0.05) in comparison with the reference food. Moreover, the blood glucose response to 15% OBS chocolate cookies was significantly lower (p<0.05) compared to other test foods.

The iAUC for each subject and GI value of the test food were calculated and are shown in Table 4.5. Glucose has the highest iAUC (199.60 ± 12.75), followed by control chocolate cookies (125.32 ± 7.27), 0% OBS chocolate cookies (104.63 ± 8.43) and 15% OBS chocolate cookies (101.16 ± 6.41). All the test foods exhibited significant difference (p<0.05) in mean iAUC when compared with the reference but no significant differences (p>0.05) were detected among all the test foods.

GI is the ranking (on a scale of 0 to 100) given to different carbohydrate-rich foods, depending on how the food affects the blood glucose response (Jenkins et al., 2008). The ranking for food with moderate GI is between 56 and 69, while low GI food is 55 or less (Schuchardt et al., 2016). The control chocolate cookies (GI = 63 ± 5) and 0% OBS chocolate
cookies (GI = 56 ± 3) were classified as moderate-GI food, whereas 15% OBS chocolate cookies (GI = 50 ± 3) fell in the low-GI food category. There were significant differences (p<0.05) in GI among all the test foods.

Table 4.5  Mean of iAUC and GI values of glucose and three formulations of chocolate cookie formulated with OBS and OBR

<table>
<thead>
<tr>
<th>Test food</th>
<th>iAUC (mmol x min/l)</th>
<th>GI value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>199.60 ± 12.75\textsuperscript{a}</td>
<td>100\textsuperscript{a}</td>
</tr>
<tr>
<td>Control (0% OBS and OBR)</td>
<td>125.32 ± 7.27\textsuperscript{b}</td>
<td>63 ± 5\textsuperscript{b}</td>
</tr>
<tr>
<td>0% OBS + 8% OBR chocolate cookie</td>
<td>104.63 ± 8.43\textsuperscript{b}</td>
<td>56 ± 3\textsuperscript{c}</td>
</tr>
<tr>
<td>15% OBS + 8% OBR chocolate cookie</td>
<td>101.16 ± 6.41\textsuperscript{b}</td>
<td>50 ± 3\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Mean ± SEM values bearing different subscript letter within the same column indicate significant differences (p<0.05).

DF plays an important role in developing low-GI food. DF is made up of highly complex substances that do not degrade in the gut which can also be defined as nondigestible carbohydrate. Different types of DF are distinguished by their water solubility which has different effects in lowering glycaemic response (Weickert and Pfeiffer, 2018). The lower GI of 0% OBS chocolate cookies is due to the substitution of 8% OBR in the formulation which consists mainly of soluble fibre pectin. Soluble fibre is well known for its gel-forming properties. This leads to the increase in viscosity of food which slows down digestion and prolongs the feeling of fullness, hence a sharp increase of blood glucose can be prevented (Abutair et al., 2016). In addition, the soluble fibre in OBR can inhibit starch gelatinization in chocolate cookies by acting as a barrier to protect starch granules against water penetration which leads to the formation of smaller starch granules. As a result, enzymatic hydrolysis of starch is limited which leads to a slower conversion rate of starch to glucose (Weickert and Pfeiffer, 2018) therefore delaying entry of glucose into the bloodstream and lowering the postprandial glucose level. Similar trends were reported by Abutair et al. (2016) who showed improvement in glycaemic response among type 2 patients using soluble fibers from psyllium.
The nature of sugar components is another factor influencing glycaemic glucose response. Incorporation of 15% OBS was shown to further lower the GI of the chocolate cookies compared to 0% OBS chocolate cookies which indicated that substitution of table sugar with OBS can reduce the postprandial glucose level. This is possibly due to higher fructose content and lower sucrose content in OBS compared to table sugar which is 100% sucrose without any additional nutrients (Insel et al., 2018). According to Bantle (2009), fructose is one of the sweetest naturally occurring sugars with a lower GI (19) in comparison to sucrose (68) and glucose (100). There were a few studies demonstrated the effect of fructose in lowering blood glucose in diabetic rats (Kwon et al., 2008), healthy subjects (Heacock et al., 2002) and subjects with type 2 diabetes (Vaisman et al., 2006). One of the reasons is the different metabolism of fructose and glucose. Fructose is primarily delivered and metabolized in the liver whereas glucose is readily absorbed in the bloodstream (Sun and Empie, 2012). Secondly, hepatic glucose uptake is stimulated by fructose. Conversion of fructose to fructose-1-phosphate in the liver stimulates glucokinase in hepatocytes, which is responsible for the uptake and storage of glucose as glycogen. The activation of glucokinase causes the uptake of unmetabolized glucose in the bloodstream into the liver (Erejuwa et al., 2012). A study by Shiota et al. (2002) revealed that small amounts of fructose with glucose load increased hepatic glucose uptake and reduced postprandial hyperglycaemia.

Low GI foods have been closely associated with controlling body weight, improving glucose tolerance and reducing the risk of type 2 diabetes. Thus, development of low GI chocolate cookies by using OBS and OBR could be useful in providing health benefits to consumers.
Figure 4.4  Mean blood glucose response of glucose and three formulations of chocolate cookies formulated with OBS and OBR.
CHAPTER 5
CONCLUSION AND FUTURE RECOMMENDATIONS

5.1 Conclusion

From the aspect of OBS and OBR characteristics, OBS is not purely a sugar solution but also contains minerals and organic acids and could be used as a nutritive sweetener to replace sucrose. Moreover, OBR contains appreciable amounts of TDF, indicating a potential source of DF.

In the present study, there were some changes in nutritional compositions of chocolate cookies due to the incorporation of both OBS and OBR. Other than fat content was not affected, there was a significant increase in both TDF and ash content with the addition of OBR. Besides, carbohydrate and sucrose content were significantly decreased as more OBS was incorporated. The addition of OBS had also altered the physical properties of the chocolate cookies. The spread ratio was found to increase significantly with the increasing levels of OBS due to the high solubility of OBS than sucrose. Meanwhile, the firmness was decreased as level of OBS addition was higher causing softer cookie.

The microstructure of the chocolate cookie was found to be affected with the incorporation of both OBS and OBR. The presence of DF in OBR absorbs more water making more surfaces of the starch granules is protected against penetration by water and thereby limit starch gelatinization as well as reduced the integrity retention of the starch granule which led to the restriction of starch susceptibility to enzymatic hydrolysis. At the same time, addition of OBS delay starch gelatinization by increasing the gelatinization temperature which will cause delay in starch swelling, amylose leaching, and limit the start of starch gelatinization.
In terms of sensory acceptability, consumers assigned the lowest sensory score for the cookie enriched with 20% OBS + 8% OBR. Since healthy bakery product with acceptable sensory properties is important to fulfil consumers’ expectation, this present study indicated that addition of 15% OBS + 8% OBR is the most effective way to produce palatable chocolate cookie without changing negatively its desirable sensory characteristics.

The in vivo glycaemic determination showed that the novel food ingredients OBS and OBR can be incorporated in selected bakery products to help reduce the peak postprandial blood glucose response and to develop a food product with low GI value.

In summary, finding of the present study demonstrated that overripe banana can be an alternative novel DF-rich and low-GI food ingredients, which could widely utilised in developing various overripe banana-based functional foods. Thus, further highlight the possibility to produce bakery products utilizing agro-industrial co-products to prevent unnecessary food waste.
5.2 Recommendations for future research

As recommendation, more researches are also needed to extend the current level of knowledge related to other potential active phytonutrient compounds such as polyphenol contents that may responsible for the effects on glycaemic response of OBS and OBR. Storage stability study of formulated products could be assessed in the aspects of microbial growth, colour changes, crispiness and firmness. In addition, long-term intervention strategy using OBS and OBR formulated product as a functional food is recommended to be validated through large scale population trials, especially for diabetes population to confirm the health benefits of overripe banana enriched food products.
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APPENDICES

APPENDIX A  HUMAN ETHICS APPROVAL LETTER

18th July 2019

Miss Ng Yee Vern
School of Health Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

JEPeM Code: USM/JEPeM/19030180
Protocol Title: Glycemic Index of Chocolate Cookies Incorporated with Overripe Banana.

Dear Miss,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code USM/JEPeM/19030180, which should be used for all communication to the JEPeM-USM related to this study. This ethical clearance is valid from 18th July 2019 until 17th July 2020.

Study Site: School of Health Sciences, Universiti Sains Malaysia.

The following researchers also involved in this study:
1. Prof. Dr. Wan Rodzi Wan Ishak
2. Dr. Tengku Alina Tengku Ismail

The following documents have been approved for use in the study.
1. Research Proposal

In addition to the abovementioned documents, the following technical document was included in the review on which this approval was based:
1. Participant Information Sheet and Consent Form (English version)
2. Participant Information Sheet and Consent Form (Malay version)
3. Data Collection Sheet – Sensory Evaluation Form

Attached document is the list of members of JEPeM-USM present during the full board meeting reviewing your protocol.

While the study is in progress, we request you to submit to us the following documents:
1. Application for renewal of ethical approval 90 days before the expiration date of this approval through submission of JEPeM-USM FORM 3(B) 2019: Continuing Review Application Form.
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.
3. Revisions in the informed consent form using the JEPeM-USM FORM 3(A) 2019: Study Protocol Amendment Submission Form.
4. Reports of adverse events including from other study sites (national, international) using the JEPeM-USM FORM 3(G) 2019: Adverse Events Report.
5. Notice of early termination of the study and reasons for such using JEPeM-USM FORM 3(E) 2019.
6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using JEPeM-USM FORM 3(C) 2019: Final Report Form.

Please note that forms may be downloaded from the JEPeM-USM website: www.jepem.kk.usm.my


Thank you.

"ENSURING A SUSTAINABLE TOMORROW"

Sincerely,

PROF. DR. HANS AMIN VAN ROSTENBERGH C
Chairperson
Jawatankuasa Etika Penyelidikan (Manusia) JEPeM
Universiti Sains Malaysia
Cost calculation for one piece of 15% OBS + 8% OBR chocolate cookie

<table>
<thead>
<tr>
<th>Items</th>
<th>Ingredients</th>
<th>Quantity per pack; Cost per pack (RM)</th>
<th>Quantity used; Cost to make one dough (RM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wheat flour</td>
<td>1 kg; 1.99</td>
<td>69.9 g; 0.14</td>
</tr>
<tr>
<td>2</td>
<td>OBR</td>
<td>150 g; 3.99</td>
<td>6.1 g; 0.16</td>
</tr>
<tr>
<td>3</td>
<td>OBS</td>
<td>200 g; 3.99</td>
<td>6.2 g; 0.12</td>
</tr>
<tr>
<td>4</td>
<td>Castor sugar</td>
<td>500 g; 2.25</td>
<td>34.8 g; 0.16</td>
</tr>
<tr>
<td>5</td>
<td>Butter</td>
<td>250 g; 4.20</td>
<td>35 g; 0.58</td>
</tr>
<tr>
<td>6</td>
<td>Margarine</td>
<td>250 g; 3.20</td>
<td>5 g; 0.06</td>
</tr>
<tr>
<td>7</td>
<td>Egg</td>
<td>10; 4.80</td>
<td>1/3 (20 g); 0.16</td>
</tr>
<tr>
<td>8</td>
<td>Baking powder</td>
<td>50 g; 1.20</td>
<td>0.5 g; 0.01</td>
</tr>
<tr>
<td>9</td>
<td>Cocoa powder</td>
<td>200 g; 7.26</td>
<td>8 g; 0.29</td>
</tr>
<tr>
<td>10</td>
<td>Corn flour</td>
<td>400 g; 1.35</td>
<td>5 g; 0.02</td>
</tr>
</tbody>
</table>

Total Cost 30.24 1.70

One cookie dough can produce 40 pieces of cookie (around 6 g per piece)

One piece of cookie = RM1.70 ÷ 40 = RM0.04
BORANG PENILAIAN SENSORI

Umur: No. Panel:
Jantina: Lelaki/Perempuan Kod Sampel:
Tarikh:

Arahan:
You will be given samples for sensory test. Please circle on the most suitable scale for all the aspects below based on your preferences. Please rinse your mouth before examine every samples.

1. Appearance
   - 1: Dislike the most
   - 2: Dislike
   - 3: Moderate
   - 4: Like

2. Texture
   - 1: Dislike the most
   - 2: Dislike
   - 3: Moderate
   - 4: Like

3. Taste
   - 1: Dislike the most
   - 2: Dislike
   - 3: Moderate
   - 4: Like

4. Flavour
   - 1: Dislike the most
   - 2: Dislike
   - 3: Moderate
   - 4: Like

5. Overall acceptance
   - 1: Dislike the most
   - 2: Dislike
   - 3: Moderate
   - 4: Like

Comment: _______________________________
APPENDIX D CLINICAL EXAMINATION FORM

CLINICAL EXAMINATION REPORT

Glycemix Index of Chocolate Cookies Incorporated with Overripe Banana
Student: Ng Yee Varn
Main Supervisor: Prof. Dr. Wan Rosli Wan Ishak
Co-researcher: Dr Tengku Alina Tengku Ismail
Date: 

Subject’s name: _____________________________________________
Address: ___________________________________________________
Sex: _______ IC Number: ________________ Age: _______ Occupation: ____________

Health History
Have you ever suffered from (“Yes” or “No” must be answered to each question)
AIDS: _______ Hepatitis: _______ Renal Disease: _______ Food allergy: ____________
Coronary artery disease: _______ Irritable bowel disease: _______ Cancer: ____________
Celiac disease: _______ Gastrointestinal disease: ____________
Any medical surgical event within the last six months: ________________________________
A) Medications: ________________________________________________________________
B) Alcohol / Smoking: __________________________________________________________
I declare that the answers given are true and complete: _____________________________
(Signature) (Date)

Medical Officers Report
Height (cm): ___________ Weight (kg): ___________ BMI (kg/m²): ___________

General Condition
Circulatory system
Pulse: ___________________ Any clinical enlargement of heart: ______________________

Abdomen & Viscera
Any enlargement of spleen or liver: ________________________________

Comments:
I hereby certify that I have examined Mr / Mrs __________________________ and that I find
him/her free/suffering from organic disease and fit/unfit to take part in the above mentioned
research project.
Name: ___________________________ Signature: ___________________________ Date: __________
APPENDIX E PARTICIPANT INFORMATION SHEET AND CONSENT FORM (MALAY)

MAKLUMAT KAJIAN

Tajuk Kajian : Indeks Glisemik biskut coklat yang diformulasikan dengan pisang ranum (Overripe banana)

Penyelidik : Prof. Dr. Wan Rosli Wan Ishak, Dr. Tengku Alina Tengku Ismail (MMC No. 36833), Ng Yee Vern

PENGENALAN

Pisang ranum merupakan pisang yang memiliki bintik coklat dan tidak digemari oleh orang ramai namun pisang yang memiliki bintik-bintik gelap ini ternyata memiliki beberapa manfaat kesihatan yang tidak diketahui oleh pengguna. Ia tidak mempunyai kesan sampingan kepada manusia jika diambil. Selain daripada itu, ia mengandungi gula semulajadi dan dipercayai berupaya membantu menurun paras glukosa dalam darah.

Indeks glisemik jenis-jenis makanan mempengaruhi paras glukosa darah. Indeks tersebut akan mengukur peningkatan paras glukosa darah dua atau tiga jam selepas pengambilan makanan. Apabila indeks glisemik digunakan untuk penyediaan makanan sihat, ia akan membantu pengawalan paras glukosa darah. Hal ini amat penting untuk individu yang menghidap diabetes, atlet dan individu yang berlebihan berat badan supaya dapat mengetahui konsep baru dalam pemakanan sihat.

Anda dijemput untuk mengambil bahagian secara sukarela dalam kajian jangka pendek ini. Sebelum anda memberi persetujuan untuk mengambil bahagian dalam kajian ini, adalah penting bagi anda membaca dan memahami maklumat kajian sebelum anda bersetuju untuk menyetarai kajian penyelidikan ini. Sekiranya anda menyetarai kajian ini, anda akan menerima satu salinan borang ini untuk simpanan anda.

Penyertaan anda di dalam kajian ini dijangka mengambil masa selama setahun. Seramai 14 subjek semuanya akan terlibat dalam kajian ini.

TUJUAN KAJIAN

Tujuan utama kajian ini adalah untuk menentukan indeks glisemik bagi produk hasilan biskut coklat yang diformulasikan dengan 5 formulasi pisang ranum yang berbeza. Hasil kajian ini memberi manfaat dalam pelbagai aspek, iaitu:
1. Menentukan indeks glisemik produk berasaskan karbohidrat terpilih.
2. Memperbanyakkan data berkaitan indeks glisemik untuk makanan terproses berasaskan karbohidrat di Malaysia.
3. Membri maklumat yang penting dan berguna untuk pasakit diabetik, kardiak dan berlebihan berat badan bagi membantu mereka memilih makanan yang mempunyai indeks glisemik yang rendah.

KELAYAKAN PENYERTAAN

Anda hendaklah seorang dewasa yang sihat.

Kriteria yang dihendaki:
1. Lelaki atau perempuan
2. Berusia antara 18 hingga 60 tahun
3. Jika perempuan, tidak hamil dan tidak menyusukan anak
4. Indeks jisim tubuh antara 18.5 hingga 24.9 kg/m²
5. Tiada sejarah pernah menghidap AIDS atau hepatitis, inflamasi saluran usus, diabetes atau penyakit jantung (angina, aritimia, atau kegagalan jantung)
6. Tiada sejarah perubatan yang akut atau melalui pembedahan pada 6 bulan terakhir.

Kriteria yang tidak dihendaki:
1. Anda akan dikeluarkan daripada penyertaan jika menggunakan ubat-ubatan
2. Anda juga akan dikeluarkan daripada penyertaan jika anda tidak boleh mengikut prosedur eksperimen atau keadaan lain yang penyelidik mendapati anda tidak sesuai untuk menyertai kajian ini
3. Anda akan dikeluarkan daripada penyertaan, jika anda menunjukkan ciri-ciri alergi terhadap makanan eksperimen yang telah diambil

PROSEDUR-PROSEDUR KAJIAN

Jika anda bersetuju untuk menyertai kajian ini, anda dikehendaki menghadiri diri sebanyak 8 kali dan berpuasa untuk tempoh semalam selama 10-12 jam, supaya sampel darah ketika berpuasa boleh diambil. Selepas tempoh tersebut, anda akan diberikan minuman glukosa (Glucolin™), dan seterusnya sampel darah akan diambil untuk selang masa 15, 30, 45, 60, 90 dan 120 minit. Sampel darah akan diambil secara suntikan pada lengan anda bertujuan untuk menganalisis tahap kandungan glukosa darah. Ujian untuk minuman glukosa sebagai makanan standard akan dijalankan sebanyak 3 kali. Anda akan diminta untuk mengambil makanan ujian yang lain (biskut coklat mengandungi 0, 5%, 10%, 15% dan 20% pisang ranum) pada masa lawatan keempat, kelima, keenam, ketujuh dan kelapan. Setiap sesi akan dijalankan seminggu sekali.
Sepanjang ujian dijalankan, anda hanya dibenarkan untuk mengambil minuman kopi, susu atau air tanpa gula. Minuman yang dipilih akan ditetapkan untuk setiap lawatan.

RISIKO

Subjek mungkin mengalami sedikit ketidakselesaan pada jari selepas titisan sampel darah diambil pada lengan.

PENYERTAAN DALAM KAJIAN

Penyertaan dalam kajian ini adalah secara sukarela. Anda boleh menolak dari menyerlai kajian ini atau menarik diri dalam kajian ini pada bila-bila masa tanpa penalti atau kehilangan faedah-faedah yang sepatutnya anda perolehi.

MANFAAT YANG MUNGKIN

1. Kajian ini akan menghasilkan maklumat indeks glisemik produk hasilan biskut coklat yang diformulasikan dengan pisang ranum.
2. Data yang diperolehi amat berguna untuk individu yang berlebihan berat badan, diabetes, penyakit kardiak dalam memilih makanan yang mempunyai indeks glisemik yang rendah.

HONORARIUM DAN INSENTIF

Honorarium akan diberikan kepada semua responden.

PERSOALAN

Sekiranya anda mempunyai sebarang soalan mengenai prosedur kajian ini atau hak-hak anda, sila hubungi:

Prof. Dr. Wan Rosli bin Wan Ishak  
Pusat Pengajian Sains Kesihatan  
USM Kampus Kesihatan, 16150 Kubang Kerian, Kelantan  
No. Tel: 09-7677749

Atau

Dr. Tengku Alina Tengku Ismail  
Department of Community Science  
Pusat Pengajian Sains Perubatan  
No. Tel: 09-7676645  
No. MMC: 36833
Sekiranya anda mempunyai sebarang soalan berkaitan kelulusan Etika atau sebarang pertanyaan dan masalah berkaitan kajian ini, sila hubungi:

En. Mohd Bazlan Hafidz Mukrim
Setiausaha Jawatankuasa Etika Penyelidikan (Manusia) USM
Bahagian Penyelidikan dan Inovasi (P&I)
USM Kampus Kesihatan.
No. Tel: 09-767 2354 / 09-767 2362
Email : bazlan@usm.my or jepem@usm.my

KERAHSIAAN
Maklumat yang anda berikan akan dirahsiaakan oleh kakitangan kajian. Ia tidak akan dedahkan secara umum melainkan jika dikehendaki oleh undang-undang. Data yang diperolehi dari kajian ini tidak akan mengenalpasti anda secara perseorangan. Hasil kajian mungkin akan diterbitkan untuk tujuan perkongsian ilmu.
Semua borang kajian dan data yang anda berikan boleh disemak oleh pihak penyelidik, Lembaga Etika kajian ini dan pihak berkuasa regulatori bagi tujuan mengesahkan prosedur dan/atau data kajian klinikal.
Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemprosesan data seperti yang dihuraikan di atas.

TANDATANGAN
Untuk dimasukkan ke dalam kajian ini, anda atau wakil sah anda mesti menandatangani serta mencatatkan tarikh halaman tandatangan (Lihat Lampiran S)
Tajuk Kajian: Indeks Glisemik biskut coklat yang diformulasikan dengan pisang ranum

Penyelidik: Prof. Dr. Wan Rosli Wan Ishak, Dr. Tengku Alina Tengku Ismail (MMC No. 36833), Ng Yee Vern

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini. Dengan menandatangani mukasurat ini, saya mengesahkan yang berikut:

1. Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini termasuk apa-apa maklumat berkaitan risiko yang ada dalam kajian dan saya telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
2. Semua soalan-soalan saya telah dijawab dengan memuaskan.
3. Saya, secara sukarela, bersetuju kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
4. Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
5. Saya telah pun menerima satu salinan Borang Maklumat dan Keizinan Peserta untuk simpanan peribadi saya.

Nama Peserta

No. Kad Pengenalan Peserta

Tandatangan Peserta atau Wakil Sah (dd/MM/yyyy)  Tariikh (Masa jika perlu)

Nama & Tandatangan Individu yang Mengendalikan Perbincangan Keizinan (dd/MM/yyyy)  Tariikh

Nama Saksi dan Tandatangan (dd/MM/yyyy)  Tariikh

Nota: i) Semua peserta yang mengambil bahagian dalam projek penyelidikan ini tidak dilindungi insuran.
### APPENDIX F MEAN BLOOD GLUCOSE RESPONSE

Mean blood glucose response of glucose as reference food and 3 formulations of chocolate cookie (control, 0% OBS + 8% OBR and 15% OBS + 8% OBR) based on time. (n=13)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Test food</th>
<th>mmol/l (mean + SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Glucose</td>
<td>5.23 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>5.34 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0% OBS + 8% OBR cookie</td>
<td>5.33 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>5.05 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>Glucose</td>
<td>6.99 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>6.27 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0% OBS + 8% OBR cookie</td>
<td>6.17 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>5.87 ± 0.13</td>
</tr>
<tr>
<td>30</td>
<td>Glucose</td>
<td>8.63 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>7.50 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0% OBS + 8% OBR cookie</td>
<td>7.25 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>6.73 ± 0.16</td>
</tr>
<tr>
<td>45</td>
<td>Glucose</td>
<td>8.84 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>7.83 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>0% OBS + 8% OBR cookie</td>
<td>7.59 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>7.06 ± 0.14</td>
</tr>
<tr>
<td>60</td>
<td>Glucose</td>
<td>7.63 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>6.78 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0% OBS + 8% OBR cookie</td>
<td>6.54 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>6.16 ± 0.11</td>
</tr>
<tr>
<td>90</td>
<td>Glucose</td>
<td>5.65 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Control (0% OBS + 0% OBS) cookie</td>
<td>5.63 ± 0.15</td>
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<tr>
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<td>0% OBS + 8% OBR cookie</td>
<td>5.64 ± 0.08</td>
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<tr>
<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>5.30 ± 0.11</td>
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<tr>
<td>120</td>
<td>Glucose</td>
<td>4.71 ± 0.08</td>
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<td>0% OBS + 8% OBR cookie</td>
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<td></td>
<td>15% OBS + 8% OBR cookie</td>
<td>4.77 ± 0.11</td>
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**APPENDIX G  iAUC CALCULATOR**

![Excel iAUC Calculator](image)

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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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</tr>
</tbody>
</table>

**DIRECTIONS:**

1. **OPTIONAL:** Enter NAME, DATE, TEST MEAL INTO cells C1..C3
2. IF times are NOT correct: enter times into cells B6..B17
3. Enter blood glucose (or other) values into cells C6..C17 (do NOT skip times and do not leave zeros at the end! ERASE unwanted values by typing /RE)
4. Enter number of places to round values into cell E17
5. PRINT: type Alt-P (ensure printer is set up first)

1 = Number of figures after decimal:

Spreadsheet is protected (cannot be changed) except for highlighted values.

---

**DATE:** 2019

**TEST:** control

**TIME VALUES**

0 4.7
15 5.2
30 6.1
45 6.5
60 6.2
90 5.3
120 5.7

**AREA:** 131.3
Effect of overripe banana pulp incorporation on nutritional composition, physical properties, and sensory acceptability of chocolate cookies

Ng, Y. V., Tengku Alina, T. I. and Wan Rosli, W. I.

1Nutrition and Dietetics Program, School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150, Kubang Kerian, Kelantan.
2Department of Community Medicine, School of Medical Sciences, Universiti Sains Malaysia Health Campus, 16150, Kubang Kerian, Kelantan.

Abstract

The intake of dietary fibre (DF) has been proven to lower the risk of chronic diseases, leading to the increasing demand for fibre-enriched bakery product. Banana is one of the most consumed fruits that exhibits rich sources of DF and provides excellent nutritional health benefits. However, overripe banana is discarded due to its low quality and appearance. Thus, the present work was aimed to determine the properties of chocolate cookies formulated with overripe banana pulp powder (OBPP) as partial replacement (0, 6, 8, and 10%) for wheat flour. Nutritional composition, physical properties, and sensory acceptability of the cookies were analysed using AOAC methods, texture profile analyser, and 7-point hedonic scaling method, respectively. Results showed that increased incorporation of OBPP significantly increased the nutritional values of chocolate cookies. Chocolate cookies formulated with 10% of OBPP recorded the highest total dietary fibre (8.21%) and ash (1.23%) contents. In texture profile analysis, the firmness of the chocolate cookies was recorded to increase slightly with increasing level of OBPP, although this was not significant. Sensory scores for the control (0%) and 6% OBPP-incorporated cookies were not significantly different for all the sensory attributes. However, the incorporation of 8% OBPP produced the highest scores in terms of aroma, flavour, and overall acceptance. In summary, the addition of 8% OBPP could be an effective way to produce nutritious and the most palatable chocolate cookies.

Keywords

chocolate cookies, overripe banana pulp, nutritional values, physical evaluation, sensory acceptability

Introduction

Recently, the incidence of chronic diseases is increasing at an unprecedented rate and becoming a major public health issue all over the world including Malaysia. Chronic diseases, including cardiovascular diseases, cancers, chronic respiratory diseases, and diabetes mellitus (DM), are the major causes of mortality globally (Basoli and Take, 2009). The prevalence of DM has been increasing too high for many countries

DF is the edible part of the plant which is resistance to enzymatic digestion and absorption in human small intestine with complete or partial fermentation in large intestine (Dhingra et al., 2012). The recommended DF intake for adults are 20 - 35 g/day (ADA, 2000). Nevertheless, the intake for DF among populations is low, ranging from only 3 - 16 g/day (Timin and Slavin, 2008; Lee and Wan Muda, 2019). The importance of DF has led researchers to look for new sources.
APPENDIX I  BRITISH FOOD JOURNAL ACCEPTANCE LETTER

British Food Journal – Decision on Manuscript ID BFJ-12-2019-0934.R2
British Food Journal <onbehalfof@manuscriptcentral.com>
Wed 4/4/2020 5:55 PM
To: yasvarn@msem.com <yasvarn@msem.com>; draina@usm.my <draina@usm.my>; weiril@usm.my; <yumei@usm.my>
01-Apr-2020

Dear Ng, Yee Vern; Tengku Ismail, Tengku Alina; Wan Ikhlas, Wan Rosli

It is a pleasure to accept your manuscript BFJ-12-2019-0934.R2, entitled “EFFECT OF OVERRIPE BANANA IN DEVELOPING HIGH DIETARY FIBRE AND LOW GLYCAEMIC INDEX COOKIE” in its current form for publication in British Food Journal. Please note, no further changes can be made to your manuscript.

Please go to your Author Centre at https://mc.manuscriptcentral.com/bfj (Manuscripts with Decisions for the submitting author or Manuscripts I have co-authored for all listed co-authors) to complete the Copyright Transfer Agreement form (CTA). We cannot publish your paper without this.

All authors are requested to complete the form and to input their full contact details. If any of the contact information is incorrect you can update it by clicking on your name at the top right of the screen. Please note that this must be done prior to you submitting your CTA.

If you have an ORCID please check your account details to ensure that your ORCID is validated.

By publishing in this journal your work will benefit from Emerald EarlyCite. As soon as your CTA is completed your manuscript will pass to Emerald’s Content Management department and be processed for EarlyCite publication. EarlyCite is the author proofed, typeset version of record fully citable by DOI. The EarlyCite article sits outside of a journal issue and is paginated in isolation. The EarlyCite article will be collated into a journal issue according to the journal’s publication schedule.

FOR OPEN ACCESS AUTHORS: Please note if you have indicated that you would like to publish your article as Open Access via Emerald’s Gold Open Access route, you are required to complete a Creative Commons Attribution Licence - CCBY 4.0 (in place of the standard copyright assignment form referenced above). You will receive a follow up email within the next 30 days with a link to the CCBY licence and information regarding payment of the Article Processing Charge. If you have indicated that you might be eligible for a prepaid APC voucher, you will also be informed at this point if a voucher is available to you (for more information on APC vouchers please see http://www.emeraldgpb.com/openpartnerships

Thank you for your contribution. On behalf of the Editors of British Food Journal, we look forward to your continued contributions to the Journal.

Yours sincerely,
Dr. Sook Yng Tan
Guest Editor, British Food Journal
tantan@msu.edu.my
Certificate of Achievement

This is to certify that

Ng Yee Vern

as the

Best Oral Presenter

in the

2nd International Conference of Food Science and Nutrition

which was held in

Management & Science University,
Shah Alam, Malaysia

5th & 6th December 2019

Assoc. Prof. Dr. Seirah Abdul Karim
Dean
Faculty of Health & Life Sciences
This certificate is awarded to

Ng Yee Vern

for excellence demonstrated during the Oral Highlights Presentation at the ASEAN Emerging Researchers Conference held on the 9th & 10th of December 2019 at Sunway University, Malaysia.

Prof. Dr. Abhi Veerakumarasivam Dr. Orakanoke Phanraksa
Co-Chairs of the ASEAN Young Scientists Network
LIST OF PUBLICATIONS

Journals


Conferences


Ng, Y.V., Tengku Alina, T.I. and Wan Rosli, W.I. (2019). Production of Natural Sweetener from Overripe Banana and Utilization of its Residue in Developing Low Glycaemic Index Chocolate Cookie. ASEAN Emerging Researchers Conference, Sunway University, Selangor, 9th – 10th December 2019. [Poster]
Winning Awards

**Best Oral Presenter Award** in 2nd International Conference of Food Science and Nutrition, 2019. Title: Effect of Overripe Banana as Potential Source of Dietary Fibre and Natural Sweetener in Developing Low Glycaemic Index Chocolate Cookie. *(Appendix J)*

**Young Researcher Award** in ASEAN Emerging Researchers Conference, 2019. Title: Production of Natural Sweetener from Overripe Banana and Utilization of its Residue in Developing Low Glycaemic Index Chocolate Cookie. *(Appendix K)*