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NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPERTIES AND POTENTIAL APPLICATION OF DABAI OLEORESIN, DABAI OIL AND DABAI KERNEL FAT IN DARK CHOCOLATE MAKING

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BY

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LIST OF ABBREVIATIONS

°C	-	Degree Celsius
min	-	Minute
h	-	hour
%	-	Percentage
mg	-	Milligram
g	-	Gram
cm	-	centimetre
kg	-	kilogram
nm	-	nanometre
mL	-	millilitre
L	-	Litre
pH	-	Potential of hydrogen
UV-Vis	-	Ultraviolet-visible
SC-CO_2	-	Supercritical carbon dioxide
SFC	-	Supercritical Fluid Centre
Mpa	-	Megapascal
rpm	-	Revolutions per minute
M	-	Molar
N	-	Normality
Kcal	-	Kilocalories

β	-	Beta
FW	-	Fresh weight
ww	-	Wet weight
SD	-	Standard deviation
SFA	-	Saturated fatty acid
MUFA	-	Monounsaturated fatty acid
PUFA	-	Polyunsaturated fatty acid
CBE	-	Cocoa butter equivalent
DC	-	Dark chocolate
D2OL2O	-	Dark chocolate with 20% dabai oleoresin and 20% dabai oil
D4OL	-	Dark chocolate with 40% dabai oleoresin
DKDO	-	Dark chocolate with 30% cocoa butter, 5% dabai oil and 5% kernel fat
LDL	-	Low-density lipoprotein
HDL	-	High-density lipoprotein
ROS	-	Reactive oxygen species
FTC	-	Ferric thiocyanate
TBA	-	Thiobarbituric acid
BHT	-	Butylated hydroxyl-toluene
$FeCl_3$	-	Ferric chloride
SPSS	-	Statistical Package for the Social Sciences
ANOVA	-	Analysis of variance

Abstract

NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPERTIES AND POTENTIAL APPLICATION OF DABAI OLEORESIN, DABAI OIL AND DABAI KERNEL FAT IN DARK CHOCOLATE MAKING

Wan Khairunisa Wan Shapie

Cocoa butter (CB) is an important ingredient in chocolate making which contribute to good quality chocolate. It is also the most expensive vegetable fat. Vegetable fats that have similar physical and chemical properties with CB can replace it in chocolate production. This study aimed to determine the suitability and acceptance of dabai oleoresin, dabai oil and dabai kernel fat in dark chocolate making as cocoa butter equivalent (CBE). Nutritional composition and antioxidant properties were also determined in chocolates containing dabai oil, oleoresin and kernel fat. Sensory evaluation such as color, aroma, flavour, aftertaste, brittleness, smoothness and overall acceptability were measured in the dark chocolate samples. The result shows dark chocolate containing 40% dabai oleoresin (D4OL) contain high moisture and high ash contents. Meanwhile, dark chocolate containing 20% dabai oil and 20% dabai oleoresin (D2OL2O) contain high protein and total available carbohydrate contents. D4OL chocolate exhibited the highest antioxidant activity based on β-carotene bleaching assay compared to dark chocolate (DC) sample. In thiobarbituric assay, DC shows the highest antioxidant activity followed by D2OL2O, D4OL and DKDO. There were significant differences in antioxidant activity ($p<0.05$) between DC, D2OL2O, D4OL and DKDO as determined by β-carotene bleaching and TBA assay. Sensory evaluation revealed that chocolate made from 30% cocoa butter, 5% dabai kernel fat and 5% dabai oil (DKDO) have higher score in overall acceptability. Findings of this study showed dark chocolate containing dabai oleoresin exhibited better antioxidant properties compared to DKDO. Overall, this study has revealed the potential application of dabai fat (dabai oleoresin, dabai oil, and dabai kernel fat) in dark chocolate making. Further, study on rheological properties of dark chocolate could help in obtaining high-quality chocolate with good texture properties.

Abstrak

KOMPOSISI NUTRISI, SIFAT ANTIOKSIDA DAN POTENSI APLIKASI OLEORESIN DABAI, MINYAK DABAI DAN LEMAK BIJI DABAI DALAM PEMBUATAN COKLAT GELAP

Wan Khairunisa Wan Shapie

Lemak/mentega koko adalah bahan penting dalam pembuatan coklat yang akan memberi kualiti yang baik untuk coklat. Ianya juga merupakan lemak sayur yang mahal. Lemak sayur yang mempunyai sifat fizikal dan kimia yang hampir sama dengan lemak koko boleh mengantikannya dalam pembuatan coklat. Kajian ini bertujuan menentukan kesesuaian dan penerimaan oleoresin dabai, minyak dabai dan lemak biji dabai di dalam pembuatan coklat gelap untuk menganti lemak koko. Komposisi nutrisi dan sifat antioksida juga ditentukan di dalam coklat gelap yang mengandungi oleoresin dabai, minyak dabai dan lemak biji dabai. Penilaian deria terhadap coklat ditentukan dari aspek warna, aroma, rasa, rasa selepas, kerapuhan, kelancaran dan penerimaan keseluruhan. Keputusan kajian menunjukkan coklat yang mengandungi 40% oleoresin dabai (D4OL) mempunyai tinggi kandungan kelembapan dan abu (mineral). Manakala, coklat yang mengandungi 20% oleoresin dabai dan 20% minyak dabai mempunyai tinggi kandungan protein dan karbohidrat. Coklat D4OL menunjukkan aktiviti antioksida yang tinggi berdasarkan asai pelunturan beta karotene berbanding sampel coklat gelap (DC). Berdasarkan keputusan kaedah thiobarbituric asid, coklat gelap (DC) menunjukkan aktiviti antioksida yang tinggi diikuti oleh D2OL2O, D4OL dan DKDO. Terdapat perbezaan signifikasi dalam aktiviti antioksida ($p<0.05$) di antara coklat gelap (DC), D2OL2O, D4OL dan DKDO. Penilaian deria menunjukkan skor yang tinggi untuk coklat yang mengandungi 30% lemak koko, 5% lemak biji dabai dan 5% minyak dabai (DKDO) dari aspek penerimaan keseluruhan. Hasil kajian ini menunjukkan coklat yang mengandungi oleoresin dabai mempunyai aktiviti antioksida yang lebih baik berbanding DKDO. Keseluruhannya, kajian ini menunjukkan potensi aplikasi lemak dabai seperti oleoresin dabai, minyak dabai dan lemak biji dabai dalam pembuatan coklat gelap. Kajian sifat rheologi dapat meningkatkan mutu kualiti coklat dengan sifat tekstur yang lebih baik.

CHAPTER 1

INTRODUCTION

1.1 Background

Canarium odontophyllum Miq. which also known locally as dabai and Sibu olive in Sarawak that belong to *Burseraceae* family have many nutrients and high in antioxidant properties (Chew et al., 2012). Normally, the seed are removed despite of it have edible kernel meanwhile the skin and pulp of dabai are consumed by local people (Chua, Nicholas, & Yahya, 2015). Local people in Sarawak usually soften the dabai flesh by soaking in lukewarm water (50°C) for 15-20 min and sprinkled with salt, dip in soy sauce and sugar to develop the taste of dabai fruit (Ding, 2011).

There are many nutrients of dabai fruit such as total carbohydrate, protein, fat, and antioxidant properties (total phenolic content, total flavonoids content and

total anthocyanin contents) (Chew, Prasad, Amin, Azrina, & Lau, 2011). Dabai is indigenous fruit in Sarawak that nutritious and have high energy. Previous study, reported dabai proximate composition such as energy (339 kcal), protein (3.8%), fat (26.2), carbohydrate (22.1%), crude fibre (22.1), moisture (41.3%), ash (2.3%), potassium (810 mg), phosphorus (65mg), calcium (200 mg) and magnesium (106 mg) values (Voon and Kueh, 1999).

To date, there are no known applications of dabai oleoresin and dabai kernel fat that have been identified. Dabai pulp oleoresin contain 43.61 g/100 g saturated fatty acid (SFA) and 41.98 g/100 g of monounsaturated fatty acid (MUFA). Previous study, showed that dabai kernel fat is highly saturated fat (60% SFA) and become solid at room temperature (Azrina et al., 2010). According to deMan and deMan (1994), dabai kernel fat contain 60% saturated fatty acid (SFA), 36-37% monounsaturated fatty acid (MUFA) and 3% polyunsaturated fatty acid (PUFA) that similar with cocoa butter. Thus, dabai kernel fat have the potential to replace cocoa butter or use as cocoa butter equivalent (CBE) (Azrina et al., 2010).

Chocolate made up of around 70% of fine solid particles in continuous fat phase such as cocoa butter and milk fat (Fernandes, Müller, & Sandoval, 2013). Chocolates are formulated with important ingredients such as cocoa butter, cocoa solids, sugar and lecithin (emulsifier) but chocolate also can be vary in formulations such as addition of fruits, nuts and cereals (Torres-Moreno, Torrescasana, Salas-Salvado, & Blanch, 2015). There are many types of chocolate such as dark, white and milk chocolates that have different composition of cocoa solids, cocoa butter and milk fat (Torres-Moreno et al., 2015).

Glicerina, Balestra, Dalla Rosa, and Romani (2016), describe dark chocolate is mainly consisting of cocoa liquor, cocoa butter and sugar. According to Department of Standards Malaysia, (2011) dark chocolate, semi-sweet chocolate or bittersweet chocolate should contain more than 35% total solid, more than 18% cocoa butter and more than 14% fat-free cocoa solids. Dark chocolate comprises of not less than 140 g kg^{-1} chocolate liquor but cocoa butter, sugar and emulsifier like lecithin or vanillin as flavourings may also be included as much as 600 g kg^{-1} (Torres-Moreno, Tarrega, Costell, & Blanch, 2012). Emulsifier like soy lecithin that is used in chocolate making comprise of 62% to 70% phospholipids and is included in chocolate formulation to hold down the plastic viscosity of the cocoa mass (Zuliani Stroppa et al., 2011).

Cocoa butter is an important major component in chocolate formation as it influence the melting characteristic of a chocolate (Zuliani Stroppa et al., 2011). Besides, the key ingredients in chocolate formulation like cocoa powder, sugar and others are binded by cocoa butter resulted in certain sensory and physical properties (Sonwai, Kaphueakngam, & Flood, 2012). Concern on high price of cocoa butter caused many researchers search out alternative for cocoa butter to be used in production of chocolate and chocolate products (Jahurul et al., 2013). Other than cocoa butter, the blend of kernels fruit and plant based oil can be substituted for cocoa butter. Examples of plant fat used are such as mango kernel fat with palm oil, and Krabok (*Irvingia Malayana*) seed fat with coconut oil (Sonwai et al., 2012; Sonwai, Ornla-Ied & Aneknun, 2015).

1.2 Problem statements

Chocolate is solid at ambient temperature (27°C) but melt at body temperature (37°C) in the mouth (Fernandes et al., 2013). According to Beckett (2000), the different composition of cocoa solids, cocoa butter and milk fat in dark, milk and white chocolate produced different composition of carbohydrate, protein and fat in chocolate products. Previous study, reported many benefits of consuming chocolates especially the dark chocolate as it has been associated with lower blood pressure and lower risk of cardiovascular disease (CVD) (Allen, Carson, Kwik-Uribe, Evans, & Erdman, 2008).

Dabai fruit consist of skin, flesh and kernel. Each part of dabai fruits contains several of nutritional composition. There are several studies that reported the nutritional composition of *Canarium odontophyllum* Miq. such as carbohydrate, protein, fat, calcium, antioxidant and many others nutrients (Chew, Prasad, Amin, Azrina, & Lau, 2011; Chin Xuan & Azrina, 2016). Dabai fruits have many potentials to be commercialized as food such as dabai crackers, pitted dabai, dabai chocolate cookies, mayonnaise dabai, dabai paste, dabai oil and cleaning products such as dabai soap (LyeYee et al., 2012).

Azrina et al. (2010) reported that dabai kernel oil contain high saturated fatty acid (SFA), hence it has been categorized as saturated fat. Previous study, reported that dabai kernel have 36% monounsaturated fatty acid (MUFA), which is higher than corn oil (26.5%) and soybean oil (22%) (Baylina et al., 2007). Hence, dabai kernel oil has higher possibility to be solid at room temperature (20-25°C) (Azrina et al., 2010). Dabai kernel fat has been recommended as a substitution for cocoa butter

and known as cocoa butter equivalent (CBE) because dabai kernel oil have fatty acid composition that similar with cocoa butter (60% SFA, 36-37% MUFA and 3% PUFA) (deMan and deMan, 1994; Azrina et al., 2010).

Dabai pulp oleoresin and dabai oil are fat extracted from supercritical fluid extraction (SFE) using carbon dioxide system. In SFE, a high-pressure extraction vessel was charged with the dried and grinded dabai pulp for extraction. Dabai pulp oleoresin have 43.61 g/100 g saturated fatty acid (SFA) and 41.98 g/100 g of monounsaturated fatty acid (MUFA). Besides, dabai pulp oleoresin is also become solid at room temperature (unpublished data, 2018).

Cocoa butter is an important ingredient in making chocolate and chocolate products and it is also the most expensive vegetable fats (Sonwai et al., 2012). Normally, the seed of dabai are removed despite of it have edible kernel meanwhile the skin and pulp of dabai are consumed by people. Discarded dabai kernel should be given a special attention as it possibility be used as non-conventional source of vegetable fat for the chocolate to help in reducing the agricultural waste. There is no published data on the making of chocolate with dabai pulp oleoresin, dabai oil and dabai kernel fat as replacement for cocoa butter. Therefore, the present study explores the use of dabai oleoresin, dabai oil and dabai kernel fat to substitute cocoa butter in chocolate making. Nutritional composition of various dabai fat mixture in formulation of dark chocolate such as dabai oleoresin, dabai oil and dabai kernel fat were determined to understand it potential as new alternative of cocoa butter.

1.3 Significance of the study

Canarium odontophyllum (CO) or also known as dabai fruits are famous in Borneo Island especially Sarawak and it is known as indigenous fruit of Sarawak as dabai has not been fully explored for its potential. *Canarium odontophyllum* (CO) fruit also known as high fat fruit where dabai kernel contain 60.8% total saturated fatty acid (SFA) and 44.4% SFA in dabai pulp that almost similar with palm oil which is 47.9% (Azrina et al., 2010). Dabai fruit have variety of nutrients and dense in energy at 339kcal, carbohydrate (22.1%), fat (26.2%) and protein (3.8%) (Voon and Kueh, 1999). The present study focus on determination of nutritional composition, antioxidant properties and acceptance of dark chocolates (Dark, D2OL2O, D4OL and DKDO) prepared using mixture of dabai fats.

Today, there is no published data on nutritional composition of dark chocolates prepared using dabai pulp oleoresin, dabai kernel fat and dabai oil chocolate. The present study determined the nutritional composition and antioxidant properties of dark chocolate variants; dark 20% dabai oleoresin 20% dabai oil chocolate (D2OL2O), dark 40% dabai oleoresin chocolate (D4OL), dark dabai kernel dabai oil chocolate (DKDO) and compared with dark chocolate (DC). This study provides new knowledge about potential of D2OL2O, D4OL and DKDO as new chocolate product using dabai oleoresin, dabai oil and dabai kernel fat as cocoa butter equivalent (CBE). Furthermore, the data obtained from this study can be used as future reference for the research on dabai oleoresin, dabai oil and kernel fat as new fat for cocoa butter equivalent (CBE).

1.4 Objectives

1.4.1 General Objective

To determine and compare nutritional composition, antioxidant properties and acceptance of DC, D2OL2O, D4OL and DKDO as CBE in chocolate making.

1.4.2 Specific Objectives

I. To determine and compare nutritional composition (ash, moisture, protein, fat, total available carbohydrate, energy) of DC, D2OL2O, D4OL and DKDO.

II. To determine and compare antioxidant properties (β-carotene bleaching assay and Thiobarbituric acid) of DC, D2OL2O, D4OL and DKDO.

III. To determine suitability and acceptance of dabai oil, dabai oleoresin and dabai kernel fat in the dark chocolate formulation.

1.5 Alternative hypotheses

I. There is significant difference in nutritional composition (ash, moisture, protein, fat, total carbohydrate and energy) of DC, D2OL2O, D4OL and DKDO.

II. There is significant difference in antioxidant properties (β-carotene bleaching assay and Thiobarbituric acid) of DC, D2OL2O, D4OL and DKDO.

III. There is significant difference in suitability and acceptance of dabai oil, dabai oleoresin and dabai kernel fat as CBE in dark chocolate formulation.

CHAPTER 2

LITERATURE REVIEW

2.1 Dabai

Dabai is popular indigenous fruits in Sarawak which known as Sibu Olive that can be found alongside the river bank at Sibu, Limbang, Kapit and Sarikei, Sarawak. *Canarium odontophyllum* Miq. fruit are harvested by farmers and local people and sold the fruits at their market (Azrina et al., 2010). Dabai fruit contains many important nutrients and phytochemicals that have many health benefits. The skin of dabai fruit is light yellow while it still unripe and turn to black/dark purple after it ripe (Faridah, Nagendra, Amin, Lau, & Azrina, 2010; Siti Hawa, Jeffrey and Fadzelly, 2013). Figure 1 shows the whole and half split dabai fruit.

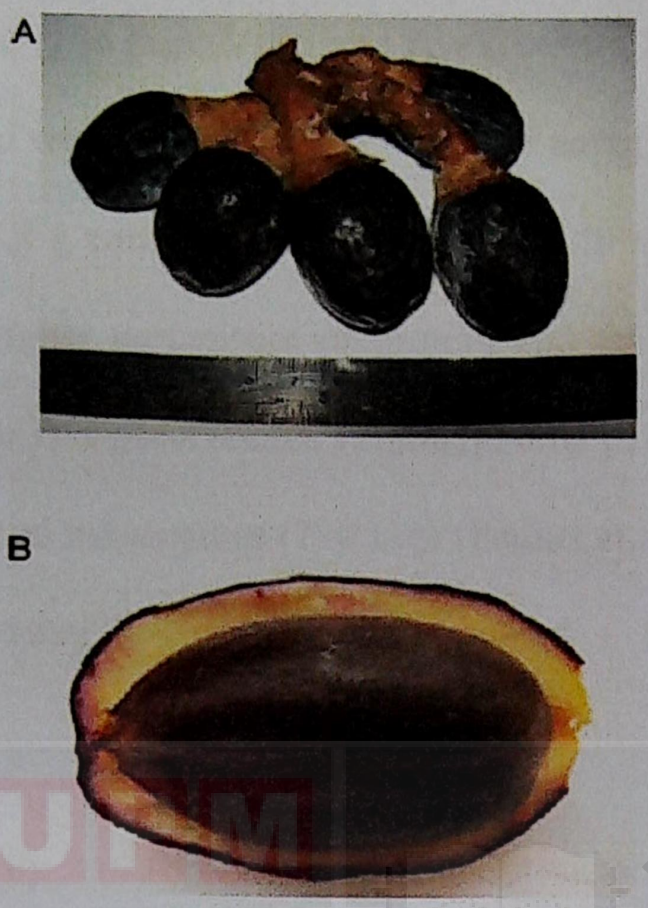

Figure 1:Whole fruit of dabai (A) and half split fruit (B)

(source: Chew et al., 2011)

Physical appearance of dabai fruits is oblong in shape which has been measured for length (3.75±0.1 cm), diameter (2.4± 0.19 cm, and weight (11.1±1.5 g) (Faridah, Nagendra, Amin, Lau & Azrina, 2010). The dabai fruit consist of 5-6% skin, 54-60% flesh and 35-40% of kernel which are inedible except for all parts (Azrina, Nurul Nadiah, & Amin, 2010). Dabai fruit have a single seed at the centre with dark purple skin and yellow pulp (Azrina et al., 2010; Nagendra, Lye, Khoo, Bao Yang, Azrina and Amin, 2011). Dabai fruit is soaked in warm water for a few minutes to soften the flesh and was eaten with condiment such as sugar, salt or soy sauce to enhance its flavor (Faridah et al., 2010).

Voon and Kueh (1999) reported the nutrients of dabai pulp which nutritious and high energy dense, which containing 339 kcal energy, 41.3% moisture, 3.8% protein, 26.2% fat, 22.1% carbohydrate, 4.3% crude fibre, 2.3% ash. Dabai fruit (pulp) also have good source of phosphorus (65 mg), potassium (810 mg), calcium

(200 mg) and magnesium (106 mg) (Voon and Kueh, 1999). Nutrient composition of dabai kernel are analysed which contains 499.4 kcal of energy, 27.1 g moisture, 10.8 g protein, 26.2 g fat, 47.2 g carbohydrate, 15.8 g crude fibre and 3.4 g ash content (Paulus et al., 2013). Higher percentage of protein and fat can be found in dabai kernel. Dabai kernel also is a good source of phosphorus (590 mg), potassium (680 mg), calcium (110 mg) and magnesium (290 mg) (Paulus et al., 2013). The moisture contents that found in dabai kernel and pulp are 79.4 ± 1.7% and 53.8 ± 0.6% (Azrina et al., 2010).

The previous study has found the antioxidant capacities of dabai fruit and ranked it from highest to lowest which are skin, flesh + (plus) skin, flesh and kernel respectively. Faridah et al., (2010), study has reported that high correlation was found between total phenolic content and total antioxidant capacity. Therefore, the skin of dabai fruit has been recommended as a main source of natural antioxidants (Faridah et al., 2010).

The previous study reported the total saturated fatty acid in dabai kernel is 60.8%. Meanwhile, palmitic, myristic, oleic and linoleic acids in dabai kernel oil are 46.4, 9.3, 35.1 and 2.8% (Azrina et al., 2010). Dabai kernel oil is saturated fat-rich oil due to its high saturated fatty acid (SFA) (60%) characteristic. Moreover, dabai kernel also high in monounsaturated fatty acid (MUFA) which is 36% compared to corn oil (26.5%) and soybean oil (22%) (Baylina et al., 2007). Composition of SFA and MUFA of dabai kernel oil make its have high tendency to be solid at room temperature (Azrina et al., 2010). Besides, dabai kernel fat has been suggested as cocoa butter equivalent (CBE) due to its similarity in fatty acid composition with cocoa butter which has 60% SFA, 36-37% MUFA and 3% PUFA (deMan and deMan, 1994; Azrina et al., 2010).

2.2 Chocolates

In worldwide, 4.6 million tonnes of cocoa beans was produced in 2013 (FAO, 2015b). According to UN Food and Agriculture Organisation, average cocoa bean production (%) by region were high in Africa (66%), followed by Asia (18%), Americas (15%) and Oceania (1%) in 1993 until 2013. Africa as world's leading cocoa producer was expected to maintain production for over decades (Sarris, 2003). Indonesia contribute 17% to the world cocoa production and this country is Asia's top producer of cocoa (FAO, 2015). Cocoa beans are rich source of polyphenols which contribute about 10% of the dry weight of cocoa bean and dark chocolate (Rusconi & Conti, 2010).

Chocolate is produced from the cocoa beans, the fruit of the cocoa tree (*Theobroma cacao L.*) from *Sterculiaceae* family (Rusconi & Conti, 2010). Cocoa solids, cocoa butter, sugar, lecithin (emulsifier) are important ingredients in making chocolate (Torres-Moreno et al., 2015). The steps to make chocolate are mixing, refining, couching, tempering, moulding and cooling (Lomonaco, Grimaldi, Aparecida, & Gonçalves, 2017). Torres-Moreno et al. (2015) reported that the energy content in chocolate is higher than 3000 kcal and have high content of fats and carbohydrates.

There are four step or process that important in chocolate production which are mixing, refining, conching and tempering (Beckett, 2000). First step in chocolate production is mixing of ingredients by time-temperature combinations in a continuous or batch mixer to get constant consistency. Secondly the chocolate is going through refining process to obtain desirable and smooth texture using both two

and five-roll refiners. Thirdly, conching process agitate the chocolate for a few hours at temperature greater than 50°C. Conching is important process to obtain desirable viscosity, flavour and texture. Fourthly, tempering can provide chocolate a desirable glossy appearance, snapping, contraction and resistance to fat bloom. According to Talbot (1999) and Beckett (2000), there are four key steps in tempering which are melting to completion (at 50°C), cooling to point of crystallisation (at 32°C), crystallisation (at 27°C) and conversion of any unstable crystals (at 29-31°C).

Dark chocolate are usually made up from milled cocoa nibs, sugar and cocoa butter (Beckett, 2008). The consumption of chocolate has protective effect on blood pressure, profile of lipid, the sensitivity to insulin, and the activation of platelets. Dark chocolate is more protective than white or milk chocolate (Fernández-murga, Tarín, García-perez, & Cano, 2011). Several studies have reported that consumption of chocolate can reduce the risk or delay the development of cancer, chronic diseases such as cardiovascular disease (CVD) and other age-related disease (Hammerstone et al., 2000; Afoakwa, 2008; Cooper et al., 2008).

Previously, a randomized crossover study of effect of dark chocolate and milk chocolates on appetite and energy intake were conducted. The study showed dark chocolates able to increase satiety, reduce the craving to eat sweet foods and decrease energy intake compared to milk chocolate (Sørensen & Astrup, 2011). Study on short administration of dark chocolate on 15 healthy subjects that was randomly assigned showed dark chocolate decrease blood pressure level and increase insulin sensitivity in healthy subjects compared to white chocolate (Grassi, Lippi, Necozione, Desideri, & Ferri, 2005). Besides, in randomised crossover study on 23 healthy subject feds with cocoa powder and dark chocolate showed cardiovascular risk can be reduced by decreasing of LDL oxidation susceptibility, increasing HDL-

cholesterol concentration, serum total antioxidant and it does not affect prostaglandin functions (Wan et al., 2001).

2.3 Cocoa Butter and Cocoa Butter Equivalent

Cocoa butter (CB) is obtained from extraction of fat in cocoa beans and it contains more than 98% of total fatty acids, linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0) (Akhter, 2016). CB have a few form of polymorphic such as α, γ, β′ and β crystals with melting points of 17, 23, 26 and 35-37°C respectively but only β crystal were used because it has unique characteristics which is high melting point (35-37 °C) in chocolate products that can give complete melting in the mouth (Jahurul et al., 2013). Besides, cocoa butter also gave brittleness, glossy, and hardness characteristics to chocolate (Oracz, Zyzelewicz, Budryn & Nebesny, 2015).

CB has a long shelf-life because it has favourable fatty acid distribution which low in C18:2 (<3%) and some of C18:3 including some presence of oxidants such as tocotrienols and tocopherols (Zyzelewicz, Krysiak, Budryn, Oracz, & Nebesny, 2014). Cocoa butter (CB) from cocoa beans is vegetable fat that is widely use in chocolate production and became an issue because of its high price compared to other vegetable fats (Bootello, Hartel, Garcés, Martínez-Force, & Salas, 2012; Jahurul et al., 2013; Shamsudin, Mohamed Ibrahim & Nooi Tan, 2006; Sonwai et al., 2012). CB that can only be produced in limited amount from cocoa bean is one of the

factors that caused the high price of cocoa butter (Zaidul, Nik Norulaini, Mohd Omar, & Smith, 2007).

Cocoa Butter Equivalent (CBE) are vegetable fats that have similar physical and chemical properties (melting temperature, melting rate, tempering necessity, crystallization temperature) with cocoa butter and widely used in chocolate production (Soekopitojo, Hariyadi, Muchtadi & Andarwulan 2010). The facts that cocoa butter has limited gloss retention, costly raw material, unsuitable melting point for others use and climate conditions become concern to the industries and confectionery to seek alternative or replacer for cocoa butter in chocolate production (Torbica, Jovanovic & Pajin, 2006). Three main group of cocoa butter alternative are cocoa butter equivalents (CBE), cocoa butter replacers (CBR) and cocoa butter substitutes (CBS). Blending and/or modifying of vegetable fats can be used to make cocoa butter alternatives. Physico-chemical characteristics of fat and oils can be modified by the hydrogenation, fractionation and interesterification process (Oracz, Zyzelewicz, Budryn & Nebesny, 2015).

Therefore, many researchers study on cocoa butter replacer (CBR) and cocoa butter equivalent (CBE) in chocolate production that use fruit other than cocoa beans such as mango kernel fat (MKF), palm oil mid-fraction (PMF) (Jahurul et al., 2014a; Jahurul et al., 2014b; Kaphueakngam & Sonwai, 2009; Sonwai et al., 2012;), sunflower hard stearins (SHSs) (Bootello et al., 2012), kokum butter as cocoa butter improver (Maheshwari & Yella Reddy, 2005; Reddy & Prabhakar, 1994), shea butter (Olajide, Ade-Omowaye, & Otunola, 2000), and sal fat (Reddy & Prabhakar, 1989).

2.4 Nutrients in chocolate

Nutrients in the food are classified as macronutrients and micronutrients. 'Macro' itself mean large molecules or compound whereas 'micro' mean small molecules or compound in foods. Macronutrients in food include are carbohydrate, protein and fat that are important in providing energy (calories) to the human body (NCCFN, 2010). Meanwhile, micronutrients in the food which are mineral and vitamin responsible in proper functioning of the body and boost healing process of wound (NCCFN, 2010). Indeed, these nutrients have given many benefits to human growth and maintain health. In general, chocolate is a type of food that contain high sugar and fat content but it can provide positive influence to human health and nutrition.

2.4.1 Moisture content

Determination of moisture content in foods is the most important analyses, however it is the most difficult because difficulty in obtaining accurate and precise results. The moisture content of the foods is various depending on types of food (Bradley, 2010). The moisture content in food greatly affect the taste, quality, consistency, shelf life and stability of foods (Mettler-Toledo, 2012). After completion remove of moisture from food, the remainder of dry food are known as total solids. The removing of water from food products is a crucial step to food manufacturer due to inexpensive filler which is water (Bradley, 2010).

Furthermore, the moisture can ensure the quality factor in preservation products and affects stability in dehydrated foods such as dehydrated vegetables and fruits and potatoes. Foods that store in packaging or shipping need to remove its moisture to ensure the quality of products. The water removal is easier depend on how the water acts within the food product. Example of three states of water in food products are adsorbed water, water of hydration and free water. The accuracy measurement of moisture content also depends on form of water within the food (Bradley, 2010).

Some precaution need to be taken while conducting moisture content determination such as exposure of food sample to the atmosphere, heat from sample grinding and high temperature. There are many methods in moisture determination such as oven drying method, microwave analyser, vacuum oven, forced draft oven and infrared drying. In oven drying methods, the sample is heated under some conditions and the moisture content is calculated from the loss weight of sample. The content of moisture can varied depend on the time and temperature of drying, type of oven used and conditions within the drying oven. The oven drying method are simple and simultaneous analysis of large amounts of samples can be conducted. However, the method might time consuming as it takes long time to remove to moisture from foods (Bradley, 2010).

Usually chocolate consist of 30% fat and only 1% of moisture (Beckett, 2008). However, previous study has reported the moisture content of chocolate may vary based on fermentation time of cocoa beans. The moisture content in chocolate varied in fermentation time for cocoa beans from 1 to 7 days are 5.15, 5.28, 5.35, 5.67, 5.80, 5.86 and 6.23% respectively (Joel, Pius, Deborah, & Chris, 2013).

2.4.2 Ash content

Ash refer to the inorganic residue left after process of combustion or oxidation of organic matter in a food sample (McClement, 2003). There are two types of ashing method use such as dry and wet ashing. Dry ashing are used for proximate analysis and a few mineral analyses meanwhile wet ashing (oxidation) are preparation for further analysis of minerals. Fresh sample need undergo dried process before proceeding to ashing method. On the other, dry foods such as cereals, bread, dried foods do not have go through drying process (Marshall, 2010). Ash content determination is importance in food analysis as it provides the total mineral content in foods. Ash determination is proximate analysis for nutritional aspect. The food products that high in mineral content cause analysis of minerals become crucial procedure. The ash content in various food products are different (Marshall, 2010).

Dry ashing are using muffle furnace in temperature range from 500-600 °C. In the presence of oxygen all the organic matters, water and volatiles substances are burned and vaporized into carbon dioxide (CO_2) and oxides of nitrogen (N_2). Phosphate, silicates, sulfates, oxides and chlorides are conversion from minerals (dry ashing). Meanwhile, wet ashing is process conducted by using oxidizing agents and acids or both to oxidize the organic substances. The fume hood is needed when acid substances is used during wet ashing. Moreover, preparation for certain elemental analysis which is wet ashing are preferable over dry ashing (Marshall, 2010).

Dry ashing and wet ashing (oxidation) can be conducted either using microwave systems or conventional method. The choice of method is depending on limitation of time, cost, sample size and purpose of ash determination. Conventional

method involves used of muffle furnace for incineration process with high temperatures and the residue can be used for certain mineral analyses. Besides, conventional dry ashing are not costly, simple to conduct and can many sample at the same time (McClement, 2003). The microwave system can cut the time taken to analyse the ash content and do not required many equipment. However, wet ashing consume time compared to dry ashing and only can analysis little number of samples (Marshall, 2010).

The ash content of chocolate can be varied depend on fermentation time of cocoa beans. A study has reported ash content of chocolate that varied in fermentation time for cocoa beans from 1 to 7 days which are 2.15, 1.97, 1.88, 2.46, 2.19, 2.14 and 2.23% respectively (Joel et al., 2013). There are variety types of mineral in chocolate such as calcium, iron, magnesium, copper, zinc, potassium etc. Calcium (mg/44 g) content in dark and milk chocolate is 14.1 mg and 84.0 mg respectively. Iron (mg/44 g) content in dark chocolate is 1.4 mg meanwhile 0.6 mg for milk chocolate. Besides, potassium (mg/44 mg) content in dark chocolate is 160.6 mg meanwhile 169.4 mg in milk chocolate. Phosphorus (mg/44 mg) content in dark and milk chocolate is 58.1 mg and 95.0 mg respectively. Meanwhile, zinc (mg/44 mg) content for dark chocolate and milk chocolate is 0.7 mg and 0.6 mg respectively. Lastly, magnesium (mg/44 g) content for dark and milk chocolate is 90.0 mg and 26.4 mg respectively (Chocolate USA, 2007; Gebhardt & Thomas, 2001; Minifie, 1989).

2.4.3 Total available Carbohydrate

Carbohydrate is nutrient in food that become source of energy to human which contribute 40-80% of total energy intake (NCCFN, 2017). Carbohydrate may be classified to their polymerisation and have three principal groups which are sugars (monosaccharides, disaccharides, polyols), oligosaccharides (malto-oligosaccharides, other oligosaccharides) and polysaccharides (starch, non-starch polysaccharides) (FAO, 2008).

The Malaysia recommended nutrients intake (RNI) recommends that 55% to 70% of total calorie intake per day for adults should be contributed from total carbohydrate (NCCFN, 2017). 'An optimum diet should consist of at least 55% of total energy coming from carbohydrate obtained from variety of food sources' (FAO, 2015a). Thus, human should consume mostly food from the source of the carbohydrate from the base of Malaysian food pyramid such as rice, noodle, bread, cereals, cereal products and tubers. Carbohydrate is function as source of energy that can obtained from foods. Carbohydrate provides 4 calories per gram (energy) (Illinois, 2008). NCCFN (2017) recommend Malaysian people to take 4 – 8 servings of carbohydrate food sources per day.

Carbohydrates also give off flavours, bulkiness, water-holding, viscosity, freeze-thaw stability, body, aromas, satiety and desirable textures in foods (BeMiller, 2010). Monosaccharides/ simple sugars are the carbohydrates that can be absorbed from the small intestine. Oligosaccharides and polysaccharides need to be hydrolysed into simple sugar for absorption and utilization process. Lactose, sucrose, starch, malto-oligosaccharides/ maltodextrins are the only carbohydrates that can be digest

by human. Starch and dietary fiber are the two types of complex carbohydrates. Example of foods that contain starch and dietary fiber are breads, cereals and vegetables. Starch is the only polysaccharides that can be digested and utilize as source of energy by human. Dietary fiber is plant cell-wall such as lignin, cellulose and hemicelluloses. Dietary fiber able to reduce serum cholesterol keep normal bowel function and reduce the postprandial hyperglycemic response (BeMiller, 2010).

Determination of total carbohydrate can be conducted through two method which are 'by difference' and the direct measurement of reducing sugar component (Cummings & Stephen, 2007). Total carbohydrate by differential method is the remainder value from subtraction of moisture, ash, protein, fat and alcohol content in food from the actual total weight of food (e.g. 100g) (Cummings & Stephen, 2007). There is limitation when using 'by differential' method because tannins, waxes, lignin, organic acids, and some Maillard products (non-carbohydrate constituents) are also included in the remainder at the end of the method (Cummings & Stephen, 2007).

Another method to determine total carbohydrate is Clegg-anthrone method (Peris-Tortajada, 2004). Clegg-anthrone method is hydrolysis process of sample/foods into carbohydrate component reducing sugars with the presence of strong acid and reacted with anthrone reagent. The reaction of this procedure will produce a blue/green colour and the absorbance is read at 630 nm.

Carbohydrate content per 100 g in dark chocolate is 55 g meanwhile 57 g in milk chocolate (Chocolate, 2007; Gebhardt & Thomas, 2001; Minifie, 1989). According to Afoakwa et al., (2007) carbohydrate content of dark, milk and white

chocolate are 63.5%, 56.9% and 58.3% respectively. Beckett (2008), has reported the carbohydrate content for milk and white chocolate which are 57g/100g and 58g/100g respectively. Another study has reported the carbohydrate content for dark (45.7g/100g) and milk chocolate (58.5g/100g) (Sørensen & Astrup, 2011).

2.4.4 Protein

Protein are found in foods from both plants and animals. Protein is amino acid molecules which bonded together by peptide bond that provide essential amino acids for growth and repair of human body tissue (NCCFN, 2017). Other functions of protein are immune function, building of lean muscle mass, making essential enzymes and hormones and source of energy when carbohydrate is not available (Illinois, 2008). Protein composed of several elements such as carbon, nitrogen, hydrogen, sulfur and oxygen. Recommended intake of protein is not same for different sex and age.

There are two type of protein that derived from food which are plant (beans, nuts, soy products) and animal protein (poultry, fish, milk, egg) based (NCCFN, 2017). 'The term "complete protein" refers to food that contain all the essential amino acids needed by body, whereas, incomplete protein refers to foods lacking in one or more essential amino acids' (NCCFN, 2017). Mostly, complete protein found from animal protein based compare to plant protein, but plant protein can be converted to complete protein with combining of complementary protein (NCCFN, 2017).

Protein analysis are conducted to determine the total protein content and content of a certain protein in a mixture. Kjeldahl method is commonly used for protein content determination in foods. There are three steps in Kjeldahl method which are digestion, neutralization and titration (Chang, 2010). Firstly, the sample are digested with sulphuric acid with the presence of catalysts (Kjeltabs). The previous digest is neutralized with alkali and distilled into a boric acid solution. Nitrogen in the sample is converted into nitrogen after the borate anions are titrated with standardized acid. The result of the Kjeldahl method represents the crude protein content of the foods.

The advantages application of Kjeldahl method are applicable to all types of foods, accurate, and inexpensive. Meanwhile, it disadvantages are poor precision compared to biuret method, time consuming, measures total organic nitrogen and corrosive reagent. Kjeldahl method only required little preparation and used smaller size of sample particles. Besides, Kjeldahl method is the preferred method for food containing high fat (Chang, 2010).

Protein are provide 4 calories per gram of energy from foods to human body (Illinois, 2008). Dark chocolate per 100 g provide 5 g of protein meanwhile milk chocolate has 7 g of protein (Chocolate USA, 2007; Gebhardt and Thomas, 2001; Minifie, 1989). According to Afoakwa et al., (2007), the protein content in dark, milk and white chocolate are 28.0%, 30.7% and 30.9%. Meanwhile, Beckett (2008) has reported protein content per 100g in milk and white chocolate which are 7g and 9g respectively. Another study has reported the protein content for dark (8.9g/100g) and milk chocolate (6.0g/100g) (Sørensen & Astrup, 2011).

2.4.5 Fat

Generally, lipids are soluble in chloroform or other organic solvents, ether but only soluble in small quantities in water. The term fats, oils and lipids are often used interchangeably. Fats are referred to those lipids that are solid at room temperature meanwhile oils are refer to lipid that in liquid form at room temperature. On the other hand, lipid is total collection of food molecules such as monoacylglycerols, diacylglycerol and triacylglycerol. Food products may contain various types of lipids but triacylglycerols and phospholipids are the most importance lipid (Min & Ellefson, 2010).

'Fat is the major determinant of the energy density of diets, providing a high 9.0 kcal/g compared with much lower 4.0 kcal/g for carbohydrate and protein' (NCCFN, 2017). All types of lipid from animal and plant based that eaten as food is known as dietary fat. Dietary fat is crucial for our body because it help in production of body hormone with contribution of essential fatty acids which are linoleic acid (LA) and α-linolenic acid (ALA) (NCCFN, 2017).

Other functions of fat are enhance the taste, texture and stability of food, source of energy, act as cushion to body organ, help in absorption of fat soluble vitamin (A, D, E, K & carotenoids), growth and development function and maintain the cell membranes structure ((Illinois, 2008). Fat deficiency is rare, but it can occur when malabsorption problem arises (NCCFN, 2017). There are several types of fat which are saturated fatty acids, trans fatty acids and unsaturated fatty acids (NCCFN, 2017). 'Both separated fats (e.g. Cooking oils, margarines, butter, shortenings, etc.)

and unseparated fat (fats in the tissues of food) contribute to the total fat in our diet' (NCCFN, 2017).

Soxhlet extraction or its modified method is a common crude fat determination. This method required a dried sample for the extraction. The fat content in food usually determined by semi-continuous solvent extraction which is soxhlet method. In semi-continuous solvent extraction, the solvent goes up in the extraction chamber for 5- 10 minutes and completely soak the sample and back to the boiling flask through siphons. The fat content is measured by weight loss of the sample or by weight of the fat removed. Soxhlet method allow soaking process occurred to the sample and not cause channeling as it can result in incomplete extraction (Min & Ellefson, 2010).

The fat content in 100 g of dark chocolate is 32 g meanwhile milk chocolate is 33 g (Chocolate USA, 2007; Gebhardt and Thomas, 2001; Minifie, 1989). The fat content in dark and milk chocolate mainly contribute by cocoa butter or milk fat. Besides, chocolate fat contributes many fatty acids and triglycerides that consist of saturated stearic, palmitic and monounsaturated oleic which do not give or increase the blood cholesterol levels (Afoakwa et al., 2007). However, the fats from cocoa butter which mainly consist of stearic triglycerides (C18:0) was excreted in stool because its less well absorbed characteristic. Cocoa butter has low bioavailability and only give minimal effect on serum cholesterol (Wollgast & Anklam, 2000; Cooper et al., 2008). The fat content for the dark, milk and white chocolate are 5.0%, 7.7% and 8.0% respectively (Afoakwa et al., 2007). Beckett (2008) has reported fat content per 100g in milk and white chocolate which are 33g for both chocolates. Another study has reported the fat content for dark (42.3g/100g) and milk chocolate (32.0g/100g) (Sørensen & Astrup, 2011).

2.5 Antioxidant Properties

Oxidative stress triggered by free radicals that interrupts the normal redox state in human body, thus causing countless human chronic diseases such as obesity, cardiovascular diseases, and certain types of cancers (Pino-Garcia et al., 2016). Antioxidant sources from plant have capacity to control the disruption and prevent the oxidative stress related diseases (Chen et al., 2016). Besides, 'flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease' (Kähkönen et al., 1999).

The previous study, reported that defatted dabai pulp was associated with huge reduction of formation of atherosclerotic plaque by significant decreasing in TC, LDL-C and lipid peroxidation levels (Faridah, Azrina, Amin, Amom, & Yuon, 2012). The delaying of atherosclerosis and reduction in coronary artery disease risk occurred because of high dietary fiber and high properties of antioxidant. Thus, the study suggested dabai defatted pulp as anti-oxidative agent and hypocholestrolemic (Faridah et al., 2012).

Defatted dabai which is by-product of dabai oil extraction have potential as source of antioxidants (Khoo, Azrina, Amin, & Faridah, 2013). Defatted dabai pulp and peel have been explored for containing major phenolic compounds which are flavonoids and anthocyanins (Khoo et al., 2012; Khoo, Azrina, Amin, & Sadek Hassan, 2015). Previous study showed that fullfat and defatted dabai pulp contain high antioxidant properties and have better effect than statin (Azrina, Amin, Zulkhairi & Faridah, 2013).

The previous study showed that, all-trans-β-carotene was greatly present in peel (69.5 ± 1.0 mg/kg), pulp (31.1 ± 0.76 mg/kg) and kernel (15.1 ± 3.0 mg/kg) respectively (Prasad et al., 2011). Besides, dabai pulp exhibit significantly higher β-carotene bleaching activity compared to peel and kernel, also high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, meanwhile dabai peel was showed significantly higher scavenging activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals. On the other hand, other study that test on β-carotene beaching assay found out that the antioxidant activity was high in dabai skin followed by skin and flesh, flesh and dabai kernel respectively (Faridah, Prasad, Amin, Yuon & Azrina, 2010).

There are various antioxidant properties found in cocoa such as polyphenol; flavonoids such as catechin, epicatechin, and procyanidins that might contribute to health or medicinal. Dark chocolate contains high level of catechin (flavan-3-ols) (Kerimi & Williamson, 2015). White chocolate cannot give contribution to polyphenol-induced advance in health aspects compared to dark and milk chocolate because white chocolate does not contain cocoa solid in it preparation (Afoakwa, 2010). Most of the dark chocolate contain higher amounts of antioxidant cocoa flavanols compared to milk chocolate. The content of milk proteins (casein) in milk chocolate might impair the absorption of procyanidins (Wollgast & Anklam, 2000). According to Afoakwa (2010), a 40-g serving of dark chocolate contribute 951 mg of cocoa flavonoids meanwhile milk chocolate contains 394 mg of cocoa flavonoids.

CHAPTER 3

METHODOLOGY

3.1 Reagents and chemicals

Petroleum ether, methanol, butylated hydroxy-toluene (BHT), boric acid, hydrochloric acid and ferric chloride ($FeCl_3.6H_2O$) were purchased from Merck (Darmstadt, Germany); ethanol, and sodium hydroxide were purchased from Friedemann Schmidt Pty. Ltd. (Brisbane, Australia); Kjeltabs Cu 3.5 (each tablet contains 7 g potassium sulphate and 0.8 g copper (II) sulphate), sulphuric acid, 93-98% from Foss (Hillerod, Denmark). β-Carotene, linoleic acid, Tween 20, and were purchased from Sigma Chemical Co. (St. Louis, MO, USA); chloroform was from Fisher Scientific (London, UK). Glucose (standard curve), perchloric acid (52%), sulphuric acid and anthrone powder were used in Clegg-Anthrone method.

Meanwhile, 1-butanol and TBA were used in Thiobarbituric acid value determination.

3.2 Apparatus and Equipment

Beaker, burette, conical flask, round bottom flask and volumetric flask were purchased from Schott Duran (Mainz, Germany); Kjeltec aluminium block digester, Kjeltec distillation unit, porcelain crucible from FOSS (Hoganas, Sweden); weighing balance from A&D Co. (Tokyo, Japan), water bath and air oven from Memmert GmbH & Co. KG (Schwabach, Germany); pH meter from Mettler Toledo Co. (Shanghai, China); Soxhlet extraction instrument, extraction thimble, extraction chamber and muffle furnace from Thermo Fisher Scientific, Inc. (Massachusetts, USA); rotary evaporator from BÜCHI Labortechnik (Flawil, Switzerland); Virtis freeze dryer from SP Scientific (Suffolk, UK); and digestion flask from FOSS (Algerie, Germany); UV-Vis spectrophotometer (SECOMAM CE. France).

3.3 Experimental Design

The flow chart of this study is shown in Figure 1. Four types of chocolate were prepared from cocoa powder, cocoa butter, icing sugar, lecithin, dabai pulp oleoresin (cocoa butter equivalent), dabai oil and dabai kernel fat as samples. Dark chocolate formulation was adapted from (Glicerina, Balestra, Dalla, & Romani, 2013) with slightly modification. Four chocolate samples were cut into small pieces and homogenized by using blender. For each chocolate samples that had been homogenized, they were kept in chiller (0°C to 4°C) prior to nutritional analysis. Freshly used portion were analyzed for ash content, moisture content, total available carbohydrate, protein, fat and antioxidant properties by several analysis methods in this chapter.

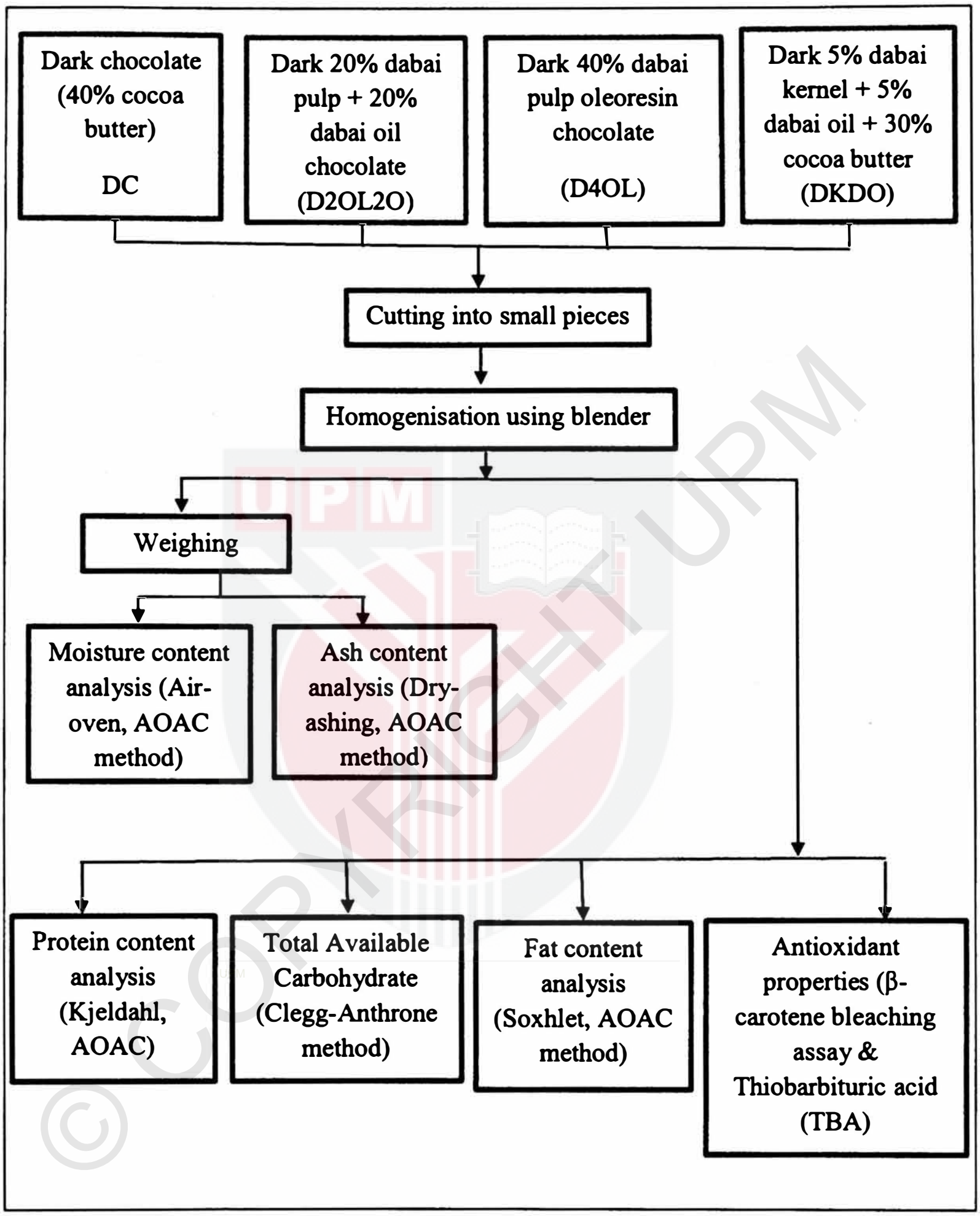

Figure 2: Flow Chart in determination of Nutritional Composition of Chocolates (DC, D2OL2O, D4OL and DKDO)

3.4 Sampling and sample preparation

3.4.1 Procurement of raw materials

Cocoa powder (CP), lecithin (Radiant Whole Food SDN BHD) and icing sugar was purchased at Jusco, AEON. Cocoa butter was purchased from online shopping (www.lazada.com.my).

3.4.2 Preparation of kernel fat

Fresh dabai fruits were obtained from Sarawak, Malaysia. The packed fruits in cool-box container were delivered to Universiti Putra Malaysia by flight. The separation of fruit started with separating of pulp and dabai kernel parts. Present study was used kernel part of dabai. Fruit nut were cracked and mashed manually by using pestle and mortal to obtain the greenish cotyledon (kernel fat). Then, this kernel fat was used as part of ingredients in dark chocolate preparation. The kernel fat was stored in sealed plastic bag before chocolate preparation.

3.4.3 Dabai Pulp Oleoresin

Freeze dried dabai pulp (62.46 kg) was grounded together to produce no less than 0.2 mm powder. The dabai pulp powder was subjected for SC-CO_2 extraction at Supercritical Fluid Centre (SFC) Universiti Putra Malaysia. The supercritical fluid extraction using carbon dioxide system is illustrated in Figure 3. A high-pressure extraction vessel is charged with the dried and grinded dabai pulp for extraction. Liquid carbon dioxide from a cylinder tank is pumped by a high-pressure pump through a heater and into the extraction vessel. An automated control valve operates with the heater to adjust the pressure at 40 MPa and temperature at 40 °C of the system to change the phase of carbon dioxide from liquid to supercritical state.

The supercritical carbon dioxide at pressure of 40 MPa and temperature of 40 °C flows through the high-pressure vessel to a first separation vessel 1 and 2 by way of the automated control valve and a heater. The automated control valve and the heater adjust the pressure and temperature of the carbon dioxide as required to precipitate the oil and oleoresin. The first separator is set at the highest pressure in the series and oleoresin precipitated in the separator 1. The 2nd separator ramp downwards in pressure and the oil is collected.

Once the pressure is reduced, the carbon dioxide will eventually become gas and exiting the separator vessel 2 before returns to the high-pressure pump and the process is recycled again by following the extraction time required. The process flow is thus circular with the supercritical fluid density being increased for extraction and decreased for precipitation. When the dabai pulp is fully extracted the circulation of carbon dioxide is halted so that the extraction vessel is emptied and recharged. The

oleoresin and oil are removed from the separation vessel 1 and 2 via a manual valve after the extraction is completed.

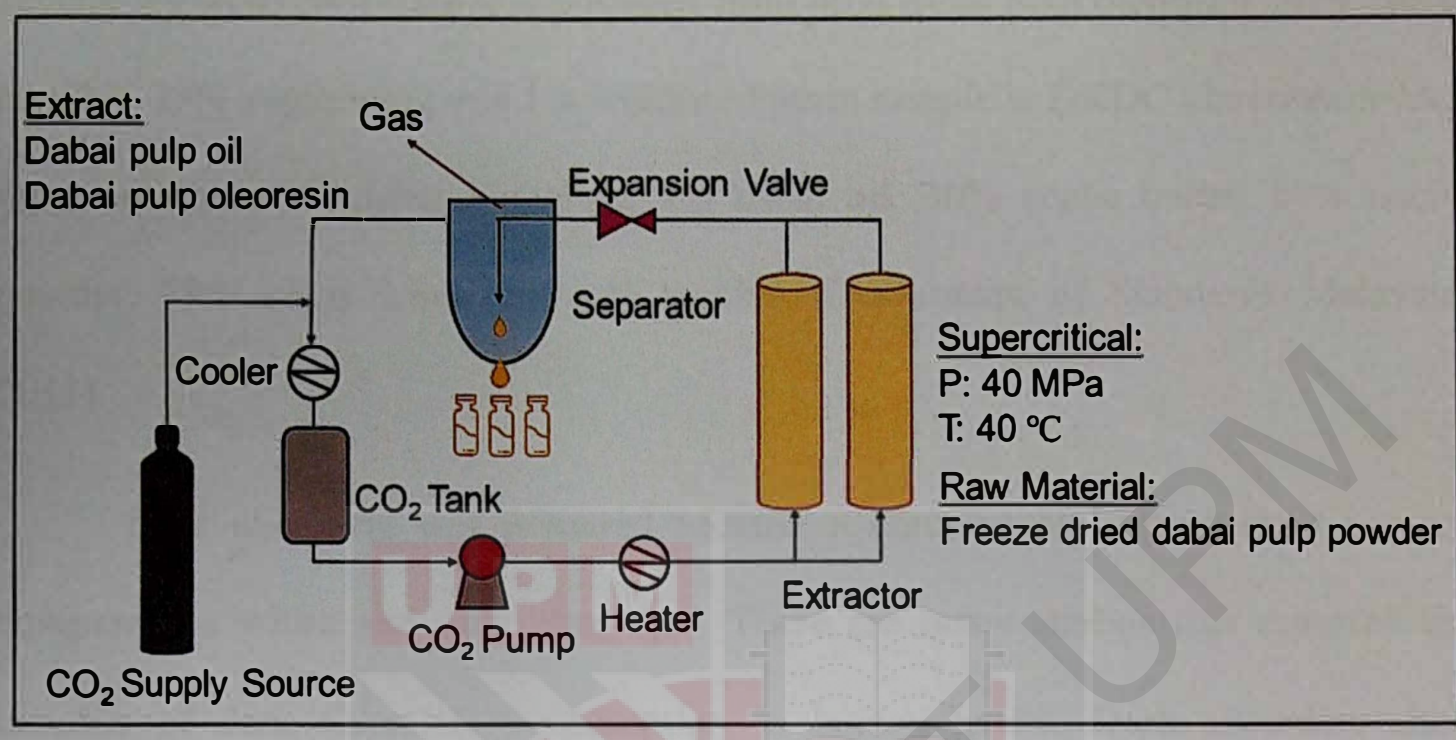

Figure 3: Schematic diagram of supercritical extraction system

3.4.3 Dark chocolates preparation

Four types of dark chocolate selected for this study includes dark (dark chocolate as standard), D2OL2O (20% dabai pulp oleoresin and 20% dabai oil chocolate), D4OL (40% dabai pulp oleoresin chocolate), DKDO (5% dabai kernel fat, 5% dabai oil with 30% cocoa butter chocolate). Each chocolate sample was prepared using different ingredients such as cocoa powder, cocoa butter, lecithin, sugar icing, dabai pulp oleoresin, dabai oil and dabai kernel fat. First sample (dark chocolate), were made up from 70% dark chocolate that consist of 40% cocoa butter, 30% cocoa powder, and others ingredients such as 29% icing sugar and 1% lecithin.

Second sample is D2OL2O chocolate which was prepared from 20% dabai pulp oleoresin, 20% dabai oil, 30% cocoa powder, 29% icing sugar and 1% lecithin. Third sample is D4OL chocolate was prepared from 40% dabai pulp oleoresin, 30% cocoa powder, 29% sugar icing and 1% lecithin. Fourth sample is DKDO chocolate which made up from 5% dabai kernel fat 5% dabai oil, 30% cocoa butter, 30% cocoa powder, 29% sugar icing, and 1% lecithin (Department of Standards Malaysia, 2011).

Dark chocolate was prepared because it contains high antioxidant content compared to white and milk chocolate. There are many studies that reported the benefits of dark chocolate consumption to human health. Dark chocolate shall contain, on a dry matter basis, more than 35% total solid, more than 18% shall be cocoa butter and more than 14% fat-free cocoa solids (Department of Standards Malaysia, 2011). Dark chocolate was prepared from 40% cocoa butter, 30% cocoa powder, 1% lecithin, and 29% icing sugar. Formulation of dabai pulp oleoresin with dabai oil and dabai kernel and dabai oil with cocoa butter chocolate was prepared by following recipe of dark chocolate with slightly modification.

The chocolates were prepared by using double boiler method. Firstly, the water was boiled in the pot above the stove. Then, the cocoa powder, icing sugar and lecithin were mixed together in bowl. After the water is boiled, aluminium bowl was put above the pot and ensure that bowl does not touch the hot water. Next, cocoa butter were melted in the aluminium bowl. After cocoa butter melted and become smooth, add the previous powder mixture into the aluminium bowl. The mixture was blended together and until smooth. Lastly, the mixture was poured into container or mould to be keep in chiller. Final product of the dark chocolate samples can be seen as ***Appendix A***.

All dark chocolate samples undergone the same sample preparation process. Samples were cut into small pieces and homogenized using a blender. Each of the dark chocolate samples were weighed. The fresh portions of chocolate samples were analyzed for ash, moisture, total available carbohydrate, protein and fat contents. Some portions were extracted for antioxidant properties determination such as β-carotene bleaching assay and Thiobarbituric acid (TBA).

3.4.4 Sample extraction

A modification of a published method was used for extraction and hydrolysis (Vinson et al., 1999) and modified by Medeiros, Marder, Wohlenberg, Funchal, & Dani, (2012). The samples (10 g) were defatted using multiple extractions with hexane. Defatting of sample is an important step because fat/ oil can disturb the process of antioxidant determination (Gordon et al., 2012). The defatted sample was mixed with solution composed of 10 ml of hydrochloric acid (HCL) 1.2 M with the addition of 90 ml 50% aqueous methanol. The final solution was heated in water bath at 90°C for 2 hours with stirring at every 30 minutes and extract obtained was filtrated. The extracted samples were used for antioxidant properties determination such as β-carotene bleaching assay and Thiobarbituric acid (TBA) value.

3.5 Determination of Moisture Content

Air oven method that adopted from AOAC International (2000) was used to determine the moisture content in chocolate samples. Firstly, an empty aluminium dish and lid were undergoing dried process for 3 hours in the oven at 105°C. Then, the aluminium dish and lid were transferred to desiccator to cool them and be weighed afterward. Ten grams of homogenized sample was weighed into the aluminium dish and was spread uniformly and evenly. The aluminium dish with samples was placed in the oven for overnight at 105°C.

After overnight, the lid was replaced before the aluminium dish with samples was removed from the oven. Then, aluminium dish was cooled in desiccator after been removed from oven and had been weighed after both of aluminium dish and sample reach the room temperature (20-25°C). This procedure was repeated until the constant weight was recorded. Samples were conducted in triplicates. The moisture content was calculated using the equation below:

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

3.6 Determination of Ash Content

The determination of ash content was determined by using drying ash method which has introduced by AOAC International (2000). For the first step, a porcelain crucible and its lid were placed in the muffle furnace at 505°C for fifteen minutes (15min) or greater than that. Secondly, the porcelain crucible and its lid were removed from the muffle furnace and placed in the desiccator to cool them. The porcelain crucible with 5 g of sample and its lid were weighed after the temperature both of porcelain crucible and the lid reached room temperature (20-25°C).

Next, the porcelain crucible and sample were ignited in the muffle furnace (already preheated for overnight at 550°C) without its lid until light grey or white ash was obtained. Lastly, the porcelain crucible and lid were placed in the desiccator to be cool down and was weighed right after the crucible and lid reach the room temperature (20-25°C). This step was repeated until the constant weight was obtained for each triplicates sample. The ash content was determined by using the equation below:

$$\text{Ash content, \% by weight} = \frac{\text{Weight of ash (g)}}{\text{Weight of sampe (g)}} \times 100$$

3.7 Determination of Total Available Carbohydrate

The total available carbohydrate content of sample was determined by using Clegg-anthrone method (Peris-Tortajada, 2004). Sample of 1 g was digested with 13 mL 52% perchloric acid to hydrolyze disaccharides, trisaccharides and higher oligomers to their component reducing sugars and was reacted with anthrone reagent under acidic condition to produce a blue/green condition. Anthrone reagent was prepared by dissolving 0.1% (w/v) anthrone in diluted sulphuric acid (sulphuric acid: water in ratio of 2.3:1.0, v/v). An aliquot (1 mL) of appropriately diluted hydrolysate was mixed with 5 mL anthrone reagent. Absorbance of the reaction mixture was measured at 630 nm against a blank after incubated in boiling water for 12 min and cooled. All samples were analyzed in triplicate. Glucose (0-100 mg/L) was used to construct a standard curve for quantification and the results are expressed as g/100 g FW of sample.

$$\text{Total available carbohydrate content (\% glucose by weight)} = \frac{25\ X\ b}{a\ X\ W}$$

a= absorbance of glucose standard working solution
b= absorbance of sample extract
W= weight of sample taken (g)

3.8 Determination of Protein Content

The determination of protein content in chocolate samples was determined using a Kjeldahl method that estimated total nitrogen content as explained in AOAC International (2000). A conversion factor (6.25) was used to convert measured nitrogen content in chocolate samples to protein content.

Firstly, 1 gram of sample was weighed into a digestion tube. Then, two Kjeltabs and 12 ml of concentrated sulphuric acid was added into the tube. The mixture was shaken gently. Next, the exhaust system was attached to the tubes in digestion rack and the rack was loaded with exhaust into the digestion block at 420°C. The digestion tube was heat up (about 60 min) until all samples were clear with blue or green solution.

The rack of tubes with exhaust still in a place was removed and had been cool for 20 minutes. Then, 30 ml of 4% boric acid with bromocresol green or methyl red indicator solution was added into a receiver flask. After that, the previous digest that already be cooled was diluted with 80 ml of distilled water and 50 ml of 40% sodium hydroxide to dilute the digest. The mixture was allowed with 4 min distillation and the distillate was titrated with a standardized 0.2N hydrochloric acid until the blue or grey end point was achieved.

The blank also conducted simultaneously with the same steps without the sample. Each sample was conducted in triplicates. The protein content was calculated by using following equation:

$$\text{Nitrogen content, \% by weight: } \frac{(T-B)x\ 0.2\ x\ 14.007\ x\ 100}{1000\ x\ W_{sample}}$$

Where, T: Titration volume for sample (ml)

B: Titration volume for blank (ml)

0.2: Normality of titrant (mol/l)

14.007: Atomic mass of nitrogen

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

Protein content, % by weight: Nitrogen content x conversion factor

3.9 Determination of Fat Content

Soxhlet method which introduced in AOAC International (2000) with modifications by Tee et al., (1996) was used to determine the lipid content of the samples. First step is sample of 10 g was weighed into an extraction thimble that placed in the extraction chamber afterward. The extraction chamber was placed under a condenser and suspended above a weighed flask which contains 180 ml of petroleum ether. After about 8 to 16 hours, the extractions of lipid were collected under reflux on a Soxhlet extraction instrument.

Then, the residue of petroleum ether was removed by using a rotary evaporator and the flask was dried in oven for an hour at 100°C. Lastly, the flask was cooled in desiccator and weighed using weighing scale. This step was repeated until the two-successive weighing reading was obtained that not differ by more than 0.1% of the mass of the sample portion obtained. Each sample conducted in triplicates. The fat content was calculated by using the equation below:

$$\text{Fat content, \% by weight} = \frac{W - W_o}{W_{sample}} \times 100$$

Where, W_o: Weight of the flask without fat (g)

W: Weight of the flask with fat (g)

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

3.10 Determination of Energy Content

The energy content was determined by using calculation based on the kilocalories of the macronutrients such as carbohydrate, fat, protein which are 4, 9 and 4 kilocalories (kcal) respectively (Whitney & Rofles, 2008). Energy content was expressed per 100 g portion of each chocolate samples (DC, D2OL2O, D4OL and DKDO).

3.11 Antioxidant properties determination

3.11.1 Beta-carotene bleaching assay

β-carotene solution was prepared by dissolving 10 mg β-carotene in 10.0 ml of chloroform (Amin, Azrina, Hock, Prasad & Kong, 2013). Then, 0.2 ml of β-carotene solution was mixed with 20 mg of linoleic acid and 200 mg of Tween 40 into round bottom flask. Then, the chloroform was removed using rotary evaporator at 40°C for 5 min. After evaporation, 50.0 ml of distilled water was added to the residue and shake vigorously to form an emulsion. Lastly, 5.0 ml of aliquots of the emulsion was pipette into the test tubes containing 0.2 ml of sample. The absorbance was read at 470 nm using spectrophotometer and test tubes was placed in a water bath at 50°C for 5 min and read again. BHT was used as standard or positive control. Meanwhile, an equal volume of ethanol by replacing the sample was used as negative control. Degradation rate and antioxidant activity, AA (%) was determined by using following formula:

$$DR = \frac{\ln \frac{a}{b}}{t}$$

Where In is natural log, a is the initial absorbance (470nm) at time 0, b is the absorbance (470nm) at 30, 60, and 120min and t is the initial absorbance (470nm) at time 0.

$$\text{Antioxidant activity, AA (\%)} = \frac{DR_{Control} - DR_{Sample/Standard}}{DR_{Control}} \text{X } 100$$

3.11.2 Thiobarbituric acid value (TBA)

The TBA test was performed according to the method of Kikuzaki and Nakatani (1993). The same samples that was prepared for FTC method were used in TBA method. The sample solution (2ml) was added 1 ml 20% aqueous trichloroacetic and 2 ml 0.67% aqueous thiobarbituric acid solution. After that, the mixture was placed in boiling water bath for 10 min. The mixture was centrifuged at 3000 rpm for 20 min. Next, the absorbance of supernatant was recorded at 532 nm on the final day of the FTC method.

3.12 Sensory evaluation

Dark chocolate samples were evaluated on appearance (color), aroma, flavour, brittleness, smoothness, aftertaste and overall acceptability (Lillah`, Ali, Imran, Ghulam & Maratab, 2017). Evaluation was performed using 9-point hedonic scale by 14 panellist. Dark chocolate samples were coded using three digit random numbers. The judges were asked to taste the four dark chocolate samples and drink plain water to rinse their palate after tasting each dark chocolate samples. Scorecard for sensory evaluation can be referred to ***Appendix B.***

3.13 Statistical analysis

The data obtain from this study were calculated and converted into mean and standard deviation (SD). The hypothesis of this study was decided either to be accepted or rejected by set the significant difference at *p*-value less than 0.05. The mean of nutritional composition, antioxidant properties and sensory evaluation of DC, D2OL2O, D4OL and DKDO were analyzed using one-way analysis variance (ANOVA) followed by post-hoc multiple comparison test (Tukey HSD) using IBM SPSS version 22.

CHAPTER 4

RESULTS & DISCUSSION

4.1 Moisture content

The moisture content of dark chocolates was assessed based on wet weight (ww) basis. According to Table 1, the moisture content of the dark chocolates ranged from 2.37- 3.54%. DKDO had the highest moisture content (3.54%), followed by D2OL2O (3.19%), and D4OL (3.14%). Meanwhile, dark chocolate (DC) had the lowest moisture content (2.37%) among the chocolate samples.

The moisture content of dark chocolate samples in descending order is DKDO > D2OL2O > D4OL > DC. The mean difference of moisture content was significantly different ($p<0.05$) among the four dark chocolate samples. However, multiple comparison showed D2OL2O was not significantly difference with D4OL.

Based on the results, the lower moisture content of dark chocolate in this study may be contributed from the usage of cocoa powder and cocoa butter. Generally, in the production of cocoa and chocolate products, cocoa bean undergo the process of fermentation, drying and roasting (Afoakwa, Quao, Takrama, Budu, & Saalia, 2013) thus, resulting dark chocolate (DC) prepared containing cocoa products (cocoa powder and cocoa butter) had the lowest moisture content compared to D4OL, D2OL2O and DKDO.

The moisture content of D2OL2O and D4OL were almost similar probably due to usage of dabai oil and oleoresin formulation. The supercritical carbon dioxide extraction of dabai oil and oleoresin did involve process such as drying and roasting henceforth, the moisture content was higher than DC. Despite of having 30% of cocoa butter in chocolate formulation, DKDO contain the highest moisture (3.55%) compared to DC. Previous study reported the moisture content in dabai kernel ranged from 27.09 – 45.38 g/100 g (Chua et al., 2015). Thus, original level of moisture in dabai kernel might contribute to high moisture content in DKDO.

Table 1: Moisture contents of four different dark chocolate samples.

Chocolate samples	Moisture (%±SD)
DC	2.37 ± 0.08^{c}
D2OL2O	3.18 ± 0.24^{b}
D4OL	3.13 ± 0.06^{b}
DKDO	3.55 ± 0.04^{a}

Values are expressed as mean± SD, n=4, values in the same column with same superscript letters are not significantly different ($p>0.05$). The values are based on wet weight (ww) basis. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

4.2 Ash content

The ash content of dark chocolate samples was assessed based on wet weight (ww) basis. Among the four samples, DC and D2OL2O showed slightly higher ash content (95.9%) followed by D4OL (95.8%) while DKDO had slightly lower ash content (95.7%). Multiple comparison test revealed that there was no significant difference detected between DC, D2OL2O, and D4OL. Further, no significant difference was detected between D4OL and DKDO. However, there was a significant difference detected between DC, D2OL2O and DKDO ($p<0.005$). The ash content of the dark chocolate samples in descending order is DC, D2OL2O, D4OL and DKDO.

Chocolate contains various kinds of minerals such as potassium, iron, copper and magnesium (USDA National Nutrient Database for Standard Reference, 2010) that contributed in the ash content. Non-fat cocoa solids or cocoa powder is produce by extraction of cocoa butter from cocoa liquor. Non-fat cocoa solid contain various kinds of minerals, vitamins, fiber and polyphenols (Katz, Doughty, & Ali, 2011). Minerals are not destroyed by heating because they have lower volatility compared to other food components. Thus, all dark chocolate samples contain high ash value as they made up from cocoa powder.

Dark chocolate (70% - 85%% cacao) provides 36 mg/100kcal of magnesium, 114 mg/100kcal of potassium, 1.90 mg/100kcal of iron (Katz et al., 2011). Ash content in dark chocolate (95.9%) for the present study are in agreement with Katz et al., (2011), since dark chocolate contain various kind of minerals. Dabai pulp provide 5.02-6.93 mg/100 g potassium, 8.77-12.05 mg/100 g sodium, 28.47-43.72 mg/100 g

calcium, 2.10-3.14 mg/100 g iron, 0.21-0.47 mg/100 g copper and 0.77-0.92 mg/100 g of zinc (Voon and Kueh, 1999). D2OL2O and D4OL also had high ash content, which might due to mineral content in dabai pulp. Meanwhile dark chocolate with 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter formulation had slightly lower ash content.

Table 2: Ash contents values of four different dark chocolate samples

Chocolate samples	Ash (%±SD)
DC	95.9±0.1[a]
D2OL2O	95.9±0.1[a]
D4OL	95.8±0.1[ab]
DKDO	95.7±0.0[b]

Values are expressed as mean± SD, n=4, values in the same column with same superscript letters are not significantly different ($p>0.05$). The values are based on wet weight (ww) basis. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

4.3 Total available carbohydrate

The total available carbohydrate of sample was determined by using Clegg-anthrone method (Peris-Tortajada, 2004). Based on the results, total available carbohydrate of dark chocolate samples in this study ranged from 51.8% to 86.8%. The results show that, D2OL2O had the highest total available carbohydrate (86.8%), followed by DKDO (79.7%), D4OL (79.2%) and DC (51.8%) respectively. The mean differences of total available carbohydrate between the four dark chocolate samples were significantly different ($p<0.05$).

The percentage of cocoa can affect the content of each nutrient in chocolate. The percentage of carbohydrate content decreases as total fat content increases depending on high content of cocoa solids in chocolate (Torres-Moreno, Torrescasana, Salas-Salvadó, & Blanch, 2015). Based on Table 3, the fat content (43.6%) and total available carbohydrate (51.8%) of dark chocolates are in agreement with (Torres-Moreno et al., 2015).

All dark chocolate sample contain >50% total available carbohydrate, therefore, carbohydrate is the main component meanwhile, fat (>40%) ranked second. It was observed that total available carbohydrate content in the D4OL and DKDO were almost same (79.2 ± 13.75 g/100 g FW and 79.7 ± 7.65 g/100 g FW respectively). D2OL2O was higher in carbohydrate compared to DC. However, all three dark chocolates which use dabai in the formulation (D2OL2O, D4OL and DKDO) have higher total available carbohydrate compared to DC.

Previous study conducted by Shakirin et al., (2012) showed that carbohydrate content in fullfat dabai pulp powder was 180.2± 0.08. In this study dabai oil and

oleoresin was extracted from fullfat dabai pulp powder via supercritical carbon dioxide extraction. Thus, the original carbohydrate content in fullfat dabai pulp powder could be contributing to high total carbohydrate in dark chocolates which used dabai in the formulation.

4.4 Protein content

The determination of protein content in chocolate samples was determined using a Kjeldahl method that estimated total nitrogen content as explained in AOAC International (2000). A conversion factor (6.25) was used to convert measured nitrogen content in dark chocolate samples to protein content.

The protein content of the four dark chocolate samples ranged from 8.1% to 9.3%. The result shows, DC contain the highest protein content (9.3%), followed by D2OL2O (9.2%), DKDO (9.1%) and D4OL (8.1%), respectively. There is no significant different between four dark chocolate samples in their protein contents.

Sørensen & Astrup, (2011) reported the protein content of dark chocolate (70% cocoa) is 8.9 g/100 g. Meanwhile, the protein content of dark chocolate in the current study is 9.34 g/100 g. The result of protein content of dark chocolate in the present study are comparable with previous study. The protein content of D2OL2O and DKDO are almost similar with DC. Meanwhile, D4OL contain the lowest protein content. Cocoa products contains high total nitrogen which might influence the determination of protein in dark chocolate.

4.5 Fat content

Soxhlet method which introduced by AOAC International (2000) with modifications by Tee et al. (1996) was used to determine the lipid content of the dark chocolate samples. The fat content of dark chocolate samples was determined based on wet weight (ww) basis.

Based on the results, the fat content of dark chocolate samples ranged from 41.2% to 43.6%. Table 3 shows, dark chocolate (DC) contains the highest fat content (43.6%), followed by D4OL (42.1%), D2OL2O (41.7%) and DKDO (41.2%), respectively. There were no significant differences in the mean values of the four dark chocolate samples.

The fat content of dark chocolate (70%) reported in previous study was 42.3 g/100 g (Sørensen & Astrup, 2011). Meanwhile, the result of fat content of dark chocolate in the present study is 43.6 g/100 g. This present study showed comparable fat content with study by Sørensen & Astrup (2011). Further, the total fat content in DC, D2OL2O, D4OL and DKDO are comparable. In this study the fat portion of each dark chocolate was kept at 40%.

Table 3: Proximate content of dark chocolate samples [in gram]

Dark chocolate samples	Fat (%±SD)	Protein (%±SD)	Total available carbohydrate (%±SD)	Energy (kcal± SD)
DC	43.6±1.2[a]	9.3±0.5[a]	51.8±20.1[b]	637.4±89.1[a]
D2OL2O	41.7±4.3[a]	9.2±1.3[a]	86.8±5.5[a]	759.4±26.6[a]
D4OL	42.1±0.8[a]	8.1±0.1[a]	79.2±13.8[ab]	727.6±58.2[a]
DKDO	41.2±0.3[a]	9.1±0.2[a]	79.7±7.7[ab]	725.6±31.5[a]

Values are expressed as mean± SD, g/100g fresh weight. Values in the same column with same superscript letters are not significantly different ($p>0.05$). The proximate composition values are based on wet weight (ww) basis with per 100 g edible portion (E.P) of dark chocolate samples for energy. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

4.6 Energy

The determination of energy content of dark chocolate samples was carried out using calculation based on the energy in kilocalories of the macronutrients such as carbohydrate, fat, protein which are 4, 9 and 4 kilocalories (kcal) respectively (Whitney & Rofles, 2008). Energy content was expressed per 100 g portion of each dark chocolate samples.

The total energy content of dark chocolate samples in this study range from 637.4 kcal to 759.4 kcal. Based on the results, D2OL2O had the highest energy

content (759.4 kcal), followed by D4OL (727.6 kcal), DKDO (725.6 kcal) and DC (637.4 kcal), respectively. There were no significant differences in the energy mean values of the four dark chocolate samples.

Sørensen & Astrup, (2011) reported energy content of dark chocolate was 597.99 Kcal. Meanwhile, energy content for the present study is 636.9 kcal. The major contribution to energy content of dark chocolate samples are fat and sugar proportion (gram) in dark chocolate formulation. It can be seen in this study that the energy content in dark chocolate that used dabai oil and oleoresin in formulation showed higher energy content than DC.

4.7 β-carotene bleaching assay

In the β-carotene bleaching assay, β-carotene undergoes rapid discoloration in the absence of an antioxidant. Heating of test emulsion in water bath at 50°C induce oxidation process by involving subtraction of H-atom from an active methylene group of linoleic acids (Noor Atiqah, Maisarah & Asmah, 2014). Linoleic acids formed a linolate free radical attacks the highly unsaturated β-carotene to replace its H-atom. The antioxidant properties in samples is be able to prevent the attack on β-carotene by counteract the linolate free radical (Noor Atiqah, Maisarah & Asmah, 2014).

Figure 4 shows antioxidant activity for the four dark chocolate samples at different time (30min, 60min and 120min). Table 4 shows D4OL exhibited the

highest antioxidant activity followed by DC, DKDO and D2OL2O. Besides, D4OL also showed significant different ($p<0.05$) in antioxidant activity at t=120 min compared to D2OL2O and DKDO except for DC. With regards to BHT, none of the antioxidant activity of dark chocolate samples were higher than BHT (standard).

The lower absorbance value of β-carotene linoleic emulsion over time are due to degradation process or bleaching of β-carotene emulsion. Thus, dark chocolate samples with the lowest degradation rate exhibit the highest antioxidant activity. The degradation rate of the dark chocolate samples can be referred to ***Appendix C***.

There are several factors that may contribute to the antioxidant activity of dark chocolate. According to Marder et al., (2012), dark chocolate (67% cocoa liquor) contain higher polyphenol content. The content of polyphenols in cocoa varied depend on soil type, climate, geographical origin, plant variety and planting region. In addition, formulation of cocoa in chocolate also can change the phenolic content of the final chocolate (Marder et al., 2012). Phenolic compound have antioxidant capacity to delay the rate of oxidation through inhibition of reactive oxygen species (ROS) and metal complexation (Duarte-Almeida, Dos Santos, Genovese, & Lajolo, 2006).

The presence of vitamin E isomers in cocoa butter has been studied previously, majority from γ-tocopherol, fewer amount of α-tocopherol, β-tocopherol and tocotrienols (93.90mg/kg, 4.20 mg/kg, 3.70 mg/kg and <10 mg/kg, respectively) (Carpenter, Hammerstone, Romanczyk, & Aitken, 1994, Undurraga & Erazo, 2001). Thus, vitamin E isomers of cocoa butter may contribute to antioxidant activity of DC and DKDO which contain cocoa butter in dark chocolate formulation.

Dabai oil contain 0.01 mg/100 g of vitamin E meanwhile dabai oleoresin contain 0.13 mg/ 100 g of vitamin E (unpublished data, 2018). Vitamin E is a group of compounds that include both tocopherols and tocotrienols, which are antioxidant. Thus, vitamin E content in dabai oleoresin might contributed to the high antioxidant activity of D4OL which contain 40% dabai oleoresin.

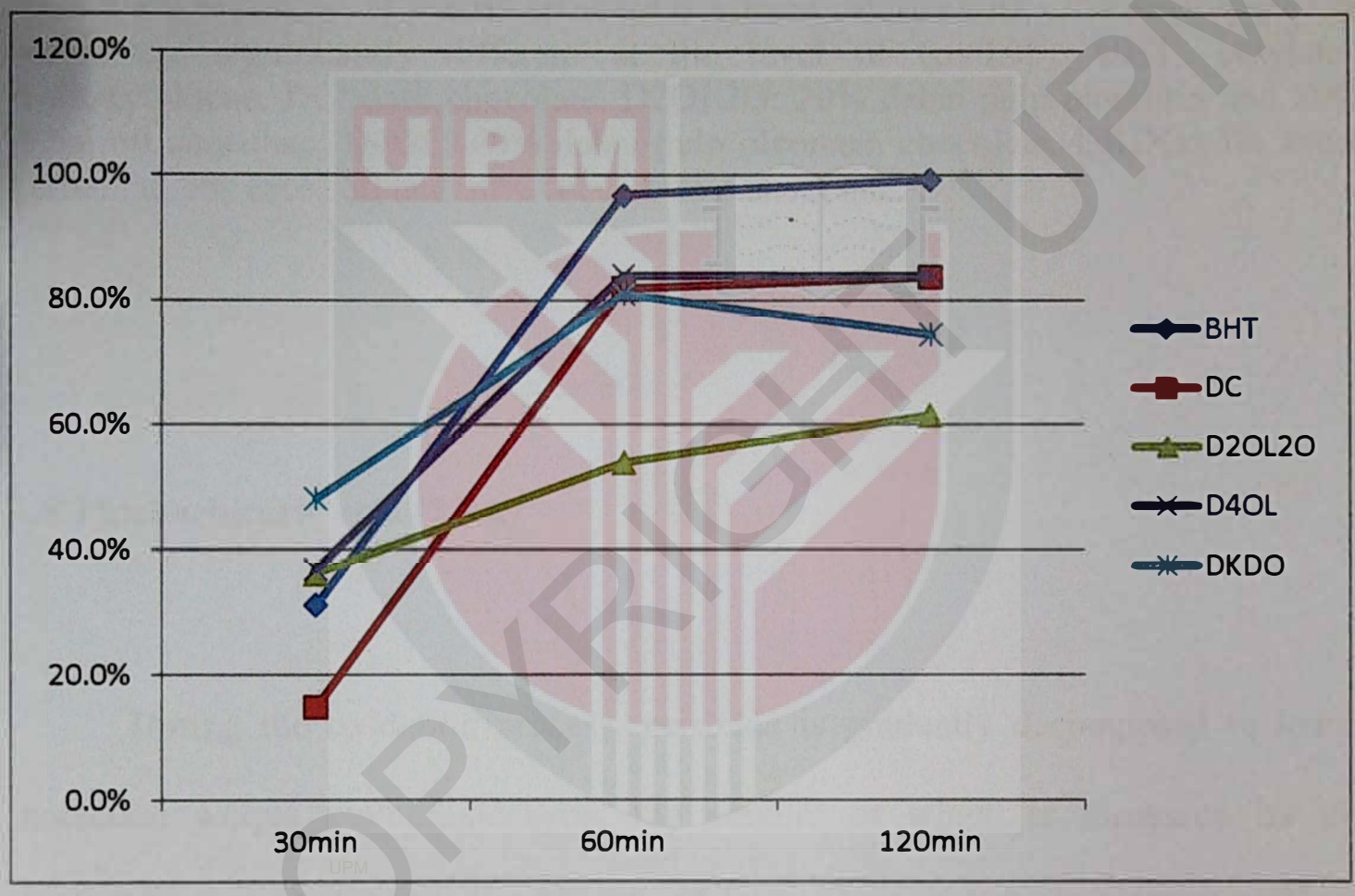

Figure 4: Antioxidant activity (%) for standard (BHT) and dark chocolate samples at 30min, 60min and 120min. BHT: butylated hydroxytoluene, DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

Table 4: Mean antioxidant activity of dark chocolate samples calculated on the basis of the rate of β-carotene bleaching at t= 120min.

Dark chocolate samples	Antioxidant activity (%)
BHT	99.27±0.21[a]
DC	83.70±2.69[b]
D2OL2O	61.50±5.39[d]
D4OL	83.90±2.17[b]
DKDO	74.27±2.92[c]

Values are expressed as mean± standard deviation. Means with different superscript letters are significantly different at the level of ($p<0.05$). BHT: butylated hydroxytoluene, DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

4.8 Thiobarbituric acid (TBA)

During the oxidation process, peroxide is gradually decomposed to lower molecular weight malondialdehyde, the amount of which is measured by the thiobarbituric acid (TBA) assay. The absorbance values of dark chocolate samples at 532nm was measured after 14 days are shown in Figure 5.

As shown in Figure 5, a low absorbance value represents a high level of antioxidant activity. The absorbance values for DC, D2OL2O, D4OL and DKDO (1.561, 2.573, 2.911 and 3.186, respectively) were higher than BHT (0.442) thus represent lower antioxidant activities compared to standard. DC contain the highest antioxidant activity followed by D2OL2O, D4OL and DKDO. Figure 5 also shows antioxidant activity of D2OL2O were slightly higher than D4OL. Meanwhile,

DKDO shows lowest antioxidant activity compared to the four dark chocolate samples.

DC and D2OL2O exhibit strong antioxidant activity in TBA assays. These results, we could therefore suggest that the consumption of DC and D2OL2O could possibly offer some protection against lipid peroxidation. Vitamin E content in dabai oil and dabai oleoresin might contribute to antioxidant activity of D2OL2O and D4OL against lipid peroxidation.

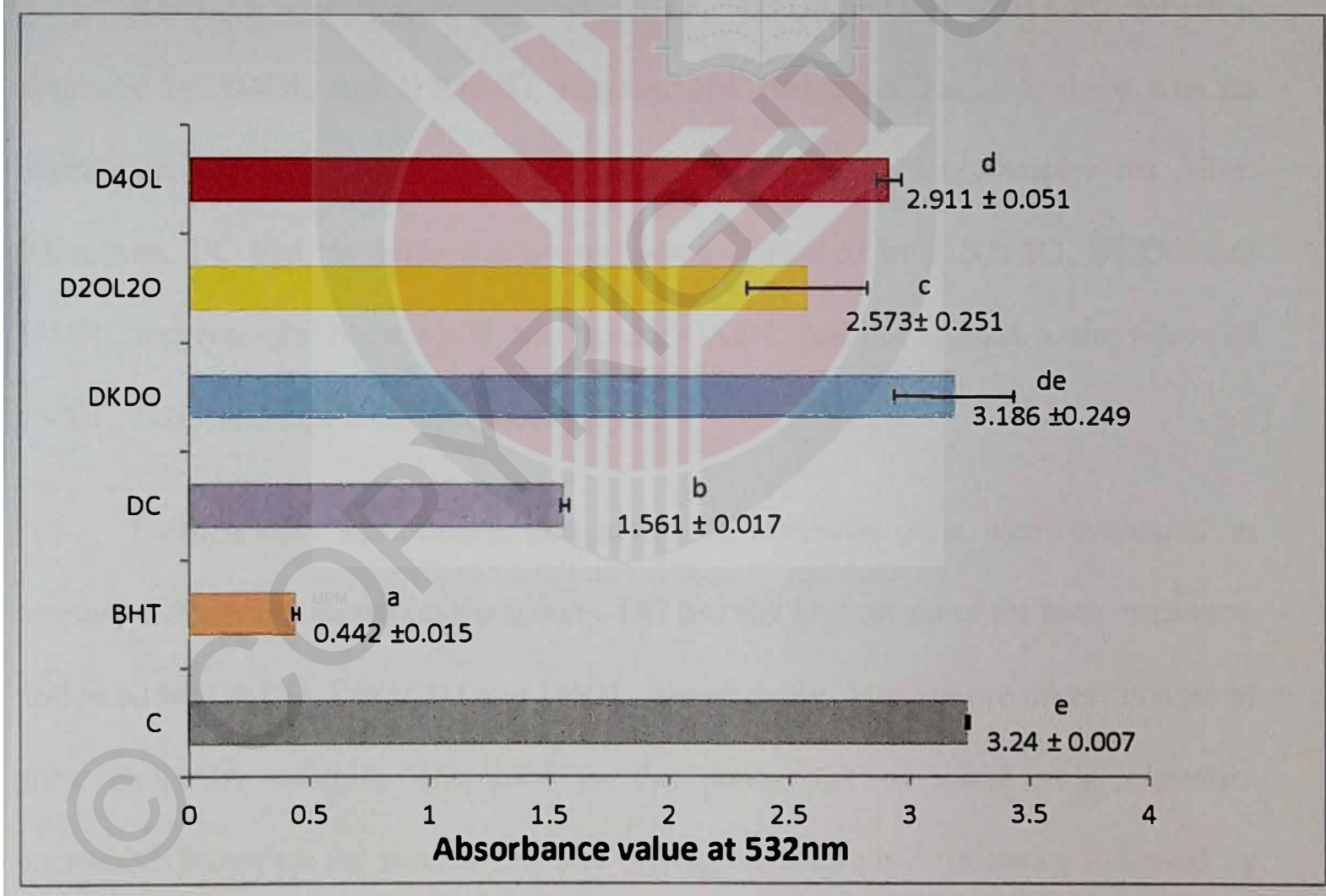

Figure 5: Amount of malondialdehyde in lipid peroxidation, measured using TBA assay.

4.9 Sensory evaluation

Dark chocolate samples were evaluated on appearance (color), aroma, flavour, brittleness, smoothness, aftertaste and overall acceptability. Analysis of variance showed that for most attributes of dark chocolate samples (DC, D2OL2O, D4OL and DKDO) were significantly different ($p<0.05$) for aroma, flavour, brittleness, and overall acceptance except for color, smoothness and aftertaste as presented in Table 5.

Based on sensory evaluation results, DC and DKDO had same score for color followed by D4OL and D2OL2O, respectively. Based on Table 5, there was no significant different ($p<0.05$) between the four dark chocolate samples for color. Moreover, DC had the highest score in aroma, followed by D2OL2O, DKDO and D4OL, respectively. Meanwhile, for flavour DKDO had the highest score, followed by DC, D2OL2O and D4OL, respectively.

Furthermore, smoothness and aftertaste attributes also were evaluated in sensory evaluation. Based on the results, DC had the highest score for both attributes, followed by DKDO, D2OL2O and D4OL, respectively. Mean score on brittleness of dark chocolate samples decreased as the percentage of dabai pulp oleoresin increased. Based on the results, DC had the highest score in brittleness, followed by DKDO, D2OL2O and D4OL. Thus, cocoa butter might contribute to brittleness characteristic in dark chocolate containing cocoa butter (DC and DKDO).

Overall, the acceptability of dark chocolate samples increased with increasing amount of cocoa butter in chocolate. The acceptability score among the four dark chocolate samples was highest in DC, followed by DKDO, D2OL2O and D4OL. Fat

content in chocolate able to give desirable physical characteristics such as gloss, melt-in-the-mouth properties, creamy texture, rich taste and snap (Norton, Fryer, Parkinson, & Cox, 2009). Chocolate manufacturing by industrial section have common processing methods such as mixing, refining, conching, tempering and depositing, moulding and demoulding. These processing methods able to give desirable sensory characteristics of chocolate such as smooth textures and removing of grittiness oral perceptions (Afoakwa, 2010).

Mixing of ingredients during chocolate production is important to obtain constant formulation consistency. D2OL2O and D4OL were perceived by panellist as somewhat gritty in chocolate texture. Size reduction of ingredient particles (such as sugar and cocoa solids) in chocolate is very crucial to obtain a smooth texture by process of refining using a three or five-roll refiner (Lucisano, Casiraghi, & Mariotti, 2006). After that, chocolate should go through the process of conching to remove unpleasant flavours which develop the chocolate aroma. Besides, conching is also essential in development of viscosity and final texture of chocolate.

Tempering process is important contribution in polymorphic forms of cocoa butter, form V, a β polymorph to give brittleness, glossy appearance, contraction and resistance to bloom in chocolate (Afoakwa,2010). In poorly, tempered chocolate, color attribute was affected as reflected light is disturbed by unstable crystal growth (Hartel, 2001). High moisture in chocolate aggregates sugar particles to cause grittiness and reduced the smoothness characteristic in chocolate (Naviglio, Ferrara, & Gallo, 2014). The higher score on smoothness for DC was in agreement with Naviglio, Ferrara, & Gallo, (2014) due to its lowest moisture content (2.37%).

Table 5: Attributes for dark chocolate sensory evaluation for each dark chocolate samples.

Dark chocolate samples	DC (standard)	D2OL2O	D4OL	DKDO
Color (mean±SD)	7.00±1.71[a]	6.64±1.74[a]	6.93±1.90[a]	7.00±1.62[a]
Aroma (mean±SD)	7.00±1.88[a]	6.26±1.14[a]	4.57±2.07[b]	5.43±1.60[b]
Flavor (mean±SD)	6.50±1.99[a]	6.14±1.92[a]	4.21±2.19[b]	6.57±1.51[a]
Brittleness (mean±SD)	6.29±1.49[a]	4.93±1.64[ab]	4.07±1.54[b]	5.79±1.37[a]
Smoothness (mean±SD)	6.14±1.75[a]	4.86±2.18[a]	4.50±1.83[a]	5.79±1.37[a]
Aftertaste (mean±SD)	6.64±1.60[a]	5.64±1.69[ab]	4.71±2.05[b]	5.86±1.10[ab]
Overall acceptability (mean±SD)	7.07±1.39[a]	5.93±1.86[a]	4.21±2.05[b]	6.43±1.16[a]

Values are expressed as mean± standard deviation. Means with different superscript letters are significantly different at the level of $p<0.05$. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

The nutritional composition, antioxidant properties and potential application of dabai oleoresin, dabai oil and dabai kernel fat in dark chocolate making was observed and determined in this study. In addition, differences of antioxidant properties were determined between DC, D2OL2O, D4OL and DKDO.

Findings show D2OL2O dark chocolate contain good nutritional composition which are high in protein and total available carbohydrate contents meanwhile D4OL contain high ash content. DKDO contain lower moisture and lower fat contents compared to dark chocolate. D4OL exhibited higher antioxidant activity compared to DC based on β-carotene bleaching assay. On the other hand, DC shows higher antioxidant activity followed by D2OL2O, D4OL and DKDO based on thiobarbituric acid test. DC, D4OL and D2OL2O could probably offer some protective effect towards lipid peroxidation.

Mainly, DC contain the highest score in several sensory attributes such as color, aroma, aftertaste, brittleness, smoothness and overall acceptability except for flavor. DKDO shows similar score with DC in color meanwhile D4OL shows almost similar result with DKDO followed by D2OL2O. D4OL shows the lowest score on taste such as aroma, flavor and aftertaste compared to DC, DKDO and D2OL2O. Besides, D4OL shows similar result in score of texture (brittleness and smoothness) with score of taste. Furthermore, DKDO is the most preferred and acceptable based on sensory evaluation (overall acceptability) after dark chocolate (DC).

The knowledge on physicochemical and sensory properties of chocolate not let us predict the consumer acceptability. The desirable sensory characteristics of chocolate such as smoothness and brittleness can be improved through process of mixing, refining, conching, tempering, moulding and demoulding. Rheological properties in dark chocolate samples should be studied to obtain high-quality products with good texture properties.

Overall, this study has revealed the potential application of dabai oleoresin, dabai oil and dabai kernel fat as cocoa butter equivalent (CBE) in dark chocolate making. Besides, DKDO are the most preferred compared to D2OL2O and D4OL based on sensory evaluation. Further, study on rheological properties of dark chocolate could help in obtaining high-quality chocolate with good texture properties.

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APPENDICES

APPENDIX A

Dark chocolate samples. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate.

APPENDIX B

SCORECARD

FOOD SAMPLE: CHOCOLATES

INSTRUCTION: Please observe and taste the sample that were given. Start from the sample on your left. Evaluate each of it based on the characteristics and scoring stated in this form. Don't forget to rinse your mouth using plain water after you taste each of the sample.

1. Dislike Extremely **2**. Dislike Very Much **3**. Dislike Moderately **4**. Dislike Slightly

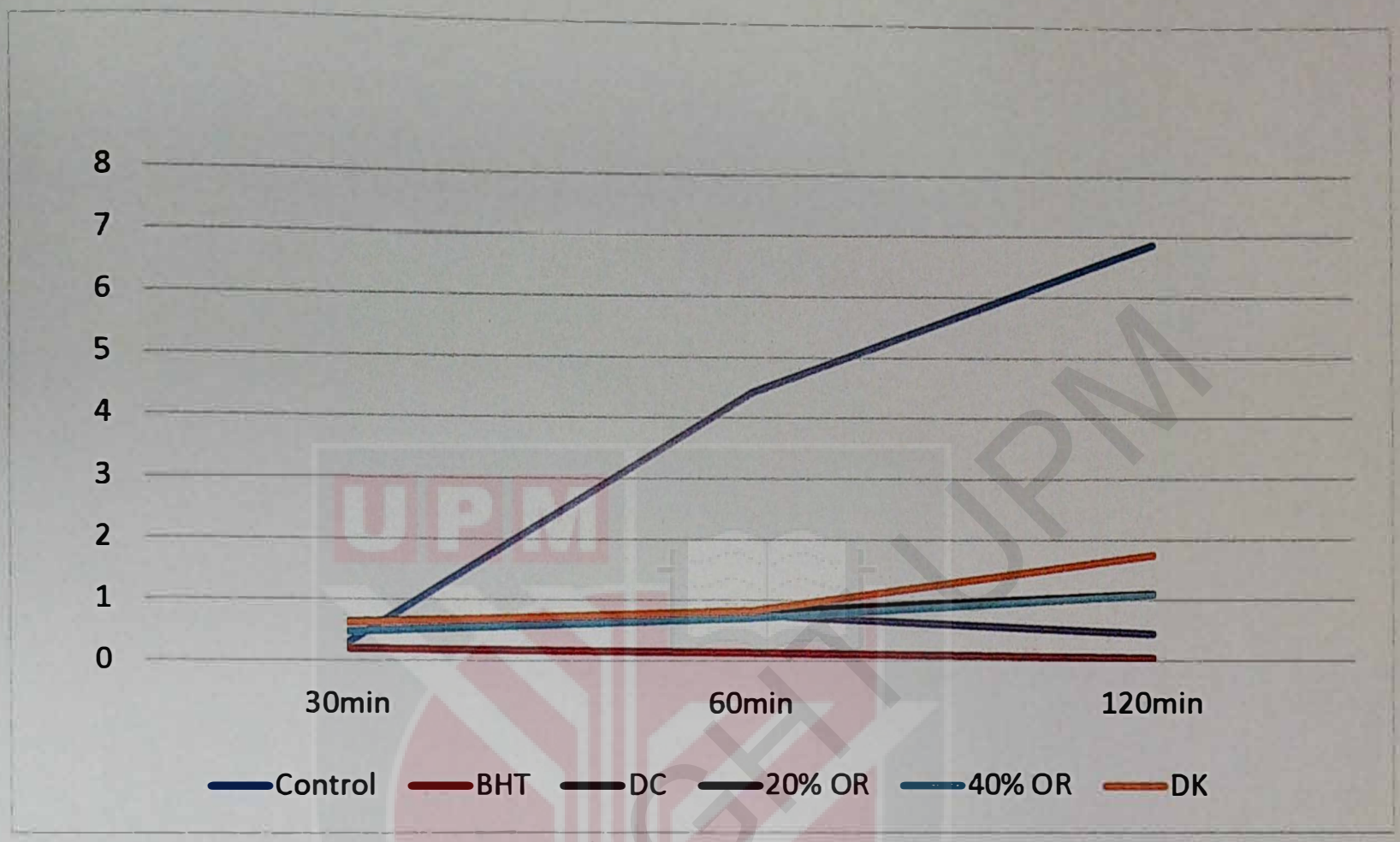
5. Neither Like nor Dislike **6**. Like Slightly **7**. Like Moderately **8**. Like Very Much

9. Like Extremely

Panel ID:
Date:

Attributes	Sample			
	132	243	354	465
Appearance: color				
Aroma				
Flavor				
Brittleness				
Smoothness				
Aftertaste				
Overall acceptability				
Comments (if have):				

APPENDIX C



Degradation rate of dark chocolate samples by β-carotene bleaching assay. BHT was used as a standard. DC: dark chocolate, D2OL2O: 20% dabai pulp oleoresin and 20% dabai oil chocolate, D4OL: 40% dabai pulp oleoresin chocolate, DKDO: 5% dabai kernel fat, 5% dabai oil and 30% cocoa butter chocolate