Nutrient: Jurnal Gizi
Volume 3, Nomor 1, Juni 2023
e-ISSN : 2798-4796
p-ISSN : 2798-480X

Pengaruh Penambahan Rumput Ular Sabah (*Clinacanthus Nutans*) Terhadap Sifat Antioksidan dan Sensory Coklat

Effect Of Adding Of Sabah Snake Grass (Clinacanthus Nutans) On The Antioxidant And Sensory Properties Of Chocolate

Mansoor Abdul Hamid[1,2*], Lim Kai Yen[1], Yeap Chi Hong[1], Mazarina Devi[3], Maryana Mohamad Nor[4]

Abstrak Penelitian ini dilakukan untuk mengidentifikasi pengaruh metode pengeringan terhadap aktivitas antioksidan daun rumput ular Sabah serta penambahan bubuk alang-alang terhadap antioksidan dan sifat sensoris cokelat. Sifat antioksidan sampel coklat ditentukan melalui uji kandungan fenolik total dan uji aktivitas penangkapan radikal DPPH. Formulasi terbaik cokelat isi rumput ular Sabah ditentukan berdasarkan uji sensori dan antioksidan. Kandungan gizi coklat ditentukan melalui metode AOAC sedangkan aktivitas air dan kekerasan cangkang coklat ditentukan dengan menggunakan water activity meter dan Texture Analyzer. Perubahan cokelat selama penyimpanan ditinjau dari aspek kadar air, aktivitas air, kekerasan, titik leleh, jumlah mikroorganisme dan skor sensori. Hasil penelitian menunjukkan bahwa coklat F2, coklat yang diisi dengan 100g bubuk rumput ular Sabah adalah formulasi terbaik dengan kepuasan panel dan aktivitas antioksidan yang lebih tinggi. Sampel ini mengandung kadar air 7,25%, abu 2,19%, lemak 30,61%, protein 5,41%, serat kasar 1,94% dan karbohidrat 52,61%. Sampel penyimpanan menunjukkan perbedaan yang signifikan ($p<0,05$) pada kadar air, aktivitas air, kekerasan dan sifat leleh setelah disimpan selama delapan minggu tetapi masih aman untuk dikonsumsi.

Kata kunci: Rumput ular Sabah, antioksidan, sensori, kandungan nutrien, stabiliti penyimpanan.

[1]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia. email: chot@ums.edu.my
[2]Functional Foods research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
[3]Prodi Pendidikan, Tata Boga, Fakultas Teknik, Universitas Negeri Malang, Jalan Semarang 5, 65145, Malang, Jawa Timur, Indonesia.
[4]Institute of Food Security and Sustainable Agriculture (IFFSA), Universiti Malaysia Kelantan, Locked Bag No.100, 17600 Jeli, Kelantan, Malaysia.

Abstract *This research was carried out to identify the effect of drying methods on the antioxidant activity of Sabah snake grass leaves as well as the addition of Sabah snake grass powder on the antioxidant and sensory properties of chocolate. Antioxidant properties of chocolate samples were determined through total phenolic content and DPPH radical scavenging activity tests. The best formulation of chocolate filled with Sabah snake grass was determined based on sensory and antioxidant test. Nutrient content of chocolate was determined through AOAC methods whereas water activity and hardness of chocolate shell were determined using water activity meter and Texture Analyzer. Changes of chocolate during storage was reviewed through the aspect of moisture content, water activity, hardness, melting point, microorganism count and sensory score. Results showed that chocolate F2, chocolate filled with 100g of Sabah snake grass powder was the best formulation with satisfaction by panels and higher antioxidant activity. This sample contain 7.25% moisture, 2.19% ash, 30.61% fat, 5.41% protein, 1.94% crude fibre and 52.61% carbohydrate. The storage sample showed significantly difference ($p<0.05$) in moisture content, water activity, hardness and melting property after kept for eight weeks but still safe to be consumed.*

Keywords: Sabah Snake grass, antioxidant, sensory, nutritional content, storage stability.

A. INTRODUCTION

Chocolate is one of the most popular food dan always been craved by the people around the world. This is because chocolate has the potential to impart enjoyment and positive emotion (El-kalyoubi et al., 2011). In brief, chocolate can be defined as the product form from the mixture of roasted cocoa bean with sugar and then grind with or wthout the addition of cocoa butter until small particle size is obtained (Bent et al., 2013). Among all types of chocolate available in the Malaysia market, filled chocolate possessed the highest percentage of sales in accordance to types of chocolate which is as high as 70.10% in the year 2016 (Euromonitor, 2016). Normally, the filling use in filled chocolate are such as nougat, toffee, fruits and nuts. Chocolate with herb filling is very limited in the market. However, since the Malaysia chocolate confectionery market has the highest growth percentage among all types of confectinery in Malaysia and there are strong interest for functional chocolate from consumers, chocolate filled with herbs can be developed.

`

In this research, Sabah snake grass was incorporated into the chocolate filling. Sabah snake grass is scientifically known as Clinacanthus nutans and it is a species of herbal plant found in South East Asia especially in Thailand, Indonesia and Malaysia (Arullappan et al., 2014). In the system of taxonomy, Sabah snake grass in classified into the kingdom of Plantae, phylum of Magnoliophyta, class of Magnoliopsida, order of Lamiales, family of Acanthaceae, genus of Clinacanthus and species of nutans (Arullappan et al., 2014). Sabah snake grass possess high medical value and is being used as source of antioxidant (Yong et al., 2013). Antioxidant neutralizeed free radicals in our body and this subsequently helps to prevent various diseases and slowed down the aging process (IFT, 2016; Siat, 2015).

However, Sabah snake grass posses moisture content as high as 80% (Moses et al., 2015). Therefore, drying is vital to lower the water activity by removing majority of the water content (Oliveira et al., 2015). Drying also helps to maintain and lengthen the shelf life of product which spoil easily such as vegetables and herbs, facilitate storage and reduce the transportation cost and packaging. However, different drying methods can affect the phytochemical contents and antioxidant of the product being dried. Thus, selection of optimum drying method is vital to preserve the bioactive compounds when drying of natural ingredients. From industry view point, cabinet drying and vacuum drying are two methods commonly employed as they are of lower cost compared to freeze drying. Basically, cabinet dryer is made up of a group of small trays which are suitable for dehydration of vegetables. On the other hand, vacuum dryer is used for drying of products sensitive to heat.

B. MATERIALS AND METHODS

a. Raw Materials

The origin of Sabah snake grass used in this research is from Sabah snake grass farm placed in Papar, Sabah. Sabah snake grass was bought from the retailer in Big Market Kota Kinabalu, Sabah. Other ingredients such as dark compound chocolate, white compound chocolate and whipping cream were purchased from Baked with Me Sdn. Bhd. All chemicals used in analysis were provided by Faculty of Food Science and Nutrition, Universiti Malaysia Sabah.

b. Pre-treatment and Preparation of Sabah Snake Grass Leaves

Pre-treatment and preparation of Sabah snake grass leaves were conducted according to Moses et al. (2014). Sabah snake grass leaves were cleaned by soaking in water for 1 min. Then, the leaves were placed under running tap

water for 30 sec and drained the excess water completely. Sabah snake grass leaves were bleached in water for 30 sec at the temperature of 98±5˚C and subsequently soaked in cold water for another 30 sec. After that, the leaves were placed in cabinet dryer (Protech FDD-720, Malaysia) at 40˚C until reach the constant moisture content. Dried Sabah snake grass leaves were ground into powder using blender (Panasonic Blender, Malaysia) and sieved through sieve with particle size 125µm. These dried leave powders were stored in sterile and air-tight glass bottle.

c. Production of Chocolate

The method of production and formulation of chocolate filled with Sabah snake grass were conducted according to Siew (2014) and Abou-Zaid and Nadir (2014) with modification. The filling formulation was shown in Table 1.

Table 1: Filling formulation for control chocolate and chocolate filled with Sabah snake grass leave

Formulation	Sabah Snake Grass Powder (g)	White Chocolate (g)	Whipping Cream (g)
Control	0	700	300
F1	50	700	300
F2	100	700	300
F3	150	700	300

To produce the fillin, white compound chocolate was chopped and melted. Then, whipping cream was added and mixed well before Sabah snake grass powder was added. Chocolate shell was prepared by pouring 1/3 melted chocolate into the mould and allowed to be set. Then, 5.0±0.1g Sabah snake grass filling placed into centre of the chocolate shell before pour another melted chocolate onto it for covering process. Allowed for another 10-15 min for chocolate to solidify before demould process take place.

d. Sensory Evaluation of Produced Chocolate

Chocolate containing different amounts of Sabah snake grass leaves powder were evaluated by 40 panels from the students of Faculty of Food Science and Nutrition, Universiti Malaysia Sabah. A piece of chocolate sample (10g) for each formulation was placed on plastic plate coded with three digit number and served at room temperature (Leite et al., 2013). The sensory

evaluation was carried out using seven scale evaluations for colour, texture, sweetness, taste, after-taste and overall acceptance. Warm water was served to rinse mouth before evaluating each sample.

e. Extraction of Phenolic and antioxidant Compounds in Chocolate

Extraction of phenolic and antioxidant compounds in chocolate was carried out according to Belščak-Cvitanović et al. (2012[b]). Chocolate with filling was crushed and 8.00±0.01g of sample was treated with 10ml n-hexane for three times. Defatted sample was then air-dried for 24 hours before 4.00±0.01g sample was extracted with 20ml methanol for two times for 30 min in ultrasonic immersion (Branson, USA). After each extraction, the solution was centrifuge for 10 minutes at 6000rpm and supernatant obtained was kept at -20˚C in freezer (Thermo Fischer, USA).

f. Determination of Total Phenolic Content of Chocolate

Determination of total phenolic content of chocolate was carried out as shown in 2.4 as for Sabah snake grass extract.

g. Determination of DPPH Radical Scavenging Activity of Chocolate

Determination of DPPH radical scavenging activity of chocolate was carried out as shown in 2.5 as for Sabah snake grass extract. The concentration of chocolate extract used was 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0mg/mL.

h. Determination of Chemical Composition of the Control Chocolate and the Best Formulation of Chocolate Filled with Sabah Snake Grass

The chemical components, moisture, ash, fat, protein and crude fiber for chocolate were determined according to the standard methods of AOAC (2005). Total carbohydrate content was calculated by difference.

i. Storage Study of the Control Chocolate and the Best Formulation of Chocolate Filled with Sabah Snake Grass

Storage study was conducted for a period of eight weeks. Chocolate samples were wrapped with aluminium foil and transferred into plastic boxes which were sterile and air- and moisture-tight (Böhme et al., 2012). The plastic boxes were kept in cold room (Pandey & Singh, 2011). The storage study included various tests, specifically, moisture content, water activity, hardness, melting point, microbiological and multiple comparison test. Each test was conducted on chocolate sampel each week for a duration of eight weeks.

`

i1. Determination of Moisture Content

Moisture content of chocolate filling and shell were determined according to AOAC (2005) method.

i2. Determination of Water Activity

Water activity of chocolate was determined according to AOAC (2005) using water activity meter (Cole Parmer, USA). Approximately 2g of sample chocolate was placed into the small cup and water activity meter was placed on top of the small cup. After five minutes, the value of water activity was shown on the screen.

i3. Determination of Hardness

Hardness of chocolate was determined according to Belščak-Cvitanović et al. (2012b) using Texture Analyzer (Stable MicroSystems, UK) equipped with a cylindrical stainless steel probe with diameter 2.0mm and mounted on 30kg load cell. Prior to measurement, the closure of the chocolate was removed using a scalpel and the filling was discarded. The shell was punctured at a speed of 1.00mm/s with a distant of 7.5mm.

i4. Determination of Melting Behaviour

Melting point of chocolate was determined according to Afoakwa et al. (2008) using Differential Scanning Calorimeter (DSC) (PerkinElmer, USA). DSC was calibrated with indium and analysing rate was 5˚C/min using empty aluminium pan as reference. About 5±0.1mg of sample was filled into the pan and sealed using sample press. This process was conducted by heating the sample at a rate of 5˚C/min from 15˚C to 55˚C in nitrogen gas.

i5. Microbiological Test

Microbiological test was conducted using total plate count (TPC) method in accordance to Gounga et al. (2008) and Xu et al. (2008). Plate count agar (PCA) and potato dextrose agar (PDA) were used. PCA media was used to detect the existence of microorganisms whereas PDA media was used to detect the existence of mold and yeast (Gounga et al., 2008; Xu et al., 2008).

i6. Multiple Comparison Test of Stored Chocolate

Stored chocolate was evaluated by 40 untrained panels from the students of Faculty of Food Science and Nutrition, University Malaysia Sabah. In this test, reference sample (fresh sample) was labelled with R and was served with stored sample for a specific duration fixed (ISO, 2010). The evaluation was carried out for colour, texture, sweetness, taste, after-taste and overall

`

acceptance. Panels were asked to give their score according to the difference that exit between sample R and stored chocolate.

j. Statistical Analysis

Analyses were carried out in triplicate and their results were subjected to statistical analysis. It comprised determination of average values and their standard deviation as well as significant difference at the level where $p<0.05$.

C. RESULTS AND DISCUSSION

a. Total Phenolic Content of Sabah Snake Grass

The total phenolic content (TPC) of Sabah snake grass dried through cabinet dryer and vacuum dryer was shown in Table 2.

Table 2: Total phenolic content of Sabah snake grass leave powder

Drying Method	Mean±Standard Deviation (mg GAE/ g dried leave powder)
Cabinet Drying	3.86 ± 0.03^{a}
Vacuum Drying	5.20 ± 0.04^{b}

1: Values are stated as mean±standard deviation, n=3.
2: Values with different superscript letters are significantly different ($p<0.05$).

Sabah snake grass leaves dried by vacuum dryer (5.20mg GAE/g dried leaves) had significantly higher ($p<0.05$) TPC content compared to cabinet dryer (3.86mg GAE/g dried leaves) because shorter drying time and higher drying rate for vacuum drying had minimized the loss of TPC by reducing the oxidation of phenolic compounds (Naknaen et al., 2015; Li et al., 2014). Similar result also had been reported by Pham et al. (2015) who studied on the effect of drying methods on physicochemical and antioxidant properties of Helicteres hirsute Lour leaves.

b. DPPH Free Radical Scavenging Activity of Sabah Snake Grass

In this research, Butylated hydroxyanisole (BHA) was used as standard for the determination of DPPH radical scavenging activity, it was found that the EC_{50} for BHA was 0.0072mg/mL. Table 3 showed the result of EC_{50} DPPH radical scavenging activity of Sabah snake grass leave samples dried through cabinet and vacuum dryer. Based on Table 3.2, the concentration of Sabah snake grass leave extract needed to achieve the EC_{50} of BHA was 1997 times for cabinet dried leaves and 928 times for vacuum dried leaves.

`

Table 3: EC_{50} DPPH free radical scavenging activity of Sabah snake grass leave powder

Drying Method	Mean±Standard Deviation (mg/mL)
Cabinet Drying	14.38±0.30[a]
Vacuum Drying	6.68±0.15[b]

1: Values are stated as mean±standard deviation, n=3.
2: Values with different superscript letters are significantly different ($p<0.05$).

Sabah snake grass leaves dried by vacuum dryer (EC_{50}= 6.68mg/mL) had significantly lower ($p<0.05$) EC_{50} value compared to cabinet dryer (EC_{50}= 14.38mg/mL) because limited oxygen content during vacuum drying had brought to lower oxidation level. In addition, shorter drying time for vacuum drying also reduced helps to reduce thermal degradation (Tanongkankit et al., 2015).

c. Sensory Quality Characteristics of Produced Chocolate

The effect of incorporation of the Sabah snake grass powder at different amount (50g, 100g and 150g) on the sensory score for several attributes, specifically, colour, aroma, texture, sweetness, taste, after-taste and overall acceptance of produced chocolate were studied and results were indicated in Table 4.

Table 4: Influence of Sabah snake grass powder on sensory characteristics of produced chocolate

Atributes	Mean Score±Standard Deviation			
	Control	F1	F2	F3
Colour	5.71±0.82[a]	5.68±0.92[a]	5.70±1.11[a]	5.70±1.02[a]
Aroma	5.20±1.12[a]	5.30±1.07[a]	5.33 ±0.94[a]	5.28±0.82[a]
Texture	5.38±0.96[a]	5.30±0.76[a]	5.08±0.57[a]	4.30±0.82[b]
Sweetness	4.89±1.17[a]	5.15±1.17[a]	5.38±1.01[a]	5.43±0.98[a]
Taste	5.15±1.18[a]	5.25±0.98[a]	5.38±1.01[a]	4.70±1.16[b]
After-Taste	4.36±1.11[a]	5.18±1.22[a]	5.28±0.99[a]	4.68±0.62[a]
Overall Acceptance	5.09±0.84[a]	5.18±0.64[a]	5.33±0.62[a]	4.75±0.59[b]

1: Values are stated as mean±standard deviation, n=40.
2: Values with different superscript letters in the same row are significantly different ($p<0.05$).

From the result obtained, it could be observed that the incorporation of Sabah snake powder at 50g or 100g into chocolate filling did not cause

any significant ($p<0.05$) deleterious effect on all organoleptic quality attributes judged as the sensory scores and overall acceptance obtained for these two formulations were above average. However, the addition of Sabah snake grass powder at 150g had caused a significant ($p<0.05$) reduction in the judging score for several attributes, specifically, texture, taste and overall acceptance. The texture and taste were negatively affected because of its coarse texture and herbal taste which subsequently caused an undesirable mouthfeel.

d. Total Phenolic Content of Chocolate

The total phenolic content (TPC) of chocolate produced was shown in Table 5.

Table 5: Total phenolic content of Sabah snake grass leave powder

Formulation	Mean±Standard Deviation(mg GAE/g chocolate)
Control	0.13 ± 0.01^{a}
F1	0.16 ± 0.01^{b}
F2	0.18 ± 0.01^{c}
F3	0.21 ± 0.01^{d}

1: Values are stated as mean±standard deviation, n=3.
2: Values with different superscript letters are significantly different ($p<0.05$).

Chocolates with Sabah snake grass leave powder filling (0.16-0.21mg GAE/g chocolate) had significantly higher ($p<0.05$) TPC content compared to control sample (0.13mg GAE/g dried leaves) because Sabah snake grass leave powder contained high content of phenolic content as reported by Siat (2015).

e. DPPH Radical Scavenging Activity of Chocolate

The DPPH free radical scavenging activity of chocolate produced was shown in Table 6.

Table 6: Free radical scavenging power (EC_{50}) of chocolate extract

Formulation	Mean±Standard Deviation (mg/mL)
Control	22.93 ± 0.18^{a}
F1	19.05 ± 0.38^{b}
F2	17.18 ± 0.27^{c}
F3	15.29 ± 0.33^{d}

`

1: Values are stated as mean±standard deviation, n=3.
2: Values with different superscript letters are significantly different ($p<0.05$).

Chocolates with Sabah snake grass leave powder filling (15.29-19.05mg/mL) had significantly lower ($p<0.05$) EC_{50} values compared to control sample (0.13mg GAE/g dried leaves) because Sabah snake grass leave powder contributed to higher content of phenolic compounds which subsequently improved the antioxidant property of chocolate produced. This is due to the fact that phenolic compounds are the contributor to radical scavenging activity (Li et al., 2014).

f. Chemical Composition

From sensory evaluation and antioxidant results, it can be concluded that chocolate fortified with an amount of 100g Sabah snake grass powder (F2) is the best formulation because this amount give desirable sensory attributes as well as high antioxidant content. The effects of incorporation of Sabah snake grass powder on the chemical composition of chocolate had been shown in Table 7.

Table 7: Chemical composition of chocolate supplemented with 50, 100 and 150g of Sabah snake grass powder.

Component	Mean±Standard Deviation (%)	
	Control	F2
Moisture	6.52±0.21[a]	7.25±0.15[b]
Ash	1.67±0.02[a]	2.19±0.01[b]
Fat	35.21±0.49[a]	30.61±0.34[b]
Protein	4.67±0.02[a]	5.41±0.01[b]
Crude fiber	0.83±0.01[a]	1.94±0.07[b]
Carbohydrate	51.10±0.39[a]	52.61±0.26[b]

1: Values are stated as mean±standard deviation, n=3.
2: Values with different superscript letters are significantly different ($p<0.05$).

Based on Table 3.6, the moisture, ash, protein, crude fiber and carbohydrate of chocolate F2 produced increased significantly ($p<0.05$) compared to control chocolate. However, the fat content of chocolate F2 was significantly lower ($p<0.05$) compared to control chocolate. This is because according to Moses et al. (2015), dried Sabah snake grass leave has a moisture content in the range of 3.53-4.88%, 10.85% mineral, 1.57% fat, 19.26% protein, 17.34% crude fiber and carbohydrate as high as 64.40%. Thus, Sabah snake grass leave can be used as a potential source of food nutrient.

`

g. Chocolate Stability

The best formulation of Sabah snake grass filled chocolate, chocolate F2 and control chocolate were stored for a duration of eight weeks. The moisture content, water activity, hardness, melting point, microbe count and sensory properties of chocolate produced were studied each week.

g1. Moisture Content

The changes in moisture content of chocolate filling and chocolate shell for control chocolate and chocolate F2 during eight weeks of storage were indicated in Figure 1 and 2.

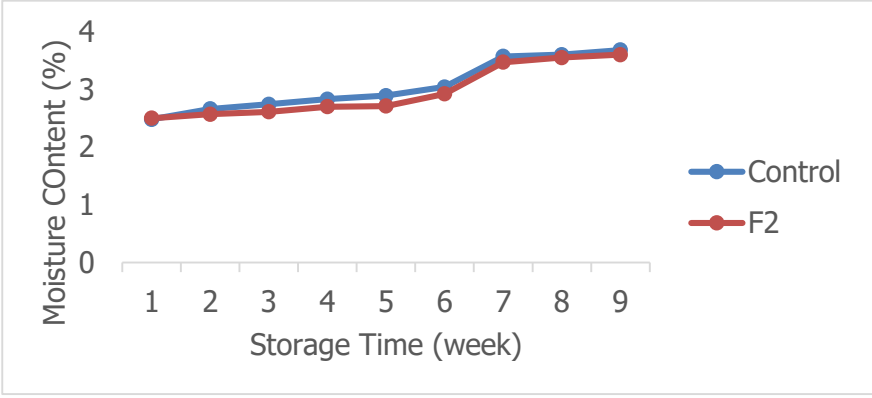

Figure 1: Moisture content of chocolate shell for control chocolate and chocolate F2 during eight weeks of storage

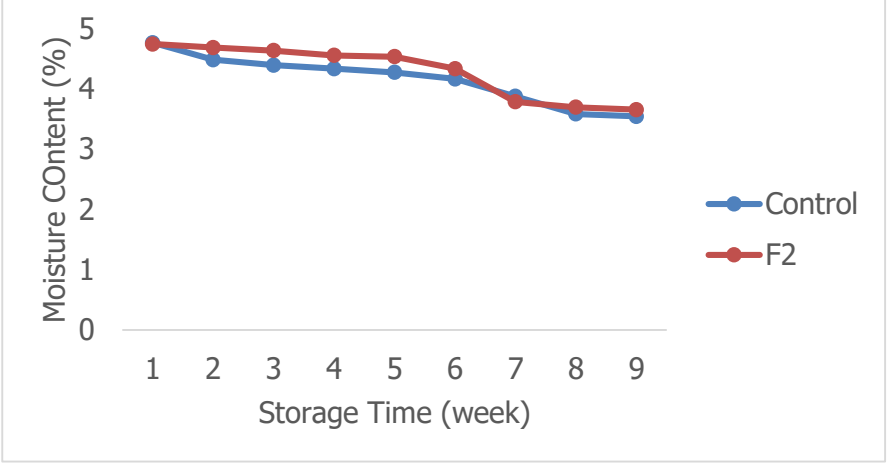

Figure 2: Moisture content of chocolate filling for control chocolate and chocolate F2 during eight weeks of storage

`

Based on Figure 1 and 2, the moisture content of chocolate shells increased throughout the eight weeks of storage whereas the moisture content of chocolate fillings decreased throughout the eight weeks of storage regardless of control sample or chocolate F2 sample. However, significant difference ($p<0.05$) in the moisture content of chocolate shell and filling for control chocolate was observed on week 3 whereas significant difference ($p<0.05$) in the moisture content of chocolate shell and filling for chocolate F2 was only being observed on week 5. This is because the addition of Sabah snake grass powder which is high in fiber content helps to restrict and slow down the movement of water molecules from the filling into the chocolate shell.

g2. Water Activity

The changes in water activity for control chocolate and chocolate F2 during eight weeks of storage were indicated in Figure 3.

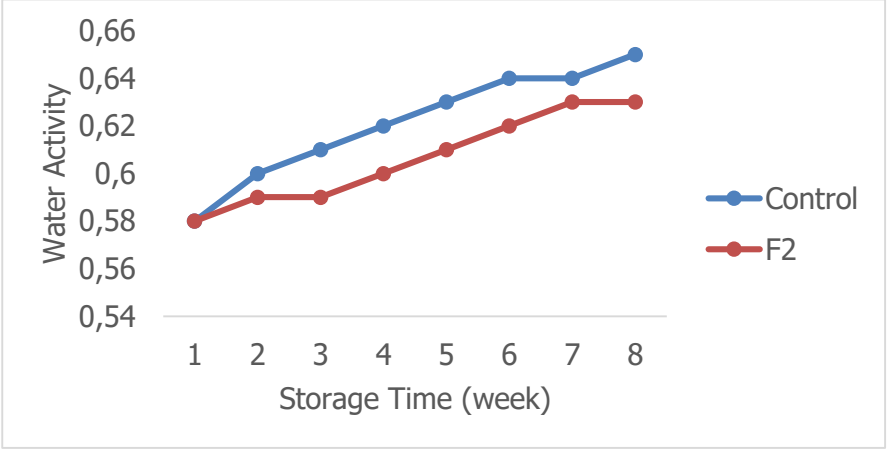

Figure 3: Water activity for control chocolate and chocolate F2 during eight weeks of storage

Based on Figure 3, the water activity of chocolate increase throughout eight weeks of storage regardless of control chocolate or chocolate F2. Water activity increased throughout the storage period due to moisture migration from the filling with higher water activity to the chocolate shell with lower water activity. Migration of moisture content occur until thermodynamic equilibrium was achieved (Sitkiewicz & Palacha, 2006). Significant rise ($p<0.05$) in water activity were detected from the second week for control chocolate and fifth week for chocolate F2. This is due to the presence of Sabah snake grass powder which helps to restrict moisture movement.

g3. Hardness

The changes in hardness for control chocolate and chocolate F2 during eight weeks of storage were indicated in Figure 4.

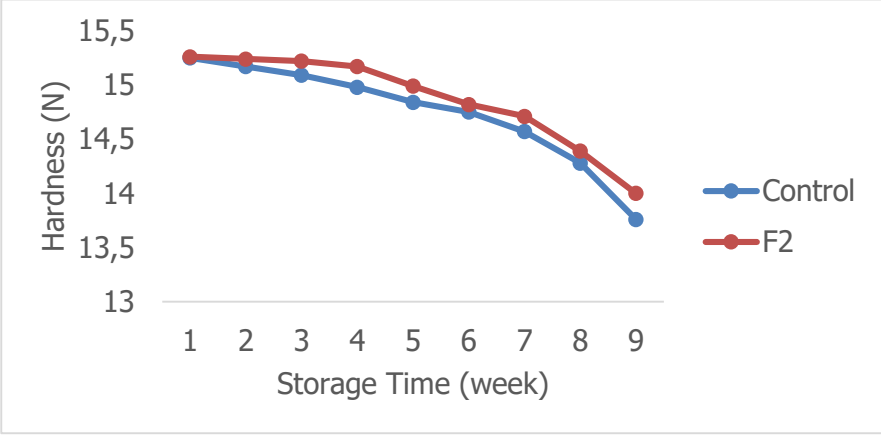

Figure 4: Hardness for control chocolate and chocolate F2 during eight weeks of storage

Based on Figure 4, the hardness for control chocolate and chocolate F2 decreases throughout eight weeks of storage. Significant decrease ($p<0.05$) in hardness were detected on the fifth week for control chocolate and seventh week for chocolate F2. Decrease in chocolate hardness was the result of migration of moisture content which subsequently weaken the chocolate shell by dissolving the sugar content in it (Talbot, 2009). Other factors that may affect the hardness of chocolate are such as chocolate formulation, technique of production, tempering, polymerisation and cooling temperature of chocolate (Machalkova et al., 2014).

g4. Melting Point

The changes in melting point for control chocolate and chocolate F2 during eight weeks of storage were indicated in Figure 5.

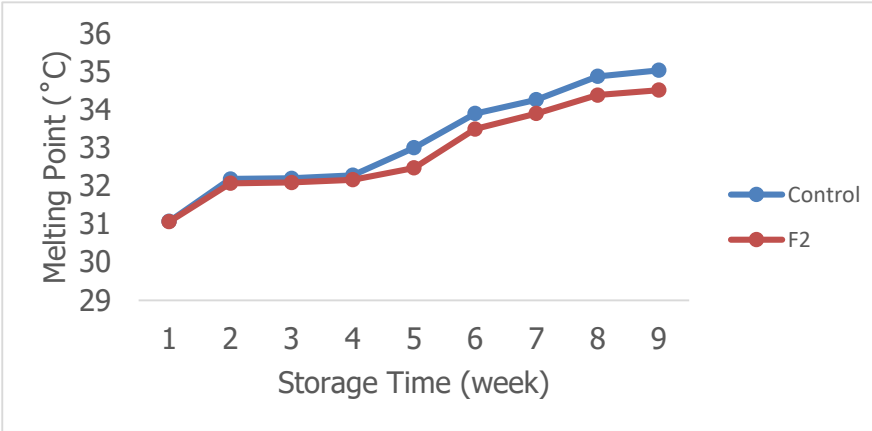

Figure 5: Melting point for control chocolate and chocolate F2 during eight weeks of storage

Based on Figure 5, the melting of chocolate increase throughout eight weeks of storage regardless of control chocolate or chocolate F2. Significant increase ($p<0.05$) in melting point for both control chocolate and seventh week were detected from the first week. Increased in melting point throughout the storage period is due to the changes in the polymorphic structure. Ali et al. (1998) reported that increased in melting point was the consequent of long storage time as stabilization of fat crystal structure occurred. Whereas Nightingale et al. (2011) reported that increased in melting point was the result of formation of tightly packed chain in chocolate samples. Additionally, the high melting point of chocolate at the end of the storage study was clearly due to the formation of form IV polymorphic structure as this form has the highest melting point among the fat crystal structure and consequently lead to fat blooming (Cussler & Moggridge, 2001).

g5. Microbe Count

The changes in microbial counts for chocolate F2 and control chocolate during eight weeks of storage were indicated in Table 8 and 9.

Table 8: Bacterial counts for control chocolate and F2 chocolate during eight weeks of storage.

Week	Total Bacteria Count (cfu/g)	
	Control	**F2**
0	<10	<10
1	<10	<10

2	$1.1x10^1$	<10
3	$9.8x10^1$	<10
4	$1.7x10^2$	1.3×10^1
5	$3.5x10^2$	1.1×10^2
6	$5.2x10^2$	2.3×10^2
7	$6.8x10^2$	3.7×10^2
8	$9.3x10^2$	4.7×10^2

Table 9: Yeast and mold counts for control chocolate and F2 chocolate during eight weeks of storage.

Week	Total Yeast and Mold Count (cfu/g)	
	Control	**F2**
0	<10	<10
1	<10	<10
2	$1.4x10^1$	<10
3	$1.2x10^2$	<10
4	$2.9x10^2$	1.6×10^1
5	$5.6x10^2$	3.3×10^2
6	$8.5x10^2$	4.9×10^2
7	$1.3x10^3$	7.9×10^2
8	$3.6x10^3$	1.2×10^3

Based on Table 8 and 9, control chocolate and chocolate F2 showed increased in microbe count throughout the eight weeks of storage. This is because high nutrient content in chocolate and long storage time had made chocolate sample a suitable for medium for microbial proliferation (Pandey & Singh, 2011). However, the increased in microbial count was lower for chocolate F2. This may due to the fact that Sabah snake grass possess antimicrobial properties (Siat, 2015). Previous research also found that incorporation of herbs into meat products had extended the shelf life of meat products (Lucera et al., 2012).

According to GCC Standardization Organization (GSO), the maximum number of total plate count allowable for chocolate products is not more than 10^6cfu/g. Table 3.7 and 3.8 had indicated that the chocolate products produced were still safe for consumption at the end of the storage study.

g6. Sensory Evaluation

`

The changes in sensory quality characteristics, specifically colour, aroma, texture, sweetness, taste, after-taste and overall acceptability of stored chocolate F2 during eight weeks of storage were indicated in Table 10 and 11.

Table 10: Changes in sensory quality characteristics of chocolate F2 during eight weeks of storage.

Week	Attributes (Mean Score±Standard Deviation)			
	Colour	**Aroma**	**Texture**	**Sweetness**
1	4.05±0.60[a]	4.15±0.74[a]	4.10±0.90[a]	4.15±0.66[a]
2	3.98±0.80[a]	4.13±0.72[a]	4.08±0.66[a]	4.10±0.55[a]
3	3.98±0.36[a]	4.10±0.63[a]	4.05±0.50[a]	4.10±0.67[a]
4	3.95±0.22[a]	4.08±0.53[a]	4.05±0.71[a]	3.90±0.74[a]
5	3.90±0.44[a]	4.05±0.50[a]	3.90±0.98[a]	3.88±0.88[a]
6	3.85±0.74[a]	4.03±0.58[a]	3.80±0.69[b]	3.85±0.77[a]
7	3.60±0.67[b]	3.90±0.67[a]	3.50±0.85[b]	3.83±0.75[a]
8	3.38±0.77[b]	3.78±0.66[a]	3.30±0.88[b]	3.63±0.49[b]

[1:] Values are stated as mean±standard deviation, n=40.
[2:] Values with different superscript letters are significantly different ($p<0.05$).

Table 11: Changes in sensory quality characteristics of chocolate F2 during eight weeks of storage.

Week	Attributes (Mean Score±Standard Deviation)		
	Taste	**After-Taste**	**Overall Acceptance**
1	4.05±0.93[a]	4.08±0.80[a]	4.18±0.64[a]
2	4.03±0.53[a]	4.03±0.58[a]	4.13±0.61[a]
3	4.00±0.45[a]	4.00±0.51[a]	4.08±0.47[a]
4	3.95±0.55[a]	3.98±0.53[a]	4.00±0.45[a]
5	3.95±0.50[a]	3.93±0.57[a]	3.98±0.42[a]
6	3.90±0.81[a]	3.88±0.79[a]	3.93±0.57[a]
7	3.80±0.65[a]	3.80±0.69[a]	3.63±0.54[b]
8	3.73±0.51[a]	3.73±0.51[a]	3.43±0.55[b]

[1:] Values are stated as mean±standard deviation, n=40.
[2:] Values with different superscript letters are significantly different ($p<0.05$).

Based on Table 10 and 11, significant decreased ($p<0.05$) in sensory score for colour, texture, sweetness and overall acceptance of chocolate were detected during the eight weeks of storage. Sensory score for colour

decreased due to the formation of white coloured splotches on the surface of chocolate. This phenomenon is known as fat blooming and it is correlated to the existence of form IV fat crystal in the chocolate (Afoakwa, 2010). Reason for sensory score for texture attribute decreased was due to its coarse texture which was the result of formation of form IV fat crystal. Decreased in sweetness score was also due to the formation of type IV fat crystal which has the potential to reduce the sweetness in chocolate (Nightingale et al., 2011). Overall acceptability of stored chocolate reduced as the result of significant changed ($p<0.05$) in colour, texture and sweetness of chocolate. This research found that these attributes can affect consumer acceptability on chocolate products.

D. CONCLUSION

Study found that vacuum drying was a better drying method compared to cabinet drying as it minimize the loss of antioxidant capacity of Sabah snake grass. Additional of Sabah snake grass powder into white chocolate and whipping cream at ratio of 10:7:3 gave the best acceptability in sensory quality of chocolate. This formulation also significantly better quality in nutrient especially ash, crude fibre and protein as well as higher level of antioxidant activity. The sample also showed better in stability with no significantly changes in physicochemical dan microbial load throughout the storage periods.

REFERENCE

Abou-Zaid, A.A. & Nadir, A.S. 2014. Quality Evaluation of Nutritious Chocolate and Halawa Tahinia produced with Moringa (Moringa oleifera) Leaves Powder. Middle East Journal of Applied Sciences. **4**(4): 1007-1015.

Afoakwa, E. O., Paterson, A., Fowler, M. & Vieira, J. 2008. Characaterization of melting properties in dark chocolates from varying particle size distribution and composition using differential scanning calorimetry. Food Research International, **41**:751-757.

Afoakwa, E.O. 2010. Chocolate Science and Technology. West Sussex: Blackwell Publishing.

Ali, A.R., Moi, L.M., Fisal, A., Nazaruddin, R. & Sabariah, S. 1998. The Application of Borneo Tallow in Dark Chocolate Shell. Journal of Food Science and Agriculture, **76**: 285-288.

AOAC. 2000. Official Methods of Analysis. Gaithersburg: Association of Official Analytical Chemist.

Arullappan, S., Rajamanickam, P., Thevar, N. & Kodimani, C.C. 2014. In Vitro Screening of Cytotoxic, Antimicrobial and Antioxidant

Activities of Clinacanthus nutans (Acanthaceae) leaf extracts. Tropical Journal of Pharmaceutical Research, **13**(9): 1455-1461.

Belščak-Cvitanović, A., Komes, D., Benković, M., Hečimović, I., Ježek, D. & Bauman, I. 2012b. Innovative formulations of chocolate enriched with plant polyphenols from Rubus idaeus L. leaves and characterization of their physical, bioactive and sensory properties. Food Research International, **48**: 820-830.

Bent, A.J., Bennion, E.B. & Bamford, G.S.T. 2013. The Technology of Cake Making. 6th edition. Dordrecht: Springer Science+ Business Media.

Böhme, B., Kretzschmar, R., Schneider, Y., Fiala, P. & Rohm, H. 2012. Effect of Alcohol in Starch-Thickened Fillings on the Storage Stability of Dark Chocolate Pralines. Journal of the American Oil Chemists' Society, **89**: 447-454.

Cussler, E.L. & Moggridge, G.D. 2001. Chemical Product Design. New York: Cambridge University Press.

El-kalyoubi, M., Khallaf, M.F., Abdelrashid, A. & Mostafa M. Eman. 2011. Quality characteristics of chocolate- Containing some fat replacer. Annals of Agricultural Science, **56**(2): 89-96.

Euromonitor International. 2022. Chocolate Confectionery in Malaysia. [Online]. http://www.portal.euromonitor.com.ezproxy.ums.edu.my/portal/analysis/tab. [2 April 2023].

GCC Standardization Organization (GSO). 2014. Microbiological Criteria for Foodstuffs. ms. 20. State of Qatar.

Gounga, M.E. & Xu, S.W. 2008. Nutritional and microniological evaluation of chocolate-coated Chinese chestnut (Castaneamollissima) fruit for commercialuse. Journal of Zhejiang University SCIENCE B, **9**: 675-683.

Hee, Y.Y. & Chong, G.H. 2015. Drying behaviour of Andrographis paniculata in vacuum drying. International Food Research Journal, **22**(1): 393-397.

Institutes of Food Technologists (IFT). 2022. What are Antioxidants? [Online]. Dlm. http://www.ift.org/knowledge-center/learn-about-food-science/food-facts/what-are-antioxidants.aspx. [23 Mac 2023].

International Organization for Standardization (ISO). 2010. ISO 5495:2005: Sensory, Methodology, Paired Comparison Test. Switzerland.

Leite, P.B., Bispo, E.D.S. & Santana, L.R.R. 2013. Sensory Profiles of Chocolate Produced from Cocoa Cultivars Resistant to Moniliophtora Perniciosa. Revista Brasileira de Fruticultura, **35**(2): 594-602.

`

Li, W.Q., Li, Q, Duan, J.L. & Xu, J. 2014. The Effects of Different Drying Methods on Nutrients and Antioxidant Activities of Agaricus bisporus. Advances in Sciences and Engineering, **6**(2): 35-39.

Lucera, A., Costa, C., Conte, A. & Nobile, M.A.D. 2012. Food Applications of Natural Antimicrobial Compounds. Frontiers Microbiology, **3**: 1-13.

Machalkova, l., Hrivna, L., Nedomova, S. & Juzl, M. 2014. The Effect of Storage and Production Method of Chocolate Confectionery on Changes in Its Quality. Potravinarstvo® Scientific Journal for Food Industry, **9**(1):39-47.

Moses, L.M., Hasmadi, M, Zaleha, A.Z. & Mohd Fadzelly, A.B. 2015. Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (Clinacanthus nutans Lind.) Leaves. International Food Research Journal, **22**(2): 661-670.

Naknaen, P., Charoenthaikij, P. & Kerdsup, P. 2015. Physicochemical Properties and Nutritional Compositions of Foamed Banana Powders (Pisang Awak, Musa sapientum L.) Dehydrated by Various Drying Methods. Walailak Journal of Science and Technology, **13**(3): 177-191.

Nightingale, L.M., Lee, S.Y. & Engeseth, N.J. 2011. Impact of storage on dark chocolate: Texture and polymorphic changes. Journal of Food Science, **76**(1):142-153.

Oliveira, S.M., Brandao, T.R.S. & Silva, C.L.M. 2015. Influence of Drying Processes and Pretreatments on Nutritional and Bioactive Characteristics of Dried Vegetables: A Review. Food Engineering Review. DOI10.1007/s12393-015-9124-0.

Pakade, V.E., Cukrowska, E. & Chimuka, L. 2013. Metal and Flavonol Content of Moringa oleifera grown in South Africa. South Africa Journal of Sciences, **109**(3): 835-837.

Pandey, A. & Singh, G. 2011. Development and storage study of reduced sugar soy containing compound chocolate. Journal of Food Science and Technology. **48**(1):76-82.

Pham, H.N.T., Nguyen, V.T., Vuong, Q.V., Bowyer, M.C. & Scarlett, C.J. 2015. Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of Helicteres hirsuta Lour. Leaves. Journal Technologies, **3**: 285-301.

Pothitirat, W., Chomnawang, M.T., Supabhol, R. & Gritsanapan, W. 2009. Comparison of bioactive compounds content, free radical scavenging and anti-acne bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia, **80**(7): 442-227.

`

Siat, Y.F. 2015. Genetic, phytochemical and bioactivity studies of Clinacanthus nutans (Burm. F.) Lindau (Acanthaceae). RMIT Univerity. Tesis Doktor Falsafah.

Siew, K.M. 2014. Kesan Penambahan Daun Moringa oleifera ke atas Ciri-ciri Antioksidan dan Sensori Coklat. Universiti Malaysia Sabah. Tesis Sarjana Muda.

Sitkiewicz, I. & Palacha, Z. 2006. Effect of Aeration Mode and Storage on Selected Physical Properties of Agar Based Confectionery Filling with Chocolate Coating. Acta Agrophysica, **7**(1): 219-230.

Talbot, G. 2009. Science and Technology of Enrobed and Filled Chocolate, Confectionery and Bakery Products. Boca Raton: CRC Press.

Tanongkankit, Y., Chiewchan, N. & Devahastin, S. 2015. Evolution of antioxidants in dietary fiber powder produced from white cabbage outer leaves: effects of blanching and drying methods. Journal of Food Science and Technology, **52**(4): 2280-2287.

Xu, C.C., Cai, Y.M., Zhang, J. & Moriya, N. 2008. Ensiling and subsequent ruminal degradation characteristics of barley tea grounds treated with constrating additives. Animal Feed Science and Technology, **141**: 368-374.

Yong, Y.K., Tan, J.J., Teh, S.S., Mah, S.H., Gwendoline, C.L.E., Chiong, H.S. & Ahmad, Z. 2013. Clinacanthus nutans Extracts Are Antioxidant with Antiproliferative Effect on Cultured Human Cancer Cell Lines. Evidence-Based Complementary and Alternative Medicine, **2013**(2013): 1-8.

`