



Effect of Moringa Leaves Powder Incorporated into Chocolate on the Quality and Stability Properties

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ABSTRACT

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This study was carried out to determine the effect of the addition of *Moringa oleifera* (Moringa) leaves powder? on the antioxidant and sensory properties of chocolate. Three formulations with different percentage of Moringa leaves powder (1, 3, and 5 percent) were studied. Through the hedonic sensory test, there is no significant difference (p less than 0.05) between the formulations for all attributes except the taste. Chocolate with 5 percent Moringa leaves showed the highest total phenolic content and antioxidant activity compared to the other formulations. The addition of moringa leaves caused decreased the chocolate hardness while increased the chocolate average particle size. After 8 weeks of storage, no bloom formation was observed on the surface of the chocolate but the hardness found to be decreased. chocolate associated with moringa leaves powder has potential with health benefit and still has good quality chocolate character.

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I. Introduction

In recent years, several functional foods were introduced into the market when consumers become interested in healthy lifestyle and nutrition (Abu-Hussein, 2017; Martirosyan & Singh, 2015). Currently, the main trend in the global confectionery market is products with functional benefits towards health such as low calorie candies and functional chocolates. Chocolate products were introduced into the functional food market because of their health benefits and exceptional taste. Although chocolate contains high levels of naturally occurring cocoa flavanols, food scientists and technologists are looking for ways to modify chocolates through innovative research. Through these initiatives the beneficial bioactive components of cocoa can be preserved (Glaberson, 2013).

Chocolates are normally consumed as chocolate confectioneries, chocolate-coated products (biscuits and ice-creams), and also other food products that contain cocoa or chocolate powder such as beverages, cakes, and snacks. Cocoa and chocolate products are special food for hundreds of years. Chocolate is produced from the seed of *Theobroma cacao* originated from South America. Now, chocolate is enjoyed all around the world (Afoakwa, 2016). The consumption of chocolate confections in the world increases annually and it was estimated approximately 5.539 million tons in the year 2010 (ICCO, 2012). Chocolate is a nutritious source of energy with high nutritional values. The health benefits of chocolate have gained the interest of scientists and nutritionists because it has the ability to lower the risk of chronic diseases such as cardiovascular diseases (CVD) and cancer (Afoakwa, 2016).

Phenolic compounds of plant origin have gained attentions because of their beneficial and functional effects including antioxidant and antimicrobial activities. Oxidation is the main process of free radical formation in foods, chemicals, and organisms. Degradation of food and chemicals are influenced by free radicals. Phenolic compounds act as free radical scavengers and protect the human body from free radicals. In addition, phenolic compounds can retard fat oxidation to increase the shelf-life of food (Cicerale, Conlan, Sinclair, & Keast, 2008). Various types of method used to determine

the phenolic compound content and antioxidant of food (Číž et al., 2010; Huang, Ou, & Prior, 2005; Wang, Jonsdottir, & Ólafsdóttir, 2009). In past research, most phenolic compounds used in the enrichment of chocolate originated from plant sources such as ginseng, green tea, *Ginkgo biloba*, guarana, red raspberry leaves and dried fruits (Belščak-Cvitanović et al., 2012; Komes, Belščak-Cvitanović, Škrabal, Vojvodić, & Bušić, 2013).

Moringa oleifera (Moringa) is a high value plant in tropical and subtropical countries such as India, Pakistan, Philippines, Thailand, and Africa. Moringa leaves are found to contain high levels of natural antioxidants (Siddhuraju & Becker, 2003). Moreover, Moringa leaves are also rich in minerals, amino acids, vitamins, β -carotene, and various phenolic compounds. It is used as traditional medicine in the prevention and treatment of several ailments such as gastric ulcer, skin disease, fever, fatigue, and bronchitis (Anwar, Latif, Ashraf, & Gilani, 2007). In previous studies, Moringa leaves were added in the development of bread (Sengev, Abu, & Gernah, 2013), cookie (Dachana, Rajiv, Indrani, & Prakash, 2010), and rice cracker (Manaois, Morales, & Abilgos-Ramos, 2013). The aim of the present study was to identify the suitability of Moringa leaf powder incorporated in chocolate and determined the antioxidant and sensory properties of chocolate enriched with dried Moringa leaf powder.

II. Materials and Methods

A. Materials

Fresh *Moringa oleifera* leaves were supplied by Borneo Moringa Sdn. Bhd., Nabawan, Sabah. Materials for chocolate preparation such as cocoa powder, cocoa butter substitute (CBS), white chocolate, castor sugar, and whipped cream were purchased from a local baking ingredients supplier.

Chemicals such as petroleum ether, sulphuric acid, potassium phosphate, copper (II) sulphate, sodium hydroxide, boric acid, bromocresol, and hydrochloric acid were purchased from Merck, Germany. Chemicals for antioxidant analysis are n-hexane, methanol, Folin-Ciocalteu reagent (FCR), sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) powder, butylated hydroxyanisole (BHA), glacial acetic acid, sodium acetate trihydrate, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid, iron (II) chloride, and iron (II) sulphate heptahydrate (Sigma, USA).

B. Preparation of *Moringa oleifera* Leaves

The preparation of *Moringa oleifera* (moringa) leaves was conducted according to Iqbal and Bhangar (2006); Vongsak et al. (2013) with some modifications. The leaves were soaked in water for 1 min to remove foreign materials. The leaves were then dried in a drying cabinet at 50°C for 16 h. The dried leaves were grinded using an electric grinder and sieved with a 125 μm sieve. The Moringa leaf powder was kept in an airtight container at 7-10°C until further use.

C. Formulation of Chocolates

The method for making the chocolates was described by Belščak-Cvitanović et al. (2012) with some modifications. The following formulation used for the production of dark chocolate: 25% cocoa powder, 45% CBS, and 30% sugar. The formulation for the filling of the chocolate developed based on combination of moringa leaf powder in range of 1 to 5%, white chocolate (65-70%) with fixed amount of whipped cream (30%). The CBS melted at 80°C and the cocoa powder and sugar were added into the molten CBS. The ingredients were mixed and the mixture was refined using a refiner for 5 h to grind the sugar and cocoa particles to the required fineness of 18-35 μm . The particle size of the mixture was tested with a Hegman Gauge. In order to produce the filling, white chocolate was grated and melted at 80°C. After that, whipped cream was added and mixed. moringa leaf powder was then added to the mixture and stirred.

The refined dark chocolate was heated and poured into plastic moulds. The moulds were tapped to remove any air bubbles in the chocolate and the moulds were inverted before the chocolate totally became firm for shell formation. The moringa filling was added into the centre of the chocolate shell using a piping bag. The filled shells were covered with the dark chocolate and tapped. The chocolates were chilled in the refrigerator at 7-10°C. The chocolates were removed from the moulds and packed into polyethylene (PP) bags. The packed chocolates were stored in the refrigerator (2-4°C) until analysis.

D. Sensory Evaluation

A hedonic test was carried out to evaluate the three chocolate formulations where 40 panellists consisting of students from the Faculty of Food Science and Nutrition, University Malaysia Sabah were chosen at random to participate in the hedonic test. The chocolates were evaluated based on colour, aroma, texture, sweetness, taste, aftertaste, and overall acceptability. A seven point hedonic scale was used in the evaluation of the chocolates (1 = dislike extremely and 7 = like extremely) (Meilgaard, Carr, & Civille, 2006).

E. Antioxidant Capacity of Chocolates

1) Preparation of Chocolate Extracts

The chocolate extracts were prepared as reported by Belščak-Cvitanović et al. (2012). The chocolate products were frozen and grinded. To remove fats from the samples, 8.00 ± 0.01 g of each chocolate sample was extracted three times with 10 mL n-hexane. The defatted samples were air-dried for 24 h to remove residual organic solvent. About 4.00 ± 0.01 g of each defatted chocolate sample was extracted with 20 mL of 80% methanol for 25 min in an ultrasonic bath (Branson 8510, Branson). The mixture was centrifuged for 10 min at 6000 rpm and the supernatant was decanted. The residual sample was extracted again and the supernatants were combined. The extract was stored in a freezer at -20 °C.

2) Determination of Total Phenolic Content (TPC)

According to a method described by Belščak-Cvitanović et al. (2012), total phenolic content of the chocolate extracts was determined by a UV-vis spectrophotometer (Lambda 35, PerkinElmer) using the Folin-Ciocalteu reagent. All measurements were performed in triplicates. Gallic acid (0.1-0.5 mg/ml) was used for the calibration and the results were expressed as mg gallic acid equivalents (GAE)/g of sample

3) DPPH Free Radical Scavenging Activity

Antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Pothitirat et al. (2009). All measurements were performed in triplicates. BHA (0.001-0.100 mg/ml) was used as a standard and the results were expressed as EC_{50} . EC_{50} is the amount of sample required to reduce the initial DPPH concentration by 50%.

4) Ferric Reducing Antioxidant Power (FRAP)

Antioxidant activity was also determined by the ferric reducing antioxidant power (FRAP) as described by Wangcharoen and Gomolmanee (2013). Iron (II) sulphate heptahydrate was used as the standard with a concentration range of 0.1 to 2.0 mM/ml and the results were expressed as mM Fe(II)/ml (mM Trolox/ml). All measurements were performed in triplicates.

F. Proximate Analysis

Moisture, ash, fat, protein, and crude fibre contents were determined as described in AOAC methods (AOAC, 2000). The total carbohydrates content was obtained by subtracting the sum of the other major ingredients (moisture, ash, fat, protein, and fibre) from 100%.

G. Determination of Physical Properties of Chocolate

1) Determination of Particle Size Distribution

The determination of the particle size distribution was done using the Zetasizer Nano S Particle Size Analyser (Zen 1600, Malvern) as described by Belščak-Cvitanović et al. (2012), with some modifications. About 0.1 ± 0.01 g of defatted chocolate was dispersed in 5 ml sunflower oil at ambient temperature (20 ± 2 °C). To ensure the particles were independently dispersed, the mixture was stirred in an ultrasonic bath for 2 min. The sample was transferred into a cuvette with a maximum height of 10 mm. Mean particle size was measured and all measurements were performed in triplicates.

2) Texture Analysis

Texture analysis was performed using a texture analyser (TA.XT plus, Stable Micro Systems) as described by Belščak-Cvitanović et al. (2012). The chocolate samples were taken out from the refrigerator and left at room temperature for 1 h before analysis. Penetration test was carried out using 2 mm cylinder probe and a 10 kg load cell. The samples were penetrated at a speed of 1.0 mm/s at a distance of 7.5 mm. The maximum force (N) needed for penetration gives an indication of the sample hardness. All measurements were performed in triplicates.

H. Storage Quality Study

1) Determination of Water Activity

Water activity of the chocolate samples was determined as described in the AOAC method (AOAC, 2000) using a water activity meter (EW-37910-45, Cole Parmer) before and after the bloom cycle test.

2) Bloom Cycle Test

Bloom cycle test was carried out to determine the shelf-life of the chocolate as described by Hodge and Rousseau (2002). The samples were stored in an incubator at a fixed cycle. Each cycle represents 24 h, where the samples were stored at 32°C for 8 h and at 20°C for 16 h. The formation of white spots on the surface of the chocolate was observed during the storage period. Water activity and texture analysis were carried out after 8 weeks of storage.

I. Statistical Analysis

All data collected were analysed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software. Analysis of variance (ANOVA) was performed for the hedonic test and the antioxidant capacity of the chocolate. The *t*-test was performed to compare between the control sample and the best formulation in terms of proximate, physical properties, and storage quality. The significant difference is $p < 0.05$.

III. Results and Discussion

A. Sensory Evaluation

Table 1 shows the mean score for sensory evaluation of the chocolates. Results from ANOVA showed that the difference in terms of mean score for each attribute is not significant ($p > 0.05$) except for the taste. There is only a significant difference ($p < 0.05$) between chocolates enriched with 1 and 5% moringa leaves powder in terms of the taste. The moringa leaves powder gave an unpleasant herbal taste to the chocolates. Although colour and texture plays an important role in the perception of chocolate, Dürschmid et al. (2006) reported that the taste and mouthfeel were the most significant sensory categories for describing chocolates. The low acceptability of the chocolate taste is associated with herbal taste of the moringa leaves powder. The grassy taste was often make limitation of the application of herbs into food as shown in this study. Similar results were reported in the development of food products with moringa leaf powder such as bread (Sengev et al., 2013), rice cracker (Manaois et al., 2013), and cookie (Dachana et al., 2010). The use of flavourings to mask the unpleasant herbal taste of the leaf powder may help to improve the acceptability of the product. Aftertaste is described as the residual taste that is left on the tongue after swallowing (Murano, 2003) but overall acceptance showed no significantly different ($p > 0.05$) among the samples so sample associated with 5% moringa leaves powder still can be accepted.

Table 1. Min Score of Hedonic SWNSORY Test of Chocolate Filling with Moringa Leaf Powder

Attribute	Moringa Leaves Powder Content (%)			
	Control	1	3	5
Colour	5.60 ^a ± 0.10	5.53 ^a ± 0.99	5.40 ^a ± 1.15	5.58 ^a ± 1.01
Aroma	6.03 ^a ± 1.02	5.93 ^a ± 1.02	5.98 ^a ± 1.10	5.85 ^a ± 1.23
Texture	5.49 ^a ± 1.08	5.35 ^a ± 1.08	5.28 ^a ± 1.13	5.18 ^a ± 1.06
Sweetness	5.15 ^a ± 1.01	5.10 ^a ± 1.01	5.18 ^a ± 0.96	5.13 ^a ± 0.85
Taste	4.67 ^a ± 1.08	4.58 ^a ± 1.08	4.50 ^{ab} ± 1.32	3.48 ^b ± 1.63
Aftertaste	5.55 ^a ± 1.33	4.75 ^a ± 1.33	4.58 ^a ± 1.28	4.55 ^a ± 1.47
Overall Acceptability	5.83 ^a ± 1.40	5.80 ^a ± 1.40	5.66 ^a ± 1.37	5.64 ^a ± 1.22

*Values superscripted with the same alphabet at same row are not significant ($p < 0.05$).

B. Antioxidant Capacity of Chocolates

1) Total Phenolic Content (TPC)

From the present study, the chocolates enriched with moringa leaf powder showed an increase in TPC as compared to the control sample. Table 2 shows the TPC of chocolate samples after the addition of moringa leaf powder. Chocolate enriched with 3% and 5% leaf powder showed a significant

difference ($p < 0.05$) in terms of TPC. The addition of 5% leaf powder increased the TPC to 13.82 ± 0.09 mg GAE/g. Increase in TPC was reported to have a positive relationship with antioxidant activity of chocolate (Komes et al., 2013). According to Cooper et al. (2008), the presence of non-fat cocoa solid (NFCS) is a good indicator to determine the total phenolic content. Therefore, theoretically, high NFCS content shows high phenolic content in chocolate. Dark chocolate showed higher TPC because of higher NFCS as compared to milk chocolate and white chocolate (Belščak-Cvitanović et al., 2012).

Table 2. The Total Phenolic Content (TPC), DPPH (EC_{50}) and FRAP Value of Control and Chocolate Filling with Moringa Leaves Powder

Chocolate Sample	TPC (mg GAE/g)	DPPH (mg/mL)	FRAP (mM Trolox/g sample)
Control	$9.31^a \pm 0.06$	$14.42^a \pm 0.09$	$8.55^a \pm 0.52$
MLP 1%	$10.20^a \pm 0.06$	$13.53^a \pm 0.10$	$9.32^a \pm 0.13$
MLP 3%	$12.10^b \pm 0.06$	$11.70^b \pm 0.16$	$11.79^b \pm 0.67$
MLP 5%	$13.83^c \pm 0.09$	$8.83^c \pm 0.01$	$14.86^c \pm 0.71$

* Values superscripted with the different alphabet at the same row are significant different ($p < 0.05$).

** MLP = moringa leaves powder

2) DPPH Free Radical Scavenging Activity

As observed for the free radical scavenging activity of the chocolate extract increased with the addition of moringa leaves powder. This shows the increased in antioxidant activity of the chocolate enriched with moringa leaves powder. Chocolate extract with 5% moringa showed the highest scavenging activity compared to other formulations. From Table 2, chocolate extract with 5% moringa required the lowest concentration, which is 8.83 ± 0.01 mg/ml to achieve EC_{50} of scavenging activity compared to the control sample. There is a significant difference ($p < 0.05$) between the control sample and the chocolate enriched with moringa leaves powder. The concentration of extracts decreased significantly ($p < 0.05$) when the percentage of moringa leaves increased except for the chocolate with 1% moringa leaf powder. This proved that the antioxidant activity of chocolate increased when the percentage of moringa leaves increased. Dark chocolate has higher antioxidant activity in terms of free radical scavenging activity as compared to other types of chocolates (Tabernerero, Serrano, & Saura-Calixto, 2006).

3) Ferric Reducing Antioxidant Power (FRAP)

FRAP of the chocolate extract increased with the addition of moringa leaf powder as shown in Table 2. Increase in FRAP value can be associated with increase in antioxidant activity of chocolate. Chocolate samples enriched with 3 and 5% moringa leaf powder showed significant increases ($p < 0.05$) in ferric reducing capability with values of 11.79 ± 0.67 and 14.86 ± 0.71 respectively as compared to 1% (9.32 ± 0.13). According to a study done by Belščak-Cvitanović et al. (2012), the ferric reducing capability of chocolate increased significantly ($p < 0.05$) after the addition of 3% raspberry leaf extract. The determination of TPC and FRAP were reported to have high correlation and the reducing capability of a sample is a direct measure of its antioxidant activity.

C. Proximate Analysis

The best formulation was chosen based on the balance between acceptability and antioxidant values of the chocolate, which is the chocolate with 5% moringa. The addition of moringa leaf powder has significantly increased the nutrition value of the chocolate. An increase in moisture content observed after addition of 5% moringa leaf powder. This increment is associated to the initial moisture content of the moringa leaf powder. The addition of 5% moringa powder increased the ash content of the chocolate samples due to the increase in mineral content. Minerals that were reported at high levels in the moringa leaves are calcium, iron, and potassium (Sengev et al., 2013). In previous studies, the addition of 5% moringa powder was reported to increase the ash content of rice cracker (Manaois et al., 2013) and flat noodle (Abilgos, 1996).

Fat content was found to be lower when the chocolate samples enriched with 5% moringa powder. The fat content of the chocolate decreased by 28.63% after the addition of 5% moringa powder. According to Manaois et al. (2013), increased in level of moringa powder decreased the fat content of rice cracker. In cookies, there is no significant increase ($p < 0.05$) in fat content after the addition of

5%, 10% and 15% moringa leaves powder (Dachana et al., 2010). The chocolate samples enriched with 5% moringa leaves powder showed an increase in protein content. The protein content of chocolate increased from 4.85% to 6.90%. Moringa leaves powder was reported to be high in protein content ($31.74 \pm 0.34\%$) and is comparable to the protein content of soya bean and milk (Satwase, Pandhre, Sirsat, & Wade, 2013). According to Manaois *et al.* (2013), the protein content of rice cracker increased after addition of 1% moringa leaves powder. Sengeev *et al.* (2013) also reported an increase in protein content of bread enriched with moringa leaves powder. In the production of flat noodles, addition of 5% moringa leaves powder increased the protein content significantly ($p < 0.05$) (Abilgos, 1996).

The addition of moringa leaves powder also increased the crude fibre content of the chocolate samples. The crude fibre content was increased by 54.09% as compared to the control sample. This is due to the high crude fibre content of moringa leaves ($19.32 \pm 0.03\%$) (Sengeev et al., 2013). The high fibre content in moringa leaves is sufficient to improve the movement of food through the alimentary canal with potential to prevent colon cancer (Satwase et al., 2013). Sengeev (2013) reported that the crude fibre content of bread increased significantly ($p < 0.05$) after the addition of moringa leaves powder. Chocolate enriched with moringa powder showed an increase in carbohydrate content of 19.39%. Sengeev (2013) mentioned that there is no significant difference ($p > 0.05$) in carbohydrate content of bread although the percentage of moringa leaves powder increased. Similar observation was reported in the development of rice cracker with moringa leaves powder (Manaois et al., 2013).

D. Physical Properties of Chocolate

1) Particle Size Distribution (PSD)

Particle size distribution (PSD) of chocolate samples increased after the addition of moringa leaves powder. Similar results were reported by Belščak-Cvitanović et al. (2012) after raspberry leaf extract added into chocolate. Large particle size of chocolate was influence the perception of mouthfeel. The particle size of the control sample was $17.85 \pm 0.49 \mu\text{m}$ whereas the chocolate enriched with 5% moringa leaves powder had a particle size of $22.82 \pm 0.34 \mu\text{m}$. The difference in particle size is not significant ($p > 0.05$). The particle size increased as the chocolate was not further refined after the addition of moringa leaves powder. However, the particle size of the chocolate samples was within the range of 18-50 μm (Afoakwa, 2016).

2) Texture Analysis

When compared to the control sample, the addition of Moring leaf powder decreased the hardness of chocolate. From the texture analysis, the hardness of the control sample was $19.52 \pm 0.27 \text{ N}$ while the chocolate sample enriched with 5% moringa leaves powder had a hardness of $13.77 \pm 0.30 \text{ N}$. There is no significant difference ($p > 0.05$) in terms of hardness of the chocolate samples. Similar results were observed by Anwar et al. (2007) when the raspberry leaves extract was added into the chocolate. Insignificant reduction ($p > 0.05$) was observed when 3% raspberry leaves extract was added into the chocolate. Decrease in hardness is affected by increase in particle size (Afoakwa, 2016).

E. Storage Quality Test

1) Water Activity

The addition of moringa leaves powder has increased the water activity (a_w) of the chocolate. The a_w value of the control chocolate was 0.51 ± 0.01 while the a_w of the chocolate enriched with moringa leaves powder was 0.57 ± 0.01 . The difference in a_w was not significant ($p > 0.05$). The increase in a_w after the addition of moringa leaves powder was associated with the high moisture content of the moringa leaves. However, both a_w values were below the critical level of 0.6 (Stahl, 2010).

2) Bloom Cycle Test

In the bloom cycle test, varying temperatures will increase the rate of fat bloom formation. One week of bloom cycle represents one month of actual storage. After 8 weeks of bloom cycle, no formation of white or gray spots was observed on the surface of the chocolate. Texture analysis showed a decrease in hardness of the chocolate after 8 weeks. The hardness of the control chocolate was $5.65 \pm 0.12 \text{ N}$ while the hardness of the chocolate enriched with moringa leaves powder was $3.39 \pm 0.17 \text{ N}$. The decreased in chocolate hardness was associated with the formation of unstable crystals due to change in temperature. The difference in hardness before and after the bloom cycle was significant ($p < 0.05$). Water activity test showed that the increase in a_w value was not significant

($p > 0.05$) for both samples after 8 weeks of bloom cycle. The a_w value of the control chocolate was 0.51 ± 0.01 while the chocolate enriched with moringa leaves powder was 0.57 ± 0.01 .

IV. Conclusion

Chocolate filling with 5% moringa leaves powder has potential to develop with significantly increased the crude fibre content up to 54% and improved the total phenolic content (TPC) and antioxidant activity (FRAP and TRAP) as compared to control. The chocolate was also stable throughout the period of storage with maintained the physicochemical characteristics and well accepted by the sensory panels.

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