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Original Article

Physicochemical characteristics, total phenolic content, antioxidant activity, and sensory acceptability of panned keto dark chocolate with monk fruit and stevia as sugar substitutes

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Abstract

This study explored the use of natural sweeteners, monk fruit (*Siratia grosvenorii*) and stevia (*Stevia rebaudiana*), as sugar substitutes in panned dark chocolate. The research evaluated the physicochemical properties (moisture, water activity, lightness, pH, and hardness), total phenolic content (TPC), antioxidant activity (DPPH and FRAP assays), and sensory acceptability of chocolate formulations using these sweeteners. The icing sugar (29.5%) was used for the control sample (F1), while it was substituted with stevia and monk fruit for F2 and F3, respectively. Results indicated that substituting sugar with monk fruit and stevia did not significantly affect the physicochemical properties. However, F2 (stevia) exhibited higher antioxidant activity and TPC (210.29 ppm IC50, 17.02 mg GAE/g FRAP, and 28.91 mg GAE/g TPC) compared to the control. The overall acceptability score for F2 (5.91) was the lowest (6.00–6.73), although the glossiness, texture, cocoa flavour, and bitterness were comparable to the control. Overall, monk fruit and stevia proved to be viable sugar substitutes in panned dark chocolate, offering higher antioxidant activity and TPC while maintaining consumer acceptability.

Keywords: physicochemical, antioxidant activity, sensory acceptability, panned dark chocolate, stevia, monk fruit

1. Introduction

A sweetener, also known as a sweetening agent, is the kind of food additive that functions to provide a sweet taste to food. Besides that, it also serves other additional purposes as preservative, texture modifier, fermentation substrate, flavouring and colouring agent, and bulking agent (Aguilar, Acosta, Rodriguez & Mazo, 2020). Natural sweeteners are sweeteners that can be obtained or derived from plants and animals and must be edible and suitable for consumption (Saraiva, Carrascosa, Raheem, Ramos, & Raposo, 2020). Based on a review article by Milbrand (Milbrand, 2023), monk fruit and stevia are some examples of well-known healthy sugar substitutes today.

Stevia, a natural sweetener from *Stevia rebaudiana* Bertoni, originates in Paraguay, Brazil, and Argentina. While there are 230 species of *Stevia*, only *S. rebaudiana* produces the sweet steviol glycosides, which are 250–300 times sweeter than sucrose but with no added calories, making it ideal for those managing diabetes, cholesterol, or cardiovascular issues (Peteliuk *et al.*, 2021). The FAO/WHO has set a safe consumption limit of 4 mg/kg body weight per day for stevia (Ashwell, 2015).

Monk fruit (*Siraitia grosvenorii*), a vine from southern China and northern Thailand, is also a popular zero-

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calorie sweetener. Its sweetness, derived from mogrosides IV and V, is 250–400 times that of sucrose, allowing for minimal usage (Muñoz-Labrador *et al.*, 2022). Monk fruit's low glycemic index prevents spikes in blood sugar, making it beneficial for diabetics (Yeung, 2023). Recent studies explore sugar replacement in confectioneries, especially chocolate, using these natural sweeteners (Ibrahim *et al.*, 2020).

Sugar plays a critical role in determining the physicochemical properties of dark chocolate, influencing factors such as texture, sweetness, moisture content, and overall stability. It affects key attributes like water activity, pH, and hardness, which contribute to the product's shelf life and sensory characteristics. Replacing sugar with alternative sweeteners can impact these properties. Studies have shown that the structure of chocolate, particularly its crystallization behaviour, is heavily dependent on the type and concentration of sugars used (Beckett, 2017). For instance, sugar contributes to the chocolate's snap and gloss due to its interaction with cocoa butter, an essential factor for high-quality dark chocolate (Frontera *et al.*, 2021).

In recent years, natural sweeteners such as monk fruit and stevia have gained attention as sugar substitutes in chocolate due to their non-caloric nature and health benefits (Muñoz-Labrador *et al.*, 2022). Stevia, derived from *Stevia rebaudiana*, is 250–300 times sweeter than sucrose and has minimal effects on blood glucose levels (Peteliuk *et al.*, 2021). Similarly, monk fruit contains mogrosides, which are 200–400 times sweeter than sucrose but have a negligible caloric impact (Yeung, 2023). This study aimed to explore the physicochemical and sensory of keto dark chocolate formulated by substitution sugar with these sweeteners.

In addition to their sweetness and minimal caloric impact, both monk fruit and stevia are known for their antioxidant properties, which can contribute to the functional benefits of food products. Monk fruit contains mogrosides, which have demonstrated antioxidant activity by neutralizing free radicals and reducing oxidative stress. Similarly, stevia is rich in steviol glycosides, which exhibit antioxidant potential that can improve the stability and nutritional profile of food systems (Peteliuk *et al.*, 2021). Incorporating these sweeteners into chocolate formulations not only addresses consumer demand for healthier options but also enhances the product's functional properties, particularly its antioxidant capacity.

2. Materials and Methods

2.1.1 Materials

Cocoa liquor, lecithin, and sweeteners have been supplied and provided by the Malaysian Cocoa Board, Cocoa Innovative and Technology Centre, Nilai, Negeri Sembilan. The three types of sweeteners that have been used in this study were refined icing sugar (Central Sugars Refinery Sdn. Bhd., Malaysia), monk fruit (Lakanto, Saraya Goodmaid Sdn. Bhd., Malaysia) and stevia (MH Food, Matahari Sdn. Bhd., Malaysia). All the sweeteners were in powder format.

2.1.2 Production of panned keto dark chocolate with monk fruit and stevia as sugar substitutes

The panned keto dark chocolate was developed using three alternative sweeteners which were icing sugar (F1), stevia (F2), and monk fruit (F3), based on the formulation in Table 1. The chocolate panning process was done by using an automated panning machine. The melted chocolate was added gradually into the centre of the panning machine. The machine kept tumbling during the processing to ensure the centre was coated entirely to build up the chocolate coating. The melted chocolate was added continuously until the desired thickness of the coating was obtained.

2.1.3 Determination of water activity

The water activity of the panned keto dark chocolate was measured by using a water activity meter (Aqualab 4TE, Meter Food Group, Washington, USA).

2.1.4 Determination of pH

The pH analysis was conducted to determine the acidity or alkalinity of the panned chocolate by using the pH meter (Fisher Scientific™ Fisherbrand™ Accumet™ AB315 pH/mV). The sample was extracted with methanol in a ratio 1:10.

2.1.5 Determination of texture profile

The texture profile of panned keto dark chocolate was evaluated by using the Texture analyser (TA. XT Plus, Texture Technologies, Scarsdale, NY). The load cell of 2000 g and compression platens (P/75) have been used and the primary TPA characteristic measured was hardness.

2.1.6 Determination of colour

The colour analysis of the panned chocolate was conducted by using the Minolta chroma meter (CR-400; Konica Minolta, Japan), using the CIE system. The symbols

Table 1. Formulations of panned keto dark chocolate samples

Ingredient	Chocolate Formulations		
	F1 (%)	F2 (%)	F3 (%)
Cocoa mass	70.0	70.0	70.0
Icing sugar	29.5	-	-
Stevia sweetener	-	29.5	-
Monk fruit sweetener	-	-	29.5
Lecithin	0.5	0.5	0.5

Note: F1 = Panned keto dark chocolate with icing sugar, F2 = Panned keto dark chocolate with stevia, F3 = Panned keto dark chocolate with monk fruit.

L*, a* and b* represent brightness, green/red colour coordinate, and blue/yellow colour coordinate, respectively.

2.1.7 Determination of proximate composition

The Association of Official Analytical Chemist (2000) procedure was used to determine the proximate composition in terms of moisture, crude protein, crude fat, ash, crude fibre, and carbohydrate.

2.1.8 Determination of antioxidant activity

The method used for the antioxidant extraction of the panned keto dark chocolate was based on Medina-Mendoza *et al.* (2023). The extraction of the sample was conducted by adding 10 mL of methanol into 1.0 g of panned keto dark chocolate and centrifuging for 15 min at the speed of 3,500 rpm to obtain a clear supernatant.

Two methods, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by Akowuah, Ismail, Norhayati, and Sadikum (2005) and ferric reducing antioxidant power (FRAP) by Benzie and Strain (1999), were used to measure the antioxidant activity of panned keto dark chocolate.

2.1.9 Determination of total phenolic content

The Folin-Ciocalteu method was utilised to determine the total phenolic content (TPC) of the panned keto dark chocolate (De Camargo, Regitani, Gallo, & Shahidi, 2015).

2.1.10 Determination of sensory acceptability

54 panellists including students and staff of both genders from Universiti Teknologi MARA (UiTM) Shah Alam as well as the Malaysian Cocoa Board, Nilai, were involved in the sensory evaluation. 9-point Hedonic scale based on several attributes including colour, glossiness, texture, cocoa flavour, sweetness, bitter aftertaste, and overall acceptance, were evaluated by the panellists.

2.1.11 Statistical analysis

Three measurements per sample were recorded for each analysis, and one-way analysis of variance (ANOVA) was used to determine whether there were significant differences in mean values between different samples, followed by Duncan's *post hoc* comparison at a 95% confidence level using IBM SPSS statistics version 28.0.

3. Results and Discussion

3.1 Physical analysis of panned keto dark chocolate

The results of the physical analysis in terms of water activity, pH, hardness from texture profile analysis, and colour coordinates L*, a*, and b*, are shown in Table 2.

Based on the data presented in Table 2, a_w showed no significant difference between F2 and F3, but these formulations had significant differences ($p<0.05$) with F1. Based on the data, the a_w for all the formulations were between 0.39 to 0.42, which were relatively low, thus microbial growth could be prevented. This result agrees with the water activity levels for hard panned coatings in a previous study, which was between 0.40 and 0.75 (Hartel, Von, Elbe, & Hofberger, 2018). Therefore, the choice from alternative types of sweeteners did not affect the water activity of the panned chocolate. According to research conducted by Norhayati, Suzielawanis, Rasma, and Khan (2013), a_w for dark chocolate must not be more than 0.5 if it is stored at 25 °C and 80% relative humidity (RH) (incubator). This is because dark chocolate with a water activity higher than 0.5 was shown to have fungal growth. However, if it was stored at 18 °C with lower RH at 60% (chiller cabinet), the fungal growth was prevented. Generally, high temperature and relative humidity stimulate and enhance the growth of fungi. A low water activity level is important in chocolate production, as it not only prevents microbial growth but also retains the product stability by preventing the chocolate cracking and the formation of fat bloom.

In terms of pH of the developed panned chocolate, there was no significant difference between F1 and F2, yet these had significant differences ($p<0.05$) with F3. In a previous study, the pH of 70% dark chocolate was slightly acidic ranging between 6.5 and 6.7 (Abdalbasit, Gasmalla, Yang, Amadou, & Xiao, 2014). Nonetheless, the developed panned chocolate had a lower pH level, which might be influenced by the pH of added sweetener in the panned chocolate. Abdalbasit, Gasmalla, Yang, Amadou, and Xiao (2014) found that stevia sweetener has an acidic nature, with a pH range from 5.95 to 6.24. This acidity can potentially impact the outcome of this study. Monk fruit sweetener, unlike other sweeteners, is not affected by changes in pH and remains stable in acidic conditions. As a result, it does not alter the pH level of the dark chocolate produced (Abdalbasit, Gasmalla, Yang, Amadou, & Xiao, 2014).

Table 2. Water activity (a_w) and pH of panned keto couverture chocolates

	F1	F2	F3
Water activity (a_w)	$0.39±0.00^b$	$0.42±0.00^a$	$0.42±0.00^a$
pH	$6.00±0.04^b$	$5.93±0.04^b$	$6.26±0.03^a$
Hardness (N)	$2835.41±68.77^b$	$2723.55±145.20^b$	$9058.43±312.89^a$
L*	$36.90±1.28^a$	$37.62±0.61^a$	$36.14±0.67^a$
a*	$2.73±0.03^a$	$2.62±0.05^a$	$2.43±0.14^b$
b*	$2.88±0.26^a$	$2.81±0.21^a$	$2.80±0.09^a$

Note: F1 = Panned keto dark chocolate with icing sugar, F2 = Panned keto dark chocolate with stevia, F3 = Panned keto dark chocolate with monk fruit. Data are presented as mean±SD (n=3). Mean values in the same row with different superscript are significantly different ($p<0.05$).

The texture profile analysis (TPA) on the hardness of the panned chocolate in Table 2 shows that F1 and F2 were not significantly different ($p<0.05$), however they were significantly different with F3. As reported by Medina-Mendoza *et al.* (2023), dark chocolate made of cocoa liquors added with sugar and cocoa butter, required 5,451 g force for hardness. However, it is reduced when the chocolate composition was added with cocoa butter equivalent. The icing sugar had anti caking agent that does not dissolve in the chocolate mixture: this makes sugar particles prone to crystal growth, and the sugar crystals are quite brittle (Hartel, Von, Elbe, & Hofberger, 2018). The crystals make the developed chocolate more brittle and less force is needed to crush it, compared to the chocolate developed with monk fruit sweetener.

In terms of colour measurements, there was no significant difference ($p<0.05$) among the formulations in terms of L* and b*. However, only F3 had a significant difference ($p<0.05$) with others in terms of a*. The darkness of the chocolate is affected by the acidity of the cocoa, as research conducted by Greweling (2012) gave a darker colour as the acidity of chocolate was reduced, and this supports the results shown in this study where no significant changes in colour were seen because there were no significant pH differences among the formulations.

3.2 Proximate analysis of panned keto dark chocolate

From the data in Table 3, there was no significant difference ($p<0.05$) among the formulations in terms of moisture content, crude protein, and ash. Hartel, Von, Elbe, and Hofberger (2018) stated that the percentage of moisture content of hard panned coating should be between 0 and 1 %. The moisture content percentage of all developed panned chocolate was within the range.

The crude protein, fat, and ash content ranges were 9.44 - 11.39, 44.55 – 46.90, and 2.09 – 2.15, respectively. The carbohydrate content showed significant differences ($p<0.05$) between all the formulations, where F2 showed the highest carbohydrate (32.48), followed by F1 and F2 with 31.87 and 30.99, respectively. According to the obtained results, the carbohydrate content in all formulations was significantly lower than the fat content. This investigation is valid since the formulated chocolate was specifically designed for the keto diet, which involves consuming a high amount of fat and a low amount of carbohydrates (Masood, 2023).

3.3 Total phenolic content (TPC) of panned keto dark chocolate

Table 4 shows TPC of panned keto couverture chocolate, at which F2 possessed significantly ($p<0.05$) the highest TPC value (28.91±0.57 mg GAE/g) while there was no significant difference in TPC between F1 and F3.

In a previous study conducted by Balcázar-Zumaeta *et al.* (2022), the TPC of 70% dark chocolate was between 15.8 and 40.55 mg GAE/g and TPC of the developed panned chocolate fell within this range. According to the researcher, the TPC level is significantly influenced by the cultivation environment and the type of cocoa beans used in production (Olugbami, Gbadegesin, & Odunola, 2014). In a separate study conducted by Medina-Mendoza *et al.* (2023), a TPC value of 18.76 mg GAE/g was obtained for dark chocolate produced from cocoa liquors, sugar, and cocoa butter. However, the addition of cocoa butter equivalent resulted in a decrease in TPC. The TPC in chocolate manufacture is mainly influenced by factors such as the formulation, type of cocoa beans, provenance, and temperature during growing, as well as the temperature during processing (Afoakwa, 2016). As a result, the TPC in the panned chocolate for all formulations developed in this study were differed from the chocolate used in previous experiments.

Table 3. Proximate analysis of panned keto couverture chocolates

Chemical component (%)	F1	F2	F3
Moisture content	$0.90±0.09^a$	$0.89±0.07^a$	$1.01±0.11^a$
Crude protein	$11.38±0.93^a$	$9.44±0.19^a$	$11.39±0.12^a$
Fat	$44.83±1.13^b$	$44.55±0.01^b$	$46.90±0.01^a$
Ash content	$2.15±0.00^a$	$2.09±0.00^a$	$2.10±0.00^a$
Crude fibre	$11.35±0.05^b$	$11.58±0.03^a$	$9.57±0.07^c$
Carbohydrate	$31.87±0.01^b$	$32.48±0.09^a$	$30.99±0.01^c$

Note: F1 = Panned keto dark chocolate with icing sugar, F2 = Panned keto dark chocolate with stevia, F3 = Panned keto dark chocolate with monk fruit. Data are presented as mean±SD (n=3). Mean values in the same row with different superscripts are significantly different ($p<0.05$).

Table 4. Total phenolic content and antioxidant activity of panned keto couverture chocolates

	F1	F2	F3
TPC (mg GAE/g)	$23.70±1.43^b$	$28.91±0.57^a$	$23.58±0.24^b$
DPPH IC_{50} (ppm)	$237.49±3.42^b$	$210.29±1.62^c$	$321.45±4.50^a$
FRAP (mg GAE/g)	$13.54±0.03^b$	$17.02±0.16^a$	$17.06±0.16^a$

Note: F1 = Panned keto dark chocolate with icing sugar, F2 = Panned keto dark chocolate with stevia, F3 = Panned keto dark chocolate with monk fruit. Data are presented as mean±SD (n=3). Mean values in the same row with different superscripts are significantly different ($p<0.05$).

3.4 Antioxidant activity of panned keto dark chocolate

Table 5 shows the DPPH scavenging activity (IC_{50}) and ferric reducing antioxidant power (FRAP) of the developed panned chocolate. Based on the data, all formulations were significantly different ($p<0.05$) in the IC_{50} value. IC_{50} is the concentration of an antioxidant-containing substance needed to scavenge 50% of initial DPPH radical. The lower the IC_{50} value, the more effective the chemical to scavenge DPPH, which indirectly proves that it has higher antioxidant activity (Balcazar *et al.*, 2022). From Table 5, F2 (210.29±1.62 ppm) obtained the significantly ($p<0.05$) highest antioxidant activity with a low IC_{50}. The high antioxidant activity in F2 was associated with the high TPC shown in the previous Table 4.

In terms of FRAP value, there was no significant difference between F2 and F3, but both showed significant difference ($p<0.05$) with F1, where F2 and F3 exhibited the highest FRAP. The FRAP indicates the strength of the reducing power of the assay as antioxidant power. The value could be classified into five categories; very low FRAP (<1 mg/L), low FRAP (1-5 mg/L), good FRAP (5-10 mg/L), high FRAP (10-40 mg/L), and very high FRAP with more than 40 mg/L (Chen, Ghazani, Stobbs, & Marangoni, 2021). Based on the classification, the developed panned chocolate had a high FRAP or antioxidant activity. The high FRAP also was contributed by the high TPC shown in Table 4. In a study conducted by Zujko and Witkowska (2011), the FRAP antioxidant activity in the experimental dark chocolate containing 46% of cocoa solids was 14.67 mmol/100 g. This suggests that the FRAP or antioxidant activity prominently depends on the quantity of cocoa solids added during the production.

3.5 Sensory acceptability of panned keto dark chocolate

Table 5 demonstrates the outcomes of a sensory evaluation test in each of its attributes, for panned keto dark chocolate. There are no significant differences shown for almost all of the attributes, excluding glossiness, sweetness, and overall acceptance.

Table 5 reveals that glossiness for F2 (6.40±1.55) obtained the highest significant acceptance score as compared to F1 (6.24±1.68) and F3 (5.69±1.84). According to Saputro, Muhammad, Sunarharum, Kusumadevi, and Irmandharu (2021), the shiny and smooth surface of chocolate is referred to as glossiness, which is also the surface’s ability to reflect light, an optical phenomenon that is always connected to a product’s appearance. Tempering is a crucial processing step that helps control gloss, which is a significant quality attribute of chocolate (Chen, Ghazani, Stobbs, & Marangoni, 2021). On the other hand, F1 ranked the highest for sweetness (6.80±1.28) and overall acceptance (6.83±1.33). The control sample or icing sugar is indeed the most familiar sweetener present, thus attracting the consumers who have been consuming them for years. In addition, Parker, Lopetcharat, and Drake (2018) reported in their study that the temporal check-all-that-apply (TCATA) method identified monk fruit and stevia as having the bitter aftertaste and control sugar as having the least bitter taste. Based on the previous studies, these natural sweeteners are sweet, but stevioside and rebaudioside can also impart bitter, metallic, and licorice-like tastes, while the mogroside from monk fruit has an aftertaste like licorice, which made them least accepted and limits the widespread commercial development (Bhattacharya, 2023; Parker, Lopetcharat, & Drake, 2018; Samuel *et al.*, 2018). Overall, F2 or panned keto dark chocolate with stevia obtained the closest result to the control sample and was more preferred compared to F3 or panned keto dark chocolate with monk fruit when comparing the overall sensory attributes.

4. Conclusions

Production of keto dark chocolate could be formulated by substituting for icing sugar other alternative sweeteners. Substitution with Stevia could increase crude fiber content, total phenolic compounds, and antioxidant activity. Sensory evaluation further highlighted that this sample appeals in terms of glossiness, sweetness, and overall acceptability. These results indicate that the incorporation of natural sweeteners like Stevia is possible for providing health benefits for consumers.

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Table 5. Sensory acceptability of panned keto dark chocolates

Attribute	F1	F2	F3
Colour	$6.85±1.27^{a}$	$7.00±1.23^{a}$	$6.93±1.24^{a}$
Glossiness	$6.24±1.68^{ab}$	$6.40±1.55^{a}$	$5.69±1.84^{b}$
Texture	$6.20±1.68^{a}$	$6.17±1.88^{a}$	$6.06±1.49^{a}$
Cocoa flavour	$6.46±1.48^{a}$	$5.96±1.54^{a}$	$5.98±1.74^{a}$
Sweetness	$6.80±1.28^{a}$	$6.11±1.63^{b}$	$5.93±1.55^{b}$
Bitterness	$6.11±2.02^{a}$	$5.44±2.29^{a}$	$6.20±1.79^{a}$
Overall acceptance	$6.83±1.33^{a}$	$5.91±1.73^{b}$	$5.93±1.60^{b}$

Note: F1 = Panned keto dark chocolate with icing sugar, F2 = Panned keto dark chocolate with stevia, F3 = Panned keto dark chocolate with monk fruit. Data are presented as mean±SD (n=3). Mean values in the same row with different superscripts are significantly different ($p<0.05$).

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