INFLUENCE OF MONK FRUIT AND STEVIA AS NATURAL SWEETENERS ON THE HARDNESS, TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, AND SENSORY EVALUATION OF OMEGA DARK CHOCOLATE

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ABSTRACT – *Nowadays, the incorporation of natural sweeteners such as stevia (Stevia rebaudiana) and monk fruit (Siraitia grosvenorii) to replace the sucrose in chocolate products has gained attention among health-conscious consumers who are increasingly seeking out healthier snacking alternatives, and high-quality chocolate while providing delectable flavour. This study aimed to determine the hardness, water activity, total phenolic content (TPC), antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and sensory profile of omega dark chocolate (enriched with 2.5% sacha inchi oil oleogel) with 29.5% natural sweeteners (monk fruit and stevia). Omega dark chocolate (ODC) formulated with sucrose (refined sugar) was used as a control. The addition of natural sweeteners had no significant impact ($p>0.05$) on the water activity ($a_w = 0.36$) of ODC compared to the control. However, the addition of natural sweeteners lowers the hardness ($p<0.05$) of ODC than control. Meanwhile, formulated ODC containing monk fruit and stevia exhibited higher TPC (27.19-28.20 mg GAE/g) and antioxidant activity (91.34-91.52%) than the control. Sensory evaluation involving 85 panellists showed that ODC samples (sucrose, monk fruit, or stevia) received similar scores ($p>0.05$) for all attributes (colour, glossiness, texture, cocoa flavour, sweetness, bitterness, and overall acceptability), and were well-accepted by consumers (range sensory scores: 6.12-7.51). Therefore, the application of monk fruit and stevia as sucrose replacers in omega dark chocolate has the potential to be a healthier option, especially regarding total phenolic content and antioxidant activity for health-conscious consumers.*

Keywords: Natural sweetener, omega dark chocolate, sensory evaluation, antioxidant, total phenolic content

INTRODUCTION

Globally, chocolate is the favourite confectionery product among consumers, with higher consumption (61%) than candies (about 39%) (Godshall, 2016). Dark chocolate is widely known as a mood booster. The main ingredient is cocoa liquor, which raises serotonin levels (the happy hormone) in the brain (Silva *et al.,* 2023). The high content of polyphenols, flavonoids and theobromine in dark chocolate provides enormous health benefits, such as anti-diabetic, anti-inflammatory, antioxidant, and anti-microbial properties. It also contains vitamins and minerals that positively modulate the human immune system and protect against cardiovascular disease, cancers, and other brain-related disorders, such as Alzheimer's disease (Samanta *et al.,* 2022).

Consumers demand functional dark chocolate with less saturated fats by incorporating vegetable oil oleogels and low-calorie sweeteners to replace sugar in chocolates (Selvasekaran & Chidambaram, 2021). World Health Organization (2015) strongly recommended limiting the free sugar intake (maximum <10% daily energy intake from added or free sugars) to prevent diabetes, obesity, hypertension and dental carries. Thus, chocolate producers and researchers are actively involved in developing low-sugar or sugar-free chocolate products with less saturated fats, which are healthier and have functional properties (Ishak *et al*., 2024; Aranguren & Marcovich, 2023). Chen *et al*. (2022) reported that the incorporation of palm sap sugar as sucrose replacer and palm oil and monoglyceride stearate oleogels as functional ingredients to produce heat-stable and bloom-resistant chocolate while introducing healthy sweeteners with low-calorie content to help reduce health risks and satisfy consumer interest.

Numerous studies have suggested that natural sweeteners as sugar replacers (sugar alcohols, high potency sweeteners, natural raw materials, sweet proteins, and low-non-digestibility carbohydrates) are added to milk and dark chocolate products (Selvasekaran & Ramalingam, 2021). For this study, natural sweeteners from natural raw materials (monk fruit and stevia) are incorporated into omega dark chocolate (ODC). Monk fruit and stevia are approved by the U.S. Food and Drug Administration (FDA) and considered 'generally recognized as safe' (GRAS) (Yeung, 2023; Perrier *et al.,* 2018). Both plant-derived sugar alternatives are non-nutritive, thus making them less or zero in calories and energy, which benefits health-conscious consumers.

Monk fruit (*Siraitia grosvenorii*) is a herbaceous plant from southern China and is popularly known as lo han kuo (Shivani *et al.,* 2022). Mogroside V (derived from the triterpenoid glycosides) is the active ingredient in monk fruit that provides a very intense sweetness level from 250 to 425 compared to sucrose (Chen *et al.,* 2022; Chéron *et al.,* 2019). Despite being an excellent substitute for artificial and refined sweeteners, the monk fruit has draw the consumers attention due to its antihyperglycemic, anti-inflammatory, anti-asthmatic, antioxidative, liver-protective, immunoregulation, and possibly anti-carcinogenic which can prevent or treat asthma, diabetes, cardiovascular disease, and cancers (Ban *et al.,* 2020).

Meanwhile, stevia is a non-nutritive natural sweetener derived from the leaves of *Stevia rebaudiana*. The active ingredient, steviol glycosides, contains stevioside and rebaudioside A, the diterpene family. Based on a study by Chéron *et al.* (2019), the relative sweetness of stevioside is 300, while the rebaudioside A is 250 to 450, compared to the sucrose with the relative sweetness of 1. Studies showed that stevia leaves contain essential amino acids, carbohydrates, protein, phenols, antioxidants, vitamins, minerals, prebiotic and anti-inflammatory properties, as well as dietary fibre, which aids in blood glucose control (Arshad *et al.,* 2022).

However, no previous studies have examined the effect of monk fruit and stevia as natural sweeteners on the quality attributes of ODC. The objectives of the study were to determine the water activity, texture, total polyphenols, antioxidant activity and sensory evaluation of ODC incorporated with natural sweeteners from monk fruit and stevia.

MATERIALS AND METHODS

Materials

Ingredients used in the production of ODC incorporated with different sweeteners were cocoa liquor (Favorich, Guan Chong Berhad, Malaysia), deodorised cocoa butter (Favorich, Guan Chong Berhad, Malaysia), refined sugar as sucrose (Central Sugars Refinery Sdn. Bhd., Malaysia), monk fruit sweetener (Lakanto, Saraya Goodmaid Sdn. Bhd., Malaysia), stevia sweetener (MH Food, Matahari Sdn. Bhd., Malaysia), sacha inchi oil oleogel and soy lecithin (Biofresh Green Sdn Bhd, Malaysia) as emulsifier. Sacha inchi oil oleogel can be obtained according to the method described by Ishak et al. (2024). The materials and ingredients used in this study were purchased from authorised suppliers in Malaysia.

Chemicals

Chemicals used in this study were: methanol, acetone and sodium carbonate (Na_2CO_3) from R & M Chemicals, Malaysia. Gallic acid, Folin-Ciocalteu, ascorbic acid and 2,2-diphenyl-1- picrylhydrazyl (DPPH) reagents were obtained from Sigma-Aldrich, USA.

Preparation of Omega Dark Chocolate with Sucrose and Natural Sweeteners

The percentage of sucrose and natural sweeteners (monk fruit and stevia) in ODC formulations were listed in Table 1. The ODC formulations were developed according to a previous study, incorporating the same percentage of natural sweeteners with sucrose (Ishak *et al.,* 2024).

Table 1: Percentage of Sucrose and Natural Sweeteners (Monk Fruit and Stevia) in Formulation of Omega Dark Chocolate

Ingredients	**DCC (%)**	**DCM (%)**	**DCS (%)**
Cocoa mass (cocoa liquor and cocoa butter)	67.5	67.5	67.5
Sacha inchi oil oleogel	2.5	2.5	2.5
Sucrose	29.5	-	-
Monk fruit sweetener	-	29.5	-
Stevia sweetener	-	-	29.5

Note: DCC = Omega dark chocolate with sucrose (control), DCM = Omega dark chocolate with monk fruit, DCS = Omega dark chocolate with stevia

Three ODC formulations were developed as follows: ODC with refined sugar (sucrose) was used as a control sample (F1); ODC with monk fruit sweetener (F2); and ODC with stevia sweetener (F3). ODC with different sweeteners were produced according to the method described by Ishak *et al.* (2024). Cocoa liquor, sucrose or sweeteners (monk fruit or stevia) and half of melted cocoa butter were mixed in a concher (Pascal Engineering, England) at 45°C for 10 minutes. Then, the chocolate mixtures were rolled three times using a triple roller mill (Pascal Engineering, England) to reduce their particle sizes to less than 30 µm. After that, the refined mixtures and the remaining cocoa butter were mixed in the concher for 6 hours at 45°C. One hour before the completion of the conching process, sacha inchi oil oleogel and soy lecithin were added to the mixtures. After conching, the liquid dark chocolate was heated for overnight at 40°C in an oven. Next, the melted dark chocolate was tempered manually on the marble slab by reducing the temperature of the chocolate (from 40 to 27°C) to obtain the most stable form of fatty acid crystals of cocoa butter (Ishak *et al.*, 2023). The tempered dark chocolate was then mixed with the remaining (about 1/3) of warm dark chocolate (40°C) to obtain the desired temperature (31 to 32°C), which would melt the unstable polymorph crystals of cocoa butter (Ishak *et al.*, 2023; Zhao & James, 2019). The tempered chocolates were poured into the polycarbonate mould and cooled at a temperature of 13±1°C for 60 minutes to solidify. The chocolates were unmoulded and stored at room temperature for further analysis (water activity, texture, total phenolic content, antioxidant activity, and sensory evaluation). Figure 1 shows the ODC formulations with sucrose and natural sweeteners (monk fruit and stevia).

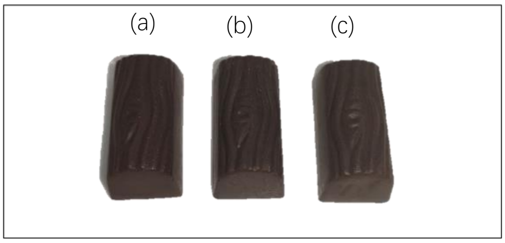

Figure 1: (a) Omega Dark Chocolate with Sucrose (DCC) as Control, (b) ODC with Monk Fruit Sweetener (DCM), and (c) ODC with Stevia Sweetener (DCS)

Methods

Determination of Water Activity

Water activity (a_w) of ODC with sucrose and natural sweeteners were measured by water activity meter 4TE (Aqualab, United States) at 23°C. Samples were crushed by pestle and mortar before being measured for water activity. The water activity for each sample was measured in triplicates (García-Alamilla *et al.*, 2017).

Determination of Texture

Knife Edge with Slotted Insert (HDP/BS) installed on a TA.XTplusC Texture Analyzer (Stable Micro Systems Ltd, U K) was used for penetration tests of ODC with sucrose and natural sweeteners. The test was carried out at 20°C using compression mode to penetrate the ODC sample with a depth of 10 mm at a rate of 1 mm/s. The maximum penetration force through the sample was recorded for the hardness value (kg) of the chocolate sample.

Antioxidant Extraction

The antioxidant extraction of the ODC with sucrose and natural sweeteners were carried out (Abu *et al.*, 2017). The antioxidant extraction was carried out by adding 30 mL of methanol into 3.0 g of grounded ODC samples with solid to solvent ratio of 1:10 (w/v) in the centrifuge tubes and vortexed for a minute (Abu *et al.*, 2017). Next, the samples were centrifuged at a speed of 3000 rpm at 25°C for 10 minutes to obtain clear supernatant. The extracts of the samples were stored in labelled tubes wrapped with aluminium foil and kept in the refrigerator for further analysis.

Total Phenolic Content

The total phenolic content (TPC) of ODC were determined using the Folin-Ciocalteu method (Camargo *et al.*, 2022). First, 0.1 g of the defatted sample (based on the antioxidant extraction method) was extracted with 10 mL of 70% acetone in a centrifuge tube by sonication in ice water for 30 minutes. Then, the sample mixtures were centrifuged for 10 minutes at a speed of 5000 rpm at 4°C to obtain the clear supernatant. After that, 100 uL of sample extracts (clear supernatant) were mixed with 7.9 mL of distilled water and 500 uL of Folin-Ciocalteu reagent. After being left in the dark for 4 minutes, 1.5 mL of 20% sodium carbonate (Na_2CO_3) was added and then set aside for another 2 hours in a dark environment. The absorbance of the solution was measured by Genesys 30 UV-Vis spectrophotometer (Thermo Fischer Scientific, USA) at 765 nm. All sample extracts were measured in triplicates. A standard gallic acid curve was plotted by preparing the dilutions at 0, 50, 100, 200, 300, 400, 500 mg/L in ethanol. The equation of the standard curve was obtained ($y = 0.001x + 0.0072$, where $R^2 = 0.9994$). The TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of sample (mg GAE/100 g sample). The TPC of ODC formulations were calculated according to Equation 1.

$$\frac{\left(\frac{10}{1000}\right) \times C}{M} \qquad (1)$$

Where,

C = The concentration is determined from the standard curve (mg/L)
M = Mass of sample (g)

DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity of ODC with sucrose and natural sweeteners were measured according to the method of Karim *et al.* (2019). Briefly, methanol was added into 0.004 g of DPPH and made up to 100 mL methanolic DPPH solution. Then, 0.01 g of standard ascorbic acid was added into a 10 mL volumetric flask and made up with distilled water to make a concentration of 1000 ppm as a stock solution of standard ascorbic acid. Then, different series of standard solutions at 0 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm were made. The stock solution and several dilutions of the ODC extracts (based on the antioxidant extraction method) were made up with methanol. Then, 1.2 mL of ascorbic acid in various concentrations (20-100 ppm) were combined with 9.0 mL of methanolic DPPH solution, and the mixtures were vortexed. They were left for 30 minutes in the dark, and then the absorbance at 517 nm were measured using Genesys 30 Visible Spectrophotometer (Thermo Fischer Scientific, USA) in triplicates. The same method was used to analyze the antioxidant activity of ODC extracts. The absorbance for the blank sample (methanolic DPPH solution) and the chocolate sample was measured and calculated according to Equation 2.

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100 \qquad (2)$$

Where,
A = Absorbance of DPPH blank
B = Absorbance of sample with DPPH

<u>Determination of Sensory Acceptability</u>
Sensory evaluation of the ODC with sucrose and natural sweeteners was conducted using a 9-point hedonic scale. A total of 85 panellists, including staff and students of both genders from the Malaysian Cocoa Board and Universiti Teknologi Mara (UiTM), were invited for the sensory evaluation of chocolate samples. Each panellist received three chocolate samples, which were placed in clear plastic containers and labelled with a three-digit code in a random order, a sensory evaluation form that contained the basic sociodemographic data, a pencil and plain water to rinse their mouth before the test for each sample. The panelists were needed to evaluate and rate the chocolate samples on a 9-point hedonic scale based on the attributes (colour, glossiness, hardness, cocoa flavour, sweetness, bitter aftertaste, and overall acceptance). Table 2 shows the degree of liking for the 9-point hedonic scale used for the sensory evaluation of chocolate samples.

Table 2: Degree of Liking for the 9-point Hedonic Scale in Sensory Evaluation of Omega Dark Chocolate

Score	Indicator
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

<u>Statistical Analysis</u>
All of the analyses were determined in triplicates except for the sensory evaluation. The data was analyzed using one-way analysis of variance (ANOVA). Differences in the means values and the statistical group were tested at a 0.05 significance level to determine the significance between the method variable and the final result. IBM SPSS Statistics 26 software was used for data analysis.

RESULTS AND DISCUSSIONS

Water Activity of Omega Dark Chocolates with Sucrose and Natural Sweeteners
The water activity (a_w) of ODC with sucrose and natural sweeteners are shown in Table 3.

Table 3: Water Activity and Hardness of Omega Dark Chocolate with Sucrose and Natural Sweeteners

Formulation	**DCC**	**DCM**	**DCS**
Water activity (a_w)	0.36 $\pm 0.00^a$	0.36 $\pm 0.01^a$	0.36 $\pm 0.00^a$
Hardness (kg)	12.55 $\pm 0.63^a$	11.35 $\pm 0.09^b$	11.23 $\pm 0.32^b$

Note: DCC = Omega dark chocolate with sucrose (control), DCM = Omega dark chocolate with monk fruit, DCS = Omega dark chocolate with stevia

Results showed that the incorporation of natural sweeteners did not change significantly ($p>0.05$) the a_w of ODC (DCM and DCS) compared to DCC. All chocolate samples have a similar a_w (0.36). Beuchat (1981) reported that a_w range of 0.3-0.4 is considered as low a_w and no microbial proliferation. Furthermore, low occurrence of lipid oxidation and enzyme activity at water activities between 0.3-0.4. The addition of sucrose or natural sweetener (30%) in the ODC formulation could also considered as a preservation technique and reduce the amount of water available for microbial growth (Beuchat *et al.*, 2013). The same author reported that the minimum a_w for microorganisms to grow is 0.60 (where cell division and physiological activities of microorganisms are impaired under a_w 0.6). Sun *et al.* (2023) also stated that the a_w for chocolate ranged from 0.3–0.5 due to the low moisture with high sugar and fat content. Therefore, due to low water activity, ODC with natural sweeteners are considered safe to consume.

Hardness of Omega Dark Chocolates with Sucrose and Natural Sweeteners

The hardness of omega dark chocolate with sucrose and natural sweeteners are shown in Table 3. Results showed that control (DCC) received the highest hardness value (12.55 kg) ($p<0.05$) compared to other chocolate samples with natural sweeteners (DCM and DCS) (11.23-11.35 kg). Sucrose substitution by natural sweeteners (monk fruit and stevia) significantly affected the hardness of ODC compared to the other sample. Similar findings have been reported in previous research indicating that the sucrose replacers (maltodextrin, inulin, fructo-oligosaccharides and trehalose) soften the texture of dark compound chocolate due to the particle-particle interactions in the chocolate system (Lim *et al.,* 2021). Chocolate texture as hardness plays an essential role not only for visual assessment but also for the melting properties in the mouth (Žuljević *et al.,* 2023). Hence, incorporation of naturals sweeteners into ODC reduced the hardness properties.

Total Phenolic Content of Omega Dark Chocolates with Sucrose and Natural Sweeteners

The total phenolic content (TPC) of ODC with sucrose and natural sweeteners are shown in Table 4. The addition of natural sweeteners (monk fruit and stevia) increased significantly ($p<0.05$) the polyphenol content of ODC samples (DCM and DCS). DCS obtained the highest TPC (28.20 mg GAE/g) and DCM at (27.19 mg GAE/g) ($p>0.05$), compared to DCC (24.96 mg GAE/g). TPC of stevia and monk fruit sweeteners ranged from (28.4-28.7 mg GAE/g sample) and (0.15-2.39 mg GAE/mg solid crude), respectively (Wuttisin & Boonsook, 2019; Ruiz *et al.,* 2015). Similar results were reported that different chocolate samples incorporated with mixtures of commercial stevia and sweeteners (maltitol and sucrose) contain TPC ranging from 25.70-30.92 mg CAE/g) (Torri e*t al.,* 2017). The TPC of ODC increased with the addition of natural sweeteners due to polyphenols in these natural sweeteners).

Table 4: Total Phenolic Content and Antioxidant Activity of Omega Dark Chocolate with Sucrose and Natural Sweeteners

	AA	DCC	DCM	DCS
DPPH inhibition (%)	93.52 ± 0.00[a]	90.33 ± 0.14[c]	91.34 ± 0.23[b]	91.52 ± 0.10[b]
TPC, mg GAE/g	-	24.96 ±0.31[b]	27.19 ±0.68[a]	28.20 ±0.71[a]

Note: AA = Ascorbic acid, DCC = Omega dark chocolate with sucrose (control), DCM = Omega dark chocolate with monk fruit, DCS = Omega dark chocolate with stevia

Antioxidant Activity of Omega Dark Chocolates with Sucrose and Natural Sweeteners

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of ODC with sucrose and natural sweeteners was shown in Table 4. The results showed that ODC with natural sweeteners (DCM and DCS) had significantly higher ($p<0.05$) free radical scavenging activity (91.34-91.52%) than the DCC (90.33%). This demonstrates that the polyphenols in each natural sweetener elevated the free radical scavenging activity in ODC. Ban *et al.* (2020) reported that the addition of monk fruit extract as a natural sweetener increased the DPPH radical scavenging activity of yogurt (approximately 50-52%) compared to plain yogurt and yogurt with sucrose (around 30-32%). Another previous study showed that guava nectar sweetened by stevia powder had the highest DPPH radical scavenging activity (32.26%) compared to other guava nectar samples (addition of neotame and sucrose) (about 30%) due to the high amount of ascorbic acid presents in stevia (Peasura & Sinchaipanit, 2022). Overall, ascorbic acid demonstrated the highest free radical scavenging activity (93.52%) compared to all chocolate samples due to its strong antioxidant and anti-inflammatory properties (Gęgotek & Skrzydlewska, 2022). Based on the results obtained, all chocolate samples exhibited high antioxidant properties (90.33-91.52%) due to the presence of epicatechin and catechin in cocoa liquor, which have potent antioxidant effects by scavenging the free radicals from body cells and prevent damage from the oxidation (Anitha *et al.*, 2021). Addition of monk fruit and stevia sweeteners, consequently extended the free radical scavenging activity of ODC.

Sensory Acceptability of Omega Dark Chocolates with Sucrose and Natural Sweeteners

The mean scores for each attribute of ODC with sucrose and natural sweeteners are presented in Table 5. Statistically, all the attributes showed insignificant difference of DCM and DCS to the DCC.

Table 5: Sensory Evaluation of Omega Dark Chocolate with Sucrose and Natural Sweeteners

Attributes	DCC	DCM	DCS
Colour	7.31±1.14	7.36±1.12	7.51±1.06
Glossiness	6.69±1.44	6.73±1.48	6.94±1.61
Hardness	7.00±1.31	7.05±1.28	7.05±1.31
Cocoa flavour	6.82±1.35	6.78±1.35	6.58±1.49
Sweetness	6.12±1.74	6.55±1.48	6.34±1.69

Bitterness	6.40±1.68	6.36±1.56	6.52±1.72
Overall acceptability	6.82±1.58	6.88±1.30	6.85±1.52

Note: DCC = Omega dark chocolate with sucrose (control), DCM = Omega dark chocolate with monk fruit, DCS = Omega dark chocolate with stevia

The mean scores for the overall acceptability of the chocolate samples (DCC, DCM and DCS) were 6.82-6.88 based on the 9-point hedonic scale. Results showed that the mean scores for overall acceptability of all chocolate samples corresponded to "like slightly" based on Table 2. The sensory evaluation result found that natural sweeteners (monk fruit and stevia) did not reduced the quality of the ODC on the colour, glossiness, texture, cocoa flavour, sweetness and bitterness ($p>0.05$) compared to control (DCC). Similar results have been reported by Lim *et al.* (2021), indicating that the addition of sucrose replacers (maltodextrin, inulin, fructo-oligosaccharides and trehalose) did not change the sensory attributes (appearance, cocoa flavour, hardness, graininess and aftertaste) of dark compound chocolate.

In the present study, no significant difference ($p>0.05$) in the hardness of chocolate samples (7.00-7.05) was observed according to the sensory evaluation, although the difference was detected by texture analyzer. The hardness of the chocolate samples was not detected by the mouth. Previous studies reported that incorporation of sucrose replacers (maltodextrin, inulin, trehalose, fructo-oligosaccharides) and natural sweetener (stevia) significantly reduced the mean score for sweetness of milk and dark chocolates due to their bitterness and strong aftertaste (Lim *et al.,* 2021; Shah *et al.,* 2010; Farzanmehr & Abbasi, 2009). However, the present study showed that the addition of stevia and monk fruit did not affect ($p>0.05$) the sweetness of ODC (6.34-6.55) compared to the DCC (6.12). Thus, ODC with stevia and monk fruit have similar sensory attributes compared to control.

CONCLUSIONS

Omega dark chocolate with monk fruit and stevia sweeteners have higher total phenolic content and antioxidant activity than the control. Sensory attributes (colour, hardness, glossiness, cocoa flavour, sweetness, bitterness and overall acceptability) of omega dark chocolate incorporated with natural sweeteners received similar scores with control. In conclusion, monk fruit and stevia sweeteners can be used as sucrose replacers to improve the total phenolic content and antioxidant activity of omega dark chocolate, which panelists prefer.

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