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Reduction of Saturated Fat in Dark Chocolate using Sacha Inchi (*Plukenetia volubilis*) Oil Oleogel

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ABSTRACT

This research studied the effectiveness of Sacha Inchi Oil Oleogel (SIOO) as a partial replacement for saturated fat in dark chocolate. Sacha inchi (*Plukenetia volubilis*) oil is high in polyunsaturated fatty acids (α-linolenic and linoleic acids) and a good source of tocopherols. This study prepared oleogels using sacha inchi oil as a base oil and food-grade beeswax as an oleogelator. Different percentages of SIOO (1%, 2.5% and 5.0%) were added in the dark chocolate. Dark chocolate without SIOO was used as a control. Fatty acid profile, total polyphenols, antioxidant activity and sensory evaluation of the formulated dark chocolates with SIOO were investigated. The incorporation of SIOO significantly ($p<0.05$) lowered the saturated fat and increased the polyunsaturated fatty acids in dark chocolate samples. This study also showed that the total polyphenols and antioxidant activity of dark chocolates enriched with 2.5 and 5.0% SIOO were significantly higher ($p<0.05$) than the other chocolate samples. Sensory evaluation showed that control and dark chocolates added with SIOO (1% and 2.5%) received similar scores for all sensory attributes. However, the highest concentration of SIOO decreased significantly ($p<0.05$) the scores for the taste and overall acceptability of dark chocolate. Therefore, the enrichment of sacha inchi oil oleogel as a functional ingredient could reduce the saturated fat and increase the polyunsaturated fatty acids and antioxidant activity of the formulated dark chocolate, which is well-accepted by consumers.

Keywords: dark chocolate, polyunsaturated fatty acid, sacha inchi oil oleogel, saturated fat

INTRODUCTION

Dark chocolate is one of the confectionery products admired by people of all ages (Merlino *et al.* 2021). Dark chocolate mainly consists of cocoa liquor followed by sugar, cocoa butter and emulsifier. The high content of cocoa liquor in dark chocolate possesses antioxidant properties due to polyphenols and flavonoids, which may provide several health benefits (Shahanas *et al.* 2019). The market demand for dark chocolate is predicted to increase from $48.29 billion to $67.88 billion by 2029 (Market Research Report 2022). However, the incorporation of solid fats such as cocoa butter equivalents, cocoa butter substitutes and cocoa butter replacers in the dark chocolate formulation increases the content of saturated fat, which may have a negative impact on people's health such as obesity, cardiovascular diseases, high cholesterol, cancer and type II diabetes (Li & Liu 2019). For that reason, consumers demand healthy and functional chocolate products with less saturated fats by increasing the content of Polyunsaturated Fatty Acids (PUFAs) compared to conventional chocolates (Selvasekaran & Chidambaram 2021).

Edible vegetable oils (soybean, canola, sunflower and corn) are high in PUFAs, which consist of linoleic (omega-6) and α-linolenic acids (omega-3) (Loganathan & Kim-Tiu 2022). Recently, oleogels developed as the latest innovative technology for oil structuring

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to entrap bulk vegetable oils within a thermo-reversible and three-dimensional gel network by food-grade oleogelators (Perţa-Crişan *et al.* 2023). At the same time, vegetable oils are transformed into solid fat by lowering the content of saturated fats, increasing the amount of PUFAs and retaining the chemical composition of the oils (Manzoor *et al.* 2022). Various edible oleogelators are commonly used as oleogel to entrap bulk vegetable oil, including waxes, lecithin, sterols, and monoacylglycerols. Beeswax is a natural wax produced by honey bees in the bee hives. Beeswax is the most common oil structure-forming agent used for the production of vegetable oil oleogels (sunflower oil, olive oil, linseed oil and canola oil) (Frolova *et al.* 2022; Issara *et al.* 2022).

Vegetable oil oleogels are frequently used in many food products such as bakery products, filling creams and ice cream to replace saturated fat while improving the food quality and nutritional value (Jing *et al.* 2022; Cabrera *et al.* 2020; Pehlivanoglu *et al.* 2018). Regarding chocolate products, Li & Liu (2019) produced dark chocolate added with corn oil-monoglyceric stearate based oleogel to replace cocoa butter partially. The authors reported that incorporating oleogels lowered the saturated fats and increased the PUFAs of dark chocolate samples. Alvarez *et al.* (2021) also stated that the replacement of cocoa butter in the milk chocolate formulations by sunflower-oil-Hydroxypropyl Methylcellulose (HPMC) reduced the saturated fats and increased the content of PUFA and Monounsaturated Fatty Acids (MUFAs).

Sacha inchi oil has high PUFAs content (linoleic acid: 34.1% and α-linolenic acid: 48.2%). Antioxidant compounds such as tocopherols (γ & β) and phytosterols (campesterol, stigmasterol & β-sitosterol) are also present in sacha inchi oil. Furthermore, total phenols, total flavonoids and total antioxidant activity of sacha inchi oil are 6.20 mg GAE/100 g, 0.34 mg rutin eq./g oil extract and 18.2–95.0 µmol TE/g), respectively (Goyal *et al.* 2022; Cisneros *et al.* 2014; Zanqui *et al.* 2016). Regarding pharmacological activity, sacha inchi oil has shown its capacity to lower total cholesterol, increase high-density lipoprotein cholesterol, and exhibit anticancer action against tumour cells (Schiessel *et al.* 2015).

To the knowledge of an author, sacha inchi oleogel has not been utilised directly to reduce the saturated fats in dark chocolate. Limited information is about the impact of oleogel-based vegetable oil on the total phenolic, antioxidant activity and sensory acceptability of chocolate products. In this study, sacha inchi oil was used as a representative of vegetable oil rich in PUFAs to develop sacha inchi oil-based oleogel with beeswax for reducing the saturated fats of dark chocolate. The objective of the present study was to investigate the effects of sacha inchi oil-based oleogel at different concentrations on the fatty acid composition, total phenolic content, antioxidant activity and sensory acceptability of dark chocolate.

METHODS

Design, location, and time

The research was conducted in the Cocoa Innovative and Technology Centre, Malaysian Cocoa Board, Negeri Sembilan and the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Selangor, Malaysia. The study was carried out from September 2022 to February 2023.

Materials and tools

Cold-pressed sacha inchi oil (Khatijah Herbs, Malaysia) and natural yellow beeswax (Personal Formula Resources, Malaysia) were used as materials to prepare Sacha Inchi Oil Oleogel (SIOO). Meanwhile, ingredients used in the production of dark chocolate were cocoa liquor, cocoa butter, icing sugar, and soy lecithin as emulsifier from Malaysian Cocoa Board, Nilai, Negeri Sembilan. Chemicals used in this study were: methanolic potassium hydroxide (KOH), n-hexane and sodium carbonate (Na_2CO_3) from R & M Chemicals, Malaysia. Meanwhile, gallic acid, Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagents were obtained from Sigma-Aldrich, United States.

Procedures

Preparation of sacha inchi oil oleogel. SIOO was prepared according to the method of Calligaris *et al.* (2020) with slight modification. Firstly, Sacha Inchi Oil (SIO) and 10% beeswax were heated by stirring them in a dark room at not more than the maximum melting temperature of beeswax (65°C) for 30 min. The heating process is completed when the beeswax is fully soluble in the SIO. SIOO was cooled overnight at room temperature (27°C) and stored at 20°C for analysis and usage in dark chocolate formulations.

Development of dark chocolate added with different percentages of sacha inchi oil oleogel. The addition of SIOO at different percentages (1.0%, 2.5% and 5.0%) in dark chocolate was conducted. Four dark chocolate formulations were prepared as follow: dark chocolate without SIOO was used as a control sample (F1); dark chocolate with 1.0% SIOO (F2); dark chocolate with 2.5% SIOO (F3) and dark chocolate with 5.0% SIOO (F4). Dark chocolate was produced according to the method described by Biswas *et al.* (2017). Dark chocolate ingredients (cocoa liquor, sugar and a quarter of melted cocoa butter) were mixed in a concher (Pascal Engineering, England) at 45°C for 5 min. After that, the particle sizes of the chocolate mixtures were reduced three times to less than 30 µm using a triple roller mill (Pascal Engineering, England). After refining, the chocolate mixtures and the remaining melted cocoa butter were mixed in concher for 6 h at 45°C. Two hours before the completion of the conching process, SIOO and soy lecithin were added to the chocolate mixtures. Afterwards, the liquid dark chocolate was tempered manually on the marble slab by reducing the temperature of the liquid chocolate from 45°C to 27°C to obtain the most stable form of fatty acid crystals of cocoa butter. The tempered dark chocolate was poured into a polycarbonate mould and cooled at 13±1°C for 60 min to solidify the chocolate. The dark chocolate was then removed from the mould and stored in a plastic container at room temperature for further analysis (fatty acid composition, total phenolic content, antioxidant activity and sensory acceptability).

Determination of fatty acid profile. The fatty acid composition of the dark chocolate added with different percentages of SIOO was determined as described by Md Ali & Dimick (1994). Gas Chromatography (GC) (Hewlett-Packard 6890, Agilent Technologies, Palo Alto, CA, USA) with a flame ionisation detector was used to analyse the fatty acid methyl esters (FAMEs) of chocolate samples. Fatty acid profiles of the chocolate sample were measured according to the chromatogram peak of the FAMEs.

Determination of total polyphenol content. The total polyphenol content of dark chocolate added with different percentages of SIOO was determined according to de Camargo *et al.* (2015). The total polyphenol content was expressed as gallic acid equivalents in milligrams per gram of sample (mg GAE/g dark chocolate) with the following formulation:

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

Where C is the concentration determined from the standard curve (mg/L), and M is the mass of the extracted sample (g).

Determination of antioxidant activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the dark chocolate added with different percentages of SIOO was measured according to Urbańska & Kowalska (2019). DPPH free radical scavenging activity of the chocolate sample was calculated using the following formula:

$$\mathit{Free\ radical\ scavenging\ activity}\ (\%) = \frac{(A_1 - A_2)}{A_1} \times 100$$

Where A1 is the absorbance of the control sample, and A2 is the absorbance of the chocolate sample. The free radical scavenging activity is recorded in percentage.

Sensory evaluation. Hedonic scale rating test was carried out to evaluate the sensory acceptability of dark chocolate added with different percentages of SIOO (da Silva *et al.* 2013). Fifty untrained panelists consist of staff (academic and laboratory) and students aged 20–55 from the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The 7-point hedonic scale was made by an unstructured 10 cm horizontal linear ruler, with both ends marked with a legend. The panels indicate the intensity of each sensory attribute for chocolate sample on the 10 cm unstructured scale (where 1="least liked" and 7="most liked").

Data analysis

Total polyphenol content and antioxidant activity of dark chocolate added with different percentages of SIOO were carried out in three replications (n=3), while fatty acid profile and sensory evaluation of dark chocolate added with different percentages of SIOO were conducted in two replications (n=2). All data obtained are analysed using one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test to determine if there was a significant difference between samples. A confidence level of 95% ($p<0.05$) was used to see significant differences between samples. All data are expressed as mean±standard deviation.

RESULTS AND DISCUSSION

The fatty acid composition of dark chocolate added with different percentages of SIOO is showed in Table 1. Palmitic and stearic acids (saturated fatty acids), oleic acid (monounsaturated fatty acid), linoleic and α-linolenic acids (PUFAs) were identified in dark chocolate samples. Saturated fatty acids (palmitic and stearic acids) are the major fatty acids in all chocolate samples due to the incorporation of cocoa butter as fat medium in the chocolate formulation. The control sample contains the highest amount of saturated fatty acids (66.12%). Results showed that adding different SIOO significantly decreased ($p<0.05$) the content of saturated fatty acids (57.02–64.11%) compared to the control sample. At the same time, the content of PUFAs (linoleic and α-linolenic acids) in dark chocolate increased significantly ($p<0.05$) with the concentrations of SIOO (from 1.0–5.0%). These findings were reasonably expected due to the high concentration of PUFAs present in SIO (Rodzi *et al.* 2022). Li & Liu (2019) reported that the addition of different corn oil-based oleogels (monoglyceric stearate, β-sitosterol and lecithin and ethyl cellulose) in dark chocolate decreased significantly ($p<0.05$) the saturated fatty acids (21.17–21.84%) compared to the control sample (dark chocolate with 100% cocoa butter) (37.86%). The authors also proved that the PUFAs (57.24–57.88%) of dark chocolate increased significantly by adding different corn oil-based oleogels. Enrichment of PUFAs from SIO-based oleogel in dark chocolate might lower heart diseases, prevent cancer risk and cardiovascular diseases, and improve high-density lipoprotein blood (Nguyen *et al.* 2020). Therefore, adding sacha inchi oil oleogels at different concentrations decreases the saturated fatty acids and enhances the content of PUFAs in dark chocolate.

Table 2 presents the Total Polyphenol Content (TPC) of dark chocolate added with different percentages of SIOO. The result showed that the TPC for dark chocolate added with 2.5 and 5.0% SIOO (27.35–28.69 mg GAE/g) were significantly higher ($p<0.05$) than F1 and F2 samples (24.39–25.32 mg GAE/g). However, the difference in TPC between F1 and F2 samples is insignificant ($p>0.05$). The lowest concentration

Table 1. Fatty acid profiles of the dark chocolate added with different percentages of sacha inchi oil oleogels

Fatty acid	Sample			
	F1	F2	F3	F4
Saturated fatty acid				
Palmitic acid (%)	28.19±0.14^{a}	27.01±0.01^{b}	25.09±0.01^{c}	23.93±0.17^{d}
Stearic acid (%)	37.93±0.21^{a}	37.10±0.02^{b}	35.36±0.07^{c}	33.09±0.29^{d}
Total saturated fatty acid (%)	66.12±0.44^{a}	64.11±0.05^{b}	60.45±0.05^{c}	57.02±0.50^{d}
Monounsaturated fatty acid				
Oleic acid (%)	30.62±0.03^{a}	28.89±0.11^{b}	29.08±0.04^{b}	27.75±0.24^{c}
Polyunsaturated fatty acids				
Linoleic acid (%)	2.90±0.02^{d}	3.54±0.03^{c}	5.90±0.00^{b}	7.53±0.09^{a}
α-Linolenic acid (%)	0.21±0.01^{d}	2.03±0.52^{c}	4.13±0.06^{b}	6.64±0.17^{a}
Total polyunsaturated fatty acids (%)	3.11±0.02^{d}	5.57±0.53^{c}	10.03±0.08^{b}	14.17±0.28^{a}
Total unsaturated fatty acid (%)	33.73±0.05^{d}	34.46±0.41^{c}	38.11±0.04^{b}	41.92±0.50^{a}

Values are mean±standard deviation (n=2)

$^{a-d}$Values in each row with different letters are significantly different ($p<0.05$).

Dark chocolate formulations containing sacha inchi oil oleogel: F1: Dark chocolate without sacha inchi oil oleogel; F2: Dark chocolate with 1.0% sacha inchi oil oleogel; F3: Dark chocolate with 2.5% sacha inchi oil oleogel; F4: Dark chocolate with 5.0% sacha inchi oil oleogel

Table 2. Total polyphenol content and free radical scavenging of the dark chocolate added with different percentages of sacha inchi oil oleogels

Sample	Total polyphenol content (mg GAE/g)	Free radical scavenging activity (%)
F1 (Dark chocolate without sacha inchi oil oleogel)	25.32±0.63^{c}	79.82±0.51^{b}
F2 (Dark chocolate with 1.0% sacha inchi oil oleogel)	24.39±2.10^{c}	79.89±3.23^{b}
F3 (Dark chocolate with 2.5% sacha inchi oil oleogel)	27.35±0.22^{b}	83.51±1.27^{ab}
F4 (Dark chocolate with 5.0% sacha inchi oil oleogel)	28.69±0.08^{a}	84.59±0.36^{a}

Values are mean±standard deviation (n=3)

$^{a-c}$Values in each column with different letters are significantly different (p<0.05)

of SIOO (1.0%) did not increase (p>0.05) the TPC of the dark chocolate (24.39 mg GAE/g). Ramos-Escudero *et al.* (2021) reported that simple phenol, isocoumarin, lignan, flavonoids, and secoiridoid are the major phenolic classes detected in 27 commercial sacha inchi oil samples. For that reason, the addition of SIOO increased the TPC of dark chocolate. To the best of our knowledge, the TPC of chocolate or any food product added with vegetable oil oleogel has not yet been investigated. Urbańska & Kowalska (2019) reported that the polyphenol content of chocolate using different roasted beans from various countries (Venezuela, Dominican Republic, Ghana, Columbia and Venezuela) ranged from 9.1–40.55 mg/g chocolate. In the present study, the TPC of all dark chocolate samples are in the range of those reported by Urbańska & Kowalska (2019). EFSA Panel on Dietetic Products, Nutrition and Allergies [NDA] (2012) and Andújar *et al.* (2012) stated that the high amount of cocoa liquor used in dark chocolate contributed to the great source of polyphenols, which provides numerous positive health benefits such as prevention of cancer, cardiovascular and inflammatory diseases as well as protection of low-density lipoprotein cholesterol against oxidative stress. It clearly showed that adding 2.5 and 5.0% sacha inchi oil oleogel significantly increased the total phenolic content of dark chocolate.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out to determine the antioxidant activity of dark chocolate added with different percentages of SIOO, as shown in Table 2. The results showed that F1 and F2 samples had the lowest (p>0.05) free radical scavenging activity (79.82–79.89%) compared to F3 (83.51%) and F4 (84.59%). A higher amount of SIOO (2.5 and 5.0%) can definitely improve the antioxidant activity of dark chocolate. To our knowledge, there has yet to be published data on the effects of vegetable oil oleogel on the antioxidant activity of chocolate or all types of food products. Ramos-Escudero *et al.* (2019) reported that 27 commercial SIO samples contain several lipid-soluble antioxidant compounds, such as tocopherols (γ- & δ-) and sterols (campesterol and stigmasterol), which attributed to the antioxidant activity. Also, polyphenolic compounds play a synergistic role with other antioxidant compounds (tocopherols and sterols) in SIO (Cárdenas *et al.* 2021; Liu *et al.* 2014). Moreover, tocopherols and polyphenols act as antioxidants and provide health benefits in preventing hypertension, atherosclerosis and certain cancers (Liu *et al.* 2014). The health benefits associated with this high radical scavenging activity of dark chocolate added with sacha inchi oil oleogel are worth further investigation.

The mean scores for each attribute of dark chocolate added with different percentages of SIOO are presented in Table 3. Results showed that the mean scores for the overall acceptability of the dark chocolate samples were 4.22–5.20, which corresponded to "neither like nor dislike" and like" based on the seven-point hedonic scale. Dark chocolate samples (F1, F2 and F3) received similar score (5.01–5.20) "like" (p>0.05) for overall acceptability. Not only that, mean scores for texture, aroma, taste, bitter aftertaste and glossiness of dark chocolate added with SIOO (1.0 & 2.5%) were similar (p>0.05) with the control sample. However, panelists gave the lowest sensory score (4.22) (p<0.05) for the overall acceptability of the F4 sample. Besides that, mean scores for different sensory attributes

Table 3. Mean scores for each sensory attribute of dark chocolate added with different percentages of sacha inchi oil oleogel

Sample	Attributes					
	Glossiness	Texture	Aroma	Taste	Bitter aftertaste	Overall acceptability
F1	5.28±1.21^{a}	5.38±1.31^{a}	5.10±1.27^{a}	4.96±1.58^{a}	4.84±1.56^{a}	5.20±1.28^{a}
F2	5.24±1.10^{a}	5.2±1.21^{a}	5.22±1.02^{a}	4.96±1.54^{a}	4.74±1.48^{a}	5.03±1.27^{ab}
F3	5.50±1.23^{a}	5.22±1.17^{a}	4.78±1.27^{ab}	4.38±1.62^{a}	4.58±1.58^{a}	5.01±1.51^{ab}
F4	5.38±1.01^{a}	5.30±1.18^{b}	4.34±1.26^{b}	3.90±0.67^{b}	3.84±0.68^{b}	4.22±0.50^{c}

Values are mean±standard deviation (n=50)

$^{a\text{-}c}$ Values in each column with different letters are significantly different (p<0.05).

Dark chocolate formulations containing sacha inchi oil oleogel: F1: Dark chocolate without sacha inchi oil oleogel; F2: Dark chocolate with 1.0% sacha inchi oil oleogel; F3: Dark chocolate with 2.5% sacha inchi oil oleogel; F4: Dark chocolate with 5.0% sacha inchi oil oleogel

(texture, aroma, taste and bitter aftertaste) of the F4 sample were the lowest (p<0.05) compared to other chocolate samples. The panelists commented that F4 sample contains strong bean and grass odours. This can be explained that SIO has a flavour profile includes a green aroma, which is associated with the presence of several volatile compounds such as hexanal, 3-pentanone and 1-penten-3-ol (Ramos-Escudero *et al.* 2021). Thus, the highest percentage of SIOO (5%) added to the dark chocolate received the lowest score for all sensory attributes except glossiness. Regarding the taste attribute of the F4 sample, their mean score was the lowest (3.90) compared to other chocolate samples due to the intense bitter flavour and a strong herbal taste after consumption, which panelists do not prefer. The finding of Espert *et al.* (2021) is in line with the present study that the taste of the milk chocolate added with 1.5 and 2% sunflower oil-hydroxypropyl methylcellulose based oleogels is dominated by a bitter taste. This suggested adding SIOO into the dark chocolate at concentrations of 1.0 to 2.5% to obtain an acceptable score for overall customer acceptance and sensory attributes, similar to standard dark chocolate.

CONCLUSION

This research uses different percentages of sacha inchi oil oleogel as a partial cocoa butter replacer to produce dark chocolate with reduced saturated fat. Enriching sacha inchi oil oleogel in dark chocolate significantly increased the content of polyunsaturated fatty acids by lowering the saturated fatty acids compared to the standard dark chocolate. Sacha inchi oil oleogel significantly improved the dark chocolate's total phenolic content and antioxidant activity compared to the standard dark chocolate. When sacha inchi oil oleogels at concentrations of 1.0% and 2.5% are used together with cocoa butter in the formulation, dark chocolate has similar attributes to standard dark chocolate. However, the panelists rated the lowest score for all sensory attributes of dark chocolate added with 5% sacha inchi oil oleogel. These findings verify that healthier dark chocolate with higher polyunsaturated fat content, total phenolic content and antioxidant activity and lower saturated fat content, as well as optimal sensory acceptability can be obtained by adding sacha inchi oil oleogel in the production of dark chocolate.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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