Food Research 8 (4) : 392 - 400 (August 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effects of sacha inchi (*Plukenetia volubilis*) oil on the fatty acid profile, total polyphenols, antioxidant activity and sensory acceptability of dark chocolate granola bar

[1,2,*]Ishak, I., [3]Ping, T.Y., [1]Khaironi, J., [1]Bakar, A.M.A., [1]Ahmad, F. and [4]Ghani, M.A.

[1]*Cocoa Downstream Technology Division, Cocoa Innovative and Technology Centre, Malaysian Cocoa Board, Kawasan Perindustrian Nilai, 71800 Nilai, Negeri Sembilan, Malaysia*
[2]*Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[4]*Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

Article history:
Received: 14 February 2023
Received in revised form: 26 April 2023
Accepted: 12 April 2024
Available Online: 27 August 2024

Keywords:
Sacha inchi oil,
Dark chocolate granola bar,
Total polyphenols,
Antioxidant activity,
Polyunsaturated fatty acids,
Sensory evaluation

DOI:
https://doi.org/10.26656/fr.2017.8(4).074

Abstract

Sacha inchi (*Plukenetia volubilis*) oil is rich in polyunsaturated fatty acids (PUFAs) and antioxidants, providing essential health benefits to humans. Dark chocolate granola bar formulations enriched with sacha inchi oil were developed. In this study, sacha inchi oil (SIO) was added to a dark chocolate granola bar (DCGB) to replace butter at different percentages (0-10%). Four DCGB formulations were developed as follows: 0SIODCGB without sacha inchi oil (0%), which was used as a control sample, 2.5SIODCGB enriched with 2.5% SIO, 5SIODCGB enriched with 5% SIO, and 10SIODCGB enriched with 10% SIO. Next, the effect of SIO on the fatty acid profile, total polyphenols, antioxidant activity and sensory evaluation of DCGB were determined. The result showed that 10SIODCGB had the highest ($p<0.05$) amount of PUFAs (28.14%) compared to 0SIODCGB (9.54%), 2.5SIODCGB (15.13%) and 5SIODCGB (25.32%). This study also showed that the total polyphenols and antioxidant activity of DCGB enriched with SIO formulations (2.5SIODCGB, 5SIODCGB and 10SIODCGB) were significantly higher ($p<0.05$) than the 0SIODCGB. Regarding correlation, TPC and ferric reducing antioxidant power (FRAP) of the DCGB enriched with SIO were strongly related ($r = 0.972$). Meanwhile, the scores for sensory attributes of all DGCB formulations were similar ($p>0.05$). Therefore, the enrichment of sacha inchi oil increased the polyunsaturated fatty acids, polyphenols and antioxidant activity in the formulated dark chocolate granola bar with well-accepted by consumers.

1. Introduction

Sacha inchi (*Plukenetia volubilis*) is native to the tropical rainforests in South America, including Peru and northwestern Brazil (Goyal *et al.,* 2022). Due to its high nutrient content and great agro-industrial potential, sacha inchi has been gaining more attention in recent years and is being cultivated in several countries. This crop was introduced to Malaysia two years ago and commercially grown in Kelantan. It is grown as a side crop by planters because it has a long lifespan of approximately 15 years, is easy to grow, and can be harvested in a short period (Harian Metro, 2021; Utusan Malaysia, 2021).

Sacha inchi has a high nutritional value and a lot of potential health benefits. It is rich in protein, fibre, and fat and contains several essential micronutrients and antioxidants. According to Torres Sánchez *et al.* (2021), sacha inchi seeds are suitable for dietary uses as they contain oil (35-60%), protein (25-30%), essential amino acids, minerals, vitamins A and E. The oil extracted from the sacha inchi kernel is highly nutritious due to its high content of polyunsaturated fatty acids (PUFAs), which consist of α-linolenic acid (47-51%) and linoleic acid (34-37%) (Niu *et al.,* 2014; Hildalgo *et al.,* 2019). The dietary intake of PUFAs is associated with several health benefits by reducing inflammation, blood pressure and triglycerides, risk factors for cardiovascular disease. Besides that, PUFAs found in sacha inchi oil (SIO) can

*Corresponding author.
Email: *izzreen@koko.gov.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

help prevent chronic inflammatory disease and dermatitis and control tumour proliferation (Cárdenas *et al.,* 2021).

At the same time, cocoa is Malaysia's fourth most crucial commodity sub-sector, followed by palm oil, rubber and timber. Malaysia is also the seventh largest cocoa producer in the world after the Netherlands, Ivory Coast, Indonesia, Germany, the United States and Ghana. Cocoa (*Theobroma cacao*) is a tropical evergreen tree in the family Malvaceae that is grown for its edible seeds (de Souza *et al.,* 2018). Roasted and ground cocoa beans are the main ingredients to produce chocolate products. Chocolate is a solid confectionery product made from cocoa, mixed with cocoa butter and powdered sugar. Based on the different proportions of cocoa solids (butter and liquor) used in a particular formulation, chocolate is classified into dark, milk and white chocolates (Shafi *et al.,* 2018). Dark chocolate consists of 50-90% cocoa solids, while milk chocolate contains only 10-50% cocoa solids. According to Montagna *et al.* (2019), dark chocolate with high cocoa liquor content has a higher phenolic content than the other types of chocolates (milk and white). Besides that, dark chocolate is rich in flavonoids that can lower blood pressure and reduce the risk of cardiovascular diseases and type 2 diabetes (Sørensen and Astrup, 2011; Haritha *et al.,* 2014).

In recent years, the production of different convenient products (fast and snack foods) has gained significant interest due to changes in people's lifestyles. Consumers prefer foods with health benefits, as well as convenient for consumption, storage and handling (Constantin and Istrati, 2018). Consequently, a granola bar that uses oats as the main ingredient is a convenient way to provide nutrients, bioactive compounds and dietary fibre to consumers (Silva *et al.,* 2013). In this study, dark chocolate and sacha inchi oil (SIO) were incorporated into the formulation to provide a functional granola bar rich in antioxidants, PUFAs, minerals and protein. No studies have evaluated the nutritional quality, antioxidant activity and sensory acceptability of dark chocolate granola bars (DCGB) enriched with SIO. Thus, this study was conducted to develop DCGB enriched with different percentages of SIO, and their fatty acid profile, phenolic content, antioxidant activity and sensory acceptability were determined.

2. Materials and methods

2.1 Materials

Oats, almonds, butter, vanilla extract and dried cranberry were purchased from the baking ingredients retail store (Bake With Yen) in Nilai, Negeri Sembilan, Malaysia. Meanwhile, pure honey and cold-pressed SIO were obtained from local suppliers through the online shopping application (Shopee Malaysia). All the ingredients were used to produce a granola bar enriched with SIO. Dark chocolate consists of cocoa solids (liquor and butter) and sugar supplied by the Malaysian Cocoa Board (Nilai, Negeri Sembilan, Malaysia) to coat the granola bar enriched with SIO.

2.2 Chemicals

Methanolic potassium hydroxide (KOH) and pure hexane were used to analyse the fatty acid profile. Meanwhile, Folin-Ciocalteu reagent, 7.5% sodium carbonate (Na_2CO_3) and gallic acid were used to determine total phenolic content. Next, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used in the free radical scavenging activity assay. Lastly, 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride, acetate buffer and ascorbic acid were used for ferric reducing antioxidant power assay (FRAP).

2.3 Production of dark chocolate granola bars enriched with sacha inchi oil

Different formulations of the dark chocolate granola bar enriched with SIO were prepared, as shown in Table 1. Firstly, oats and almonds were roasted in an oven at 180°C for 10 mins. Afterwards, the roasted oat and almond were transferred to a large bowl and mixed well. Then, butter, SIO, pure honey and vanilla extract were combined and heated at 60°C for 5 mins. The mixture was poured into the bowl with roasted oats and almonds and mix well. Dried cranberries were also added. The oat mixtures were transferred to the aluminium pan and refrigerated at 4°C. After an hour, the block of granola mixture was removed from the pan and cut into small sizes. Next, the granola bars were coated with dark chocolate and set aside at room temperature. Lastly, granola bars containing SIO coated with dark chocolate were stored in polyethene zip bags at 4°C for further analysis. In this study, SIO was added at various percentages to replace butter in the granola bars. 0SIODCGB was the DCGB with no SIO added (0%) that was used as control, 2.5SIODCGB was the DCGB added with 2.5% SIO, 5SIODCGB was the DCGB added with 5% SIO, and 10SIODCGB was the DCGB added with 10% SIO.

2.4 Fatty acid composition of dark chocolate granola bars enriched with sacha inchi oil

The fatty acid profile of the dark chocolate granola bar (DCGB) enriched with SIO was characterised in terms of fatty acid methyl esters (FAMEs) developed by Md. Ali and Dimick (1994). Oil extraction using petroleum ether was conducted for 8 hrs at 70°C to obtain lipids from the different DCGB formulations

Table 1. Composition of dark chocolate granola bar enriched with sacha inchi oil formulations.

Ingredients (g)	0SIODCGB	2.5SIODCGB	5SIODCGB	10SIODCGB
Oat	38	38	38	38
Almond	13	13	13	13
Pure honey	28	28	28	28
Butter	10	7.5	5.0	0
Sacha inchi oil	0	2.5	5.0	10
Vanilla extract	1.0	1.0	1.0	1.0
Dried cranberry	10	10	10	10

Dark chocolate is used as a coating for all granola bar formulations. Dark chocolate granola bar enriched with sacha inchi oil formulations: 0SIODCGB: Control without sacha inchi oil, 2.5SIODCGB: 2.5% sacha inchi oil, 5SIODCGB: 5.0% sacha inchi oil, 10SIODCGB: 10.0% sacha inchi oil.

(International Union of Pure and Applied Chemistry, 1987). The apparatus containing the DCGB sample was equipped with the Soxhlet machine (M-Top, Seoul, Korea). About 0.1 g of the DCGB sample was mixed with hexane (1.0 mL) and 1 M sodium methoxide solution (1.0 mL) in a test tube with a screw cap. Then, it was mixed at high-speed mixing using a vortex for 10 s and held for 10 mins until double-layer solutions were formed. The mixture of glycerol and non-methylated fatty acid elements accumulated in the bottom layer of the solution. Meanwhile, the top layer containing FAMEs must be pipetted out carefully without removing the mixture solutions from the bottom phase. The solution consisting of FAMEs was injected into gas chromatography equipped with a flame ionisation detector (GC-2010 Shimadzu, Kyoto, Japan) and an integrator (C-R6A Chromatopac, Kyoto, Japan). The measurements of fatty acid compositions were conducted on a capillary silica polar column HP-5 (30 m × 0.32 mm; film thickness, 0.25 µm), and the oven temperature was set at 40°C for 5 mins then accelerated up to 220°C at 20°C/min. Simultaneously, the injector and the detector temperatures were fixed at 250 and 270°C, respectively. Fatty acid profiles of the DCGB enriched with SIO formulations were measured according to the chromatogram peak of the FAMEs. The results were reported as the relative percentage of each fatty acid in the different formulated DCGB enriched with SIO.

2.5 Determination of total phenolic content of dark chocolate granola bars enriched with sacha inchi oil

The total phenolic content (TPC) of DCGB enriched with SIO was determined using the Folin-Ciocalteu method (de Camargo *et al.,* 2015). Firstly, a 0.1 g defatted sample was extracted with 70% acetone (10 mL) in a centrifuge tube and sonicated in ice water for 30 mins. Then, it was centrifuged at a speed of 5000 rpm at 4°C for 10 mins to obtain the clear supernatant. After that, 7.9 mL distilled water and 500 uL Folin–Ciocalteu reagent was added to 100 uL sample extract and mixed thoroughly. The mixture was left for 4 mins in a dark place, and then 1.5 mL of sodium carbonate (Na_2CO_3) (20%) was added to it. Next, the mixture was kept aside in a dark place for 2 hrs. Finally, the absorbance was read at 765 nm using UV-Vis Spectrophotometer (Agilent Technologies Cary 60, USA). A standard gallic acid curve was constructed by preparing the dilutions at 0, 50, 100, 200, 300, 400, 500 mg/L in ethanol, and the standard curve equation was y = 0.001x + 0.011, where R^2 = 0.9982. The TPC was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg GAE/g sample). The TPC in all DCGB enriched with SIO formulations was calculated by using the formula:

TPC is expressed as gallic acid equivalent (GAE) in DCGB enriched with SIO (g) .

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

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

2.6 Determination of antioxidant activity of dark chocolate granola bars enriched with sacha inchi oil

2.6.1 Antioxidant extraction

The antioxidant extraction from the DCGB enriched with SIO formulations was determined according to the method of Naviglio *et al.* (2014). The antioxidant extraction was carried out by adding 10 mL of methanol into 0.2 g of granola bar sample in a centrifuge tube and vortexed for 1 min. Next, it was centrifuged at a speed of 4000 rpm at 25°C for 3 mins to obtain the clear supernatant. The extract of the DCGB enriched with SIO was stored in an opaque bottle for further analysis.

2.6.2 DPPH free radical scavenging activity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the DCGB enriched with SIO extract was measured according to the method described by Akowuah *et al.* (2005). The DPPH reagent was prepared by mixing 3.49 mg of DPPH radical with methanol and filling up to 100 mL in a volumetric flask. 0.6 mL of sample extract was mixed with 2.0 mL of DPPH reagent and allowed to stand in darkness for an

https://doi.org/10.26656/fr.2017.8(4).074

hour. After that, the absorbance was measured at 517 nm by using UV-Vis Spectrophotometer (Agilent Technologies Cary 60, USA). The percentage inhibition of DPPH by DCGB enriched with SIO extract was calculated by using the following formula:

$$\% \, inhibition = \frac{A - B}{A} \times 100$$

Where A = the absorbance of the DPPH reagent, B = the absorbance of the DCGB enriched with SIO was measured after an hour of reaction with the DPPH reagent.

2.6.3 Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) activity was determined according to the method of Benzie and Strain (1999) with slight modifications. Firstly, sodium acetate buffer (300 mM, pH 3.6), TPTZ (10 mM), and iron (III) chloride (20 mM) were mixed in a ratio of 10:1:1 (v/v/v) to make the FRAP reagent. The 300 mM acetate buffer reagent was prepared by mixing 0.31 g of sodium acetate trihydrate with 1.60 mL of glacial acetic acid and then diluted to 100 mL with distilled water. At the same time, the 10 mM TPTZ solution was prepared by dissolving 0.31 g of TPTZ radical in 40 mM hydrochloric acid. The 20 mM $FeCl_3 \cdot 6H_2O$ solution was prepared by adding 0.54 mL $FeCl_3 \cdot 6\ H_2O$ with 100 mL distilled water. Then, 0.5 mL of DCGB sample extract and 2.5 mL FRAP reagent were mixed and placed in a dark place for an hour. After that, absorbance was recorded at 595 nm using Epoch™ Microplate Spectrophotometer (BioTek Instruments, USA) and the results were expressed as milligram ascorbic acid equivalents (AAE) per gram of sample (mg AAE/g DCGB). The standard curve was plotted using the ascorbic acid standard in water (0.01-0.10 mg/mL).

Ferric reducing antioxidant power (FRAP) = $C \times V / M$

Where C = The concentration is determined from the standard curve (mg/mL), V = Sample extract solution volume (mL) and M = Mass of sample extract (g)

2.7 Sensory evaluation of dark chocolate granola bars enriched with sacha inchi oil

Sensory evaluation of the DCGB enriched with SIO was conducted using a seven-point hedonic scale. A total of 30 panellists (laboratory staff and undergraduate students) from the National University of Malaysia (UKM) were invited for the sensory evaluation. Each panellist received four DCGB enriched with SIO samples (0SIODCGB, 2.5SIODCGB, 5SIODCGB and 10SIODCGB) labelled with a three-digit code, a sensory evaluation form, a pencil and plain water to rinse their mouth before the test for each sample. The panellists were requested to score on a seven-point hedonic scale from 1 to 7 (7 = strongly like, 6 = very like, 5 = like, 4 = neither like nor dislike, 3 = dislike, 2 = very dislike, 1 = strongly dislike) on each attribute of the DCGB enriched with SIO (aroma, flavour, texture, chewiness, stickiness, bitter aftertaste and overall acceptability) (Aigster *et al.,* 2011; Abidin *et al.,* 2020).

2.8 Statistical analysis

TPC and antioxidant activity of DCGB enriched with SIO were carried out in three replicates (n = 3). On the other hand, the sensory evaluation and fatty acid profile of the DCGB enriched with SIO were conducted in two replicates (n = 2). Data are reported as min±standard deviation. One-way analysis of variance (ANOVA) was used to determine whether there were significant differences in mean values between different samples, followed by Tukey's honestly significant differences (HSD) multiple rank test at a 95% confidence level using Minitab 21.0 version. The Pearson correlation coefficient was used to study the correlation between TPC and antioxidant activity ($p<0.05$) of the DCGB enriched with SIO. Microsoft Excel version 2016 was also used to calculate the obtained results' average values and create graphs.

3. Results and discussion

3.1 Fatty acid profile of dark chocolate granola bars enriched with sacha inchi oil

Table 2 shows the fatty acid composition of DCGB enriched with SIO. The predominant fatty acid in the DCGB enriched with SIO was oleic acid which comprised 28.67-38.19%, followed by palmitic acid (18.83-22.70%), stearic acid (18.34-20.53%) and linoleic acid (9.46-17.89%). This study also showed that only α-linoleic acid was found in 5SIODCGB (8.62%) and 10SIODCGB (10.26%). Furthermore, 10SIODCGB recorded the highest amount of PUFAs (28.14%) ($p<0.05$) compared to the other formulations, indicating that the addition of 10% SIO in the DCGB contributed to the highest content of PUFAs.

As shown in Table 1, oleic acid is the primary fatty acid found in DCGB enriched with SIO which is contributed by the main ingredients of the formulation (oat, dark chocolate and butter). According to Batalova *et al.* (2019), oleic acid is the dominant fatty acid in oat (33.5-36.7%). Meanwhile, dark chocolate and butter contain 25.99 and 22% oleic acid, respectively (Ergönül *et al.,* 2010; Rutkowska and Adamska, 2011). Oleic acid is a monounsaturated fatty acid that can lower the risk of coronary heart disease, cardio-metabolic risk, obesity, type 2 diabetes and hypertension. In addition, oleic acid has also been reported to decrease the low-density

Table 2. Comparison of fatty acid composition for dark chocolate granola bar enriched with sacha inchi oil.

Fatty acid	Formulation			
	0SIODCGB	2.5SIODCGB	5SIODCGB	10SIODCGB
	Saturated fatty acids (%)			
Palmitic acid (16:0)	22.70±0.04[a]	20.13±0.01[b]	19.13±0.07[c]	18.83±0.09[c]
Stearic acid (18:0)	20.53±0.03[a]	20.54±0.03[a]	19.54±0.03[b]	18.34±0.08[c]
Total saturated fatty acids (%)	51.16±0.35[a]	45.70±0.10[b]	43.29±0.08[c]	42.55±0.02[c]
	Monounsaturated fatty acids (%)			
Oleic acid (18:1)	38.19±0.43[a]	36.05±0.12[a]	30.69±0.07[b]	28.67±0.06[b]
Total monounsaturated fatty acids (%)	39.30±0.44[a]	39.17±0.11[a]	31.28±0.06[b]	29.31±0.06[b]
	Polyunsaturated fatty acids (%)			
Linoleic acid (18:2)	9.46±0.07[d]	15.13±0.01[c]	16.81±0.01[b]	17.88±0.03[a]
α-Linolenic acid (18:3)	ND	ND	8.62±0.02[b]	10.26±0.11[a]
Total polyunsaturated fatty acids (%)	9.54±0.07[d]	15.13±0.01[c]	25.32±0.13[b]	28.14±0.08[a]
Total unsaturated fatty acids (%)	48.84±0.35[c]	54.30±0.01[b]	56.71±0.08[a]	57.45±0.02[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). Dark chocolate granola bar enriched with sacha inchi oil formulations: 0SIODCGB: Control without sacha inchi oil, 2.5SIODCGB: 2.5% sacha inchi oil, 5SIODCGB: 5.0% sacha inchi oil, 10SIODCGB: 10.0% sacha inchi oil. ND: Not detected.

lipoprotein (LDL) cholesterol and increase the high-density lipoprotein (HDL) cholesterol concentration in the blood (Arsic *et al.,* 2019; Nogoy *et al.,* 2020).

Like flaxseed oil, SIO has a remarkable quantity of α-linolenic acid or omega-3. SIO contains significantly higher α-linolenic acid (47.04%) than soybean (1.8%) and corn oils (1.4%) (Carrillo Terán *et al.,* 2018). Other than that, omega-6 (linoleic acid) is also present (34.2%) in the SIO (Gonçalves *et al.,* 2018). These fatty acids have been proven to have high nutritional value that can reduce total blood cholesterol and LDL cholesterol. Moreover, they play an important role in the formation of tumours and different ageing processes (Maszewska *et al.,* 2018; Matthäus, 2008). It can be shown that the addition of SIO increased significantly ($p<0.05$) the PUFA level of the DCGB formulation. Moreover, the unsaturated fatty acids (mono and poly) of DCGB enriched with SIO formulations (2.5SIODCGB, 5SIODCGB and 10SIODCGB) were significantly higher ($p<0.05$) than in the control sample (0SIODCGB). Betancur Hoyos *et al.* (2016) also reported that adding sacha inchi into a prototype food (convenience food) had a higher content of unsaturated fatty acids than food without sacha inchi. In conclusion, the inclusion of SIO (5 and 10%) in DCGB increases the content of PUFAs content.

3.2 Total polyphenol content of dark chocolate granola bars enriched with sacha inchi oil

The TPC of DCGB enriched with SIO formulations are shown in Table 3. TPC for 2.5SIODCGB, 5SIODCGB and 10SIODCGB (6.069-6.416 mg GAE/g sample) were significantly higher ($p<0.05$) than 0SIODCGB (4.920 mg GAE/g sample). However, the TPC for 2.5SIODCGB, 5SIODCGB and 10SIODCGB are insignificant ($p>0.05$). Results showed that the addition of SIO increased the amount of phenolic content in DCGB formulations. Štěrbová *et al.* (2017) reported that the sacha inchi seeds contain high phenolic content (5.318 mg GAE/g sample). In addition, the phenolic profiles of SIO revealed five main classes, namely simple phenols, isocoumarin, lignan, flavonoids and secoiridoids (Ramos-Escudero *et al.,* 2021). Among the phenolic compounds, secoiridoids accounted for 40.96% of the TPC, followed by flavonoids (14.40%), lignans (19.69%), isocoumarins (10.56%) and phenyl alcohols (14.39%). The phenolic compounds are essential in inhibiting PUFA oxidation and the off-flavours development in oil (Nadeem *et al.,* 2017). Moreover, phenolic compounds have a beneficial effect against several diseases, such as hypertension and atherosclerosis and can prevent certain cancers and enhance immune response (Dahri *et al.,* 2017). The results also indicated a similar outcome to Medina-Mendoza *et al.* (2021) where SIO has increased phenolic content and chocolate samples' antioxidant capacity. Therefore, the addition of SIO at any concentration (2.5-10%) enhances the TPC of the DCGB formulations.

3.3 Antioxidant activity of dark chocolate granola bars enriched with sacha inchi oil

3.3.1 Free radical scavenging activity assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was conducted to determine the antioxidant activity of DCGB enriched with SIO, as shown in Table 3. The results showed that 0SIODCGB and 2.5SIODCGB had the lowest ($p>0.05$) free radical scavenging activity (48.86-50.70%) compared to

https://doi.org/10.26656/fr.2017.8(4).074

Table 3. Total phenolic content and antioxidant activity in dark chocolate granola bar enriched with sacha inchi oil.

Formulation	Total phenolic content (mg GAE/g sample)	Free radical scavenging activity (%)	Ferric reducing power (mg AAE/g)
0SIODCGB	4.920±0.538[b]	48.86±0.49[c]	0.067±0.004[b]
2.5SIODCGB	6.416±0.153[a]	50.70±2.32[c]	0.116±0.006[a]
5SIODCGB	6.023±0.115[a]	57.78±0.86[b]	0.097±0.012[a]
10SIODCGB	6.069±0.361[a]	66.94±1.42[a]	0.111±0.017[a]

Values are presented as mean±SD, n = 3. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). Dark chocolate granola bar enriched with sacha inchi oil formulations: 0SIODCGB: Control without sacha inchi oil, 2.5SIODCGB: 2.5% sacha inchi oil, 5SIODCGB: 5.0% sacha inchi oil, 10SIODCGB: 10.0% sacha inchi oil.

5SIODCGB (57.78%) and 10SIODCGB (66.94%). Indeed, higher concentrations of SIO (from 0% to 10%) can increase the antioxidant activity of DCGB formulations. These findings were mainly attributed to the high tocopherol content, which plays a vital role as an antioxidant in SIO and has synergistic interactions with phenolics (Liu *et al.,* 2014; Cárdenas *et al.,* 2021). Antioxidant compounds (tocopherols and polyphenols) can lower the risk of cardiovascular disease, chronic inflammatory diseases, and dermatitis as well as have anti-tumour potential. In conclusion, the highest concentration of SIO (10%) contributed to the highest free radical scavenging activity of DCGB formulation.

3.3.2 Ferric reducing antioxidant power assays

Table 3 shows the FRAP of the formulated DCGB enriched with SIO. The FRAP test measures the reducing potential of an antioxidant present in the samples through the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) (Payne *et al.,* 2013). As shown in Table 3, the FRAP activity of 2.5SIODCGB, 5SIODCGB and 10SIODCGB (0.097-0.116 mg AAE/g) was significantly higher ($p<0.05$) than the control sample (0SIODCGB) (0.067 mg AAE/g). This might be due to the higher phenolic content found in DCGB enriched with SIO formulations (2.5SIODCGB, 5SIODCGB and 10SIODCGB) than in the control sample without SIO (0SIODCGB). However, the reduction power of DCGB enriched with SIO at different percentages showed no significant difference ($p>0.05$).

Meanwhile, Table 4 shows the highly positive correlation ($r = 0.972$) between the FRAP assay and the TPC. It shows that the reducing activity of DCGB enriched with SIO positively related to the phenolic compounds. Ishak *et al.* (2020) reported that the reducing power of chia seed oil that is rich in PUFAs is closely associated with the carotenoid ($r = 0.998$) and phenolic compounds ($r = 0.93$). However, a weak positive correlation ($r = 0.397$) was observed between the free radical scavenging activity and TPC of DCGB enriched with SIO. It indicated that the phenolics are the main contributor to the reducing power of DCGB enriched with SIO.

Table 4. Pearson correlation of phenolic content and antioxidant activity for dark chocolate granola bar enriched with sacha inchi oil.

Correlation coefficient (r)	TPC	DPPH	FRAP
TPC	1.000	0.397**	0.972**
DPPH	0.397**	1.000	0.490**
FRAP	0.972**	0.490**	1.000

**A significant difference ($p<0.05$) for the correlation value between total phenolic content (TPC), free radical scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) assays of dark chocolate granola bar enriched with sacha inchi oil.

3.4 Sensory evaluation of dark chocolate granola bars enriched with sacha inchi oil

The mean scores for each attribute of DCGB enriched with SIO formulations are presented in Table 5. The mean scores for the overall acceptability of the DCGB enriched with SIO were 4.4-5.1 based on the seven-point hedonic scale. Results showed that the mean scores for sensory acceptability of the different DCGB enriched with SIO formulations corresponded to "neither like nor dislike" and like". Overall, 10SIODCGB (DCGB with 10% sacha inchi oil) obtained the lowest score (4.267) for overall acceptability compared to other formulations (mean score: 5.1-4.73). This might be due to the nutty flavour and bitterness in 10SIODCGB, which panellists do not prefer. Bueno-Borges *et al.* (2018) and Cisneros *et al.* (2014) reported that the taste attributes of sacha inchi seeds are astringent, bitter and strong nutty flavour. Furthermore, the flavour profiles of the sacha inchi oil are grassy and nutty and have herbal odours (Gutiérrez *et al.,* 2019; Ramos-Escudero *et al.,* 2021). To solve these problems, sacha inchi nuts are roasted to eliminate these off-flavours and possibly anti-nutritional factors (Cisneros *et al.,* 2014). Besides that, the roasting process also contributed to the oxidative stability and antioxidant activity of the SIO (Chirinos *et al.,* 2016).

The addition of 5% SIO into the DCGB formulation (5SIODCGB) received the highest sensory acceptability score (5.1) compared to other samples. Medina-Mendoza *et al.* (2021) reported that the chocolate incorporated

Table 5. Mean scores for each attribute of dark chocolate granola bar enriched with sacha inchi oil.

Sample	Attribute						
	Aroma	Flavour	Texture	Chewiness	Stickiness	Bitter aftertaste	Overall acceptability
F1	5.03±0.93[a]	4.67±1.49[a]	4.77±1.07[a]	4.47±1.41[a]	4.40±1.28[a]	4.30±1.42[a]	4.97±1.16[ab]
F2	5.23±0.94[a]	4.93±1.34[a]	5.03±1.27[a]	4.63±1.35[a]	4.60±0.93[a]	4.40±1.55[a]	4.73±1.29[ab]
F3	4.97±1.03[a]	4.77±1.50[a]	4.90±1.21[a]	4.73±1.11[a]	4.63±1.25[a]	4.77±1.59[a]	5.10±1.21[a]
F4	4.67±1.06[a]	4.33±1.52[a]	4.50±1.31[a]	4.63±1.35[a]	4.47±1.17[a]	4.13±1.57[a]	4.27±1.29[b]

Values are presented as mean±SD, n = 30. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). Dark chocolate granola bar enriched with sacha inchi oil formulations: 0SIODCGB: Control without sacha inchi oil, 2.5SIODCGB: 2.5% sacha inchi oil, 5SIODCGB: 5.0% sacha inchi oil, 10SIODCGB: 10.0% sacha inchi oil.

with 4.5% SIO had the highest score ($p<0.05$) for colour, snap, gloss and acceptability. Furthermore, Cecchi *et al.* (2019) also agreed that the incorporation of olive oil did not affect the aroma, taste and flavour of granola bar formulations. However, no statistically significant differences were recorded in aroma, flavour, texture, chewiness, stickiness and bitter aftertaste of all DCGB formulations ($p>0.05$). This suggested that adding SIO into the DCGB formulations must be not more than 5% to obtain an acceptable score for overall customer acceptance.

4. Conclusion

Four formulations of dark chocolate granola bars enriched with sacha inchi oil are developed in this study. Sacha inchi oils were added at different percentages (0-10%) to replace butter in the dark chocolate granola bar formulations. The enrichment of sacha inchi oil in the dark chocolate granola bar formulations significantly improved the content of polyunsaturated fatty acids (linoleic and α-linolenic acids). At the same time, the saturated fatty acids were reduced significantly by increasing the concentrations of sacha inchi oil in the dark chocolate granola bar formulations compared to the control sample (without sacha inchi oil). Sacha inchi oil also significantly enhanced the total phenolic content, reducing the power and free radical scavenging activity of the dark chocolate granola bar formulation compared to the control sample. Evaluation of sensory attributes demonstrated average acceptance by the panellists for all the developed dark chocolate granola bars added with sacha inchi oil formulations, as no differences were detected among the samples. However, the sensory acceptability of the dark chocolate granola bar formulation enriched with 5% sacha inchi oil was the most preferred by the panellists compared to other formulations. Therefore, this study has proved that sacha inchi oil can be applied as a functional ingredient in dark chocolate granola bar formulation by improving the polyunsaturated fatty acids, total phenolic content, and antioxidant activity and does not affect the sensory quality of the final product. In future studies, sacha inchi oil can be applied to other chocolate products for improvement in the amount of polyunsaturated fatty acids, total polyphenols and antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The research work was funded by the Development and Pre-Commercialisation of Cocoa Functional Food Products under RMK-12: PTJ120712.

References

Akowuah, G.A., Ismail, Z., Norhayati, I. and Sadikun, A. (2005). The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chemistry,* 93(2), 311-317. https://doi.org/10.1016/j.foodchem.2004.09.028

Arsic, A., Stojanovic, A. and Mikic, M. (2019). Oleic acid - Health benefits and status in plasma phospholipids in the Serbian population. *Serbian Journal of Experimental and Clinical Research,* 20(2), 3-8. https://doi.org/10.1515/sjecr-2017-0077

Batalova, G.A., Krasilnikov, V.N., Popov, V.S. and Safonova, E.E. (2019). Characteristics of the fatty acid composition of naked oats of Russian selection. *IOP Conference Series: Earth and Environmental Science,* 337, 012039. https://doi.org/10.1088/1755-1315/337/1/012039

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Betancur-Hoyos, E.D., Urango-Marchena, L.A. and Restrepo-Betancur, L.F. (2016). Effect of adding sacha inchi (*Plukenetia volubilis* L.) seeds to a prototype of convenience food draft, on the nutritional composition and sensory acceptance. *Journal of Medicinal Plants Research,* 10(29), 435-441. https://doi.org/10.5897/JMPR2016.6064

Bueno-Borges, L.B., Sartim, M.A., Gil, C.C., Sampaio, S.V., Rodrigues, P.H.V. and Regitano-d'Arce, M.A.B. (2018). Sacha inchi seeds from sub-tropical cultivation: Effects of roasting on antinutrients, antioxidant capacity and oxidative stability. *Journal of Food Science and Technology,* 55(10), 4159-4166. https://doi.org/10.1007/s13197-018-3345-1

Cárdenas, D.M., Rave, L.J.G. and Soto, J.A. (2021). Biological activity of sacha inchi (*Plukenetia volubilis* Linneo) and potential uses in human health: A review. *Food Technology and Biotechnology,* 59(3), 253-266. https://doi.org/10.17113/ftb.59.03.21.6683

Carrillo, W., Quinteros, M.F., Carpio, C., Morales, D., Vásquez, G., Álvarez, M. and Silva, M. (2018). Identification of fatty acids in sacha inchi oil (*Cursive plukenetia volubilis* L.) from Ecuador. *Asian Journal of Pharmaceutical and Clinical Research*, 11(2), 379-381. https://doi.org/10.22159/ajpcr.2018.v11i2.15515

Cecchi, L., Schuster, N., Flynn, D., Bechtel, R., Bellumori, M., Innocenti, M., Mulinacci, N. and Guinard J.-X. (2019). Sensory profiling and consumer acceptance of pasta, bread, and granola bar fortified with dried olive pomace (Pâté): A byproduct from virgin olive oil production. *Journal of Food Science,* 84(10), 1750-1783. https://doi.org/10.1111/1750-3841.14800

Chirinos, R., Zorrilla, D., Aguilar-Galvez, A., Pedreschi, R. and Campos, D. (2016). Impact of roasting on fatty acids, tocopherols, phytosterols, and phenolic compounds present in *Plukenetia huayllabambana* seed. *Journal of Chemistry*, 2016, 6570935. https://doi.org/10.1155/2016/6570935

Cisneros, F.H., Paredes, D., Arana, A. and Cisneros-Zevallos, L. (2014). Chemical composition, oxidative stability and antioxidant capacity of oil extracted from roasted seeds of Sacha-inchi (*Plukenetia volubilis* L.). *Journal of Agricultural and Food Chemistry,* 62(22), 5191-5197. https://doi.org/10.1021/jf500936j

Constantin, O.E. and Istrati, D.I. (2018). Functional properties of snack bars. In Lagouri, V. (Ed.). Functional Foods. p. 75-87. London, United Kingdom: IntechOpen. Retrieved from https://www.intechopen.com/chapters/63677

Dahri, N.C., Ho, L.-H., Tan, T.-C. and Mustafa, K.A. (2017). Composition, physicochemical, and physical properties of rolled oats snack bars formulated with green banana flour. *World Applied Sciences Journal,* 35(8), 1361-1372.

de Camargo, A.C., Regitano-d'Arce, M.A.B., Gallo, C.R. and Shahidi, F. (2015). Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin. *Journal of Functional Foods,* 12, 129-143. https://doi.org/10.1016/j.jff.2014.10.034

Ergönül, P.G., Ergönül, B. and Seçkin, A.K. (2010). Cholesterol content and fatty acid profile of chocolates mostly consumed in Turkey. *CyTA–Journal of Food,* 8(1), 73-78. https://doi.org/10.1080/19476330903205033

Naviglio, D., Ferrera, L. and Gallo, M. (2014). Extraction and characterization of free nonpolar lipid fraction of chocolate using a rapid analytical procedure. *European Scientific Journal,* 10(10), 208-215.

Gonçalves, N.D., Prata, A.S., Ribeiro, A.P., Rodrigues, R. and Chaves, F.C.M. (2018). Analysis of fatty acid composition of sacha inchi oil, presented at 1st International Congress on Bioactive Compounds and 2nd International Workshop on Bioactive Compounds. Brazil: Food Design and Health, Faculty of Food Engineering of the State University of Campinas.

Goyal, A., Tanwar, B., Sihag, M.K. and Sharma, V. (2022). Sacha inchi (*Plukenetia volubilis* L.): An emerging source of nutrients, omega-3 fatty acid and phytochemicals. *Food Chemistry,* 373(B), 131459. https://doi.org/10.1016/j.foodchem.2021.131459

Gutiérrez, L.-F., Sanchez-Reinoso, Z. and Quiñones-Segura, Y. (2019). Effects of dehulling sacha inchi (*Plukenetia volubilis* L.) seeds on the physicochemical and sensory properties of oils extracted by means of cold pressing. *Journal of the American Oil Chemists' Society,* 96(11), 1187-1195. https://doi.org/10.1002/aocs.12270

Harian Metro. (2021). Herba Amazon Tumbuh di Jelebu. Retrieved on August 30, 2022 from Harian Metro Website: www.hmetro.com.my/agro/2021/02/676569/herba-amazon-tumbuh-di-jelebu. [In Bahasa Malaysia].

Haritha, K., Kalyani, L. and Rao, A.L. (2014). Health benefits of dark chocolate. *Journal of Advanced Drug Delivery,* 1(4), 184-195.

Hildalgo, L.E.R., Rogel, C.J.V. and Bermeo S.M.B. (2019). Characterization of sacha inchi seed oil (*Plukenetia volubilis*) from 'Canton San Vicente, Manabí, Ecuador', obtained by non-thermal extrusion processes. *La Granja: Revista de Ciencias de la Vida,* 30(2), 70-79. https://doi.org/10.17163/lgr.n30.2019.07

Ishak, I., Ghani, M.A. and Yuen, J.Z. (2020). Effects of extraction solvent and time on the oil yield, total phenolic content, carotenoid and antioxidant activity of Australian chia seed (*Salvia hispanica* L.) oil. *Food Research*, 4(Suppl. 4), 27-37. https://doi.org/10.26656/fr.2017.4(S4).006

International Union of Pure and Applied Chemistry. (1987). Standard Methods for the Analysis of Oils, Fats and Derivates. 7th ed. Oxford, UK: Blackwell Scientific.

Liu, Q., Xu, Y.K., Zhang, P., Na, Z., Tang, T. and Shi, Y.X. (2014). Chemical composition and oxidative evolution of Sacha inchi (*Plukentia volubilis* L.) oil from Xishuangbanna (China). *Grasas Y Aceites,* 65 (1), e012. https://doi.org/10.3989/gya.075713

Maszewska, M., Florowska, A., Dłużewska, E., Wroniak, M., Marciniak-Lukasiak, K. and Żbikowska A. (2018). Oxidative stability of selected edible oils. *Molecules,* 23(7), 1746. https://doi.org/10.3390/molecules23071746

Matthäus, B. (2008). Virgin grape seed oil: Is it really a nutritional highlight? *European Journal of Lipid Science and Technology,* 110(7), 645-650. https://doi.org/10.1002/ejlt.200700276

Md. Ali, A.R. and Dimick, P.S. (1994). Thermal analysis of palm mid-fraction, cocoa butter and milk fat blend by different scanning calorimetric. *Journal of the American Oil Chemists' Society*, 71(3), 299-302. https://doi.org/10.1007/BF02638056

Medina-Mendoza, M., Rodriguez-Pérez, R.J., Rojas-Ocampo, E., Torrejón-Valqui, L., Fernández-Jeri, A.B., Idrogo-Vásquez, G., Cayo-Colca, I.S. and Castro-Alayo, E.M. (2021). Rheological, bioactive properties and sensory preferences of dark chocolates with partial incorporation of Sacha inchi (*Plukenetia volubilis* L.) oil. *Heliyon,* 7(2), e06154. https://doi.org/10.1016/j.heliyon.2021.e06154

Montagna, M.T., Diella, G., Triggiano, F., Caponio, G.R., de Giglio, O., Caggiano, G., di Ciaula, A. and Portincasa, P. (2019). Chocolate, "Food of the gods": History, science, and human health. *International Journal of Environmental and Research Public Health*, 16(24), 4960. https://doi.org/10.3390/ijerph16244960

Nadeem, M., Imran, M., Taj, I., Ajmal, M. and Junaid, M. (2017). Omega-3 fatty acids, phenolic compounds and antioxidant characteristics of chia oil supplemented margarine. *Lipids in Health and Disease,* 16, 102. https://doi.org/10.1186/s12944-017-0490-x

Niu, L., Li, J., Chen, M-S. and Xu, Z-F. (2014). Determination of oil contents in Sacha inchi (*Plukenetia volubilis*) seeds at different developmental stages by two methods: Soxhlet extraction and time-domain nuclear magnetic resonance. *Industrial Crops and Products*, 56, 187-190. https://doi.org/10.1016/j.indcrop.2014.03.007

Nogoy, K.M.C., Kim, H.J., Lee, Y., Zhang, Y., Yu, J., Lee, D.H., Li, X.Z., Smith, S.B., Seong, H.A. and Choi, S.H. (2020). High dietary oleic acid in olive oil-supplemented diet enhanced omega-3 fatty acid in blood plasma of rats. *Food Science and Nutrition,* 8 (7), 3617-3625. https://doi.org/10.1002/fsn3.1644

Payne, A.C., Mazzer, A., Clarkson, G.J.J. and Taylor, G. (2013). Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food Science and Nutrition,* 1(6), 439-444. https://doi.org/10.1002/fsn3.71

Ramos-Escudero, F., Morales, M.T., Escudero, M.R., Muñoz, A.M., Chavez, K.C. and Asuero, A.G. (2021). Assessment of phenolic and volatile compounds of commercial Sacha inchi oils and sensory evaluation. *Food Research International,* 140, 110022. https://doi.org/10.1016/j.foodres.2020.110022

Rutkowska, J. and Adamska, A. (2011). Fatty acid composition of butter originated from North-Eastern region of Poland. *Polish Journal of Food and Nutrition Sciences,* 61(3), 187-193. https://doi.org/10.2478/v10222-011-0020-x

Shafi, F., Reshi, M. and Bashir I. (2018). Chocolate processing. *International Journal of Advanced Biological Research,* 8(3), 408-419.

Silva, E.C., Sobrinho, V.S. and Cereda M.P. (2013). Stability of cassava flour-based food bars. *Food Science and Technology,* (*Campinas*)*,* 33(1), 192-198. https://doi.org/10.1590/S0101-20612013005000025

Sørensen, L.B. and Astrup, A. (2011). Eating dark and milk chocolate: A randomized crossover study of effects on appetite and energy intake. *Nutrition and Diabetes,* 1(12), e21. https://doi.org/10.1038/nutd.2011.17

Štěrbová, L., Čepková, P.H., Viehmannová, I. and Huansi, D.C. (2017). Effect of thermal processing on phenolic content, tocopherols and antioxidant activity of sacha inchi kernels. *Journal of Food Processing and Preservation,* 41(2), e12848. https://doi.org/10.1111/jfpp.12848

Torres Sánchez, E.G., Hernández-Ledesma, B. and Gutiérrez, L-F. (2021). Sacha inchi oil press-cake: Physicochemical characteristics, food-related applications and biological activity. *Food Reviews International,* 39(1), 148-159. https://doi.org/10.1080/87559129.2021.1900231

Utusan Malaysia. (2021). Kelantan Galak Penanaman Sacha Inchi untuk Pasaran Luar Negara. Retrieved on 13 December, 2022 from Utusan Malaysia Website: www.utusan.com.my/nasional/2021/02/kelantan-galak-penanaman-sacha-inchi-untuk-pasaran-luar-negara. [In Bahasa Malaysia].

