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Research Article

Nutritional, Antioxidant and Glycemic Response of Dark Chocolate Prepared with Sacha Inchi Oil

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ABSTRACT

This study aimed to enhance the nutritional and functional quality of dark chocolate by incorporating Sacha Inchi Oil (SIO), a healthier fat alternative derived from *Plukenetia volubilis*, which is rich in polyunsaturated fatty acids and antioxidants. The potential of SIO as a Cocoa Butter Equivalent (CBE) was evaluated in dark chocolate formulations containing 1%, 3%, and 5% SIO. Proximate composition and antioxidant capacity were analysed using standard methods (soxhlet, kjeldahl, total polyphenol content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and FRAP). Significant differences ($p<0.05$) were observed in fat, fiber, moisture, and energy contents, with the 5% SIO formulation showing reduced fat and energy levels, increased fiber and moisture, and the highest antioxidant activity. Additionally, a non-randomized controlled trial involving ten healthy participants was conducted to determine the Glycemic Index (GI) and Glycemic Load (GL) of the samples. While the control and 1% SIO samples exhibited high GI, the 3% and 5% SIO formulations were classified as medium GI, and all samples demonstrated low GL. The 5% SIO chocolate had the lowest glycemic response. These findings indicate that SIO can improve the nutritional profile, antioxidant properties, and glycemic response of dark chocolate, supporting its application as a functional ingredient in the development of healthier chocolate products, particularly for health-conscious populations in developing countries.

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INTRODUCTION

Chocolate is favoured all around the world and among the most widely consumed confectionery products due to its unique taste and sensory attributes (Medina-Mendoza *et al.* 2023). In 1753, the cocoa plant was named Theobroma cacao by Carl Linnaeus, meaning "food of the gods". With its origins tracing back to the ancient Maya civilization, where cacao was not only consumed as a beverage but also used medicinally to treat fever, cough, and pregnancy discomfort (Montagna *et al.* 2019).

Chocolate derived from the cacao tree, is a versatile delicacy commonly formulated with cocoa solids, sugar, milk, and emulsifiers in varying ratios, resulting in dark, milk, and white chocolate variants (Amoah *et al.* 2022). Dark chocolate which contains higher cocoa solids (with cocoa content typically ranging from 50% to 90%) are typically known for its richer polyphenol and flavonoid content contributing to its antioxidant capacity (Lima *et al.* 2020; Samantha *et al.* 2022). These bioactive compounds of polyphenols that are found in chocolates such as catechins, flavonol, anthocyanins, and procyanidins have been associated with its potential protection against the development of various metabolic diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes cancer and others (Rudrapal *et al.* 2022).

Cocoa Butter (CB) plays a crucial role in chocolate manufacturing as the main fat by forming a structural network that stabilizes other ingredients like cocoa, sugar, and lecithin.

The combination is essential as it will produce signature smooth texture, glossy appearance and slow melt of the chocolate (Medina-Mendoza *et al.* 2023; Sun *et al.* 2020). However, cocoa butter is predominantly composed of saturated fats (palmitic and stearic acids), thus the excessive intake of it is associated with the increased risk of non-communicable and chronic diseases, including obesity and cardiovascular disease (Didar 2021; WHO 2021). Additionally, CB is among the most expensive ingredients in chocolate manufacturing, prompting the industry to explore more health-conscious and cost-effective alternatives (Selvasekaran & Chidambaram 2021). Therefore, it has led to the incorporation of vegetable fats as Cocoa Butter Substitutes (CBS), which can mimic the properties of CB. However, the use of CBE typically limited to 5% of the total chocolate formulation to maintain its quality and the compliance with regulatory standards (Sadowska-Rociek 2022).

Previous studies have investigated the incorporation of bioactive compounds into chocolate matrices, including *Plukenetia volubilis* (commonly known as sacha inchi), a plant species indigenous to the Amazon region. Sacha Inchi Oil (SIO), extracted from the seeds of *Plukenetia volubilis*, is native to the Peruvian Amazon and is rich in polyunsaturated fatty acids omega 6 (linoleic acid) (34%) and omega 3 (linolenic acid) (51%) as well as monounsaturated fatty acids, tocopherols, phytosterols, and polyphenols (Quispe-Chambilla *et al.* 2024; Medina-Mendoza *et al.* 2021; Purba *et al.* 2021). Recent studies have demonstrated that components in Sacha Inchi Oil (SIO) contribute to improved lipid profiles, enhanced antioxidant activity, and beneficial effects in regulating blood glucose levels (Alayón *et al.* 2019; Cheong *et al.* 2024; Quispe-Chambilla *et al.* 2024). These findings may suggest that reformulating dark chocolate with SIO may offer dual benefits by improving nutritional quality while modulating glycemic response and satiety (Alayón *et al.* 2019; Cheong *et al.* 2024; Quispe-Chambilla *et al.* 2024).

Studies on the nutritional and functional effects of incorporating sacha inchi oil in the dark chocolate formulation particularly concerning glycemic index and response and detailed antioxidant capacity remains limited. Therefore, this study aims to evaluate the nutritional composition, total polyphenol content, antioxidant properties, and value of glycemic index and glycaemic response of dark chocolate partially substituted with SIO at different concentrations (1%, 3%, and 5%).

METHODS

Design, location, and time

This study involved two components: a laboratory-based experiment and a non-randomized controlled trial. The laboratory research aimed to evaluate the nutritional composition and antioxidant properties of dark chocolate incorporated with Sacha Inchi Oil (SIO), while the human study assessed the glycemic index and glycemic load of the formulations. Ethical approval for the human study was obtained from the UniSZA Human Research Ethics Committee (UHREC) with approval number UniSZA/UHREC/2023/545, and written informed consent was obtained from all participants prior to data collection. The research was conducted in Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, Terengganu, Malaysia. The study was conducted from October 2023 to December 2023.

Materials and tools

Ingredients used in the production of dark chocolate with Sacha Inchi Oil (SIO) were cocoa butter, cocoa mass, cocoa powder (80%), whey powder, palm sugar, soy lecithin, salt, vanilla, and SIO, all of which were purchased from local supplier and grocery market in Kuala Nerus, Terengganu, Malaysia. Chemicals used in this study were: Methanolic Potassium Hydroxide (KOH), n-hexane, Sodium Carbonate (Na_2CO_3), gallic acid, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH),quercetin, aluminum chloride, sodium nitrate, trichloroacetic acid, potassium ferricyanide, and ferric chloride purchased from Merck, Germany. Tools that were used in this study include Soxhlet extractor, Kjeldahl digestion and distillation unit, SOXTHERM fat extractor, Fibertherm system, spectrophotometer and digital glucometer.

Purposive sampling was used to determine Glycemic index of the chocolate samples where 10 participants were selected, as recommended by ISO 26642:2010 for Glycemic Index (GI) testing. All participants tried four types of dark chocolate samples: control (no oil), and those with 1%, 3%, and 5% sacha inchi oil. Participants were selected based on specific criteria: aged

18–25 years, males and females, normal Body Mass Index (BMI), no chronic illness, no dark chocolate allergy, and normal glucose tolerance.

Procedure

The dark chocolate was prepared based on a modified method from Gondalez-Bario *et al.* (2020). Four types of chocolate containing 80% cocoa mass were made: one control with only cocoa butter, and three samples with 1%, 3%, and 5% Sacha Inchi Oil (SIO) replacing part of the cocoa butter in the recipe. Cocoa butter was first melted using the double boiling method. Once fully melted, two-thirds of the cocoa mass was added, followed by cocoa powder to ensure even mixing. The remaining cocoa mass was then incorporated. Other ingredients such as whey powder, palm sugar, SIO, soy lecithin, salt, and vanilla were added, and the mixture was stirred continuously at temperatures between 65°C and 90°C. After mixing, the chocolate was cooled to 27°C, poured into plastic molds, and stored in a chiller until solidified.

Sample extraction was performed following the method of Medina-Mendoza *et al.* (2021), with slight modifications. The fat content of the dark chocolate samples was measured using the Soxhlet extraction method. About 5 g of the sample was weighed and placed in a beaker, then 10 mL of hydrochloric acid was added, and the mixture was boiled for 30 minutes. The sample was filtered, and the beaker was rinsed with distilled water to transfer any residue onto the filter paper. The filter paper with the residue was dried overnight. The extraction beaker was cleaned, dried, and weighed before a piece of cotton wool was added to the thimble, which was then placed in the beaker. Around 150 mL of petroleum ether was added, and the beaker was placed in the SOXTHERM system. After extraction, the beaker was dried in an oven at 105°C overnight, then weighed to determine the fat content. The fat percentage was calculated using a specific formula.

$$Fat\ (\%) = \frac{(W3 - W2)}{W1}\ \text{X}\ 100$$

W1=weight of the sample, W2=weight of the empty extraction beaker, and W3=weight of the extraction beaker with fat.

Protein content was measured using the Kjeldahl method. A 1.0 g sample was placed into a digestion tube with a Kjeltabs Cu 3.5 catalyst tablet and 15 mL of sulfuric acid. The tube was heated in the KJEDATHERM system at 380°C for 2 hours and 50 minutes for digestion. After digestion, the tube and a flask with 25 mL of 1% boric acid and 4 drops of methyl red indicator were placed into a distillation unit. The system automatically added 70 mL of sodium hydroxide and 50 mL of distilled water. The resulting distillate was then titrated with hydrochloric acid until the solution turned pink. The volume of acid used was recorded. Let A be the volume of HCl used for the sample and B for the blank. The nitrogen and protein contents were then calculated using a specific formula.

$$Nitrogen\ (\%) = \frac{0.1\ x\ (A - B) x\ 14.01\ x\ 100}{Weight\ of\ sample\ (g)\ x\ 1000}\ x\ 100$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Crude fiber was analyzed following the method by Zarinah *et al.* (2018). Fiber bags and crucibles were first dried at 105°C and cooled in a desiccator before weighing. Then, 1 g of dark chocolate was placed into a dried fiber bag. A glass spencer was used to defat the sample with 15 mL of petroleum ether, repeated three times. The defatted sample in the fiber bag was inserted into the Fibertherm system, where sulfuric acid, sodium hydroxide, and distilled water were added automatically. After the process, the glass spencer was rinsed with distilled water to remove any remaining sample. The fiber bag was placed into a crucible, dried at 105°C overnight, cooled, and weighed. It was then ashed overnight in a muffle furnace at 525°C, and the final weight of the crucible and ash was recorded.

$$Fiber\ (\%) = \frac{(W3\ -\ W2 - W4)}{W1}\ x\ 100$$

Ash content was determined using the dry ashing method. An empty crucible was first dried, cooled, and weighed. Then, 2 g of the sample was added to the crucible and placed in a muffle furnace at 550°C overnight. After ashing, the crucible was cooled and weighed again. Let: W0=weight of empty crucible, W1=weight of crucible with ash and W2=weight of crucible with sample.

$$Ash\ (\%) = \frac{(W1 - W0)}{(W2 - W0)}\ x\ 100$$

Carbohydrate and energy content was calculated using the formula from (Kassegn *et al.* 2018). Moisture content was measured using the oven drying method. A crucible was first dried in the oven for 15 to 30 minutes, cooled in a desiccator, and weighed. About 5 g of sample

was added, and the initial weight was recorded. The crucible was then dried in the oven at 105°C overnight and weighed again. Let: M=weight of empty crucible, M1=weight of crucible + sample before drying and M2=weight of crucible + sample after drying.

$$Moisture\ (\%) = \frac{(M1 - M2)}{(M1 - M)}\ x\ 100$$

Total Phenolic Content (TPC) was measured using the Folin-Ciocalteu method and was expressed as mg of gallic acid equivalent per gram of sample (mg GAE/g), calculated using the gallic acid calibration curve. Total Flavonoid Content (TFC) was determined by mixing 500 µL of sample extract with 100 µL each of aluminium chloride and sodium nitrate, followed by 4.3 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 415 nm. Quercetin was used as the standard, and TFC was expressed in mg quercetin equivalent per gram of sample (mg QE/g), based on the quercetin calibration curve.

DPPH antioxidant activity was tested by preparing DPPH solution (0.005 g in 100 mL methanol). A 100 µL of extract was mixed with 100 µL methanol and diluted into five concentrations (1:1, 1:2, 1:5, 1:10, 1:20). Each was then combined with 3.9 mL of DPPH solution. A control was prepared with only methanol and DPPH. All tubes were kept in the dark for 30 minutes, and absorbance was measured at 517 nm. Antioxidant activity was expressed as $IC_{50.}$ The IC_{50} (half maximal inhibitory concentration) is defined as the concentration of the extract required to inhibit 50% of the radical activity. The IC_{50} value is a widely used indicator of antioxidant potency, with lower IC_{50} values indicating higher free-radical scavenging activity, calculated from a graph of percentage inhibition versus concentration. A0 representing the absorbance of the control, while A1 represents the absorbance of the sample.

$$\%\ \text{of inhibition}\ = \frac{(A0 - A1)}{A0}\ x\ 100$$

The FRAP reading was done using a spectrophotometer. 1 mL of the sample extract was mixed with 1 mL phosphate buffer and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 25 minutes. After cooling, 1 mL of 10% trichloroacetic acid was added. Then, 1 mL of this mixture was combined with 1 mL distilled water and 0.2 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm. Antioxidant power was calculated from a gallic acid calibration curve and expressed as mg GAE/g.

For glycemic index determination, consent form was obtained from each of the participants before commencement of the study. All participants required to fast overnight (10 to 12 hours) before each test session. They joined four sessions, each spaced 3 days apart. On each test day, a fasting blood sample was collected, followed by consumption of dark chocolate (containing 15 g of available carbohydrate) with 250 mL of water. They had to finish the chocolate within 10–12 minutes. Blood samples were then taken at 15, 30, 60, and 120 minutes after eating. During the 2-hour test, participants were not allowed to eat or engage in any intense physical activity.

The subjects were given 50 g of reference food. The GI was calculated using the Incremental Area Under the Curve (IAUC) method.

$$GI = \frac{\text{IAUC test food}}{\text{IAUC reference food}}\ x\ 71$$

Data analysis

All data were analyzed using IBM SPSS Statistics version 25.0. Descriptive statistics (mean±SD) were calculated for proximate composition, antioxidant capacity, glycemic index, and glycemic load. One-way ANOVA was used to compare the chocolate formulations, with $p<0.05$ considered statistically significant. Repeated measures ANOVA was used to assess glycemic response across time points.

RESULTS AND DISCUSSION

The proximate composition of all dark chocolate samples formulated with varying percentages of SIO is presented in Table 1. According to the post hoc Tukey test, result for content of fat, fiber, moisture, and energy differed significantly ($p<0.05$) among the samples, whereas protein, ash, and carbohydrate contents showed no significant differences.

Result revealed fat content decreased with the increasing SIO concentrations, from 49.42±1.14% in the 1% SIO sample to 41.12±2.77% in the 5% SIO sample. This reduction is likely due to the partial substitution of cocoa butter, which is naturally rich in saturated fats, with SIO, which is known to contain high levels of unsaturated fatty acids such as linolenic and linoleic acids (Ishak *et al.* 2024;

Table 1. Proximate analysis of dark chocolate formulations with different percentage of SIO

Sample	Control DC	DC with 1% SIO	DC with 3% SIO	DC with 5% SIO
Fat (%)	48.49±1.47[b]	49.42±1.14[b]	47.20±0.96[b]	41.12±2.77[a]
Protein (%)	11.72±0.50[a]	11.83±0.31[a]	11.61±0.20[a]	11.71±0.10[a]
Fibre (%)	10.21±2.34[a]	12.27±0.62[a,b]	11.84±3.07[a,b]	18.32±3.33[b]
Ash (%)	3.21±0.07[a]	3.16±0.05[a]	3.19±0.03[a]	3.27±0.01[a]
Moisture (%)	1.18±0.18[b]	1.15±0.04[b]	1.71±0.11[c]	0.78±0.04[a]
Carbohydrate (%)	25.20±4.06[a]	22.16±1.02[a]	24.44±3.31[a]	24.81±5.89[a]
Energy (kcal)	584.04±4.73[b]	580.74±7.78[b]	569.00±13.00[b]	517.47±10.34[a]

Values are expressed as mean±SD (n=4); Same row with different subscript are significantly different where ($p<0.05$); SIO: Sacha Inchi Oil; DC: Dark Chocolate

Medina-Mendoza *et al.* 2021). Carbohydrate content showed no significant difference across all formulations, ranging from 22.16±1.02% to 25.20±4.06%, aligning with findings that carbohydrate levels in dark chocolate are more influenced by sugar content than cocoa butter (Caponio *et al.* 2022). Fiber content revealed to be significantly increased with the increased of SIO percentage, peaking at 18.32±3.33% in the 5% SIO sample compared to 10.21±2.34% in the control sample. Recent studies have reported that Sacha Inchi seeds contain approximately 6.61% total dietary fiber, comprising both soluble and insoluble fractions, which may contribute to the observed increase in total dietary fiber when incorporated with cocoa butter (Narváez *et al.* 2025). In addition, although the cocoa mass content remained constant, the increase in measured dietary fiber following the addition of Sacha Inchi Oil (SIO) is likely due to changes in the microstructure of the fat matrix rather than a true increase in fiber content. SIO, being lipid-based, does not contribute fiber directly, but its incorporation may enhance hydrophobic and hydrogen bonding interactions between cocoa-derived fibers and lipid molecules (Medina-Mendoza *et al.* 2021).

Result for energy content was found to be decreased significantly with the increasing of SIO %, from 584.04±4.73 kcal in the control to 517.47±10.34 kcal in the 5% SIO sample. This trend corresponds with the reduction in total fat in the chocolates, as fat contributes to 9 kcal/g whereas 4 kcal/g contributed from proteins and carbohydrates (Soekatri *et al.* 2021). Study by Medina-Mendoza *et al.* (2021) also demonstrated that replacing saturated fats in chocolate will also reduce overall energy density.

As for protein content, result remained stable across all formulations, between 11.61% and 11.83%, indicating no significant impact from SIO substitution. The finding suggests that the substitution of lipid component in the chocolate does not alter the overall protein component. This is consistent with previous findings that protein levels in chocolate are primarily influenced by cocoa mass content rather than cocoa butter (Zielińska *et al.* 2020). The moisture content of the dark chocolate samples ranged from 0.78% to 1.71%, with the 3% SIO sample showing the highest value. The increase in moisture could be due to the interaction of SIO with hydrophilic and hydrophobic compounds in the chocolate matrix. These include hydrogen bonding between water molecules and hydrophilic components such as cocoa fibers and sugars, as well as hydrophobic interactions between the lipid chains in SIO and non-polar regions of proteins or fats. That could possibly influence the emulsion properties and water-binding capacity which may influence the stability, texture and shelf life of the chocolate. Although slight increase in moisture content falls within acceptable range not more than 2% thus suggesting the addition of SOI will not compromise the stability of the chocolate as well its rheological and hardness of the chocolate (Quispe-Chambilla *et al.* 2022). These findings highlight the importance of optimizing SIO concentration to balance functional improvements with physicochemical quality attributes. Ash content also showed minimal variation (3.21–3.27%) among the samples. Sacha Inchi Oil (SIO) is a known source of minerals including phosphorus, potassium, and magnesium. However, its addition in small percentages may not have significantly altered mineral levels (Sethuraman *et al.* 2020).

Total phenolic content, total flavonoid content, and antioxidant capacity of dark chocolates were assessed spectrophotometrically

and the results obtained were presented in Table 2. The total phenolic content of dark chocolate ranged between 7.62±0.36 to 10.05±0.00, with dark chocolate with 5% SIO was the highest and dark chocolate with 1% SIO was the lowest. Results for total flavonoid content of the dark chocolates with different percentage of SIO ranged between 50.74±1.43 in 1% SIO incorporation to 152.60±0.24 in dark chocolate with 5% SIO incorporation. SIO is rich in antioxidant likely contributing to the observe increased in phenolic content of the samples where phenolic compounds are well known for their antioxidant potential which may influence by the type and proportion being used in the formulation. This synergistic interaction between cocoa matrix and SIO may suggests that reformulating dark chocolate with SIO not only improved its nutritional profile but also contributes positively to the antioxidant properties as well as enhancing its nutritional properties (Cárdenas *et al.* 2021).

Results for antioxidant capacity of dark chocolate samples with varying percentages of Sacha Inchi Oil (SIO) was evaluated using DPPH and FRAP assays (Table 2). The IC_{50} value indicates the concentration of chocolate extract required to reduce 50% of DPPH radicals. A lower IC_{50} corresponds to a higher antioxidant activity (Medina-Mendoza *et al.* 2021). Dark chocolate with 5% SIO showed the strongest antioxidant capacity (IC_{50}=0.89±0.17), significantly different from the control sample (IC_{50}=1.34±0.00, $p<0.05$). As the SIO percentage increased from 1% to 5%, the antioxidant activity also improved, suggesting that SIO incorporation boosts the scavenging capacity due to its high polyphenolic content (Štěrbová *et al.* 2017). This improvement is likely attributed to the rich biochemical profile of SIO. Sacha Inchi seed extract predominantly contains lipids including polyunsaturated fatty acids such as α-linolenic acid and linoleic acid, which are known to possess anti-inflammatory and antioxidant properties. In addition, SIO contains monounsaturated oleic acid, saturated fatty acids like palmitic and stearic acids, as well as a notable concentration of antioxidants such as α- and δ-tocopherols (vitamin E isomers), flavonoids, lignans, phenolic compounds, and plant sterols including β-sitosterol and stigmasterol. These bioactive compounds, particularly tocopherols and polyphenols, are effective in neutralizing free radicals by donating electrons or hydrogen atoms, thus enhancing the chocolate's total antioxidant potential (Cárdenas *et al.* 2021).

Contrary to the DPPH results, FRAP values decreased with increased SIO incorporation. The control sample had the highest ferric reducing antioxidant power (98.92±0.00 mg GAE/g), while the 5% SIO sample showed the lowest (90.49±1.23 mg GAE/g). This difference may be attributed to the pH dependency of the FRAP assay, which is typically conducted at acidic conditions (pH 3.6) (Zhong & Shahidi 2015). Previous research found that FRAP values are higher at alkaline pH levels (Apak *et al.* 2016), suggesting that polyphenol stability and redox potential are strongly influenced by pH. Moreover, it has been reported that the FRAP assay predominantly detects hydrophilic antioxidants, such as gallic acid, caffeic acid, or ascorbic acid, whereas lipophilic antioxidants are less effectively to FRAP values due to limited solubility and reactivity in the aqueous acidic assay medium (Berker *et al.* 2013). Therefore, the incorporation of SIO rich in unsaturated fatty acids and lipid-soluble antioxidants may result in dilution or displacement of water-soluble antioxidant compounds, thereby reducing overall FRAP reactivity.

Furthermore, pearson's correlation analysis demonstrated a strong negative correlation between TPC and DPPH (r^2=-0.708), TFC and DPPH (r^2=-0.822), TPC and FRAP (r^2=-0.665),

Table 2. TPC, TFC, and antioxidant capacity of DCs

Sample	Control DC	DC with 1% SIO	DC with 3% SIO	DC with 5% SIO
TPC (mg GAE/g)	8.48±0.01^{a}	7.62±0.36^{a}	9.72±0.36^{b}	10.05±0.0^{0b}
TFC (mg QE/g)	56.47±0.95^{b}	50.74 ±1.43^{a}	145.35±0.32^{c}	152.60±0.24^{d}
DPPH (IC50)	1.34±0.00^{b}	1.19±0.02^{a,b}	1.074±0.01^{a,b}	0.89±0.17^{a}
FRAP (mg GAE/g)	98.92±0.00^{b}	97.16±0.03^{b}	96.21±1.59^{b}	90.49±1.23^{a}

Values are expressed as mean±SD (n=4); Same row with different subscript are significantly different where ($p<0.05$); SIO: Sacha Inchi Oil; DC: Dark Chocolate; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power

TFC and FRAP (r^2=-0.748). These correlations support the finding that higher phenolic and flavonoid contents are associated with greater antioxidant activity, particularly in DPPH assay.

A total of 10 participants were involved in this study. Among them, 2 participants (20%) were males while the remaining 8 (80%) were females. The mean Body Mass Index (BMI) of the participants was 21.27±1.69 kg/m². Figure 1 illustrates the glycemic response of Dark Chocolate (DC) formulations containing different percentage of SIO over 120 minutes following its consumption.

A significant interaction was observed over time, F(3.77, 135.54)=2.75, p=0.034. The control dark chocolate demonstrated higher peak in blood glucose concentration after 15 minutes consumption and gradually followed decline. In contrast, chocolates containing 3% and 5% SIO showed a blunted glycemic response, where lower glucose peaks and a more stable curve over 120 minutes. As for sample with 5% SIO, result demonstrated lowest blood glucose levels at each time point, suggesting enhanced glycemic regulation. Although, post hoc Bonferroni analysis found no significant differences between formulations at each time point (p>0.05). These findings suggest that partial substitution of SIO may enhance the glycemic response that possibly attributed to the high unsaturated fat content of SIO, particularly alpha-linolenic acid (omega-3), which is known for their ability to slow gastric emptying and carbohydrate absorption also their ability to improve insulin sensitivity and reduce postprandial glycemia (Adeyemi & Olayaki 2018; Eraky *et al.* 2018). These findings align with result for fibre, TPC and TFC content where, chocolate sample with 5% of SIO had the highest fibre content and significantly different with control. Clinical study by Abd Rahman *et al.* (2023) also reported that adding 15 ml of SIO to a high-fat breakfast will reduced postprandial hyperglycemia thus enhanced insulin sensitivity in individuals with higher triglycerides and glycemic responses.

The Glycemic Index (GI) reported that control and 1% SIO formulations recorded the highest GI values (71.00±5.76 and 71.01±11.48, respectively), while 3% and 5% SIO formulations had lower GI values (69.94±11.57 and 67.68±8.98) (Table 3). The incorporation of higher amounts of Sacha Inchi Oil (SIO) appears to attenuate glycemic impact, potentially due to its high content of omega-3 fatty acids and polyphenolic compounds, which may enhance insulin sensitivity and modulate carbohydrate metabolism (Alayón *et al.* 2019; Rojanaverawong *et al.* 2023). Nevertheless, the GI of the control was lower than that of 3% SIO. This anomaly may be due to individual variation and external factors such as stress, hydration status, and hormonal fluctuations, which can influence glucose metabolism (Loneman *et al.* 2022; Sharma *et al.* 2022; Umpierrez & Pasquel 2017). In addition, several polyphenols present in SIO may inhibit the activity of α-glucosidase, a key digestive enzyme responsible for the breakdown of complex carbohydrates, thereby delaying glucose absorption and lowering postprandial blood glucose levels (Ćorkovic *et al.* 2022).

Glycemic Load (GL) reflects the quality and quantity of carbohydrates. The GL values were 10.65±0.86 (control), 10.65±1.72 (1% SIO), 10.49±1.74 (3% SIO), and 10.15±1.35 (5% SIO),

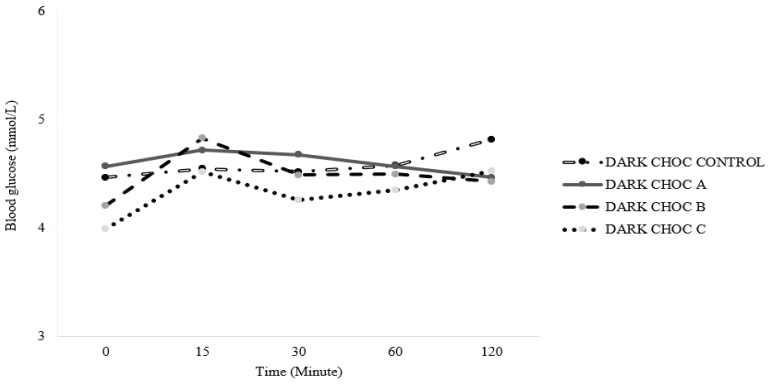

Figure 1. Glycemic response of dark chocolate prepared with different percentages of sacha inchi oil as a partial substitution of coa butter

Table 3. GI of DCs prepared with different percentage of SIO

DC samples	GI (Mean±SD)	df	F-statistics	*p*
DC control	71.00±5.76			
DC with 1% SIO	71.01±11.48	3	0.259	0.854
DCwith 3% SIO	69.94±11.57			
DC with 5% SIO	67.68±8.98			

DC: Dark Chocolate; SIO: Sacha Inchi Oil; SD: Standard Deviation; GI: Glycemic Index

Table 4. GL of DCs prepared with different percentage of SIO

DC samples	GI (Mean±SD)	df	F-statistics	*p*
DC control	10.65±0.86			
DC with 1% SIO	10.65±1.72			
DCwith 3% SIO	10.49±1.74			
DC with 5% SIO	10.15±1.35	3	0.259	0.854

DC: Dark Chocolate; SIO: Sacha Inchi Oil; SD: Standard Deviation; GL: Glycemic Load

with the lowest observed in the 5% SIO group (Table 4). These results support the potential of higher SIO incorporation to lower the glycemic impact of dark chocolate products.

CONCLUSION

Incorporating Sacha Inchi Oil (SIO) into dark chocolate, particularly at 5%, effectively lowered the glycemic index while enhancing nutritional quality through increased fiber, reduced fat, and improved antioxidant properties. All formulations maintained a low glycemic load, with the 3% SIO variant showing the highest antioxidant activity. These findings highlight the potential of SIO as a functional ingredient for developing healthier chocolate. Further studies on sensory acceptance and long-term health benefits are recommended.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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