PHYSICOCHEMICAL, TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, AND SENSORY ACCEPTABILITY OF MILK AND DARK CHOCOLATES FILLED WITH SACHA INCHI GANACHE

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ABSTRACT – *Ganache consists of cocoa butter, white chocolate and dairy cream that can be used as a chocolate filling. Nowadays, several plant seeds are incorporated into the ganache to improve the flavours, shelf life and nutritional composition. Sacha inchi (Plukenetia volubilis* L.*) is a seed that contains essential amino acids, polyunsaturated fatty acids, minerals, polyphenols and dietary fibre. In this study, ganache added with 10% sacha inchi was developed as a filling for milk and dark chocolates. The effect of sacha inchi ganache on the physicochemical, total phenolic content, antioxidant activity and sensory acceptability of milk and dark chocolates was evaluated. Milk and dark chocolates filled with ganache (without sacha inchi) were used as control samples throughout the analysis. Incorporating sacha inchi ganache decreased* significantly *($p<0.05$) the moisture and water activity of milk and dark chocolates compared to the control samples. Furthermore, the crude fibre content of milk and dark chocolates filled sacha inchi ganache were higher than the control samples. However, no significant differences ($p>0.05$) in hardness for all chocolate samples. Dark chocolate filled with sacha inchi ganache contains the highest total phenolic content and antioxidant activity compared to other chocolate samples. In terms of sensory evaluation, the mean scores for overall acceptability of the milk and dark chocolates filled with sacha inchi ganache corresponded to "like", which are similar ($p>0.05$) with control samples. Therefore, the addition of sacha inchi ganache improves the crude fibre, antioxidant activity and total phenolic content of milk and dark chocolates, which is well-accepted by consumers.*

Key words: Dark chocolate, milk chocolate, sacha inchi ganache, antioxidant activity, sensory acceptability

INTRODUCTION

Chocolate is favoured by people of all ages (Barišić *et al.,* 2019). Its popularity mainly depends on the pleasant taste and the positive emotions (joy, pleasure and calm) evoked after consumption (Konar *et al.,* 2016; Magagna *et al.,* 2017; Meier *et al.,* 2017). Three main types of chocolates, namely dark, milk and white, differ in their content of cocoa solids, milk fat and cocoa butter (Afoakwa *et al.,* 2007). Dark chocolate can be distinguished by the different percentages of cocoa solids, ranging from 47% cocoa (semi-sweet dark chocolate) to 90% cocoa (bitter dark chocolate) (Yanus *et al.,* 2014). Milk chocolate can comprise up to 20% (w/w) milk powder, which contains lactose, milk fat, and proteins that replace some of the cocoa liquor (Beckett, 2008). Meanwhile, white chocolate is made of sugar, milk powder, cocoa butter, lecithin, and vanillin, where the particles of sugar and milk powder are covered by cocoa butter as a continuous fat phase (Lončarević *et al.,* 2021).

Ganache consists of cocoa butter, white chocolate and dairy cream. Ganache can be used as a glaze, filling for pastries or chocolate bonbons (Saglio *et al.,* 2018; Wybauw, 2010). Ganache is a multiphase emulsion system composed of two immiscible liquids consisting of aqueous and fat phases formed between white chocolate and cream (Dias *et al.,* 2015; Merachli *et al.,* 2021). The ganache developed as a filling in chocolate needs to be flowable and have high stability during shelf life (Barbosa-Cánovas *et al.,* 2020; Talbot, 2009).

The Ministry of Plantation, Industries and Commodities (MPIC) aims to increase the export value of cocoa-based downstream products from RM7 billion to RM14 billion in 2022. The purpose of MPIC's strategy is to increase the cocoa market abroad, especially in Europe like Germany, Belgium, France, Spain and Switzerland (Jalil, 2022). Therefore, Malaysia should produce more cocoa-based downstream products, such as chocolates which have a great potential to be exported globally (Jalil, 2022).

Furthermore, most cocoa industries shifted their interest to producing healthier and more innovative chocolate products (Selvasekaran & Chidambaram, 2021). For instance, phytosterol esters are added to dark chocolate to lower cholesterol levels (Botelho *et al.* 2014). According to Ghorghi et al. (2023), the incorporation of hybrid gel based on beeswax-grape seed oil oleogel decreased the saturated fatty acid content of compound chocolate. Besides that, compound milk chocolate can also be fortified with chia seed oil to increase the polyunsaturated fatty acids (Razavizadeh & Tabrizi, 2021). Meanwhile, white chocolate does not contain cocoa liquor, thus lacking bioactive compounds such as polyphenols, flavonoids, catechin and fibres (Lončarević *et al.*, 2018). For that reason, dark and milk chocolates filled with sacha inchi ganache were developed to produce chocolate products with improved nutritional value.

Sacha inchi (*Plukenetia volubilis* L.) has gained attention in recent years due to their high in unsaturated fatty acids (linoleic and α-linolenic acids), proteins (essential amino acids), tocopherol, phytosterols and polyphenols (Kim & Joo, 2019). According to Del-Castillo *et al.* (2019), sacha inchi could increase high-density lipoprotein (HDL) cholesterol which is known as the "good" cholesterol to protect against heart attack and stroke. Nuts (walnut) and seeds (passion fruit seed) are commonly used as an ingredient in chocolate products (Yeo & Thed, 2022; De Clercq *et al.*, 2017). However, no previous studies have examined the effect of the sacha inchi ganache as a filling in milk and dark chocolates. Goyal *et al.* (2022) stated that consumers had limited knowledge about the health benefits of sacha inchi. Therefore, this study was conducted to develop milk and dark chocolate filled with sacha inchi ganache. The formulated chocolates were analysed for pH, water activity, moisture content, texture, crude fibre, total phenolic content, antioxidant activity and sensory acceptability.

MATERIALS AND METHODS

Materials

White chocolate callets (Callebaut), whipping cream (Anchor), invert sugar (Trimoline), sacha inchi (InchaOrganics), carrageenan and soy lecithin (EvaChem), cocoa butter and cocoa liquor (Favorich), refined sugar (Central Sugars Refinery) and skimmed milk powder (MyLacta) were used as the raw materials for developing milk and dark chocolates filled with sacha inchi ganache. Sacha inchi was grounded in a grinder (Pensonic PB-426, Malaysia) for 30 seconds. Then, the grounded sacha inchi was stored in a plastic container. All the ingredients were purchased from Bake with Yen, Nilai, Negeri Sembilan, Malaysia.

Milk and dark chocolates filled with sacha inchi ganache

<u>*Preparation of sacha inchi ganache*</u>

Ganache with sacha inchi (GSI) was developed using a method adapted from previous studies (Izzreen *et al.*, 2022; Dias *et al.*, 2015) with a few modifications by incorporating the sacha inchi and cocoa butter. Meanwhile, ganache without sacha inchi (GWSI) was also developed. About 10% of whipping cream was replaced by sacha inchi in formulation (GSI) compared to the GWSI (no replacement for whipping cream). The ganache formulations (GSI and GWSI) are presented in Table 1.

Table 1: Ganache formulations with and without sacha inchi

Ingredient	Ganache (g)	
	Ganache with sacha inchi (GSI)	**Ganache without sacha inchi (GWSI)**
White chocolate	48.0	48.0
Whipping cream	24.8	34.8
Cocoa butter	10.0	10.0
Invert sugar	7.0	7.0
Sacha inchi	10.0	0.0
Carrageenan	0.2	0.2
Total	100	100

Firstly, the invert sugar and whipping cream were heated together at 70°C by using a hot plate. After reaching the desired temperature, white chocolate was gradually poured into the hot mixture of whipping cream and invert sugar. Then, cocoa butter (in liquid form) was added to the cream and chocolate mixtures. Grounded sacha inchi and carrageenan were then added into the mixture and mixed vigorously using a hand blender (Panasonic MX-GS1, Malaysia) to obtain a smooth texture. The emulsion mixture was homogenized with a digital homogenizer IKA T25 (Ultra Turrax, Germany) at 10000 – 12000 revolutions per minute (rpm) for 30 seconds. Finally~~,~~ the sacha inchi ganache was poured into a plastic container and stored overnight in the chiller at 4°C.

<u>*Preparation of milk and dark chocolates*</u>

Dark chocolate consisting of cocoa butter, cocoa liquor, sugar and soy lecithin was produced according to the method of Abdul Halim *et al.* (2019). Meanwhile, milk chocolate contains cocoa, sugar, skimmed milk powder and soy lecithin was prepared based on the method of Lapčíková *et al.* (2022). Dark and milk chocolate formulations are shown in Table 2.

Table 2: Formulations of dark and milk chocolates

Ingredients (%)	Dark chocolate	Milk chocolate
Cocoa	54.5	40.5
Sugar	45.0	33.0
Soy Lecithin	0.5	0.5
Skimmed Milk Powder	0.0	26.0
Total	100	100

Sources: Abdul Halim et al. (2019); Lapčíková *et al.* (2022)

Cocoa butter, cocoa liquor and sugar (dark chocolate) and cocoa butter, cocoa liquor, sugar and milk powder (milk chocolate) were mixed separately in a concher (Pascal Engineering, England) for two minutes. After mixing, those ingredients were refined twice using a triple roller mill (Pascal Engineering, England) to produce a fine cocoa mixture with particle sizes ranging from 18 to 35 µm. After refining, the cocoa mixtures were mixed with soy lecithin in a concher for 6 h at 45°C.

Preparation of chocolate shell

The liquid chocolate was tempered manually on the marble slab according to the method of Biswas *et al.* (2017). The tempering temperatures of liquid milk (26°C) and dark chocolates (27°C) were achieved and reheated to 32°C for moulding. The tempered chocolate was poured into the moulds and vibrated the moulds to remove air bubbles. The surface of the mould was scrapped with stainless steel scrapper. The mould was overturned and shaken in a circular motion in different directions (clockwise and anticlockwise) to remove excess chocolate and even the thickness of the chocolate wall. Then, the chocolate was cooled in an overturned position until half set. The excess chocolate was removed by scrapping with a scrapper. The chocolate shell was cooled in a refrigerator until fully set.

Preparation of milk and dark chocolates filled with ganache (with or without sacha inchi)

The ganache (with or without sacha inchi) was filled inside the chocolate shell until almost full about 2 mm from the edge, so still have space to close the shell (as shown in Figure 1) using a disposable piping bag. Milk and dark chocolates were poured into the cavity moulds to cover the ganache. The chocolate samples were stored in the chiller at 5 – 12°C for 30 minutes. Then, the dark and milk chocolates were demoulded. All chocolate samples (Figure 2) were labelled and stored in an air-tight container for further analysis. Chocolate samples were labelled as follows - F1: Milk chocolate filled with ganache (without sacha inchi); F2: Milk chocolate filled with 10% sacha inchi ganache; F3: Dark chocolate filled with ganache (without sacha inchi); F4: Dark chocolate filled with 10% sacha inchi ganache. Milk and dark chocolates filled with ganache (without sacha inchi) (F1 and F3) were used as control samples. Meanwhile, milk and dark chocolates filled with sacha inchi ganache (F2 and F4) were compared to the control samples for pH, moisture content, water activity, texture, crude fibre, total polyphenols, antioxidant activity and sensory evaluation.

Figure 1: Ganache (with or without sacha inchi) filled into the chocolate shell

Figure 2: Dark (left) and milk chocolates (right) filled with ganache (with or without sacha inchi)

Determination of pH of milk and dark chocolates filled with sacha inchi ganache

The pH of dark and milk chocolates filled with sacha inchi ganache were measured according to the method of Rad *et al.* (2014). Firstly, all chocolate samples were melted on a hot plate at 40 ± 2°C until the ganache and chocolate became homogenous using a stirring rod. Then, 10 g of melted chocolate samples were added to 90 mL of distilled water. The mixture of chocolate with ganache and distilled water was filtered by filter paper (Whatman 4, diameter 125 mm). The pH value of chocolate filtrate was measured for three replications by pH meter (Eutech pH 2700, United States).

Determination of water activity of milk and dark chocolates filled with sacha inchi ganache

Water activity (A_w) of dark and milk chocolates filled with sacha inchi ganache were measured by Aqualab water activity meter 4TE (Wisconsin, United States) at 25°C. Chocolate samples were crushed by pestle and mortar to homogenise the chocolate with ganache before being measured by the water activity meter. The water activity for each chocolate formulation was measured in triplicates (Saglio *et al.,* 2018).

Determination of moisture content of milk and dark chocolates filled with sacha inchi ganache

The moisture content of milk and dark chocolates filled with sacha inchi ganache were analysed based on the method of García-Alamilla *et al.* (2017). About 1 g of chocolate sample was dried in an oven at 103°C for 24 hours. The moisture content was calculated according to Equation 1:

$$\text{Moisture content (\%)} = \frac{(W1 - W2)}{W1} \text{ x } 100\% \qquad (1)$$

where,
W1 represents the weight of chocolate sample before drying, W2 represents the weight of chocolate sample after drying.

Determination of texture of milk and dark chocolates filled with sacha inchi ganache

Table-top type universal tester (Shimadzu Model AGS-J, Japan) provided with RheoMeter software and equipped with 250 N load cell was used for penetration tests of milk and dark chocolates filled with sacha inchi ganache. The test was carried out at 20°C using TA2/1000 stainless steel probe to penetrate the chocolate sample with a depth of 6 mm at a rate of 1 mm/s. The hardness of the chocolate samples was recorded as the force (N) under the force–time curve at the defined penetration distance (Medina-Mendoza *et al.,* 2021; Wahyuni *et al.,* 2021).

Determination of crude fibre of milk and dark chocolates filled with sacha inchi ganache

Crude fibre of milk and dark chocolates filled with sacha inchi ganache were carried out by AOAC official method 962.09 - ceramic fibre filter method (AOAC 2000). Chocolate samples were grounded in the grinder (Pensonic PB-426, Malaysia) for 30 seconds until homogenous. About 2 g of grounded chocolate samples were digested in 50 mL of 1.25% sulfuric acid (H_2SO_4). The solution was allowed to boil for 30 min. Next, the solution was filtered by boiling water for washing and transferred to another extraction beaker. The sample filtrate was digested in 50 mL of 1.25% sodium hydroxide (NaOH). Subsequently, the sample solution was heated in an oven for 30 minutes, followed by filtration and drying. Finally, the sample residue was transferred into a muffle furnace at 550°C for 30 minutes. The measurements were carried out in triplicates (n=3), and the crude fibre results of milk and dark chocolates filled with sacha inchi ganache obtained were reported in percentage.

Determination of total phenolic content of milk and dark chocolates filled with sacha inchi ganache

The extraction of milk and dark chocolates filled with sacha inchi ganache were carried out according to the method of Adamson *et al.* (1999). Chocolate samples were grounded in the grinder (Pensonic PB-426, Malaysia) for 30 seconds until homogenous. Firstly, 10 mL of methanol (70% v/v) was added to a 2.0 g grounded chocolate sample. The chocolate mixture was shaken for 30 minutes at room temperature. This step was repeated twice. Then, the chocolate extracts were mixed thoroughly (using a stirring rod) and filtered for total phenolic content (TPC) and antioxidant activity. TPC was determined by the Folin-Ciocalteu method with some modifications (Wolosiak *et al.,* 2011; González-Molina *et al.,* 2008). About 15 μL of the chocolate extract was mixed with 12 μL of Folin-Ciocalteu reagent (2M), 170 μL of distilled water and 30 μL of sodium carbonate solution. All solutions were shaken and stored in the dark for 1 hour. About 220 μL of the mixture was pipetted into the 96-well plate. The absorbance of the solution was measured by UV-Vis spectrophotometer (Epoch BioTek, United States) at 750 nm. All chocolate extract samples were measured in triplicates. The calibration curve was plotted using standard gallic acid. The results were expressed in mg of gallic acid equivalent per 100 g sample (mg GAE/100 g).

Determination of antioxidant activity of milk and dark chocolates filled with sacha inchi ganache

DPPH radical scavenging activity

The DPPH radical scavenging activity of dark and milk chocolates filled with sacha inchi ganache extracts were measured according to the method of Poliński *et al.* (2022). Briefly, 0.5 mL of chocolate extract was added to 1.5 mL of methanol and 0.5 mL of DPPH methanolic solution (304 µmol/L). The mixture was then shaken and stored in the dark for an hour. The absorbance of the solution was measured at 595 nm by a microplate spectrophotometer (Epoch BioTek, United States). The DPPH radical scavenging activity of the dark and milk chocolates filled with sacha inchi ganache can be determined based on Equation 2:

$$(\%) = \frac{(A1 - A2)}{A1} \text{ x } 100\% \quad (2)$$

where,
A1 represents blank absorbance and A2 is the absorbance for sample

Determination of sensory evaluation of milk and dark chocolates filled with sacha inchi ganache

A consumer acceptability test was conducted to determine consumer preference regarding the degree of liking and selection of choices for milk and dark chocolates filled with sacha inchi ganache. For the seven-point hedonic scale determining the overall liking of the chocolates, untrained panellists rated each chocolate sample as 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7 = like very much. Eight attributes of the chocolates were assessed, including glossiness, texture, smoothness, aroma, bitter taste, sweetness, bitter aftertaste and overall acceptance. The sensory test involved 45 untrained panellists consisting of students and staff from the Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. These panellists were considered a representative of the target users or potential consumers of the chocolate product (Aminah, 2004). Each chocolate sample was labelled with a three-digit random code number and was served at room temperature (Espert *et al.,* 2021).

Statistical analysis

All experimental results were conducted in triplicates and expressed as mean ± standard deviation. The statistical analysis were carried out using MINITAB software version 19. Data was analysed by one-way analysis of variance (one-way ANOVA) at a 95% confidence level to find if there was any significant difference (p<0.05) between the sample means by using Tukey HSD and Fisher LSD post-hoc tests.

RESULTS AND DISCUSSIONS

pH value of milk and dark chocolates filled with sacha inchi ganache

The pH values of milk and dark chocolates filled with sacha inchi ganache are shown in Table 3. The results showed that all chocolate samples are slightly acidic, ranging from pH 5.95 to 6.46. The pH values of milk chocolates filled with ganache (with or without sacha inchi) were significantly higher (p<0.05) than those of dark chocolate samples. According to Gunaratne *et al.* (2019), dark (70% cocoa) and milk chocolates (added with 6 g of sucrose) are slightly acidic with pH values of 6.40 and 6.91, respectively, where milk chocolate has a higher pH value (p<0.05) than the dark chocolate. In addition, Izzreen *et al.* (2022) proved that the pH value of white chocolate ganache is acidic (6.71 ± 0.01). Elrofaei *et al.* (2021) stated that whole milk powder has a pH range of 6.6 – 6.7, thus lowering the pH value of milk chocolate. Overall, dark chocolate filled with sacha inchi ganache (F4) showed the lowest pH value (5.95 ± 0.01) compared to other chocolate samples.

Table 3: pH, water activity, moisture, hardness and crude fibre of milk and dark chocolates filled with sacha inchi ganache

Formulation	pH	Water activity (A_w)	Moisture (%)	Hardness (N)	Crude fibre (%)
F1	6.40 ± 0.03^a	0.77 ± 0.01^b	9.53 ± 0.02^b	11.78 ± 2.8^a	12.17 ± 0.04^c
F2	6.46 ± 0.04^a	0.71 ± 0.01^d	8.31 ± 0.01^d	13.47 ± 2.98^a	17.54 ± 0.06^a
F3	6.02 ± 0.02^b	0.81 ± 0.00^a	9.74 ± 0.01^a	11.15 ± 0.41^b	8.07 ± 0.07^d
F4	5.95 ± 0.01^c	0.73 ± 0.00^c	9.45 ± 0.01^c	10.79 ± 0.54^{ab}	15.33 ± 0.06^b

Values are expressed as mean ± standard deviation (n=3).
$^{a-c}$ Mean values with different superscript letters within the column are significantly different (p<0.05).
Formulations: F1: Milk chocolate filled with ganache (without sacha inchi); F2: Milk chocolate filled with 10% sacha inchi ganache; F3: Dark chocolate filled with ganache (without sacha inchi); F4: Dark chocolate filled with 10% sacha inchi ganache.

Water activity of milk and dark chocolates filled with sacha inchi ganache

The water activity (A_w) of milk and dark chocolates filled with sacha inchi ganache are shown in Table 3. Results showed that the addition of sacha inchi ganache decreased significantly (p<0.05) the A_w of milk and dark chocolates (A_w 0.71 – 0.73) compared to the chocolates without sacha inchi ganache (A_w 0.77 – 0.81). Overall, milk chocolate filled with sacha inchi ganache had the lowest A_w (p<0.05) compared to the other formulations. The A_w value for all chocolate formulations are above 0.70. A previous study showed that white chocolate ganache formulations contained an A_w value within the range of 0.71 – 0.72 (Izzreen *et al.*, 2022). Confectionery products with high A_w (above 0.70) encourage the growth of fungi during storage (Barbosa-Cánovas *et al*., 2020). For example, mycotoxigenic Aspergillus (fungi) most can adapt in an environment with reduced A_w of confectionery products (less than 0.80). Some species of xerophilic fungi and osmophilic yeasts can grow at A_w ranging from 0.60 to 0.70. Mycotoxin production by certain fungi requires a minimum A_w of 0.80 (Beuchat *et al.*, 2013). Xerophilic moulds (*Aspergillus chevalieri*, *A. Candidus* and *Wallemia sebi*) are microorganisms that can grow in several types of foods (chocolate fudge, snack bars, nuts, some dried fruits and raw cane sugar) within the range value of water activity (0.65 – 0.75) (Beuchat *et al.,* 2013). Therefore, it can be expected that xerophilic moulds could grow in the dark and milk chocolates filled with sacha inchi nut ganache during storage.

Moisture content of milk and dark chocolates filled with sacha inchi ganache

Table 3 shows the moisture content of milk and dark chocolates filled with sacha inchi ganache. Results showed that incorporating sacha inchi ganache decreased significantly (p<0.05) the moisture content of milk and dark chocolates. Formulation 2 reported the lowest moisture content (8.31 ± 0.01%), followed by formulation 4 (9.45 ± 0.01%), formulation 1 (9.53 ± 0.02%) and formulation 3 (9.74 ± 0.01%). Overall, dark chocolate filled with ganache (without sacha inchi) obtained the highest values of water activity and moisture content. Amevor *et al.* (2018) reported that the partial replacement of cocoa powder with different concentrations of cashew nut (75% and 90%) decreased the moisture content (p<0.05) of chocolate spread (1.00 and 0.90%, respectively) compared to the control (100% cocoa powder without cashew nut) with a moisture content of 1.40%. The sacha inchi nuts are roasted to eliminate the antinutrients and bitter taste flavour as well as increase the antioxidant activity before incorporating into the ganache (Bueno-Borges *et al.,* 2018). According to Tiefenbacher (2017), the roasting process can preserve the sacha inchi by deactivating enzymes and reducing the moisture content to inhibit the growth of fungi and bacteria. Moisture content is one of the essential characteristics in determining the shelf life of food products (Yanti *et al.*, 2022). Therefore, the moisture content of dark and milk chocolates can be reduced significantly by incorporating sacha inchi ganache as a filling.

Texture of milk and dark chocolates filled with sacha inchi ganache

Table 3 shows the hardness of milk and dark chocolates filled with sacha inchi ganache. Results showed that no significant (p>0.05) were observed in the hardness values (10.79 – 13.47 N) of chocolates incorporated with sacha inchi ganache compared to the control samples. Seçuk & Seçim (2022) found that there was no statistically significant difference in the hardness (4.1 – 4.2 N) of bitter chocolate filled with chilli peppers (powdered and seed oil) ganache and control sample (bitter chocolate without chilli pepper ganache) during storage for 15 days at 16 - 18°C. However, no similarity in the hardness values of the chocolate samples was found between the present study with Seçuk & Seçim (2022) due to the differences in the temperatures, operation of the texture equipment during measurement, chocolate mould and preservatives or additional ingredients used in the chocolate formulation (Seçuk & Seçim, 2022). Generally, the texture and appearance of chocolate are major attributes in the sensory acceptability of the consumer (Afoakwa, 2010). Therefore, milk and dark chocolates filled with sacha inchi ganache have similar hardness values to control samples.

Crude fibre of milk and dark chocolates filled with sacha inchi ganache

The crude fibre content of milk and dark chocolates filled with sacha inchi ganache are presented in Table 3. Results showed that the addition of sacha inchi ganache increased significantly (p<0.05) the crude fibre content of milk and dark chocolates compared to control samples. Formulation 2 showed the highest crude fibre content (17.54 ± 0.06%) followed by formulation 4 (15.33 ± 0.06%), formulation 1 (12.17 ± 0.04%) and formulation 3 (8.07 ± 0.07%). The increase in the crude fibre content of chocolate formulations (F2 and F4) is due to the high amount of fibre present in sacha inchi which ranged from 6.59 - 13.86 g/100 g (Goyal *et al.,* 2022; Kyaw *et al.,* 2019). Takeyama & Fukushima (2013) reported that sacha inchi contains total dietary fibre (about 81%), which consists of insoluble dietary fibre (72.4%) and soluble dietary fibre (9.0%). In addition, the mixtures of 10% *Cyperus esculentus* nuts and 40% sweet potato to replace 50% wheat flour significantly increased the fibre content of the biscuit formulation (2.58%) compared to the biscuit made with 100% wheat flour (1.77%) (Ani, 2021). Kaczmarczyk *et al.* (2012) stated that the daily intake of fibre from food can lower the risk of serious health problems, such as obesity, heart problems, diabetes, and colon cancer. Therefore, adding sacha inchi into ganache as a filling significantly increases the crude fibre content of milk and dark chocolates.

Total phenolic content (TPC) of milk and dark chocolates filled with sacha inchi ganache

Table 4 shows the total phenolic content of milk and dark chocolates filled with sacha inchi ganache. Adding sacha inchi ganache as a filling increased significantly (p<0.05) the total phenolic content of the milk and dark chocolates compared to the control samples. A previous study by Goyal *et al.* (2022), Carillo *et al.* (2018) and Chirinos *et al.* (2013) reported that sacha inchi seed contains higher phenolic content (64.8 – 91.5 mg GAE/100 g) than the sacha inchi oil (6.20 mg GAE/100 g). Dark chocolate filled with sacha inchi ganache obtained the highest total phenolic content (12.21 ± 0.68 mg GAE/g) compared to other chocolate formulations (3.57 ± 0.26 - 10.54 ± 0.21 mg GAE/g). According to Toker *et al.* (2018), the total phenolic content of dark chocolate ranges from 12 - 15 mg GAE/g. Previous authors reported that the total phenolic content of dark chocolate (578.64 ± 5.04 mg CAE/100 g chocolate) is higher than that of milk chocolate (160.46 ± 6.58 mg CAE/100 g chocolate). A similar finding by Miller *et al.* (2008) reported that the content of cocoa polyphenols, especially flavanols in dark chocolate, are five times higher than milk chocolate. This result has been linked to the amount of cocoa liquor used in the chocolate formulation. A high amount of cocoa liquor used in the dark chocolate formulation contributed to the higher phenolic content than milk chocolate. Therefore, dark chocolate filled with sacha inchi ganache contains the highest phenolic content compared to other chocolate formulations.

Table 4: Total phenolic content and free radical scavenging activity of milk and dark chocolate filled with sacha inchi nut ganache

Formulation	Total phenolic content (mg GAE/g)	Free radical scavenging activity (%)
F1	3.57 ± 0.26^{d}	76.05 ± 1.29^{d}
F2	4.27 ± 0.18^{c}	79.41 ± 1.32^{c}
F3	10.54 ± 0.21^{b}	88.04 ± 0.31^{b}
F4	12.21 ± 0.68^{a}	90.42 ± 0.30^{a}

Values are expressed as mean ± standard deviation (n=3).
$^{a-d}$ Mean values with different superscript letters within the column are significantly different (p<0.05).
Formulations: F1: Milk chocolate filled with ganache (without sacha inchi); F2: Milk chocolate filled with 10% sacha inchi ganache; F3: Dark chocolate filled with ganache (without sacha inchi); F4: Dark chocolate filled with 10% sacha inchi ganache.

Radical scavenging activity of milk and dark chocolates filled with sacha inchi ganache

Table 4 shows the DPPH radical scavenging activity of milk and dark chocolates filled with sacha inchi ganache. Results showed that incorporating sacha inchi ganache significantly enhanced (p<0.05) the antioxidant activity of milk and dark chocolates. Dark chocolate filled with sacha inchi ganache had the highest (p<0.05) antioxidant activity (90.42 ± 0.30) compared to other chocolate formulations (76.05 ± 1.29% - 88.04 ± 0.31%). Several previous studies reported that sacha inchi exhibits antioxidant activity due to the polyphenolic compounds, phytosterols and tocopherols (Rodzi & Lee, 2022; Keawkim *et al.*, 2021). The DPPH radical scavenging assay is used to determine the antioxidant activity of food products based on the mechanism of single electron transfer (Kittibunchakul *et al.*, 2022). Generally, antioxidants are found in certain foods and may prevent oxidative damage by donating an electron to free radicals, thereby reducing their reactivity (Beckett, 2019). The present study found that dark chocolate filled with sacha inchi ganache had the highest antioxidant activity. Based on the result, dark chocolate samples (with or without sacha inchi ganache) reported a higher antioxidant activity than the milk chocolate formulations. Dark chocolate has a higher antioxidant capacity than milk chocolate due to the polyphenol compounds such as catechin, epicatechin, anthocyanin and procyanidin (Barišić *et al.*, 2020). It can be concluded that sacha inchi ganache increases the antioxidant activity of milk and dark chocolates.

Sensory evaluation of milk and dark chocolates filled with sacha inchi ganache

The mean scores for each attribute of milk and dark chocolate formulations are presented in **Error! Reference source not found.**.

Table 5: Mean scores for each sensory attribute of milk and dark chocolates filled with sacha inchi ganache

Attribute	Formulation			
	F1	F2	F3	F4
Glossiness	5.16 ± 1.30^{a}	5.38 ± 1.28^{a}	5.63 ± 1.16^{a}	5.54 ± 1.15^{a}
Texture	5.67 ± 1.19^{a}	5.36 ± 1.32^{ab}	5.57 ± 1.20^{ab}	4.91 ± 1.44^{b}
Smoothness	5.8 ± 1.11^{a}	5.28 ± 1.43^{ab}	5.57 ± 1.07^{ab}	4.89 ± 1.37^{b}
Aroma	5.6 ± 1.21^{a}	5.27 ± 1.36^{a}	5.41 ± 1.16^{a}	5.21 ± 1.40^{a}
Bitter Taste	5.00 ± 1.64^{a}	4.83 ± 1.61^{a}	5.11 ± 1.21^{a}	5.08 ± 1.48^{a}
Sweetness	5.09 ± 1.83^{a}	5.04 ± 1.54^{a}	4.98 ± 1.18^{a}	4.88 ± 1.34^{a}

Bitter Aftertaste	5.11 ± 1.51[a]	4.88 ± 1.60[a]	5.11 ± 1.43[a]	4.50 ± 1.71[a]
Overall Acceptance	5.48 ± 1.19[a]	5.07 ± 1.51[a]	5.56 ± 1.16[a]	5.04 ± 1.55[a]

Values are expressed as mean ± standard deviation (n=45).
[a-b] Mean values with different superscript letters within the row are significantly different (p<0.05).
Formulations: F1: Milk chocolate filled with ganache (without sacha inchi); F2: Milk chocolate filled with 10% sacha inchi ganache; F3: Dark chocolate filled with ganache (without sacha inchi); F4: Dark chocolate filled with 10% sacha inchi ganache.

The mean scores for the overall acceptability of the milk and dark chocolate formulations were 5.04 - 5.56 based on the seven-point hedonic scale. Results showed that the mean scores for overall acceptability of the milk and dark chocolate formulations corresponded to “like”. Results showed no statistically significant differences in overall acceptance, bitter aftertaste, sweetness, bitter taste, aroma and glossiness of all chocolate formulations (p>0.05). This suggested that adding sacha inchi ganache as filling did not affect the sensory attributes of the milk and dark chocolates compared to control samples. However, formulation 4 (dark chocolate filled with sacha inchi ganache) received the lowest attribute scores for texture (4.911) and smoothness (4.889), which corresponded to “neither dislike nor like”. Meanwhile, panellists gave the highest scores for texture and smoothness of milk chocolate (with or without the addition of sacha inchi ganache) in the range of 5.278 - 5.822 (corresponding to like) due to the incorporation of milk-based ingredients that contributed to the softness of milk chocolate (Lapčíková *et al.*, 2022). Due to the strong bitter taste, sacha inchi seeds are roasted before consumption, leading to the removal of antinutrients (Bueno-Borges *et al.*, 2018). As a result, sensory attributes (bitter taste and bitter aftertaste) of milk and dark chocolates with sacha inchi ganache received similar scores (p>0.05) compared with control samples. Therefore, all chocolate formulations have similar sensory qualities in terms of glossiness, aroma, bitter taste, sweetness, bitter aftertaste, and overall acceptance. However, adding sacha inchi ganache as a filling lowers the mean score for texture and smoothness of milk and dark chocolates.

CONCLUSIONS

The present study evaluated the impact of sacha inchi ganache as filling on the quality (physicochemical, total phenolic content and antioxidant activity) and sensory attributes of milk and dark chocolates. At the same time, milk and dark chocolates filled ganache without sacha inchi were used as control samples. The obtained results concluded that sacha inchi ganache decreases moisture and water activity, which might prolong the shelf life of milk and dark chocolates. Furthermore, milk and dark chocolates filled with sacha inchi ganache obtained higher total phenolic content and antioxidant activity than the control samples. Overall, dark chocolate filled with sacha inchi ganache was selected as the best formulation in terms of health benefits based on the highest total phenolic content and antioxidant activity compared to other chocolate samples. Sensory attributes (glossiness, aroma, bitter taste, sweetness, bitter aftertaste and overall acceptance) of milk and dark chocolates filled with sacha inchi ganache obtained similar scores with control samples. In conclusion, sacha inchi ganache can be used as a functional filling base to improve the crude fibre, total phenolic content and antioxidant activity of milk and dark chocolates, which panellists prefer.

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