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Research article

Rheological, bioactive properties and sensory preferences of dark chocolates with partial incorporation of Sacha Inchi (*Plukenetia volubilis* L.) oil

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ABSTRACT

We studied the effect of substituting partially, cocoa butter (CB) with Sacha Inchi (*Plukenetia volubilis* L.) oil (SIO) on rheology, bioactive properties, and sensory preferences in potentially functional chocolate. For this 70% dark chocolates were prepared and the CB was substituted with 1.5%, 3%, and 4.5% of SIO. Hardness and viscosity of the SIO-chocolates were significantly reduced compared to the control (5451 ± 658 g; 17.01 ± 0.94 Pa s, respectively). Total phenolic content remained constant while the antioxidant capacity increased up to IC_{50} of 2.48 ± 0.10 as the content of SIO increased. The Casson yield stress and Casson plastic viscosity decreased as the amount of SIO increased. Chocolates with 4.5% SIO had a similar color, better glossiness, preferable snap attributes, and were more accepted (7.50 ± 0.08) compared to the control ($p < 0.05$), measured with a hedonic scale. Then, SIO can improve the bioactive properties of dark chocolates obtaining a potentially functional food with acceptable physicochemical characteristics. SIO can be considered as a new cocoa butter equivalent.

1. Introduction

The raw material for the manufacture of dark chocolates are cocoa beans (*Theobroma cacao* L.), valuable for their essential nutrients and functional properties (Żyżelewicz et al., 2018). Among others, the "Criollo" cocoa variety is considered as the finest variety. It is characterized as an aromatic, mild-tasting with low bitterness seed (Castro-Alayo et al., 2019; Ascrizzi et al.,2017; Qin et al., 2016). Due to its high cocoa content (>35%), dark chocolate it is considered the product derived from cocoa that has the highest content of polyphenols (Toker et al., 2018) correlated with their catechin, epicatechin, and procyanidin contents. Then, dark chocolate is recognized as an alternative antioxidant in the human diet (Todorovic et al., 2015).

Dark chocolate is a mixture of cocoa liquor and other components, surrounded by cocoa butter (CB) (Toker et al., 2018). It is a highly caloric product that contains many carbohydrates and fats, mainly CB, as well as a unique taste and texture. CB is expensive and its price increases due to the low productivity of cocoa beans and its high industrial demand (Watanabe et al., 2021). CB is the ingredient that gives chocolate its proper gloss, snap, taste (Rodriguez Furlán et al., 2017) and texture, are considered as its appearance attributes (Ostrowska-Ligęza et al., 2019). These properties are influenced by the rheology of chocolate, a science that studies quality parameters such as viscosity, Casson yield stress and Casson plastic viscosity (Glicerina et al., 2013; Beckett, 2009; Bahari and Akoh, 2018).

In order to improve the production of chocolates (economically and technologically), various types of chocolate are currently being produced, incorporating various proportions of CB and other vegetable oils: illipe, palm, sal, shea, kokum gurgi, and mango kernel (Talbot, 2009); which modify the physical characteristics of the final product (Silva et al., 2017). These vegetable fats are the so-called cocoa butter equivalents (CBE). A good CBE must be compatible with CB and not alter its physical properties when used in the manufacture of chocolate (Bahari and Akoh, 2018). Therefore, CBE must contribute with a desirable texture

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(Gregersen et al., 2015). Researchers in food and chocolate manufacturers are using CBE to reduce production costs, improve physical properties and reduce health risks for consumers (Watanabe et al., 2021). Different types of CBE have been used, including enzymatically modified vegetable oils (Watanabe et al., 2021; Zhang et al., 2020; Bahari and Akoh, 2018; Kadivar et al., 2016), considering the maximum permitted level (5%) required by the European regulation (Abdul Halim et al., 2019). In this regard, Abdul Halim et al.,. (2019) suggest that substituting 4.5% coconut oil (CNO) for CB improves the chocolate's appearance.

In the Peruvian Amazon, Sacha inchi (*Plukenetia volubilis* L.) is grown, known as Inca peanut, an important source of phenolic compounds and high antioxidant capacity (Chirinos et al., 2013). So, its use as an ingredient in food processing can improve the healthy properties of these. Given the growing demand for natural CBE, we believe it is necessary to propose alternative local sources to those already existing. Furthermore, as there are few CBE in the Peruvian market for the manufacture of chocolates, it is necessary to use other vegetable oils from local raw materials. The objective of this study is to evaluate the rheological, bioactive properties and sensory preferences of Criollo cocoa-dark chocolates with partial substitution of CB with Sacha Inchi oil (SIO).

2. Materials and methods

2.1. Materials

Pure SIO and sugar were purchased from a local market. Ingredients such as CB and Criollo cocoa beans were provided by the Cooperativa de Servicios Múltiples Aprocam (Bagua-Amazonas-Perú) and sent to the Agroindustrial Biotechnology Laboratory (LBA) from the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM).

2.2. Chemicals

All chemicals and standards: Folin-Ciocalteu's phenol, gallic acid, sodium carbonate (-)-epicatechin ≥ 98% (HPLC); (-)-catechin ≥ 97% (HPLC) from green tea, and petroleum ether ≥ 90%, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Diessenhofen, Germany), except Methanol HPLC grade (JT Baker, Deventer, The Netherlands).

2.3. Dark chocolate making process

Dark chocolates were made according to the recipe of Aprocam, considering the formula in Table 1, developed by Abdul Halim et al.,. (2019). In brief, Criollo cocoa beans of uniform size were selected. The roasting process was carried out in a tray dryer (Fischer Agro, Peru) at 110 °C, for 2 h. Cocoa nibs were obtained during the winnowing (Imsa, DC-C, Peru) from roasted beans. Next, the nibs were ground with an industrial stone roller (Prosol SAC, Tritur-50, Peru) to obtain the cocoa liquor. To carry out the conching process, all the ingredients were added to a two-roller refiner (Premier, PG508, India) for 12 h. Then, the SIO was added to the mixture in different percentages according to Table 1 and left for a remaining 1 h. The blend was tempered between 28 to 34 °C. Next, 45 g of the blend was put to the chocolate bar mold and kept for 15 min at 18 °C. Finally, it was wrapped in aluminum foil and stored at 8 °C until later physicochemical and sensory analysis.

2.4. Chemical analysis

2.4.1. Phenolic extracts

Dark chocolates were defatted using the Soxhlet extraction according to Zhou et al.,. (2015) with some modifications. In brief, 5 g of dark chocolates were defatted using petroleum ether ≥90% in a fat extractor (JP-Selecta, Det-Grasa N, Spain). Then, 1 g of defatted chocolate was mixed with 10 mL of an 80% methanolic solution, after vortexing for 1 min, the sample was taken to an ultrasound bath for 10 min at 30 °C and centrifuged at 3000 rpm for 10 min (Gültekin-Özgüven et al., 2016). The supernatant was placed in vials and kept refrigerated.

2.4.2. Antioxidant capacity

The antioxidant capacity was determined according to Żyżelewicz et al.,. (2018), Żyżelewicz et al.,. (2016), Scherer and Godoy (2009), and Brand-Williams et al.,. (1995) with some modifications. A methanolic solution (80%) of DPPH radical (solution B) was prepared to weigh 0.005 g of DPPH radical in 100 mL of this methanolic solution. Additionally, another 80% methanolic solution was prepared for the dilutions of the extract (solution C). Subsequently, dilutions of extract + methanolic solution C were prepared in the concentrations of 1:1, 1:2, 1:5, 1:10 and 1:20 (solution A). Seven test tubes containing 0.1 mL of solution A and 3.9 mL of solution B were prepared. The control was 0.1 mL of solution C and 3.9 mL of solution B. The absorbance of the samples was measured at 517 nm in a spectrophotometer (Unico, S2100, United States), after 30 min of reaction in the dark. The percentage of DPPH radical inhibition (I %) was calculated from Eq. (1), where Abs_0 and Abs_1 were the control absorbance and the absorbance of the sample; respectively. The extract concentration that produced 50% inhibition (IC_{50}) of the DPPH radical was calculated from the graph of percent inhibition versus extract concentration (Oke et al., 2009).

$$I\% = \left(\frac{Abs_0 - Abs_1}{Abs_0}\right)*100 \qquad (1)$$

2.4.3. Total phenolic content

Total phenolic content (TPC) in extracts was determined according to the Folin–Ciocalteu's procedure of Singleton et al.,. (1999) and Pantelidis et al.,. (2007), 0.05 mL of diluted extract and 0.45 mL water were mixed with 2.5 mL of 1:10 diluted Folin–Ciocalteu's phenol reagent, followed by 2 mL of 7.5% (w/v) sodium carbonate. After 5 min at 50 °C, absorbance was measured at 760 nm (Unico, S2100, United States). TPC was estimated from a standard curve of gallic acid and results were expressed as mg gallic acid equivalents/g of sample (mgGAE/g). The calibration curve ($y = 0.1073 + 0.0009x$) was prepared from the dilutions of gallic acid in a range of 0–2500 ppm ($R^2 = 0.9952$)

2.4.4. Quantification of epicatechin and catechin by UHPLC

Quantification of epicatechin and catechin in dark chocolate samples was performed according to Coklar and Akbulut (2017) and Demir et al.,. (2014) with some modifications. For that, an Agilent 1290 Infinity Series UHPLC system equipped with a G7167B mutisampler, G7104A flexible

Table 1. Formula of dark chocolates containing different percentages of SIO.

Ingredient (%)	Chocolate			
	Control	Chocolate A	Chocolate B	Chocolate C
Criollo cocoa liquor	70	70	70	70
Sugar	25	25	25	25
CB	5	3.5	2	0.5
SIO	0	1.5	3	4.5

pump, G7116B column oven, and a G7117B diode array detector was used. Before the injection, the extracts were filtered through a 0.45 µm pore size x 33 mm syringe filter (Merck, Millex, Germany). The separation was achieved using a reversed-phase C18 column (5 µm, 250 × 4.6 mm i.d.). The mobile phase was (A) water/acetic acid (98:2) and (B) water/acetonitrile/acetic acid (78:20:2). The flow rate was 0.75 mL/min and the gradient was as follows: 10–14% B (5 min), 14–23% B (11 min), 23–35% B (5 min), 35–40% B (14 min), 40–100% B (3 min), 100% B isocratic (3 min), 100–10% B (3 min) and 10% B isocratic (4 min). The detector was set to 280 nm. The column temperature was 40 °C. The epicatechin and catechin were quantified by comparison with peak areas of each standard. The data was analyzed using the Chemstation software.

2.5. Physical analysis

2.5.1. Texture

Texture (hardness) was determined using a Texture Analyzer (Brookfield, CT3 Texture Analyzer, United States) equipped with a load cell of 25 kg and a TA2/1000 stainless steel probe. All measures were kept at room temperature (20 °C) for 1 h before measuring. The following parameters were used: pre-test speed was set at 0.5 mm/s, test speed at 2.0 mm/s, product vol at 0.7 mm × 55 mm x 50mm, penetration depth 3mm, time set at 1–2 min, test speed at 50 mm/s, load cell at 25 kg, and trigger force at 20 g. The hardness (grams) was reported. All measurements were conducted at room temperature (18 ± 2 °C).

2.5.2. Rheology

The rheological properties of each sample were measured using a rheometer (Anton Paar, Model MCR 92, Austria) equipped with a concentric cylinder. Samples were first melted at 50 °C for 60 min and put into a cup. Rheological measurements were taken at 40 °C. The measurement cycle started with a preconditioning at 40 °C for 60 s. The measurements were processed with the equipment software (Rheo-Compass) which is based on Casson Model (Equation 2), where σ(Pa) is the yield stress, $\sigma_0$is Casson yield stress (Pa), $K_1$is Casson plastic viscosity (Pa.s) and γ(s-1) is the shear rate (Abdul Halim et al., 2019).

$$\sigma^{0.5} = (\sigma_0)^{0.5} + K_1(\gamma)^{0.5} \tag{2}$$

2.6. Sensory preferences

According to Abdul Halim et al., (2019), the sensory profile of the chocolate was carried out to determine the consumer preference towards all SIO-chocolates compared to the control. About 50 students from the Facultad de Ingeniería y Ciencias Agrarias (FICA) were selected as panelists for the sensory test (30 males and 20 females). The range of age from the panelists was 20–30 years old. The chocolate samples were kept at room temperature (18 ± 2 °C) for 1 h before the evaluation. In order to obtain the consent of the panelists to participate in the sensory tests, they were informed about the research objectives and the composition of the chocolate through an induction talk conducted before the test. Subsequently, they were given the questionnaire and they wrote their name as a sign of their consent to participate. Then each panelist tasted all the enriched chocolates including the control. For that, they were given four types of chocolate samples (2.5 × 1.3 × 0.7 cm, each) with three repetitions, randomly distributed. Each panelist filled a questionnaire to score from 1 ("extremely dislike") to 9 ("extremely like") using hedonic scales for attributes like taste, color, snap, gloss, and acceptability.

2.7. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using a Minitab Ver 17 Software (USA). Tukey's test was used to determine the significant differences at a level of $p < 0.05$. All experimental data were obtained in triplicates.

3. Results and discussion

3.1. Total phenolic content and antioxidant capacity

The total phenolic content (TPC) reported by Toker et al., (2018) in dark chocolate ranged between 12 and 15 mgGAE/g. In the same way, Calva-Estrada et al., (2020) reported a range of 8.94–21.17 mg GAE/g in Latin American chocolates. In our study, the range of the TPC was 16.53 ± 0.91–18.76 ± 0.15 mg GAE/g for the control and chocolates (Table 2), the analysis of the groups formed by the Tuckey test, shows that these values were similar for all the chocolates and the control. Then, the partial substitution of CB with SIO (chocolate A, B, and C) did have a significant increase in TPC ($p < 0.05$). Fernández-Romero et al., (2020) reported a reduction in the content of TPC in 46.88%, during the roasting of Criollo cocoa at 110 °C for 50 min. Therefore, we believe that although roasting may have reduced the TPC of the chocolates, they remained within a considerable range compared to Toker et al., (2018) and Calva-Estrada et al., (2020).

The IC_{50} parameter represents the concentration of chocolate extract capable of reducing the initial concentration of DPPH radical by 50% (Bordiga et al., 2015); therefore, small IC_{50} values mean a high antioxidant capacity. The antioxidant capacity of chocolates samples increased as the SIO content increased ($p < 0.05$). In a previous study performed by Summa et al. (2006), the effect of roasting on the antioxidant capacity of Ghanaian cocoa was evaluated, it was shown that the roasting process had a significant effect on melanoidins (anti-radical components of molecular weight between 5 and 10 kDa), increasing the concentration of anti-radical components. Chirinos et al., (2013) affirm that the range value of TPC for the sixteen Sacha Inchi Peruvian cultivars was within the 0.65–0.80 mg GAE/g seed. Then, due to the possible loss of TPC and antioxidant capacity during processing conditions, we tried to increase or at least minimize the loss using SIO as a substitute for CB. In our experiment, the antioxidant capacity were improved, probably due to the incorporation of SIO. We think that the roasting could have produced melanoidins and the SIO could have contributed compounds that are not TPC but that have antioxidant capacity; then, this hypothesis must be confirmed with more in-depth studies.

Several studies have confirmed the important role of dark chocolate polyphenols in human health, highlighting their beneficial effects in the treatment of cardiovascular diseases (Fanton et al., 2020; Żyżelewicz et al., 2018; Greenberg, 2015; Kwok et al., 2015), but there are still few studies that report the phenolic profile of chocolates (Martini et al., 2018). In the study carried out by Nascimento et al., (2020) in fine and artisan chocolates (from 28 to 100% of cocoa content), catechin and epicatechin contents were found ranging from 0.047-0.60 mg/g and 0.048–1.68 mg/g, respectively; concluding that chocolates with a high cocoa content are a good source of antioxidant compounds. In the present study, the catechin and epicatechin content of the chocolates ranged from 0.40 ± 0.01–0.43 ± 0.01 mg/g and 3.31 ± 0.01–3.74 ± 0.02 mg/g, respectively (Table 2), this results were higher than those found by Nascimento et al., (2020), Calva-Estrada et al., (2020) (0.45–1.03 mg/g of epicatechin, 0.06–0.25 mg/g of to catechin) and Martini et al., (2018) (2.03 mg/g of epicatechin and 0.66 mg/g of catechin). So we consider that our chocolates represent an excellent source of antioxidants. These chocolates could be considered products with functional potential, despite the imminent degradation of catechin and epicatechin as a consequence of roasting as reported by Fernández-Romero et al., (2020) (epicatechin 72.77% and catechin 24%).

3.2. Texture and rheological properties of dark chocolates

Vegetable oils known as CBE have a chemical composition and physical properties similar to CB (Talbot, 2012). This explains why some studies focus on the use of CBE to improve other properties of chocolates such as its rheological properties, texture, fat bloom stability, particle size distribution, thermal behavior and sensory profile

Table 2. TPC and antioxidant capacity of dark chocolates.

Chocolate	TPC (mg GAE/g)	Catechin (mg/g)	Epicatechin (mg/g)	Antioxidant capacity (IC_{50})
Control	18.76 ± 0.15^{a}	0.43 ± 0.01^{a}	3.74 ± 0.02^{a}	5.67 ± 0.23^{a}
Chocolate A	17.06 ± 1.03^{ab}	0.42 ± 0.01^{ab}	3.36 ± 0.01^{b}	4.38 ± 0.07^{b}
Chocolate B	16.53 ± 0.91^{b}	0.40 ± 0.01^{b}	3.31 ± 0.01^{c}	3.34 ± 0.18^{c}
Chocolate C	17.71 ± 0.25^{ab}	0.43 ± 0.01^{a}	3.74 ± 0.02^{a}	2.48 ± 0.10^{d}

Values are mean ± SD (n = 3).
Same column with different subscripts are significantly different ($p < 0.05$).

Table 3. Rheological properties of dark chocolates.

Chocolate	Hardness (g)	Casson plastic viscosity (Pa.s)	Casson yield stress (Pa)
Control	5451 ± 658^{a}	17.01 ± 0.94^{a}	2.08 ± 0.02^{a}
Chocolate A	3503 ± 844^{b}	14.65 ± 0.82^{b}	1.78 ± 0.04^{b}
Chocolate B	5562 ± 422^{a}	17.89 ± 0.27^{a}	1.81 ± 0.02^{b}
Chocolate C	4739 ± 587^{ab}	16.08 ± 1.20^{ab}	1.60 ± 0.02^{c}

Values are mean ± SD (n = 3).
Same column with different subscripts are significantly different ($p < 0.05$).

(Watanabe et al., 2021; Zhang et al., 2020; Abdul Halim et al., 2019; Bahari et al., 2018; Biswas et al., 2017; Kadivar et al., 2016). Texture determines the physical structure of a material and its mechanical and surface properties. It is measured through the hardness of the final product, understood as the force necessary to achieve a certain deformation (Ostrowska-Ligęza et al., 2019; Torbica et al., 2016). Table 3 represents the impact of the partial substitution with SIO for CB on the texture of the chocolates. In our study, the partial incorporation of SIO reduced the hardness of the chocolates A and C. The study carried out by Bahari and Akoh (2018) suggests that illipe butter and the middle palm fraction can be used to make dark chocolate with the same rheological properties and texture of a chocolates made with only CB. The opposite occurs with the use of SIO, since chocolates with lower hardness are produced with this substitute. However, our results agree with Torbica et al. (2016) and Kadivar et al. (2016), who found that the use of CBE has a softening effect on CB and therefore on chocolate, we think it is possible to use SIO as CBE, without altering the basic stander properties of the chocolate.

From a rheological point of view, dark chocolate has a complex behavior, that is, it has an apparent elastic limit and a plastic viscosity that are strictly dependent on the manufacturing process (Glicerina et al., 2013). The addition of SIO also decreased significantly the values of Casson yield stress and Casson plastic viscosity of all chocolates' samples ($p < 0.05$), these results agree with those obtained by Abdul Halim et al.,. (2019). According to Sasaki et al.,. (2012), large β′ crystals disturb the fine crystal network formed by βV crystals when both crystallize, which affects rheological properties and reduces viscosity. Then, the decreasing of Casson yield stress and Casson plastic viscosity was due to the formation of β′ in SIO-chocolates.

3.3. Sensory preference

The texture and appearance of chocolate are key attributes in the choice and acceptability of the consumer (Ostrowska-Ligęza et al., 2019), it is acceptable to use a maximum of 5% CBE on the total weight of the chocolate (Talbot, 2009). A good dark chocolate is dark brown in color and gloss (Afoakwa, 2010). Table 4 shows the results of the sensory analysis of the chocolates containing three percentages of SIO (1.5%, 3.0%, and 4.5% SIO). In our study, chocolates B and C had similar color appearance ($p > 0.05$) compared to the control (7.47 ± 0.15, 7.51 ± 0.01 and 7.34 ± 0.09, respectively). Which means that the SIO substitution did not affect the color of the chocolate. In contrast, there was a significant difference ($p < 0.05$) in gloss between the control and the chocolates containing SIO. Chocolates B and C scored the highest glosss compared to the control (7.52 ± 0.04, 7.60 ± 0.07, and 7.21 ± 0.15, respectively). For snap attributes, there was a significant difference ($p < 0.05$) between the control and the chocolate C (7.21 ± 0.10 and 7.57 ± 0.08, respectively). Most of the panelists considered that the snap of chocolate C was the best one among the other chocolates. According to Afoakwa et al. (2008), the tempering process during the production of chocolates determines the gloss and snap, then, we think this characteristic was improved by the SIO. In the same way, chocolate C was significantly preferable ($p < 0.05$) than the control and chocolate A (7.54 ± 0.05, 7.17 ± 0.04, and 6.56 ± 0.19, respectively). The chocolate taste is influenced by its ingredients composition. Therefore we think that SIO increased the taste of the chocolates. Finally, chocolates B and C were more accepted compared to the control and chocolate A (7.41 ± 0.15 and 7.50 ± 0.08 vs 7.17 ± 0.11 and 6.65 ± 0.08, respectively). These results agree with those reported by Abdul Halim et al. (2019), who used CNO as

Table 4. Sensory preference test.

Chocolate	Taste	Color	Snap	Gloss	Acceptability
Control	7.17 ± 0.04^{b}	7.34 ± 0.09^{a}	7.21 ± 0.10^{b}	7.21 ± 0.15^{b}	7.17 ± 0.11^{b}
Chocolate A	6.56 ± 0.19^{c}	6.83 ± 0.09^{b}	6.43 ± 0.08^{c}	6.91 ± 0.10^{c}	6.65 ± 0.08^{c}
Chocolate B	7.32 ± 0.04^{ab}	7.47 ± 0.15^{a}	7.28 ± 0.09^{b}	7.52 ± 0.04^{a}	7.41 ± 0.15^{ab}
Chocolate C	7.54 ± 0.05^{a}	7.51 ± 0.01^{a}	7.57 ± 0.08^{a}	7.60 ± 0.07^{a}	7.50 ± 0.08^{a}

Values are mean ± SD (n = 3).
Same column with different subscripts are significantly different ($p < 0.05$).
Each panelist was asked to score from 1 ("extremely dislike") to 9 ("extremely like") using hedonic scales.

CBE, suggesting that the replacement of CNO at 4.5% improves the appearance and rheological behavior of chocolate.

4. Conclusion

In our study, we found that the partial substitution of the cocoa butter with SIO increased the antioxidant capacity of the chocolates, though the levels added did not influence the amount of TPC compared to the control. Nonetheless, the rheological behavior and the texture of SIO-chocolates were minor compared to the control, they were within the acceptable parameters for dark chocolates. Finally, the partial substitution of CB with SIO in chocolates improved consumer preferences towards sensory analysis. Chocolates containing 4.5% of SIO, rated the highest scores of color, snap, gloss, and acceptability. Therefore, it can be said that SIO can be considered as an acceptable CBE. We consider that this work will contribute to the search for new CBE that, in addition to improving the rheological properties of chocolate, will also improve its bioactive properties to be considered a chocolate with functional potential.

Declarations

Author contribution statement

Marleni Medina-Mendoza: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Roxana J. Rodriguez-Pérez, Elizabeth Rojas-Ocampo: Performed the experiments.

Llisela Torrejón-Valqui, Guillermo Idrogo-Vásquez: Analyzed and interpreted the data.

Armstrong B. Fernández-Jeri: Contributed reagents, materials, analysis tools or data.

Ilse S. Cayo-Colca: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Efraín M. Castro-Alayo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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