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Enhancing the oxidative stability of flaxseed oil using food-grade O/W Pickering emulsions stabilized by antioxidant-rich cocoa byproduct particles

Ivan Chóez-Guaranda [a], Yohanna Mosi-Roa [b], Manuel Chacón-Fuentes [b], Karla Garrido-Miranda [c], Mauricio Opazo-Navarrete [b], Jonathan Coronel-León [a,d,e], César Burgos-Díaz [b,*]

[a] Centro de Investigaciones Biotecnológicas del Ecuador, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador
[b] Agriaquaculture Nutritional Genomic Center (CGNA), Temuco, Chile
[c] Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco, Chile
[d] Facultad de Ingeniería en Mecánica y Ciencias de la Producción, ESPOL Polytechnic University, ESPOL, Campus Gustavo Galindo, Km. 30.5 Vía Perimetral, Guayaquil, 090902, Ecuador
[e] Institute of Agrifood Research and Technology (IRTA), Food Safety and Functionality Programme, Monells, Spain

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ABSTRACT

Pickering emulsions with antioxidant properties are of great interest in developing encapsulation systems for compounds that are highly susceptible to oxidation. In this study, the fabrication of antioxidant O/W Pickering emulsions using cocoa bean shell (CBS) particles with dual functionality, as both Pickering emulsifiers and interfacial reservoirs of polyphenols, for the encapsulation of flaxseed oil was demonstrated. The antioxidant-rich CBS was pretreated to obtain Pickering stabilizers, which were characterized in terms of chemical composition and antioxidant activity using gravimetry, liquid chromatography, and spectrophotometry. O/W Pickering emulsions were then prepared by microfluidization using various concentrations of CBS particles (0.5–5 % w/w) and characterized in terms of droplet size, microstructure, and physical and oxidative stability. The results showed that CBS particles contain valuable macronutrients, including proteins (18.21 %) and fibers (57.75 %), as well as flavonoids, phenolic acids, and methylxanthines, which exhibit remarkable antioxidant activity. The obtained Pickering emulsions exhibited high resistance to creaming and a reduction in droplet size as CBS particle concentration increased, which was consistent with the TURBISCAN Stability Index (TSI) data. Moreover, emulsions stabilized with ≥4.0 % (w/w) CBS particles showed high stability against creaming over 42 days of storage at room temperature. Furthermore, oxidative stability exhibited similar trends, as indicated by OXITEST values and the measurements of primary (Peroxide values) and secondary (TBARS) oxidation products over time. These findings could contribute to the development of flaxseed oil Pickering emulsions with enhanced oxidative stability and health-promoting properties for food applications.

1. Introduction

The cocoa industry generates large amounts of byproducts, with around 20 tons of residues produced for every ton of dry cocoa bean obtained. This residual biomass mainly consists of cocoa pod husk (CPH), cocoa mucilage (also known as pulp), and cocoa bean shell (CBS) (Sánchez et al., 2023). Among these, CBS can be utilized to produce functional food ingredients due to its content of carbohydrates, proteins, dietary fibers, fats, phenolic compounds, antioxidants, and vitamins (Manzano et al., 2017; Quijano-Avilés et al., 2019; 2020, 2021; Rojo-Poveda et al., 2020). Accordingly, there is a significant opportunity to valorize this agri-food byproduct through innovative applications.

In this context, an interesting approach is the use of the CBS byproduct to produce solid amphiphilic particles with emulsifying properties. Previous studies have demonstrated that byproducts rich in proteins, fibers, and antioxidants are excellent candidates for producing

* Corresponding author.
E-mail address: cesar.burgos@cgna.cl (C. Burgos-Díaz).

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natural emulsifiers and amphiphilic particles to stabilize particle-based emulsions (Burgos-Díaz et al., 2022, 2024; Joseph et al., 2019; 2020). The use of particle-stabilized emulsions, known as Pickering emulsions, has attracted considerable attention due to their exceptional stability and functionality. This type of colloidal system refers to emulsions that are stabilized not by conventional emulsifier molecules, but by solid colloidal particles (Burgos-Díaz et al., 2020; Wu et al., 2024). Pickering emulsions exhibit more stability than surfactant-stabilized emulsions due to the attachment energy of solid particles at oil-water interfaces is much higher than that of surfactants (Boostani et al., 2024). Additionally, they exhibit low toxicity and high resistance to coalescence (Burgos-Díaz et al., 2024). The stability of these systems is mainly influenced by factors such as particle wettability, particle concentration, solid particle morphology, and oil volume fraction (Burgos-Díaz et al., 2023; Gu et al., 2024).

Given the need to minimize surfactant concentrations in food products, these colloidal systems are highly desirable for food manufacturers (Boostani et al., 2024). Proteins, polysaccharides, and polyphenols are among the biomolecules that have been widely explored for the development of food-grade Pickering emulsions (Karaca et al., 2024). However, in recent years, the use of Pickering emulsifiers derived from agri-food byproducts, such as oilseeds, fruits, and legumes, has attracted great interest due to their techno-functional properties and low cost (Burgos-Díaz et al., 2022). For instance, the byproducts from agri-food waste have been used as Pickering particles due to their ability to adsorb at the oil-water interface and stabilize emulsions without the use of conventional surfactants (Burgos-Díaz et al., 2023; Muiz et al., 2023; Riseh et al., 2024). These amphiphilic solid particles are biodegradable, non-toxic, and provide extra functional advantages such as high nutritional content, and antioxidant or antimicrobial properties, depending on their source. In addition, many plant-based Pickering particles possess intrinsic antioxidant properties due to their polyphenol content and other antioxidant compounds, such as those found in cocoa particles and other agri-food byproducts. For instance, protein-based nanoparticles enriched with phenolic antioxidants have been demonstrated to improve the oxidative stability of food-grade O/W Pickering emulsions (Chen et al., 2024). Similarly, the combination of zein and chitosan nanoparticles grafted with resveratrol has shown to stabilize edible O/W Pickering emulsions with minimal lipid oxidation and a synergistic antioxidant effect, effectively scavenging DPPH and ABTS free radicals (Ren et al., 2023). Therefore, antioxidant-rich Pickering particles can serve a dual function by enhancing both the physical stability of the emulsion and the oxidative stability of bioactive compounds encapsulated in the oil phase.

Among their applications in the food industry, Pickering emulsions are widely used for the encapsulation and controlled release of bioactive compounds such as flavors, vitamins, and probiotics, protecting them from degradation during processing and ensuring their gradual release during digestion (Karaca et al., 2024; Cassani & Gomez-Zavaglia, 2024; Hui et al., 2024). Recent research has effectively used solid-stabilized emulsions to encapsulate edible oils and enhance their oxidative stability by preventing lipid oxidation, thereby improving their shelf life (Li et al., 2022). Therefore, Pickering emulsions can be effective systems for protecting food-grade oils, particularly those rich in polyunsaturated fatty acids (PUFA), by safeguarding these sensitive oils from oxidation and thereby extending their shelf life.

One of the most important vegetable sources of PUFA is flaxseed, which stands out for its high oil content (approximately 40 % by weight). >50 % of the total fatty acids are composed of α-linolenic acid (Piornos et al., 2017). Given its composition, flaxseed oil is emerging as an important functional food ingredient due to its high content of ω−3 fatty acids. Regular consumption of ω−3-rich oils has been associated with improved cardiovascular health, enhanced cognitive function, and a reduced risk of chronic diseases such as arthritis and certain types of cancer (Kar et al., 2023). However, ω−3-rich oils, such as flaxseed oil, are highly susceptible to oxidative rancidity due to the multiple double bonds in their fatty acid chains (Piornos et al., 2017). Therefore, oil encapsulation represents a promising strategy to delay oxidative rancidity in highly unsaturated oils.

Considering the above-mentioned aspects, this study describes a novel approach using antioxidant-rich CBS particles with dual functionality, whereby the particles serve as physical stabilizers and interface-anchored reservoirs of natural antioxidants in Pickering emulsions, which may help prevent the oxidation of encapsulated edible oils. The intrinsic emulsifying and antioxidant properties of these Pickering stabilizers were explored for the first time in the stabilization of oil-in-water (O/W) CBS-based Pickering emulsions. Moreover, CBS particles were obtained through cost-effective and scalable processing techniques suitable for industrial applications, avoiding chemical synthesis and expensive methodologies. These findings will provide a deeper understanding of the application of a novel Pickering emulsifier with antioxidant properties for stabilizing O/W emulsions.

2. Material and methods

2.1. Treatment of cocoa bean shell as Pickering stabilizers

CBS was provided by Maquita Cushunchic-MCCH, a nonprofit organization located in Guayaquil, Guayas, Ecuador. The cocoa byproduct was dried at 45 °C in a convection oven VWR (Pennsylvania, USA), ground in a coffee grinder Hamilton Beach (North Caroline, USA), and sieved through a 500 μm aperture mesh. Then, CBS was defatted with hexane (1:10, w/v), stirred for 2 h, and filtered. The defatting process was repeated twice to remove as many lipid compounds as possible. After that, CBS was processed at 10,000 rpm in a rotor mill Fritsch Mill Pulverisette 14 (Indar-Oberstein, Germany) fitted with sieve rings with trapezoidal perforation. Then, milled material was sieved through a 125 μm aperture mesh to obtain a fine cocoa powder (Pickering stabilizer).

2.2. Oil extraction and fatty acids quantification of flaxseed

Flaxseeds were provided by Centro de Genómica Nutricional Agroacuícola (CGNA) located in Temuco, Araucanía, Chile. Flaxseed oil was obtained by cold pressing using a laboratory-scale mechanical press (Andes Organics, AOP-6X, Santiago, Chile). After extraction, the flaxseed oil was collected, filtered and stored in an amber glass bottle at 4 °C under dark conditions.

The fatty acids of the flaxseed oil were converted into their corresponding fatty acid methyl esters (FAMEs) according to the AOAC Official Method 996.06. The chromatographic analysis of fatty acids was performed according to Khan and Runcorn (2013) using gas chromatography (Nexis GC-2030) coupled to mass spectrometry with electron ionization Shimadzu (Kyoto, Japan). A polar capillary column DB-23 (60 m × 0.25 mm × 0.25 μm) containing (50 %-Cyanopropyl)-methylpolysiloxane was used as the stationary phase, and high purity helium as the carrier gas (1 mL/min). The injection of the samples was done at 250 °C using splitless mode. The initial oven temperature was 50 °C for 1 min, then it was increased to 175 °C at 25 °C/min, and 230 °C at 5 °C/min. The final temperature was maintained for 15 min. The temperature of the MSD transfer line and the ion source were set to 250 °C and 200 °C, respectively. The ionization voltage was 70 eV, and the data compounds were collected using the full scan mode in the mass range from 40 to 400 m/z. The concentration of fatty acids was estimated by integrating the peak area of the extract and the corresponding standards. Data processing was performed in the Shimadzu LabSolutions software version 4.5. Table S1 presents the relative abundances of six fatty acids identified in flaxseed oil.

2.3. Characterization of CBS particles

2.3.1. Proximate composition

The proximate composition of CBS particles was determined

gravimetrically following the method described by Burgos-Díaz et al. (2024). The proximate composition includes moisture (NCh 841 Of.78), total ash content (AOAC Official Method 942.05), total fat content (AOAC Official Method 920.39), total protein content (AOAC Official Method 930.03), and total dietary fiber content (AOAC Official Method 985.29). The carbohydrate content was determined by the difference. The results of gravimetric analysis were presented as mean ± standard deviation in terms of dry weight.

2.3.2. Determination of soluble and insoluble fraction of CBS

The soluble and insoluble fractions were determined according to the method described by Burgos-Díaz et al. (2024). For this, 5 % (w/w) of CBS particles were mixed with deionized water and agitated for 20 min in a multivortex (Heidolph, Schwabach, Germany). Subsequently, the samples were centrifuged at 10,000× *g* for 1 h in a centrifuge (Gyrozen Co., Ltd., Daejeon, Korea). The supernatant containing the soluble fraction, and the pellet containing the insoluble fraction in water, were collected. The percentages of soluble and insoluble fractions were then determined by gravimetric analysis.

2.3.3. Determination of size distribution and zeta potential

The particle size, zeta potential, and polydispersity index of CBS particles were determined in the Microtrac MRB Nanotrac Wave II equipment (Pennsylvania, USA). For this, 500 µL of CBS were dispersed in deionized water (1 %, w/v) and added to the analyzer's cuvette. The measurements were done at pH 7, 25 °C, and 1.47 of refractive index.

2.3.4. Microstructure observation of CBS particles

The microstructure of the particles was estimated in an optical microscope Olympus BX40 (Tokyo, Japan) equipped with a digital camera. Additionally, the microstructural properties of CBS particles were observed with a scanning electron microscope (SEM) SU3500 (Hitachi, Japan) at 12 kV, 50 Pa vacuum, and Backscattered Electron signal (BSE).

2.3.5. Water contact angle (WCA) of CBS particles

The wettability of CBS particles was qualitatively estimated from the water contact angle (WCA) measurements according to Burgos-Díaz et al. (2022), with some modifications. A compact layer of CBS was prepared by applying the powder sample onto a clean glass slide pre-adapted with double-sided tape. Afterward, a drop of deionized water was placed on the surface of the CBS layer, and the WCA was measured. Water droplet images were obtained using a Leica MZ10 F modular stereo microscope (Nanterre, France) and the WCA was measured in degrees using the GNU Image Manipulation Program (GIMP 2.10.38).

2.4. Antioxidant activity of CBS particles

2.4.1. Total phenolic content assay

The total phenolic content was estimated using the Folin-Ciocalteu method as reported by Burgos-Díaz et al. (2022), with slight modifications. Thus, 1 g of CBS particles was mixed with 10 mL of 60 % ethanol (1:10, w/v), agitated for 60 min in a multi-vortex mixer, and centrifuged at 10,000× *g* for 15 min. The supernatant was collected and filtered through a 0.22 µm membrane. After filtration, 200 µL of the extract or standard was mixed with 1000 µL of a 10 % (v/v) Folin-Ciocalteu reagent solution and left to stand for 5 min. Then, 800 µL of 7.5 % (w/v) sodium carbonate was added. The reaction mixture was incubated at 45 °C for 15 min, after which 300 µL of the reaction product was transferred to a microplate. Absorbance was measured at 765 nm using a microplate reader (Vermont, USA). The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight.

2.4.2. DPPH radical scavenging capacity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to Burgos-Díaz et al. (2022), with minor modifications. Briefly, CBS particles (1 g) were extracted with 10 mL of 80 % ethanol, agitated for 60 min, and centrifuged at 10,000× *g* for 15 min. The filtered supernatant (500 µL) was mixed with 500 µL of 0.15 mM DPPH solution, incubated for 30 min in darkness, and measured at 517 nm. Ascorbic acid served as a positive control. The radical scavenging capacity (RSC) was calculated as $RSC = [(A_0 - A_1) / A_0] \times 100$, and results were expressed as IC_{50} (mg/mL), indicating the concentration needed to scavenge 50 % of DPPH radicals.

2.4.3. ABTS radical cation inhibition assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation inhibition activity was measured following the procedure described by Viteri et al. (2022). For this, an ABTS radical cation stock solution was prepared by reacting 7 mM ABTS with 3.6 mM potassium persulfate and incubating the mixture in darkness for 24 h. After incubation, the stock solution was diluted in ethanol to obtain a final concentration of 156 µM. Then, 50 µL of the extract or standard was mixed with 150 µL of the 156 µM ABTS radical cation solution. The reaction was incubated for 30 min in darkness, and absorbance was measured at 732 nm using a multi-mode microplate reader. The results were expressed as micromoles of Trolox equivalents (TE) per 100 g of dry weight.

2.4.4. Ferric reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power (FRAP) was measured according to the methodology reported by Hozzein et al. (2020), with minor modifications. For this, a FRAP reagent mixture was prepared using a 10:1:1 (v/v/v) ratio of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution dissolved in 40 mM hydrochloric acid, and 20 mM ferric chloride dissolved in water. Then, 20 µL of the extract or standard was mixed with 180 µL of the FRAP reagent in a microplate. The reaction was incubated for 20 min in darkness, and absorbance was measured at 600 nm using a multi-mode microplate reader. The results were expressed as micromoles of Trolox equivalents (TE) per 100 g of dry weight.

2.4.5. Oxygen radical antioxidant capacity (ORAC) assay

The oxygen radical antioxidant capacity (ORAC) was measured according to Opazo-Navarrete et al. (2021), with minor modifications. Briefly, CBS particles (0.5 g) were extracted with 20 mL of 50 % acetone, agitated for 60 min, and centrifuged at 4000× *g* for 15 min. The filtered supernatant (25 µL) was mixed with 150 µL of fluorescein solution, and the reaction was initiated with 25 µL of AAPH. Fluorescence was measured at 485 nm (excitation) and 528 nm (emission) after 30 min at 37 °C. Results were expressed as micromoles of Trolox equivalents (TE) per 100 g of dry weight.

2.5. Polyphenols quantification of CBS particles by HPLC

Polyphenols, including flavonoids and phenolic acids, were extracted following Nyarko et al. (2023) with some modifications. CBS particles (1 g) were mixed with 10 mL of 0.1 N HCl (1:10, w/v), agitated for 30 min, and centrifuged at 5000× *g* for 30 min. The supernatant was purified using C18 solid-phase extraction (SPE) and filtered (0.22 µm). Then, 2 mL of the final extract was deposited in an amber vial for injection into the HPLC. Chromatographic analysis was performed using an HPLC Shimadzu LC-2050C (Kyoto, Japan) with a photodiode array (PDA) detector and a Nucleodur C18 column (4.6 × 250 mm, 5 µm) from Machenery-Nagel (Düren, Germany). The mobile phase was a mixture of 3 % acetic acid in water (eluent A) and acetic acid, acetonitrile, and water (3:25:72, v/v) (eluent B). The elution gradient started with 100 % eluent A at 1 mL/min for 5 min, followed by a gradual increase to 70 % eluent B over 35 min increasing by 10 % every 5 min, then 80 % for 5 min, 85 % at 1.2 mL/min for 10 min, and 90 % at 1.2 mL/min for 20 min. The injection volume for the extract and standards was 20 µL. The column oven temperature was maintained at 25 °C, and UV detection ranged from 240 to 370 nm. The concentration of polyphenols was

estimated by integrating the peak areas of the extract and the corresponding standards.

2.6. Preparation of Pickering emulsion

The emulsions were prepared following the method described by Burgos-Díaz et al. (2024). The CBS particle concentrations varied from 0.5 to 5 % (w/w), and the flaxseed oil content was 20 % (w/w). The pH of the aqueous phase was set at 7 using a 0.1 M buffer phosphate solution. A pre-emulsion was first formed through high-speed homogenization, followed by microfluidization. CBS powder was dispersed in the buffer solution under agitation for 20 min, then processed using an Ultra-Turrax IKA T25 (Staufen, Germany) at 6000 rpm for 2 min. The speed was increased to 8000 rpm while flaxseed oil was gradually added over 2 min, then further increased to 12,000 rpm for 10 min. The pre-emulsion was then homogenized at 600 bars using a High-Pressure Homogenizer GEA Panda Plus 1000 (Parma, Italy). The emulsions were submitted to 3 passes through the chamber. The final emulsions were stored at 4 °C.

2.7. Characterization of Pickering emulsions

2.7.1. Microstructure observation of Pickering emulsions

An estimation of the droplet size of Pickering emulsions stabilized with CBS particles was observed in an optical microscope Olympus BX40 (Tokyo, Japan) equipped with a digital camera. Furthermore, the interfacial microstructure of Pickering emulsions was visualized using a confocal laser scanning microscopy (CLSM) Olympus Fluoview 1000 (Tokyo, Japan), and the images were acquired using the FV-ASW (v. 1.7) software. The Pickering emulsions were dyed with a solution of Fast Green FCF (10 mg/mL) before scanning.

2.7.2. Droplet size distribution

The droplet size measurements of Pickering emulsions were determined by the methodology described by Burgos-Díaz et al. (2022). The emulsions were diluted in a 1:4 ratio using a solution of 10 % sodium dodecyl sulphate and agitated in a magnetic stirrer for 7 h. After that, the diluted emulsions were centrifuged at 220×g for 5 min in an Eppendorf Centrifuge 5810 R (Hamburg, Germany). The creams corresponding to the oil drops in the upper phase were collected and diluted in a 1:50 ratio using 0.1 M buffer phosphate solution. The size distributions of oil drops were determined in the Microtrac MRB Nanotrac Wave II apparatus (Pennsylvania, USA). The refractive index was set at 1.47 for the droplet and 1.33 for the aqueous phase. The distribution of the droplets was characterized by their volume-averaged diameter.

2.7.3. Creaming index of Pickering emulsions

The stability of Pickering emulsions was evaluated by tracking the creaming index (CI) over 45 days, following the method outlined by Burgos-Díaz et al. (2024). For this, 5 mL of each O/W emulsion was placed into a glass tube and sealed to avoid evaporation. The samples were observed and photographed every 7 days. The CI percentage was calculated using the following equation: $CI\ (\%) = (Hs/He) \times 100$, where Hs is the height of the serum layer, and He is the total height of the emulsion. Hs and He were measured in centimeters using the GNU Image Manipulation Program (GIMP 2.10.38).

2.7.4. Apparent viscosity of Pickering emulsions

The apparent viscosity of Pickering emulsions was determined at 25 °C using a VISCO™ rotational viscometer (ATAGO®, Japan), employing an A2L setting (composed of A2 spindle type and l-type beaker) at 250 rpm.

2.7.5. Turbiscan stability index and back-scattering profiles

The Turbiscan stability analyzer (MICROTRAC, Pennsylvania, USA) was used to evaluate the Pickering emulsion stability. Briefly, 20 mL of Pickering emulsion was placed into a cylindrical glass bottle and scanned from bottom to top for 1 h at room temperature (25 ± 1 °C). The emulsions remained stable during the determination process.

2.8. Oxidative stability of Pickering emulsions

2.8.1. OXITEST analysis

The oxidation test of the O/W Pickering emulsions prepared by microfluidization was carried out according to AOAC Official Method Cd 12c-16 using an OXITEST reactor (VELP Scientifica, Milan, Italy). For this, 15 g of Pickering emulsion was weighed and distributed uniformly into the sample cells. The device temperature and the oxygen pressure were set at 90 °C and 6 bar, respectively. The oxidative stability values of the samples were determined by the Oxisoft software and reported as induction period (IP) values.

2.8.2. Peroxide value (PV) assay

Pickering emulsions were stored in glass tubes at 45 °C for 28 days to accelerate oxidation. Lipid oxidation was measured every 7 days following the procedure described by Riernersman and Romero (2016) with minor modifications. For analysis, 0.1 g of emulsion was mixed with 1 mL of chloroform-methanol (70:30, v/v), agitated for 30 s, and centrifuged at 12,000× *g* for 8 min. Then, 75 µL of the chloroform phase was combined with 4.95 mL of chloroform-methanol, 25 µL of ammonium thiocyanate (30 %, w/v), and 25 µL of ferrous chloride (0.35 %, w/v). After a 15-min incubation at room temperature, 300 µL of the reaction product was placed in a 96-well plate, and absorbance was measured at 500 nm using a Biotek Synergy HTX microplate reader. Results were expressed as millimoles of peroxide equivalents per kilogram of sample using the equation $y = 0.0147x + 0.0132$ ($R^2 = 0.9982$) from the ferric chloride calibration curve (0–90 Fe^{3+} µg).

2.8.3. Thiobarbituric acid-reactive substances (TBARS) assay

The secondary lipid reaction products of emulsions were measured every 7 days according to the procedure reported by Burgos-Díaz et al. (2020) with minor changes. For this, 5 mL of distilled water was mixed with 0.1 g of emulsions and agitated in a vortex for 30 s. Then, 100 uL of the emulsion solution was mixed with 2 mL of thiobarbituric acid (TBA) solution, agitated in a vortex for 1 min, and incubated at 90 °C for 15 min. After centrifugation at 10,000 × *g* for 10 min in a Gyrozen Co., Ltd. apparatus (Daejeon, Korea), 300 µL of the reaction product was placed into a 96-well plate. The absorbance was measured at 520 nm against the blank in a Biotek Synergy HTX multi-detection microplate reader (Vermont, USA). The results were expressed as milligrams of malondialdehyde (MDA) equivalents per kg of sample according to the equation $y = 0.2611x+0.0305$ ($R^2 = 0.9987$) obtained from the MDA calibration curve (0.25 – 10 µg/mL).

2.9. Statistical analysis

All data were analyzed using Statgraphics Centurion 19 statistical software (Rockville, USA). Differences were evaluated using one-way analysis of variance (ANOVA), and comparisons among means were conducted using the Tukey test with a significance threshold of 0.05. All assays were carried out in triplicate and results were shown as mean ± standard deviation.

3. Results and discussion

3.1. Chemical characterization of CBS particles

The proximate analysis and physical properties of CBS particles are shown in Table 1. The CBS powder exhibits a high protein content (18.21 ± 0.28 g/100 g) and dietary fibers (57.75 ± 0.00 g/100 g). These values are consistent with previous data on cocoa bean shell, which reported protein content ranging from 10.30 % to 27.40 %, and dietary

Table 1
Proximate composition and physical properties of CBS particles.

Parameter	CBS
Proximate composition	
Protein (g/100 g)	18.21 ± 0.28
Fat (g/100 g)	1.99 ± 0.14
Dietary fiber (g/100 g)	57.75 ± 0.00
Ash (g/100 g)	9.68 ± 0.14
Carbohydrates (g/100 g)	12.37 ± 0.00
Physical properties	
Soluble fraction (%)	21.45 ± 0.06
Insoluble fraction (%)	78.55 ± 0.06
Mean size (nm)	1.24 ± 0.02
Polydispersity index	0.30 ± 0.13
Zeta potential (mV)	−26.9 ± 1.05
Form	irregular

CBS: cocoa bean shell. Proximate composition are reported on a dry matter basis.

fiber content ranging from 13.8 % to 66.33 % (Rojo-Poveda et al., 2020; Sánchez et al., 2023; Soares & Oliveira, 2022). These amphipathic macronutrients play a crucial role in the stabilization of particle-based emulsions. Pickering stabilizers or solid particles containing proteins strongly adsorb at the oil-water interface due to their amphiphilic nature (Cassani & Gomez-Zavaglia, 2024). Protein-containing particles exhibit specific amphiphilic properties and outstanding emulsification capabilities, making them highly valuable in Pickering emulsions (Xie et al. 2025). Moreover, dietary fibers create a protective mechanical barrier around the oil droplets due to their natural structural properties preventing coalescence and separation (Costa et al., 2019). Additionally, the presence of proteins and dietary fibers enhances the emulsion's properties by increasing protein content, improving texture, promoting nutrient bioavailability, and supporting digestive health (Li et al., 2024). Thus, these components serve a dual role by stabilizing the emulsion and enhancing the nutritional value of the encapsulated products.

On the other hand, the CBS powders contain a high percentage of insoluble fraction (78.55 ± 0.06 %). This is significant since the term "Pickering particles" refers to the water-insoluble fraction of the powder able to stabilize emulsions by adsorbing at the oil-water interface (Cassani & Gomez-Zavaglia, 2024). These results were comparable to previously reported values for agri-food byproducts used as Pickering stabilizers, including cocoa powder, which has been reported to contain an insoluble fraction ranging from 66.44 % to 83.37 % (Burgos-Díaz et al., 2022; Joseph et al., 2020). Our findings could be attributed to the defatting process, which effectively removes nonpolar components, including lipids and fats while concentrating other components such as proteins, carbohydrates, dietary fibers and ashes (Senarathna & Malalgoda, 2024). Furthermore, CBS particles exhibit an irregular and fragmented morphology (Fig. 1A), lacking uniform or spherical geometry. They have a mean size of <5 μm and a zeta potential of 26.9 ± 1.05 mV (absolute value). The zeta potential establishes the surface charge of a particle and indicates particle stability in a suspension. Higher absolute values often suggest greater stability, reducing the chance of particle aggregation (Feng et al., 2020). Although this parameter provides additional information about the CBS particles, in Pickering emulsions, particle size is generally much larger than the range of electrostatic or steric repulsions generated by conventional amphiphilic molecules, such as surfactants or proteins. When particulate stabilizers are anchored at the oil-water interface, they form a robust interfacial layer that prevents droplet coalescence through mechanical means, providing exceptional stability. Therefore, particle size and concentration are key parameters for controlling droplet size in Pickering emulsions rather than electrostatic repulsions.

On the other hand, the formation of Pickering O/W emulsions is highly influenced by the hydrophobicity of Pickering particles, which can be qualitatively estimated through water contact angle (WCA) measurements that assess the interaction between the water droplet and the surface of solid particles (in this case, CBS). Thus, the WCA properties of CBS particles were evaluated to determine their hydrophilic (WCA < 90°) or hydrophobic (WCA > 90°) characteristics. Hydrophilic particles are more effective at stabilizing O/W emulsions while hydrophobic particles are better suited for stabilizing W/O emulsions (Burgos-Díaz et al., 2023). As shown in Fig. 1(B), CBS particles exhibited a WCA of <90°, indicating their hydrophilic nature. In general, particles with a contact angle in the range of $15° < \theta < 90°$ should stabilize better O/W emulsions. These results were higher than data reported for other agri-food byproducts used as Pickering stabilizers, with WCA values ranging from 42.2° to 77.5° (Burgos-Díaz et al., 2022). Similar results (75±7°) have been observed for lignin-rich particles extracted from a cocoa byproduct via chemical treatment and used as colloidal particles for O/W Pickering emulsion stabilization (Cuthill et al., 2021). Thus, the present results support preliminary studies on this cocoa byproduct as a potential stabilizer for formulating O/W emulsion systems.

Furthermore, interestingly no relationship was observed between pH and the wettability values of CBS particles (Fig. 1B), despite a zeta potential value (−26.9 mV) indicating that they are highly charged particles and that pH changes should have affected their wettability. This is important since various studies have demonstrated that pH adjustments can change the surface charge (zeta potential) as well as the

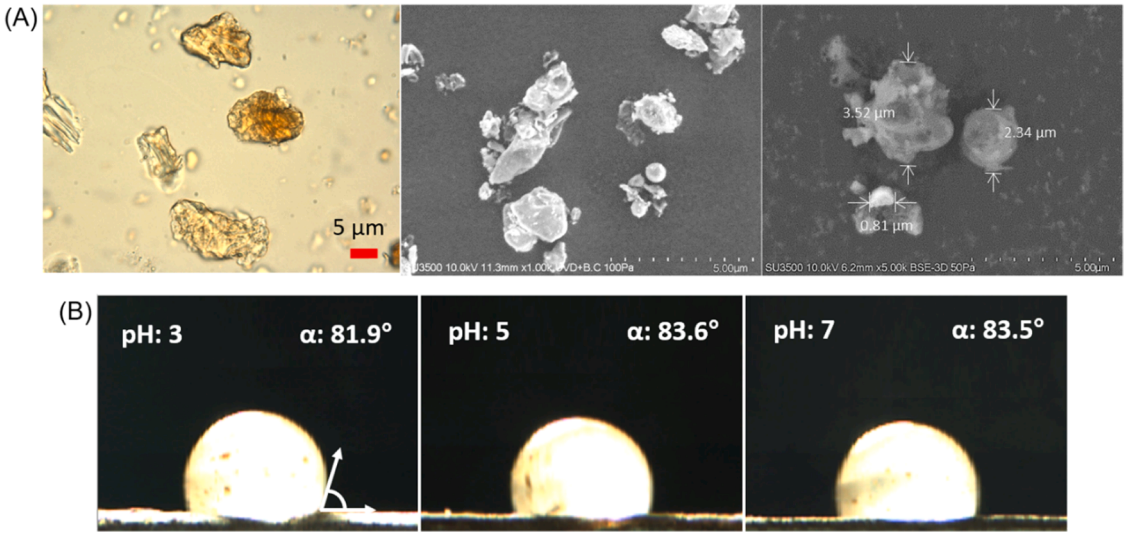

Fig. 1. Optical and SEM micrographs acquired at 40x magnification of CBS particles (A). Water contact angle of CBS particles film at different pH (B).

hydrophilicity and hydrophobicity of colloidal particles, thereby affecting their emulsifying properties (Ahmed et al., 2021; Seiler et al., 2024).

3.2. Antioxidant activity and individual polyphenols content of CBS particles

The total phenolic content (TPC) and antioxidant activity, assessed using ABTS, FRAP, ORAC, and DPPH assays, along with the individual polyphenol content detected in CBS particles, are presented in Table 2. The results indicate that CBS particles are rich in total phenolic compounds (3908.45 mg GAE/100 g), confirming the antioxidant potential of this cocoa byproduct. Notably, CBS particles exhibit the highest antioxidant capacity against oxygen radicals, as demonstrated by the ORAC assay (44,703.42 μmol TE/100 g), followed by the ABTS (8,370.31 μmol TE/100 g) and FRAP (46,448.67 μmol TE/100 g) assays. It should be noted that a low IC_{50} value (0.88 mg/mL) was observed (Fig. S1), indicating the concentration required to achieve 50 % inhibition of the DPPH radical. The high TPC in CBS was consistent with previous studies on cocoa byproducts, which reported comparable values (312 - 9495 mg GAE/100 g) in cocoa bean shells and highlighted their potential as a natural source of antioxidants (Rojo-Poveda et al., 2020). Regarding individual polyphenols, the predominant flavonoids were epicatechin (121.64 mg/100 g) and catechin (12.92 mg/100 g), while rutin was present in smaller amounts (1.66 mg/100 g). Among the phenolic acids, the most abundant were syringic acid (2.30 mg/100 g) and vanillic acid (1.67 mg/100 g), with smaller amounts of caffeic acid, chlorogenic acid, and coumaric acid. Additionally, the methylxanthines theobromine (183.96 mg/100 g), caffeine (109.56 mg/100 g), and theophylline (6.86 mg/100 g) were also detected. Finally, vanillin (8.96 mg/100 g), an aromatic compound relevant to the food industry, was identified. These results confirm that CBS particles are rich in bioactive compounds, particularly polyphenols and methylxanthines. These results are within the content range of previous data on cocoa bean shells reported for epicatechin (21 – 3497 mg/100 g), caffeine (4 – 421 mg/100 g), and theobromine (39 – 1350 mg/100 g), but differ for catechin (80 – 616 mg/100 g) and theophylline (10 – 30 mg/100 g) (Balentic et al., 2018; Rojo-Poveda et al., 2020; Sánchez et al., 2023).

Table 2
Total phenolic content, antioxidant activity (ABTS, FRAP, ORAC, DPPH) and individual polyphenols content of CBS particles. The values are reported on a dry matter basis.

Parameter	CBS
TPC (mg GAE/100 g)	3908.45 ± 32.75
ABTS (μmol TE/100 g)	8370.31 ± 79.00
FRAP (μmol TE/100 g)	46,448.67 ± 75.49
ORAC (μmol TE/100 g)	44,703.42 ± 1557.90
IC_{50}, DPPH (mg/mL)	0.88 ± 0.05
Flavonoids (mg/100 g)	
Catechin	12.92 ± 0.16
Epicatechin	121.64 ± 0.08
Rutin	1.66 ± 0.05
Phenolic acids (mg/100 g)	
Caffeic acid	0.08 ± 0.00
Chlorogenic acid	0.25 ± 0.05
Coumaric acid	0.08 ± 0.01
Syringic acid	2.30 ± 0.03
Vanillic acid	1.67 ± 0.06
Methylxanthines (mg/100 g)	
Caffeine	109.56 ± 0.18
Theobromine	183.96 ± 0.19
Theophylline	6.86 ± 0.02
Other phenolic compounds (mg/100 g)	
Vanillin	8.96 ± 0.29

CBS: cocoa bean shell. TPC: total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: ferric reducing antioxidant power; ORAC: oxygen radical antioxidant capacity; The IC_{50} represents the concentration of CBS that inhibits DPPH activity by 50 %.

Although scientific data on the antioxidant activity of cocoa bean shells assessed through ABTS, DPPH, FRAP, and ORAC assays is limited, the antioxidant activity of CBS particles could primarily be attributed to the presence of epicatechin, catechin, theobromine, and caffeine, as these were the compounds found in the highest concentrations. Flavonoids, particularly epicatechin and catechin, have demonstrated significant antioxidant activity due to their chemical structures, which facilitate electron donation and free radical neutralization. These compounds have multiple hydroxyl groups attached to their aromatic rings, which enhance their ability to act as electron donors, effectively neutralizing reactive oxygen species (ROS) and mitigating oxidative stress (Munteanu & Apetrei, 2022; Widowati et al., 2024). Alternatively, Catechin, caffeine, and theobromine have been shown to scavenge various free radicals, including DPPH and ABTS radicals, as well as reduce lipid peroxidation (TBARS formation). Among these, catechin demonstrates superior effectiveness compared to methylxanthines (Ademola et al., 2024). In contrast to flavonoids, methylxanthines are alkaloids characterized by a purine ring structure, whose primary mode of action is the inhibition of adenosine receptors (Monteiro et al., 2016). Nevertheless, methylxanthines such as caffeine and theophylline also have the ability to modulate endogenous antioxidant enzymes (Rahmati Darvazi et al., 2018), bind metal ions, scavenge hydroxyl and alkoxyl radicals, as well as inhibit lipid peroxidation, thereby reducing oxidative damage (Janitschke et al., 2021). Furthermore, the antioxidant activity may be associated with the presence of syringic acid and vanillic acid, as both phenolic compounds have demonstrated strong free radical-scavenging capabilities (Singh et al., 2021; Surya et al., 2023). In this context, the notable antioxidant capacity reported in the present study can be predominantly attributed to the combined antioxidant mechanisms of flavonoids and xanthine derivatives. Overall, the antioxidant capacity suggests that CBS particles could be used as natural stabilizers in emulsions, reducing the need for synthetic additives (Burgos-Díaz et al., 2023). These findings highlight CBS particles as a rich source of polyphenols and methylxanthines, exhibiting notable antioxidant activity.

3.3. Characterization of Pickering emulsion

3.3.1. Effect of CBS particles concentration on emulsion droplet size

Fig. 2(A) presents micrographs of emulsions stabilized by CBS particles at different concentrations (0.5–5 % w/w). As expected in this type of colloidal system, the droplet size of the O/W emulsions decreased as CBS particle concentration increased from 0.5 to 5 % (w/w), confirming enhanced Pickering emulsion stability at higher particle concentrations. As the particle concentration increases, more interfacial area is covered, leading to smaller droplets with reduced creaming, in accordance with the well-known Stokes' law. This phenomenon is consistent with our previous study using other Pickering stabilizers (Burgos-Díaz et al., 2020, 2022, 2024).

On the other hand, Fig. 2B shows that the particle size distribution of the droplet of emulsions (O/W) decreases with increasing particle concentration. Therefore, as observed in Fig. 2A and B, when the particle concentration is low, the oil droplets are much larger. This is attributed to an insufficient number of particles to fully cover the interface of newly formed oil droplets during homogenization, leading to coalescence and even oiling off in the emulsion. In contrast, at high concentrations, surface coverage is sufficient to produce smaller droplets (Schröder et al., 2018).

Fig. 3 presents CLSM images of emulsions stabilized by CBS particles. The micrographs clearly show that the oil droplets are effectively coated with a layer of accumulated CBS particles (bright red ring), confirming the successful formation of O/W Pickering emulsions. The particles anchored at the oil-water interface act as a strong physical barrier, effectively preventing droplet coalescence through steric hindrance and providing high storage stability (Burgos-Díaz et al., 2024; Ramos et al.,

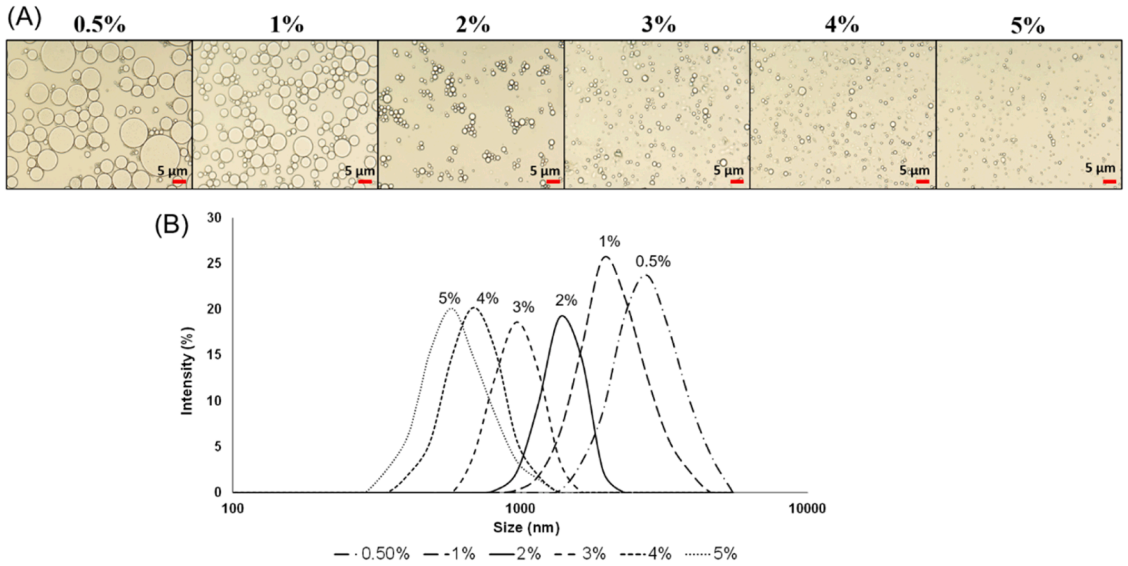

Fig. 2. Optical micrographs acquired at 40x magnification of the O/W Pickering emulsions stabilized with CBS particles at different concentrations (0.5 – 5.0 %, w/w) (A). Droplet size distribution of the O/W Pickering emulsions stabilized with CBS particles at different concentrations (0.5 – 5 %, w/w) (B).

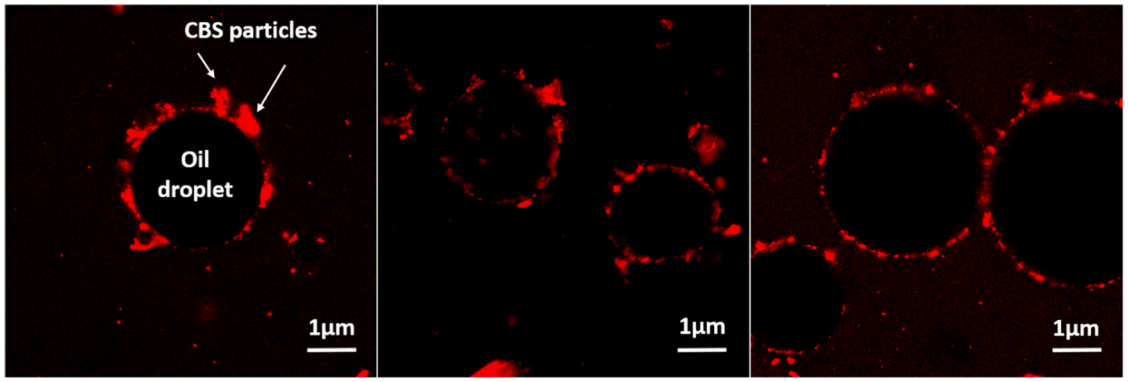

Fig. 3. CLSM micrographs acquired at 40x magnification of the O/W Pickering emulsions stabilized by CBS particles at 5 %, w/w.

2024). The thickness of this layer is typically much greater than the range of electrostatic or steric repulsions generated by conventional amphiphilic molecules such as surfactants and proteins. This result is consistent with our previous study using agri-food byproducts and protein nano-aggregates as Pickering stabilizers (Burgos-Díaz et al., 2020, 2024). In both studies, CLSM images showed that oil droplets were covered with a dense layer of accumulated particles (similar to Fig. 3), confirming that O/W emulsions are stabilized by Pickering solid particles.

The combined presence of fibers, proteins, and polyphenols in CBS particles can create a synergistic effect that enhances the stability and functionality of Pickering emulsions. In this context, polyphenols play a dual role by modifying the interfacial properties of protein-based particles, thereby promoting emulsion stability and reducing lipid oxidation (Najari et al., 2024). Polyphenols can induce conformational changes in protein structures, which helps prevent droplet aggregation and flocculation, thereby improving the physical stability of the emulsions (Tang et al., 2025). For instance, colloidal particles composed of tannic acid (antioxidant) and ovalbumin (protein) enhance interfacial wettability through non-covalent interactions, making them effective emulsifiers for stabilizing Pickering emulsions, as reported in a previous study (Chen et al., 2023). Furthermore, Pickering particles formed from ternary complexes of proteins-polysaccharides- polyphenols have shown improved stability and functionality (Najari et al., 2024). These interactions facilitate the formation of complex interfacial structures, leading to enhanced emulsifying properties and more robust emulsions. Particularly, cellulose nanofibers (CNFs) obtained from cocoa shells create a physical barrier and a network, acting as an obstacle between contiguous droplets and preventing droplet coalescence in Pickering emulsions (Gómez Hoyos et al., 2023). Comparable stabilization effects have been reported through the use of plant protein nanoparticles (Xie et al., 2025), protein/polysaccharide/polyphenol complexes (Najari et al., 2024), and plant protein–polyphenol particles (Huang et al., 2022), which contribute to the formation of denser and more compact interfacial layers, thereby enhancing emulsion stability.

3.3.2. *Effect of CBS particle concentration on the physical stability of Pickering emulsions*

The emulsion stability was evaluated by the creaming index (CI) and visual observation of Pickering emulsions. As shown in Fig. 4A, the creaming index values gradually decreased as the CBS concentration in the emulsion increased from 0.5 % to 5 %. These observations indicated that increasing the concentration of the particles gradually improved the emulsion stability. The emulsions stabilized by CBS at a concentration of ≥5 % (w/w), no creaming behavior was detected after 42 days, indicating that this sample exhibited the greatest stability during storage compared to the other samples. In addition, all emulsions were stable against coalescence, as no macroscopic oil leakage was observed at the top of the tubes (Fig. 3). This behavior is attributed to the fact that, at low particle concentrations, the particles are sparsely distributed along

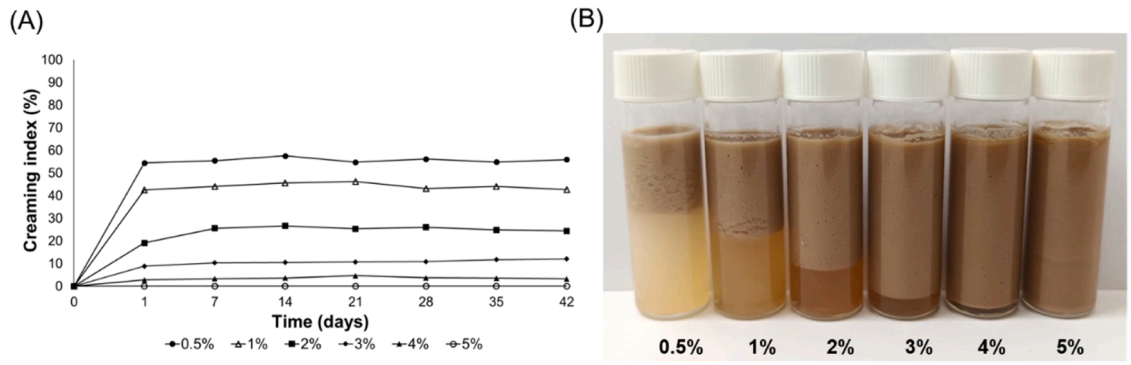

Fig. 4. Creaming index of O/W Pickering emulsions stabilized with CBS particles at different concentrations (0.5 – 5 %, w/w) (A). Images of the O/W Pickering emulsions after storage for 42 days (B).

the oil–water interface, providing insufficient coverage of the oil phase and leading to a highly unstable Pickering emulsion (resulting in greater creaming). In contrast, as the particle concentration increases, the oil droplets in the emulsion become fully covered, resulting in greater emulsion stability. Previous studies using cocoa powder as a Pickering stabilizer have reported a similar qualitative trend in emulsion stabilization, where increasing particle concentration at a constant droplet fraction leads to greater interfacial coverage and smaller droplet sizes (Joseph et al., 2020). This behavior is consistent with our previous study using agri-food byproducts, such as camelina press-cake and lupin

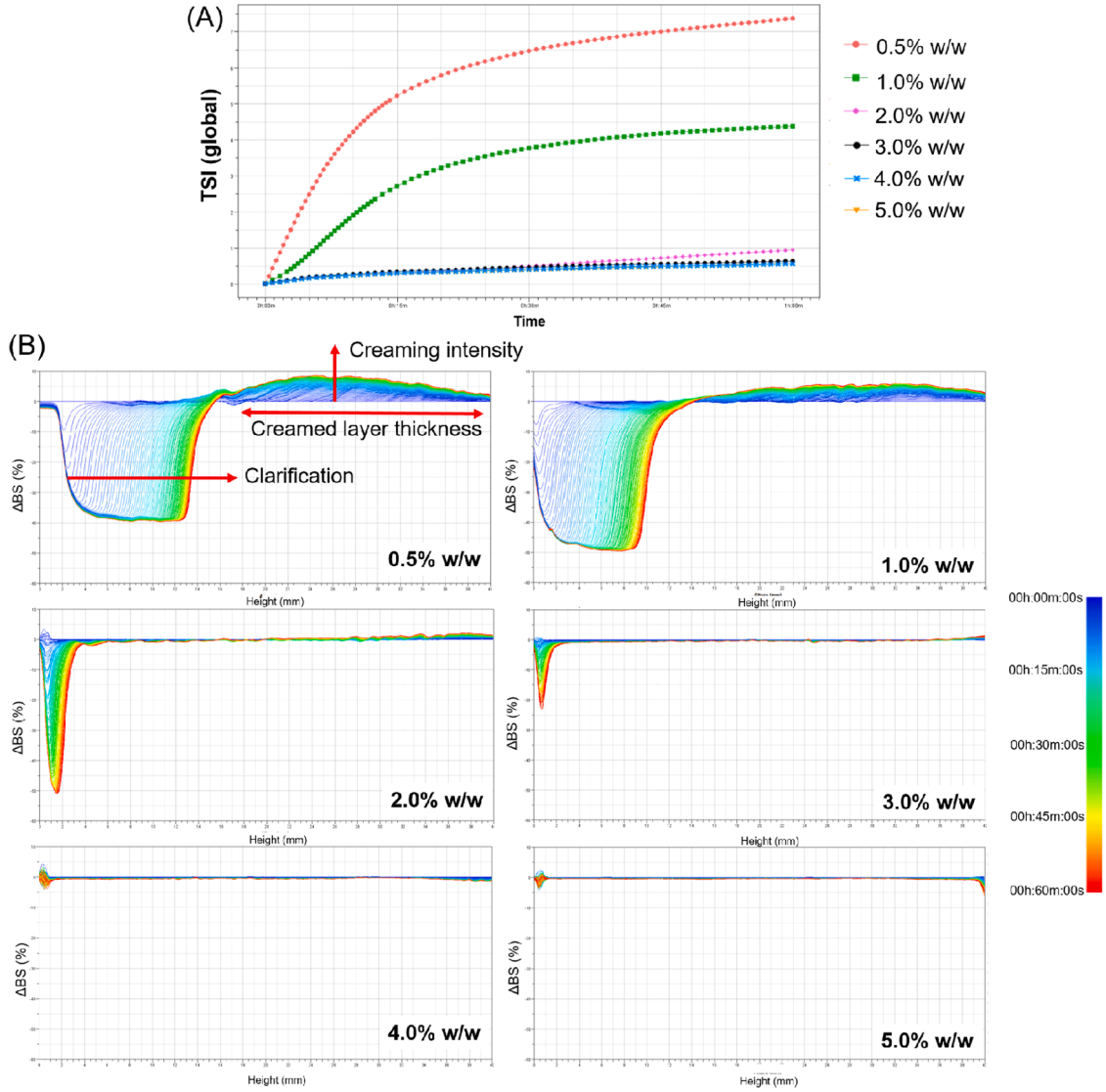

Fig. 5. Turbiscan stability index (TSI) (A), and difference of backscattered light (ΔBS) (B) of O/W Pickering emulsions stabilized with CBS particles at different concentrations (0.5 – 5 %, w/w).

byproducts (Burgos-Díaz et al., 2024). Moreover, the viscosity of the O/W CBS-based Pickering emulsions increased with the particle concentration: 32.23 ± 0.61 cP (0.5 %); 58.02 ± 0.45 cP (1 %); 113.85 ± 2.70 cP (2 %); 196,17 ± 3.10 cP (3 %); 274.60 ± 4.50 cP (4 %); 409.80 ± 7.92 cP (5 %), which aligns with previous findings (Joseph et al., 2020). At concentrations ≥4 % (w/w), the strong aggregation of CBS particles resulted in highly viscous emulsions, and in some cases, even gelation. Overall, these Pickering stabilizers induced high viscosity in the emulsions due to their robust aggregated state, which naturally delayed gravity-driven phenomena. CBS contains a significant proportion of proteinaceous amphiphilic insoluble particles, which may promote the bridging flocculation of the emulsion droplets, thus increasing the emulsion viscosity. Additionally, this effect could be attributed to the presence of excess unabsorbed particles in the aqueous phase that enhance the connectivity of the medium, increasing its viscosity. This hinders droplet movement and contributes to the stability of the Pickering emulsions (Xie et al., 2025).

3.3.3. The emulsion stability evaluation by TURBISCAN

The destabilization kinetics (creaming, sedimentation, flocculation, coalescence, phase separation, and Ostwald ripening) of the emulsion can be evaluated using the TURBISCAN analyzer. Fig. 5(A and B) illustrates the effect of CBS particle concentration on the Turbiscan Stability Index (TSI) and the backscattered light (ΔBS) percentages of O/W Pickering emulsions. Regarding the global TSI analysis, the values gradually decreased as the CBS concentration increased from 0.5 % to 5 % (Fig. 5A). A higher TSI value indicates lower stability in the system (Yue et al., 2022). This suggests that the emulsion with a lower concentration of Pickering particles (CBS) had the lowest stability, while higher concentrations improved the stability of the Pickering emulsion. In general, a higher particle concentration in emulsions is associated with greater stability. Additionally, emulsions with higher concentrations of CBS particles exhibit a gel-like structure, which increases their viscosity. This increase in viscosity may have slowed the movement of emulsion droplets, resulting in more stable Pickering emulsions. Some studies have reported that the apparent viscosity of Pickering emulsions can prevent flocculation and coalescence, thereby enhancing their stability (Feng et al., 2020; Yue et al., 2022).

Fig. 5(B) shows the backscattered (ΔBS) light intensity of Pickering emulsions stabilized with different CBS concentrations. As observed, the ΔBS intensity at the bottom of emulsions stabilized with 0.5 % and 1 % CBS decreased over time, indicating the occurrence of clarification and significant gravitational migration. Additionally, the droplet concentration at the top of the emulsion increased over time. This rise was attributed to the flotation of dispersed particles, leading to the creaming phenomenon (Yue et al., 2022).

The emulsions containing 2 % and 3 % of CBS exhibited the smallest change in ΔBS over time compared to those stabilized with lower CBS concentrations, indicating an increase in emulsion stability. Additionally, the emulsions with the highest stability corresponded to the 4 % and 5 % samples. These results are consistent with the creaming index values (Section 3.3.1) and droplet size measurement (Section 3.3.2.), suggesting that an optimal concentration of CBS particles leads to high emulsion stability. Yang et al. (2023) reported that Pickering emulsions with uniform droplets and lower droplet mobility exhibit less variation in the ΔBS intensity.

3.3.4. Oxidative stability of Pickering emulsion stabilized by CBS

The lipid oxidative stability of O/W Pickering emulsions stabilized with different concentrations of CBS particles was assessed by measuring the formation of primary (PV) and secondary (TBARS) lipid oxidation products, along with the OXITEST method (Fig 6). In this study, the OXITEST method was an effective method to compare the oxidative stability of flaxseed oil Pickering emulsions at different concentrations of CBS. Fig. 6A illustrates the induction period (IP) values, defined as the 'stability time' before lipid oxidation (Li et al., 2022), as a function of

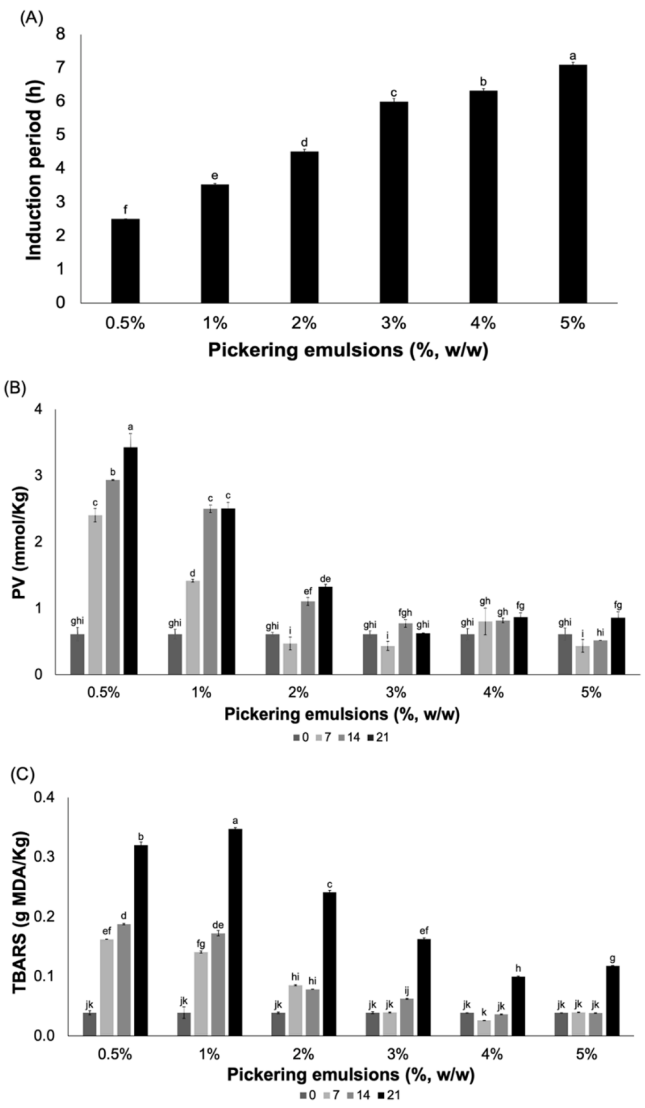

Fig. 6. Oxidative stability measured by OXITEST automatic reactor (A), Peroxide value assay at 21 days (B), and TBARS assay at 21 days (C) of O/W Pickering emulsions stabilized with CBS particles at different concentrations (0.5 – 5 %, w/w). Different letters indicate significant differences ($p \leq 0.05$) among all concentrations of Pickering emulsions.

different concentrations of CBS particles (0.5–5 %, w/w). The results showed a clear positive correlation between the CBS particles concentration and the induction period. At the lowest concentration (0.5 %), the induction period was 2.51 h, while at higher concentrations, it increased progressively. For instance, the emulsion system at a higher concentration (5 %) exhibited an induction period of 7.10 h, which was 3.21 times longer than that of pure flaxseed oil (2.21 h). This indicates that higher concentrations of CBS particles significantly enhance the oxidative stability of the resulting O/W Pickering emulsions, delaying the onset of lipid oxidation and extending their storage life. The statistical analysis confirmed that increasing the concentration of CBS particles led to significantly longer induction periods ($p < 0.05$), with the highest concentration exhibiting the most pronounced protective effect. Similar trends have been observed for polysaccharide/polyphenol composite particles, which improved both the physical and oxidative stability of O/W Pickering emulsions as particle concentration increased. Notably, these composite particles enhanced stability by forming a network structure that restricts oil droplet mobility and limits oxygen penetration, effectively reducing lipid oxidation and extending the emulsion storage time (Fang et al., 2024).

Regarding primary (PV) and secondary (TBARS) lipid oxidation products, Fig. 6B shows that PV values were highest at lower CBS particle concentrations and decreased as the concentration increased. Consequently, at 0.5 %, PV peaked by day 7, whereas at higher concentrations (e.g., 4 % and 5 %), it remained significantly lower throughout the 21-day storage period. These findings demonstrated that higher concentrations of CBS particles effectively inhibit the formation of primary oxidation products (hydroperoxides) over time. Statistical analysis confirmed that PV values at higher concentrations were significantly different from those at lower concentrations ($p < 0.05$), demonstrating the protective role of these Pickering emulsions in reducing oxidative stress in lipid-containing systems. Similarly, TBARS values decreased significantly with increasing CBS particle concentrations over time, as illustrated in Fig. 6C. Across all concentrations evaluated, TBARS values increased from day 0 to day 21, consistent with the expected progression of lipid oxidation during storage. Lower particle concentrations (0.5–1 %, w/w) exhibited higher TBARS values, particularly on day 21, whereas higher concentrations (3.0–5 %, w/w) resulted in significantly lower values, indicating enhanced oxidative stability. Notably, a slight increase in TBARS was observed in the 1 % CBS group compared to the 0.5 % group on day 21. This unexpected variation may be attributed to the sensitivity of the TBARS assay and/or experimental variability, rather than to a genuine decline in oxidative stability at higher CBS concentrations. Overall, Pickering emulsions formulated with higher particle concentrations exhibited superior protection of flaxseed oil against oxidation throughout the storage period. These findings demonstrated the inhibition of secondary oxidation products such as malondialdehyde (MDA), which is primarily responsible for the development of undesirable flavors in food lipids (Piranavatharsan et al., 2023). Comparable PV and TBARS trends have been reported for oligosaccharide/starch nanoparticles, which enhanced the oxidative stability of food-grade O/W Pickering emulsions by inhibiting primary and secondary lipid oxidation products as nanoparticle concentration increased (Li et al., 2022). It is important to mention that in this study, the Pickering emulsion stabilized with 0.5 % CBS was used as the "control sample" because it is the minimum concentration at which an acceptable emulsion can be formed. At lower concentrations, phase separation was considerably high, making it difficult to measure the samples, which affects the PV and TBARS results. Likewise, pure oil cannot be measured either, as it does not include all the components of the colloidal system, such as water.

In particular, the development of an antioxidant-rich interface composed of phenolic compounds can significantly improve the oxidative stability of Pickering emulsions (Keramat et al., 2022). In fact, the combination of polyphenols with proteins and polysaccharides to develop composite nanoparticles has been shown to form a dense protective layer around the oil droplets, thereby providing antioxidant protection against DPPH and ABTS free radicals, and preventing the volatilization of the encapsulated components in O/W Pickering emulsions (Ren et al., 2023). Additionally, as the concentration of polyphenol-rich nanoparticles increased, the droplet size of the emulsions significantly decreased, resulting in improved physical stability and preventing droplet aggregation (Ren et al., 2023). In this regard, the antioxidant capacity of CBS particles can be attributed to the presence of flavonoids, such as catechin, epicatechin, and rutin. These polyphenols likely confer upon CBS particles the ability to act as effective antioxidant stabilizers by adsorbing, as water-insoluble particles, directly at the oil-water interface (Keramat et al., 2022). Furthermore, water-soluble compounds such as chlorogenic acid, syringic acid, vanillic acid, and caffeic acid may also play a crucial role in the barrier properties of the resulting O/W Pickering emulsions. These phenolic acids may function as antioxidant agents within the interfacial layer of CBS particles, scavenging water-soluble pro-oxidants such as oxygen and transition metal ions (Keramat et al., 2022). Similarly, the alkaloids theobromine (slightly soluble in water), as well as theophylline and caffeine (highly soluble in water), could provide antioxidant capacity comparable to that of polyphenols, since these methylxanthines have demonstrated the ability to reduce lipid peroxidation markers such as TBARS (Monteiro et al., 2016; Zhang et al., 2024). Overall, these findings suggest that increasing the concentration of CBS particles, which are rich in polyphenols and methylxanthines, effectively results in a more robust interfacial layer, thereby enhancing the oxidative stability of flaxseed oil O/W Pickering emulsions.

4. Conclusion

This study demonstrated that cocoa byproducts can be effectively used to develop solid-stabilized emulsions with antioxidant properties. The Pickering emulsions exhibited long-term storage stability, maintaining a consistent creaming index value for at least 42 days. Both the creaming and Turbiscan stability index of the emulsions were influenced by the CBS concentration. Confocal microscopy confirmed that CBS particles were absorbed in the oil droplet interface, evidencing the stabilization of Pickering-type emulsions. Notably, flaxseed oil emulsions stabilized with CBS particles significantly improved oxidative stability by delaying lipid oxidation. Emulsions prepared with higher CBS concentrations exhibited enhanced oxidative stability and improved emulsion characteristics (those stabilized at concentrations of $\geq$4 %, w/w). In fact, the emulsion system with 5 % CBS particle concentration exhibited an induction period 3.21 times longer than that of pure flaxseed oil. Therefore, using food-grade Pickering stabilizers with intrinsic antioxidants offers a promising alternative for creating effective wall materials to encapsulate oils and other sensitive bioactive compounds. This study presents a novel strategy for food-grade oil emulsions, offering excellent oxidative stability and protection during storage, with potential for incorporation into a variety of food products.

Ethical statement

This research did not involve the use of animals or humans.

CRediT authorship contribution statement

Ivan Chóez-Guaranda: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Yohanna Mosi-Roa:** Methodology, Formal analysis. **Manuel Chacón-Fuentes:** Writing – review & editing, Methodology, Formal analysis. **Karla Garrido-Miranda:** Resources, Methodology. **Mauricio Opazo-Navarrete:** Writing – review & editing, Methodology. **Jonathan Coronel-León:** Writing – review & editing, Visualization, Resources. **César Burgos-Díaz:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101027.

Data availability

Data will be made available on request.

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