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Pickering emulsion stabilized by biocomposite nanoparticles as a protective system for β-carotene

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Growing consumer demand for natural compounds in various industries has driven the search for alternatives to synthetic ingredients and materials. This study evaluated a Pickering emulsion, stabilized by chitosan–*Yucca baccata* extract nanoparticles, for protecting β-carotene, a highly sensitive lipophilic compound. The emulsion was prepared by incorporating β-carotene into the oil phase (BC-PE), and then subjected to environmental stressors, including UV radiation, oxidative stress, high temperature, and prolonged storage. A simple emulsion stabilized with Tween 20 was used as a control to compare β-carotene retention. The BC-PE was incorporated into an amaranth-based plant beverage to evaluate β-carotene stability after heat treatment and storage to simulate industrial processing conditions. The results showed that the BC-PE significantly improved β-carotene retention under all tested stress conditions, with retention exceeding 85% after heat exposure and over 90% under oxidative stress and UV radiation, respectively. By contrast, the simple emulsion exhibited substantial losses, with retention dropping below 60%. The incorporation of BC-PE into amaranth milk contributed to the protection of β-carotene during pasteurization and storage, preserving its content effectively, thereby enhancing the nutritional value of the beverage. Additionally, the BC-PE provided colloidal stability by preventing phase separation. These results highlight the dual functionality of PE as both protective systems and colloidal stabilizers, offering a promising approach for enriching functional foods with bioactive compounds that are sensitive to environmental factors.

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Sustainability spotlight

Industrial practices often lead to significant degradation of sensitive natural compounds like vitamin A or β-carotene during food processing and storage. Current strategies implies adding higher quantities of the bioactive molecules or use of synthetic additives. Our work offers a sustainable advancement by developing a novel Pickering emulsion (PE) stabilized by chitosan–saponin-rich *Yucca baccata* extract composite nanoparticles. This innovative proposal protects β-carotene, demonstrating significantly higher retention compared to synthetic emulsifiers under various stressors. Critically, it also functions as a natural colloidal stabilizer, preventing phase separation in plant-based beverages. This research directly aligns with UN SDG 3 by improving nutrient delivery and SDG 12 by fostering natural, sustainable food innovations and reducing reliance on synthetic additives, paving the way for enhanced, "clean-label" functional foods.

Introduction

Emulsions are commonly used in various industries, including cosmetics, personal care, pharmaceuticals, and food. The market for surfactants and stabilizers has shown long-term growth and is projected to continue expanding in the coming years. A key driving factor for this trend is the rising demand for emulsions based on natural ingredients. Consequently, several research efforts have focused on identifying alternatives to synthetic emulsifiers, especially those suitable for products intended for direct human consumption or application.[1] In this context, alternative emulsifying systems, such as Pickering emulsions (PE), have broadened the scope of the search to include molecules, composites, and materials previously not classified as emulsifiers.

Pickering emulsions are colloidal mixtures of immiscible liquids that are stabilized by solid particles at the interface. This stabilization mechanism prevents Ostwald ripening; a destabilizing phenomenon commonly observed in simple emulsions. Unlike simple emulsions, which rely on molecular surfactants to reduce interfacial tension, PEs benefit from strong particle attachment at the interface, which prevents coalescence and phase separation.[2] This unique stabilization mechanism makes Pickering emulsions appealing for industrial applications where natural and biocompatible stabilizers are required.

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Initial reports on Pickering emulsions based on natural particles primarily focused on the use of proteins and some polysaccharides. These studies emphasize their significant potential for texture modification and the immobilization of bioactive compounds for controlled release. In particular, the ability of PEs from natural materials to carry bioactive compounds has been documented.[3,4]

Chitosan has been reported to be used in materials capable of stabilizing PE systems. It is a polysaccharide with unique chemical characteristics that is widely recognized for its ability to form structured materials and distinctive functional properties. Although chitosan is considered to have poor interfacial properties, which limits its use as an emulsifier in simple emulsions, diverse types of solid chitosan particles have been used to produce PE, particularly in combination with other compounds.[5,6] Several studies have demonstrated the versatility of chitosan-based particles in Pickering emulsions. For instance, chitosan has been co-assembled with zein, quercetin, and quaternary ammonium compounds to form antibacterial Pickering emulsions.[7] In another study, mixtures of chitosan and essential oil–tannin particles produced Pickering emulsions that exhibited both antibacterial and antioxidant properties.[8] As a drug delivery platform, Pickering emulsions comprising chitosan nanoparticles and soy protein isolate have been used to effectively deliver *Rosa roxburghii* extract for the treatment of precancerous gastric lesions.[9] Likewise, self-aggregated chitosan particles have been used to encapsulate curcumin, achieving high encapsulation efficiency and controlled release.[10] They reported that, beyond functioning as a drug-loading system with specific encapsulation efficiency and release profiles, the Pickering emulsion retained curcumin and could transport and protect lipophilic bioactive substances in oil-in-water emulsions.

Yucca baccata extract is a natural product derived from the *Yucca* plant, which is reported to have a high steroidal saponin content. These compounds exhibit multiple functional properties, including emulsification, antibacterial, and antioxidant activity.[11] Despite its high saponin content, few studies have investigated the use of *Yucca baccata* extract in simple emulsion formulations. Simple emulsions prepared with *Yucca schidigera* extract—or another species in the same genus—have been reported, and its potential as a natural surfactant has been successfully evaluated.[12] However, it is important to consider the instability phenomena that may occur over time in these simple emulsions.

Pickering emulsions have emerged as potential protective systems and delivery vehicles for lipophilic bioactive compounds, especially those that are prone to degradation, such as vitamin A and its precursor, β-carotene. These emulsions are stabilized by solid particles instead of surfactants and can provide greater stability against environmental stressors such as oxygen, heat, and light. However, Pickering emulsions are complex systems composed of water, oil, and stabilizing solid particles. Their performance depends on several formulation parameters that must be standardized for each application. Parameters such as the nature of the solid particles, the oil phase, and the bioactive compound require evaluation to ensure the formation of a stable and effective delivery system.[13] Therefore, Pickering emulsions could be a viable alternative for protecting valuable lipophilic bioactive compounds from degradation and enhancing their functionality in food or pharmaceutical matrices.

Despite the recent surge in studies utilizing Pickering emulsions based on chitosan to encapsulate and protect β-carotene, most of these approaches involve the use of synthetic or highly engineered stabilizers, such as complexes of chitosan, phytic acid and cyclodextrin, or require energy-intensive processing conditions (*e.g.* high-pressure homogenization of self-aggregated chitosan particles). While these systems have proven to be effective, scalability and sustainability issues may emerge.[14,15] More recently, Meng *et al.* reported the use of a combination of chitosan and polyphenols extracted from marine macroalgae to form Pickering emulsions with enhanced thermal and oxidative stability. While showing potential, the system involves marine-derived bioactives and exhibits high viscosity due to strong intermolecular interactions, which may hinder processing and application versatility.[16]

To date, there has been limited research on the use of natural plant-based extracts with intrinsic emulsifying and antioxidant properties in combination with chitosan. This study evaluates the use of a Pickering emulsion stabilized by natural composite nanoparticles, made of chitosan and saponin-rich *Yucca baccata* extract,[17] as a stabilizing system for transporting and protecting β-carotene. Bioactive compounds such as vitamin A and its provitamin forms, particularly β-carotene, play a critical role in human health. These compounds contribute to essential physiological functions, including gene expression, immune response, vision, and notably, the proper growth and development of children. However, β-carotene is highly susceptible to degradation due to its molecular structure, which contains conjugated double bonds, making it vulnerable to oxidative, thermal, and photodegradation. Additionally, as a fat-soluble compound, it has low water solubility, limiting its bioaccessibility and often leading to instability during digestion and reduced absorption.[18] In this context, we evaluated the protection of β-carotene in PE stabilized with composite nanoparticles (nCsYBE), which are made with chitosan and saponin-rich extract, against environmental conditions such as UV exposure, oxidative stress (induced by hydrogen peroxide), temperature, and storage. The effectiveness of this protection was also tested by incorporating the β-carotene PE (BC-PE) into amaranth milk to determine its stability and functionality during pasteurization and storage.

This study investigates the effectiveness of a unique PE system, stabilized by a natural nanocomposite, for protecting a lipophilic labile bioactive substance against various environmental stressors. Building upon these demonstrated protective capabilities, the study subsequently examines its functionality in food products during laboratory-scale production processes. This research establishes a foundation for linking the development of Pickering emulsions with their use as carriers of bioactive compounds in food, thereby enhancing their practical application in the food industry.

Experimental

Reagents

β-Carotene (BC) powder (synthetic, purity ≥93%, catalog number C9750), tripolyphosphate (TPP), and polyoxyethylene sorbitan monolaurate (Tween 20) were all obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Chitosan was provided by Primex (Iceland), with a viscosity average molecular weight (M_v) of 200 kDa and a degree of deacetylation of 82% determined by conductimetry. Saponin-rich extract was obtained from the stems of *Yucca baccata* (reference specimen 25075, herbarium of the Universidad de Sonora), as reported previously.[17] Medium-chain triglycerides (MCT, Wellthy brand, 100% coconut oil derived) and unprocessed amaranth seeds were acquired from local distributors. For all processes and reactions, deionized type I water (18.2 MΩ cm resistivity at 25 °C) was used, unless stated otherwise. All the required analytical chemicals and solvents were reagent grade and obtained from recognized commercial distributors.

Composite nanoparticles of chitosan and *Yucca baccata* extract

The nCsYBE composite nanoparticles were prepared according to our previous report.[17] A 0.4% (w/v) solution of chitosan and a 0.4% (w/v) solution of YBE were mixed at a ratio of 1 : 1 (v/v) and stirred for 10 minutes at room temperature to obtain a homogeneous solution (Sol 1). A TPP aqueous solution (Sol 2) was prepared at 0.02% (w/v). Solutions 1 and 2 were subjected to ultrasonic atomizing separately, and the resulting mists were conducted with an air stream to a mixing chamber containing 20 mL of water. The composite nanoparticles formed within this chamber and the resulting nCsYBE were collected in the water.

The obtained nanoparticles were composed of 66% chitosan, 34% saponin-rich *Yucca* extract, and less than 0.01% TPP. Dynamic light scattering (DLS) revealed that they had a mean hydrodynamic diameter of approximately 235 nm (PDI 0.8) and a zeta potential of +36.9 mV.

Preparation of β-carotene loaded pickering emulsion

To prepare the BC-PE system, the β-carotene was mixed with MCT oil at 0.2% (w/v). The oil phase containing β-carotene was mixed with the aqueous phase containing nCsYBE at 2% (w/v) at $\varphi = 0.5$ oil volume fraction. The Pickering emulsion was formed with an Ultra-turrax® homogenizer (model T25, IKA Labortechnik, Staufen, Germany) at 20 000 rpm for 2 min. In the final emulsion, the concentrations were nCsYBE at 1% (w/v) and β-carotene 0.1% (w/v). These final concentrations reflect the dilution of the initial oil and aqueous phases during emulsion preparation.

Physicochemical characterization of BC-PE

The amount of β-carotene in the PE was measured by spectrophotometry as previously reported.[19] Briefly, 0.2 mL of BC-PE was mixed with 1 mL of ethanol to break the emulsion, followed by the addition of 1.5 mL of *n*-hexane. The mixture was vigorously stirred and then left to stand for phase separation. The upper phase, which contains β-carotene dissolved in *n*-hexane, was carefully extracted and transferred to a volumetric flask. This extraction procedure was repeated until the hexane phase was colorless. The total volume collected was measured, and its absorbance recorded at 450 nm using a UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent Technology, Kansas, USA), using *n*-hexane as blank. A standard calibration curve was prepared using known concentrations of β-carotene dissolved in *n*-hexane, and this calibration curve was used to determine the β-carotene content of the emulsion samples. Subsequently, the loading efficiency (LE) and load capacity (LC) of β-carotene in the BC-PE were calculated using eqn (1) and (2); where W_f is the measured quantity of β-carotene in the BC-PE, W_0 is the mass of β-carotene added to form the PE, and W_{PE} is the weight of the BC-PE.

$$\mathrm{LE}(\%) = \frac{W_f}{W_0} \times 100\% \quad (1)$$

$$\mathrm{LC}(\%) = \frac{W_f}{W_{PE}} \times 100\% \quad (2)$$

The morphology of the droplets in the PE was analyzed using an inverted microscope (XSB 1A, AmScope, CA, USA) equipped with a 9 MP digital camera. A drop of β-carotene-loaded PE was deposited onto a microscope slide, and a smear was prepared with another slide. All observations were conducted at 25 °C. The image was captured at a magnification of 25× and processed using AmLite software (version 20180326, AmScope, CA, USA).

Droplet size was measured using a Möbiuζ instrument (Wyatt Technology, Santa Barbara, California, USA) at room temperature (25 °C). Measurements were taken 24 hours after PE preparation and subsequently weekly for 4 weeks. Each sample was diluted in water at a 1 : 4 ratio and loaded into a cuvette. The Möbiuζ system uses a 45 mW single-longitudinal-mode laser that operates at a wavelength of 532 nm. Measurements were performed at a scattering angle of $\theta = 163.5°$. All experiments were conducted in quadruplicate. Data collection and analysis were carried out using Dynamics software, version 7.10.1.21 (Wyatt Technology).

Samples were stored under controlled conditions at 4 °C and protected from light. Emulsion stability over time was assessed using the emulsification index (EI). Photographs and measurements were taken on days 1, 7, 14, 21, and 28 to document visual changes. The EI was calculated as previously described.[20] The total height (TH) of the liquid mixture and the height of the emulsified layer (HEL) were measured, and the emulsification index (EI) was calculated using eqn (3):

$$\mathrm{EI}(\%) = \frac{\mathrm{HEL}}{\mathrm{TH}} \times 100\% \quad (3)$$

Stability of β-carotene-loaded pickering emulsions

The stability and protective capacity of BC-PE were evaluated through a series of analytical procedures. For comparison, a simple emulsion (SE) containing 2% (v/v) Tween 20 as the

surfactant, MCT as the oil phase, and 0.2% (w/v) β-carotene was prepared and used as a control. Both types of emulsions were subjected to the same experimental conditions.

Photostability. The photostability of the emulsions was evaluated by placing samples in transparent vials and expose them to 23 W UV light (115 V, 0.20 A) for 4 hours at room temperature. The exposure was done inside a dark storage chamber. Aliquots of 0.2 mL were extracted from the samples every hour to measure the retention of β-carotene.

Thermal stability. The thermal stability of the emulsions was evaluated by sealing the samples in light-protected vials and incubating them in a water bath at 85 °C. Aliquots of 0.2 mL were collected at 15-minute intervals for one hour. The β-carotene content in each aliquot was then quantified to assess its stability under thermal conditions.

Hydrogen peroxide oxidation. The stability of both types of emulsions under oxidative stress conditions induced by hydrogen peroxide was assessed. Previously reported methodology was used with slight modifications.[21] Briefly, the aqueous phase of each sample contained 10% (v/v) of H_2O_2. Once the emulsions were prepared, samples were stored in the dark for 8 hours, and aliquots were taken every 2 hours for β-carotene quantification.

Storage stability. The long-term stability of the emulsions was assessed by sealing samples in light-protected vials and storing them under controlled conditions at 4 °C. Aliquots were collected weekly for 28 days and the β-carotene content was quantified to monitor its retention and stability during storage.

BC-PE functional tests

The protective effect of the nCsYBE-based PE on β-carotene during the processing and storage of a model food was evaluated using amaranth milk. For this purpose, the BC-PE was incorporated into freshly prepared amaranth milk to create a fortified product. Then, the plant beverage was subjected to a thermal process that simulated pasteurization and storage to evaluate its shelf life.

Production of amaranth milk with β-carotene. Amaranth milk (AM) was prepared using 100 g of amaranth seeds and 1 L of water as follows. First, the seeds were soaked for 8 hours in tap water, rinsed, and stirred. They were then mixed with 250 mL of drinking water at 70 °C and processed using a hand blender. The remaining 750 mL of water was added stepwise. Finally, the obtained blend was passed through a cloth sieve. To prepare testing samples (AM_{PE}), 25 mL of freshly prepared amaranth milk was added to 50 mL graduated tubes along with 0.1 mL of the BC-PE (containing 0.1% (w/v) β-carotene). This mixture was then homogenized at 20 000 rpm for 5 minutes. Amaranth milk with the same β-carotene concentration (0.1% w/v), added directly, without nanoparticles or surfactant to provide stability or protection, was used as a control reference (AM_C). It was calculated that both fortified milks (AM_{PE} and AM_C) would result in a final β-carotene concentration of 4 μg mL^{-1} in the plant-based beverage. A sample of AM without added β-carotene was reserved to determine the proximate composition of the vegetable beverage.

Storage stability of AM_{PE}. Samples of AM_{PE} (25 mL) in covered and sealed graduated tubes were stored at 4 °C for 7 days. After that time, the carotene concentration was determined using high-performance liquid chromatography (HPLC) as described below.

Storage stability of AM_{PE}. The thermal process simulating pasteurization involved heating the AM_{PE} sample in sealed graduated tubes at 80 °C for 30 minutes, followed by a thermal shock at 4 °C to maximize the reduction of microbial burden of the plant-based milk.[22] The carotene of the thermally treated milk was quantified by HPLC.

β-Carotene quantification by HPLC. Since AM is a complex mixture naturally containing carotenes and similar molecules that could interfere with the direct β-carotene determination method as used for BC-PE and simple emulsions, its determination by HPLC was necessary. A previously reported method was used with minimal modifications as detailed.[23,24] Carotenes in the samples were extracted using methanol and tetrahydrofuran (THF) under red light at room temperature. An aliquot (~0.5 g) of each sample was carefully weighed and mixed with 5 mL of methanol, then allowed to stand for 1 hour in a shaking incubator at room temperature. The samples were centrifuged at 3500 rpm for 10 minutes at 4 °C, and the upper layer of methanol was transferred to a 25 mL volumetric flask. The samples were then extracted with 5 mL of THF and vortexed; this step was repeated four times. After each extraction, the THF layer was recovered and combined in the volumetric flask containing methanol. The final volume was adjusted to 25 mL with THF. An aliquot of the extract (5 mL) was taken and dried under N_2 in a water bath at 40 °C. The dried residue was resuspended in 1 mL ethanol, and 20 μL of the total extract was injected into an HPLC system (Agilent Technologies 1220 Infinity, Palo Alto, CA, USA) equipped with a Microsorb C18 column (100-3, 100 × 4.6 mm, Netherlands) and an Agilent Technologies 1260 photodiode array detector. Total carotenoid content (α + β, μg/100 mL) was analyzed by reverse-phase HPLC using an isocratic mobile phase of acetonitrile : methanol : tetrahydrofuran (58 : 35 : 7) with UV-VIS detection at 460 nm at a flow rate 1.0 mL min^{-1}. The column temperature was maintained at 35 °C, and the total run time was 15 minutes.

Amaranth milk composition. The proximate composition of the amaranth milk, AM_C, and AM_{PE} after temperature and storage tests was determined. Moisture and ash contents were determined according to the Association of Official Analytical Chemists.[25] Total protein content was quantified using the micro Kjeldahl method with a conversion factor from total nitrogen to protein equal to 6.25 and expressed as protein percentage.[26] Lipid content was determined by low-field nuclear magnetic resonance (LF-NMR) using a NMR spectrometer (Minispec mq-20, Bruker, Massachusetts, USA).[27] Total carbohydrate content was calculated by the difference (nitrogen-free extract [Nifext] fraction).

Color characterization. The optical properties of the samples were determined by an instrumental color measurement method using a CR 400 digital colorimeter (Konica Minolta, New Jersey, USA).[28] The values of L^* (lightness/brightness),

a^* (redness/greenness), and b^* (yellowness/blueness) were recorded. The colorimeter was calibrated with a white standard in the form of a plate ($L = 97.79$, $a = -0.53$ and $b = +2.28$). A volume of 10 mL of each sample (AM, AM_C, and AM_{PE}) was placed in a Petri dish and measurements were taken. Hue angle (°hue) and chromaticity (C^*) were calculated from the a^* and b^* values according to eqn (4) and (5).[29]

$$^{\circ}\text{hue} = \arctan b\left(\frac{b^*}{a^*}\right) \quad (4)$$

$$C^* = \sqrt{a^{*2}} + b^{*2} \quad (5)$$

The color difference (ΔE) was calculated using the obtained data, with the sample control (AM) designated as subscript 1 and the samples with β-carotene (AM_C or AM_{PE}) designated as subscript 2 (eqn (6)).

$$\Delta E = \sqrt{(a_2^* - a_1^*) + (b_2^* - b_1^*) + (L_2^* - L_1^*)} \quad (6)$$

Colloidal stability of amaranth milk. The stability of samples AM, AM_C and AM_{PE} was evaluated by a previously reported method with slight modifications.[30] Samples were stored in glass tubes at 4 °C for 15 days, during which physical changes were observed and recorded to assess the stability of the plant beverages. Photographs were taken on day 1, day 7, and day 15 to document the progression of any visible changes in the samples during the storage period. For the visual stability the ratio of the volume of creaming (c) or sedimentation (s) to the total volume of liquid was calculated using the following equation (eqn (7)).[31]

$$\text{VS} = 100 - \sqrt{s^2 + c^2} \quad (7)$$

Statistical analysis

All results are expressed as means ± standard deviation, based on triplicate measurements. A one-way analysis of variance (ANOVA) was performed to determine significant differences between the respective values, and averages were compared by the Tukey–Kramer test with a $p \leq 0.05$. The statistical calculations were performed using OriginPro 2019 software (OriginLab).

Results and discussion

β-Carotene pickering emulsion characterization

The composite nanoparticles nCsYBE can effectively stabilize PE under the experimental conditions used.[17] The incorporation of β-carotene into the oil fraction, at the concentration used, apparently did not modify their stabilizing capacity. The β-carotene content in the emulsion was directly quantified by UV-visible spectrophotometry since there are no other substances in the formulation that could interfere with the measurement. A calibration curve was constructed using a reagent grade β-carotene standard. The resulting equation was $y = 0.2390x - 0.0123$, with a value of 0.999 of coefficient of determination (R^2). Once the β-carotene content was determined, the loading efficiency (LE) and loading capacity (LC) of BC-PE were calculated, yielding values of 100.7 ± 0.4% and 0.2 g per 100 mL, respectively. The results indicate the effective retention of β-carotene in the PE, showing that all the β-carotene was incorporated into the oil droplets surrounded by the composite nanoparticles. This suggests that the concentration of nanoparticles and the fraction of oil used in the system were sufficient to form stable PE.

These results are comparable to and slightly higher than those previously reported in the literature. For example, Pickering emulsions stabilized with chitosan–phytic acid–cyclodextrin nanoparticles at a concentration of 2% w/v and a phase oil fraction of 50% showed an encapsulation efficiency (EE) of 97.66 ± 1.18%.[15] Similarly, another study using complex nanoparticles composed of propylene glycol alginate, zein, and tea saponins for nutraceutical encapsulation reported an EE range of 89.86 ± 1.33% to 97.39 ± 0.76%, depending on whether microfluidization was applied.[32] If conditions are not appropriate for efficient encapsulation, the β-carotene will not be in the emulsion. This will result in greater exposure of the droplet surfaces to the external environment and chemical degradation due to light, heat, oxygen, and other external stresses. The results indicate complete β-carotene incorporation into the emulsion, with no release outside the oil phase of the system and suggest that the nanoparticle-stabilized system successfully retained the bioactive compound within the emulsion.

To assess the stability of the BC-PE the droplet size was measured over 28 days (Fig. 1A). The hydrodynamic size of the oil droplets in BC-PE was about 2.8 μm (±0.2). Their size did not change significantly over time indicating that the Pickering emulsion remained stable during storage. Microscopic analysis allowed visualization of the emulsion droplets within a single focal plane. As shown in Fig. 1B, most droplets exhibited a well-defined spherical shape and relatively uniform size, indicating a stable and homogeneous emulsion.

The influence of particle concentration on emulsion stability and droplet size has been well-documented. For example, in a study evaluating chitosan complex nanoparticle concentrations ranging from 0.5% to 1.5% (w/v), larger droplet sizes were observed at lower nanoparticle concentrations, while a smaller droplet size of 7.23 ± 0.08 μm was achieved at 1.5%.[32] In addition, the process parameters also have an influence. In the same study, pressures were evaluated, and a smaller droplet size was observed at higher pressures. As the pressure was increased from 50 to 150 MPa, the droplet size slightly decreased from 6.75 ± 0.05 to 6.27 ± 0.02 μm. In the preliminary study, the effect of nanoparticle concentration on the emulsification index and emulsion stability was assessed by monitoring droplet size over time. Results showed that higher nanoparticle concentrations led to an increased emulsified phase, while droplet size remained constant throughout storage, indicating improved stability.[17] These suggests that higher nanoparticle concentrations and the homogenization process contribute to better stabilization by forming a denser interfacial layer, reducing droplet coalescence and maintaining a uniform size. Several

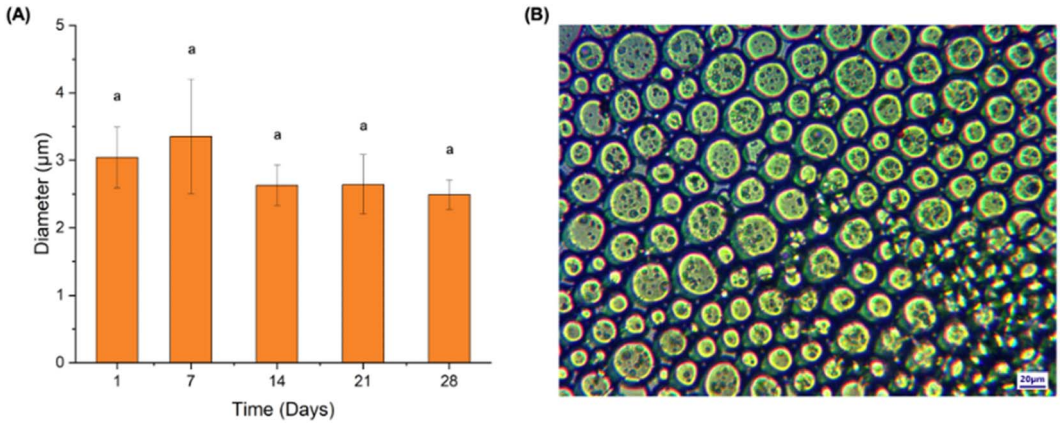

Fig. 1 (A) Droplet size in BC-PE over time measured by DLS, and (B) 25× microscopy of the droplets at day 28. Values are expressed as mean ± standard deviation. Samples with different lowercase letters indicate significant differences ($p < 0.05$) according to the Tukey test.

factors influence the stability of the system, including the concentration and size of the nanoparticles and the oil fraction. In this case, the concentration and size of the nanoparticles used were sufficient to place them at the interface between the droplets with β-carotene and prevent coalescence between the droplets for four weeks while maintaining the spherical shape.

The emulsification index (EI) is another key parameter for evaluating emulsion stability. EI measurements of BC-PE were taken weekly over a four-week period. Also, photo images were used to visually monitor any changes in the appearance of the PE over time (Fig. 2). Regardless of storage time, all PE remained stable after 28 days, with no significant variations in EI (~70%) and no visible signs of phase separation compared to day 1. These results correlate with droplet size and reflect a similar phenomenon to the one previously discussed, where the selected conditions effectively contribute to the stability of the system.

β-Carotene protection in BC-PE

The evaluation of the PE as a protective system for β-carotene involved subjecting the samples to a series of simulated external stress conditions that the bioactive compound could encounter during processing and storage. Due to its highly unsaturated structure, β-carotene is a highly reactive compound. Its extensive π-conjugation system makes the molecule electronically rich, allowing electrons to delocalize across the polyene chain. While this conjugation gives β-carotene its characteristic orange-red color and antioxidant capacity, it also makes it particularly sensitive to degradation. The two primary degradation pathways are oxidation and isomerization.[33] Experimental conditions included exposure to UV light, high temperature (85 °C), an oxidizing agent (H_2O_2), and storage conditions (7 days at 4 °C in the dark). A simple emulsion of MCT including β-carotene in the oil phase stabilized with Tween 20 was prepared and analyzed under the same conditions as comparative benchmark.

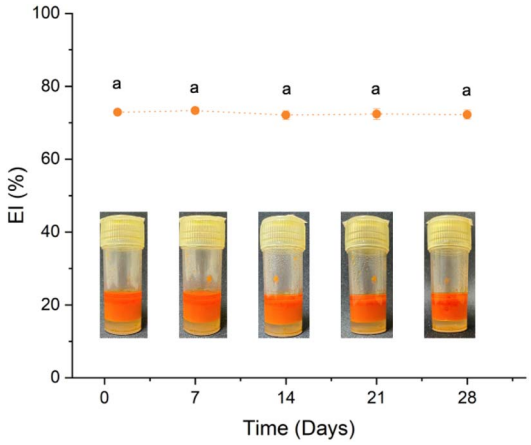

Fig. 2 The emulsification index and the appearance of the PE with β-carotene as a function of time. Values are expressed as mean ± standard deviation. Samples with different lowercase letters indicate significant differences ($p < 0.05$) according to the Tukey test.

β-Carotene is characterized by its high degree of instability when exposed to ultraviolet light (UV). UV irradiation, which is commonly used as sterilization method in food processing and other industries, can occur naturally through exposure to sunlight. When BC-PE is exposed to UV for 4 hours, the final reduction of β-carotene reached 15% (Fig. 3A). In contrast, the SE emulsion showed a constant decrease of β-carotene content, with significant differences at all measured time points, reaching a 29.1 ± 2.6% loss of β-carotene at the end of the experiment.

The electromagnetic radiation in the UV range primarily affects the conjugated double bonds of the β-carotene, promoting oxidation and breakdown. According to the observed results, it appears that the composite nanoparticles surrounding the emulsion droplets act as a physical barrier that limits UV penetration and protects β-carotene from degradation. The size and composition of the nCsYBE could interfere with the UV transmission reducing its rate of interaction with the labile β-carotene bonds. Simple emulsions depend on surfactants, molecules or macromolecules, which do not typically interfere with UV radiation. Therefore, no UV protection is expected for this type of emulsions. Pickering emulsions, in contrast, have numerous particles attached to the colloidal interface and, depending on factors such as size, form,

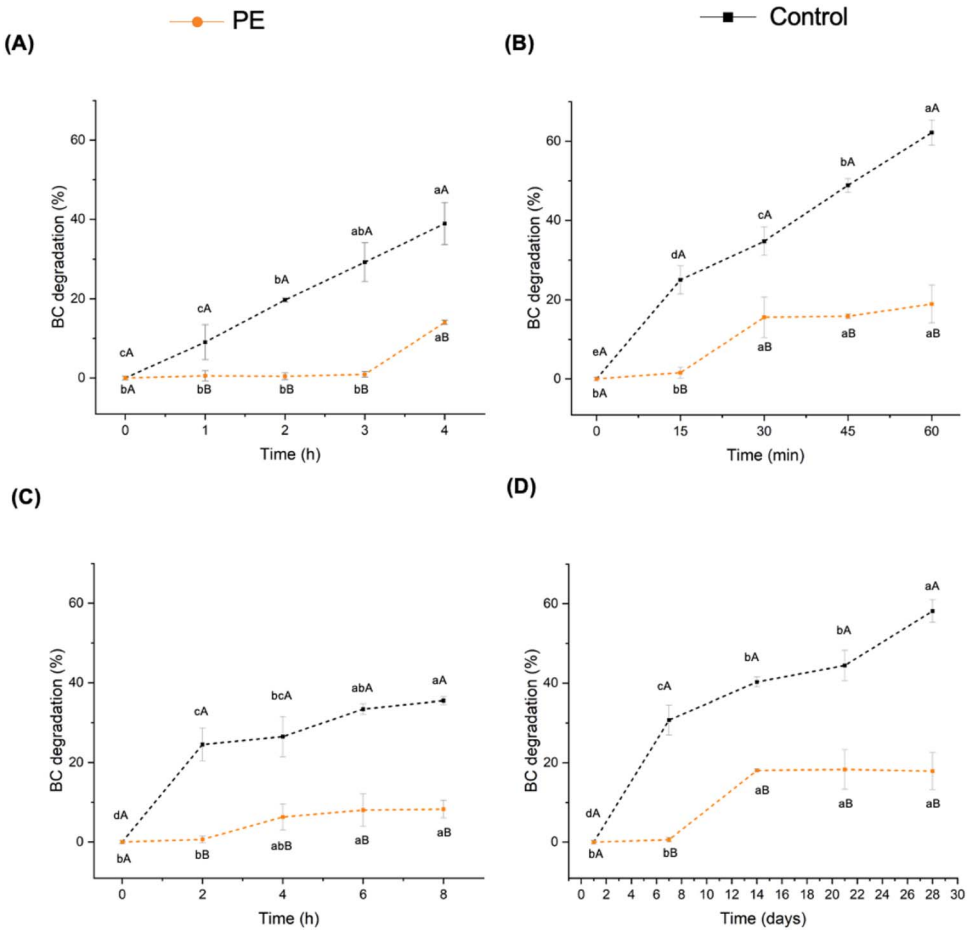

Fig. 3 BC-PE stability and protection test: (A) UV light, (B) temperature, (C) oxidation, and (D) storage. Values are presented as mean ± standard deviation. Samples with different lower-case letters (a, b, c, d and e) indicate significant differences ($p < 0.05$) when compared between measurements of the same emulsion. Samples with different capital letters (A and B) are significantly different ($p < 0.05$) when compared between emulsions, BC-PE and SE control at the same time of measurement.

composition, and others, could form a dense layer surrounding the oil droplets. This configuration creates a physical barrier and contributes to the scattering and absorption of UV radiation.[34] This optical behavior reduces the amount of radiation that reaches the interior of the droplet, enhancing the photostability of the immobilized β-carotene. It has been reported that the protection provided by Pickering emulsions can be related to the presence of nanoparticles in the system. Furthermore, the protective performance of such systems depends heavily on process parameters, which directly influence emulsion stability and the extent of bioactive compound retention.[35]

These results were superior to those obtained in a previous study by Meng *et al.*[16] which reported approximately 60% degradation of β-carotene after UV exposure. In contrast, the present system demonstrated significantly enhanced photostability, indicating an improved level of protection. Another study employing a combination of chitosan and synthetic compounds achieved comparable levels of UV shielding;[15] however, the present formulation relies solely on natural components, avoiding the use of synthetic additives. This underscores the product's ability to provide effective UV protection while maintaining a clean-label profile. Furthermore, in contrast to conventional emulsions, which are deficient in UV-barrier properties, the composite nanoparticles within our Pickering emulsion not only stabilize the system but also function as an effective UV-blocking layer. The combination of particle morphology and natural composition appears to enhance both the physical barrier effect and overall emulsion stability, which has the potential to result in greater bioactive retention over time.

The thermal stability of β-carotene was evaluated by subjecting the BC-PE to heat treatment of 85 °C for one hour. This temperature is higher than the reported degradation temperature for β-carotene (40 °C).[36] In such conditions, the content of β-carotene in the BC-PE remained stable during the first 15 min of exposure (Fig. 3B). Then, it degraded at a constant rate, reaching 20% of β-carotene loss at the end of the treatment. Conversely, the simple emulsion exhibited a significantly higher degradation rate. After 30 minutes, the β-carotene content decreased 50%, and by the end of the treatment, it had reached up to 65.3 ± 3.8% of degradation.

The kinetic energy of the molecules increases with the temperature. When heat rises, the molecular agitation disrupts the structured water network surrounding nonpolar molecules, thereby weakening these interactions.[37] Additionally, such energy surplus could be used to modify susceptible bonds in labile molecules. Under these conditions, simple emulsions become unstable and β-carotene inside the oil droplets may become exposed and susceptible to thermal degradation. In the BC-PE the reduced degradation rate of β-carotene could be related to a phenomenon observed in PE made with nCsYBE. Rheological analysis of a temperature ramp experiment demonstrated that such PE increased its storage modulus upon heating.[17] This effect was attributed to the displacement of water molecules from the interface and among the nanoparticles in close interaction with the oil droplets upon heating. Such water molecules displacement led to closer interaction among the nCsYBE and between these nanoparticles and the oil droplets where they are attached within the PE, reinforcing solid-viscosity behavior of the emulsified system at elevated temperatures. The incipient gel structure formed reduces the effects of the kinetic energy in the system, thereby lowering the β-carotene degradation rate.

Similar findings were reported in a previous study using chitosan and seaweed extract-based Pickering emulsions, where thermally labile compounds also exhibited enhanced protection under heat treatment.[16] The study demonstrated the emulsion system's effectiveness in protecting encapsulated actives against degradation at elevated temperatures, validating the protective role of the PE structure. In the current system, based on nCsYBE, we have achieved comparable β-carotene retention and have also reported a unique thermal response: a strengthening of the emulsion's viscoelastic properties upon heating. This behavior, associated with the formation of an emerging gel structure, indicates a distinct protective mechanism that further restricts molecular mobility and enhances thermal stability.

The oxidative stability of BC-PE was evaluated by simulating its exposure to reactive oxygen species using hydrogen peroxide. This analysis aimed to assess the capacity of the proposed PE to protect β-carotene from oxidative degradation. The rate of β-carotene in the BC-PE at experimental conditions was low. After 8 hours of treatment the BC-PE retained 92.4 ± 2.3% of its initial content (Fig. 3C). The degradation rate of β-carotene in SE was considerably higher. In this emulsion, there was a marked degradation (>20%) of β-carotene within the first two hours of oxidizing treatment. The degradation of β-carotene reached 35.5 ± 1.0% by the end of the experiment.

The protection against oxidation of the BC-PE could be related to the nCsYBE layers covering the oil droplets hindering the diffusion of the oxidizing reactive species into interface. Similar observations have been explained as result of nanoparticles forming a protective barrier that helps keep the β-carotene inside the droplets, preventing its exposure to peroxides around the emulsion droplets and providing structural stability.[14] The composition of the composite nanoparticles used to prepare the BC-PE could also play a role. For example, the degradation rate of chitosan at the oxidizing agent concentration used is relatively very low. Since the composite nanoparticles are attached to the interface, the effect of the oxidizing agent will be first and more probably on their components. Thus, the nCsYBE stabilize the BC-PE and also act as sacrificial material under oxidizing conditions, thereby reducing the degradation of β-carotene in the oil phase.

Regardless of its intended application, a key functional parameter of any emulsion is the stability of the system on storage. The capacity of a PE system to protect lipophilic bioactive compounds is closely related to the stability that retains the compounds of interest within the oil droplets. The stability of BC-PE over a four-week storage period was evaluated. The results showed that β-carotene levels remained stable during the first two weeks, with no significant degradation and full retention of the compound (Fig. 3D). A degradation rate was observed after two weeks, reaching a β-carotene degradation of less than 20% by the end of the experiment. The SE exhibited a notably higher degradation rate, resulting in a total β-carotene loss of 49.5% by the fourth week.

The observed results can be related to the different stabilization mechanisms of simple emulsions and PE. Simple emulsions, which are stabilized by surfactants, amphiphilic molecules, rely primarily on hydrophobic interactions and are susceptible to physical instabilities, such as coalescence and Ostwald ripening. These time-dependent phenomena compromise the integrity of the emulsion during storage, which could lead to the exposure of the bioactive compounds to external stressors and accelerate their degradation.[13] In contrast, PE is stabilized by the persistent adsorption of the solid nanoparticles at the oil–water interface forming layers that cover the oil droplets.[34] This structural feature enhances the physical stability of the PE systems and effectively shields the encapsulated β-carotene from environmental factors, resulting in improved retention and protection.

A previous study on Pickering emulsions stabilized with chitosan–phytic acid–β-cyclodextrin nanoparticles reported results that are consistent with the present findings.[15] These results indicate the stability of the Pickering emulsions across various environmental conditions, including storage. The system relied on chemically complex stabilizers to enhance emulsion performance. Despite the simplified composition of our Pickering emulsion, its efficacy is demonstrated by retaining over 80% of β-carotene after four weeks of storage. This outcome validates that a minimalistic, clean-label approach can effectively preserve sensitive bioactive compounds over an extended period.

The results presented indicate that the BC-PE provided effective β-carotene protection against UV light, high temperature, oxidative degradation, and storage time. The structural characteristics of the BC-PE, particularly the role of the chitosan–*Yucca baccata* extract nanoparticles at the interface, appear to play a key role in stabilizing and protecting β-carotene under various environmental conditions. These findings suggest that such emulsions could be promising carriers in functional food formulations or nutraceutical applications where the stability of sensitive bioactive compounds and their controlled release are critical.

BC-PE functionality tests on model food

To assess the potential for utilizing the stability and protective capacity of the Pickering emulsion containing β-carotene in food applications, the emulsion (BC-PE) was incorporated into an amaranth-based beverage. The beverage was then evaluated for its functional properties under simulated processing conditions, including thermal treatment (pasteurization) and subsequent storage to mimic shelf life. The BC-PE fortified amaranth milk (AM_{PE}) was formulated to have a 4 μg per mL β-carotene concentration, which is within the range of vitamin A intake from other dietary sources. This amount provides 25% of the established Nutritional Reference Value (NRV) for a 200 mL serving of the beverage.[38,39] Considering that amaranth seeds naturally contain carotenes, the β-carotene quantification for these samples was performed using HPLC. Additionally, α-carotene was also quantified, and the Estimated Average Requirement (EAR) was calculated. Amaranth milk with β-carotene added directly at the same final concentration was used as benchmark reference (AM_C).

The quantification of carotenoids and EAR for the heat- and storage-treated AM_{PE} samples is presented in Table 1. Data for AM and AM_C are also included. The results confirm that AM contained carotenoids prior to fortification, which is consistent with previous reports indicating that amaranth seeds naturally contain carotenoids.[40]

No significant differences in β-carotene content were observed in the fortified samples after the treatments. The stored AM_{PE} had a similar β-carotene content to that of the AM_C. This suggests that the Pickering emulsion effectively preserved the bioactive compound throughout storage, maintaining its content consistently. After heating and thermal shock, which simulated milk pasteurization, the AM_{PE} sample also had the same amount of β-carotene as the AM_C sample. This indicates that the nCsYBE helped to preserve the β-carotene added to AM despite the simulated process treatments. These results are consistent with those previously discussed for BC-PE, with similar protection levels.

The EAR values indicate an improvement in the nutritional profile of amaranth milk with the addition of bioactive compounds, particularly β-carotene. This contributes to the recommended daily requirement. Current industrial practices often involve adding bioactive compounds without protection. This means that the amount listed on the nutrition label may not reflect the amount retained after processing and storage. Many bioactive compounds are highly susceptible to degradation under the extreme conditions used to ensure food safety.[41] The results obtained show that Pickering emulsions stabilized by chitosan–*Yucca baccata* extract nanoparticles can protect sensitive bioactive substances under processing conditions that simulate industrial operations. This provides a practical approach for maintaining the recommended dietary intake of nutritionally significant lipophilic compounds.

Table 1 Content of β-carotene and contribution to Estimated Average Requirement (EAR) of amaranth milk (AM), amaranth milk with β-carotene (AM_C) and amaranth milk with BC-PE (AM_{PE}) after different treatments (heating and storage). Values are presented as mean ± standard deviation. Data on the same column with the same capital letter superscript are not different, $p < 0.05$

Sample	Treatment	β-carotene (μg/100 mL)	EAR
AM	None	115.6 ± 7.7^{B}	9.6 ± 0.6
AM_C	None	339.9 ± 50.1^{A}	28.3 ± 4.2
AM_{PE}	Heating	362.2 ± 42.5^{A}	30.2 ± 3.5
AM_{PE}	Storage	363.9 ± 32.2^{A}	30.3 ± 2.7

Functional properties

The proximate composition of the amaranth milk and fortified samples is shown in Table 2. As would be expected in a plant-based beverage, moisture accounted for the largest proportion with no significant differences between samples. The second most abundant component in amaranth milk is carbohydrates. Its content was slightly higher in temperature-treated AM_{PE} and lower in AM_C. This difference is explained by the different amounts of lipids in the samples.

With respect to the protein content of the samples, no statistically significant differences were observed among the amaranth beverage sample, the control, and the treatments. This was expected since the treatment beverages contained the addition of BC-PE or β-carotene alone, which was not expected to influence protein content. Regarding the lipid content, the AM sample had the lowest lipid content, derived solely from the amaranth seed used in its preparation. Conversely, the AM_C exhibited the highest fat content, associated with the added β-carotene in MCT as a carrier. Slightly lower fat content was found in the AM_{PE} sample that was stored. The fat reduction could be associated with reported effects of storage on lipids.[42,43] The fat content reduction in the heat-treated AM_{PE} sample is more notable, indicating the effect of temperature on the fat content of the final product. These findings suggest that the observed variation in lipid content among the samples can be primarily attributed to the presence or absence of added MCT. There were no significant differences in ash content between samples, indicating that neither β-carotene nor PE influenced this nutritional component. The values presented are determined by the composition of the vegetable drink, specifically amaranth. These data indicate differences in fat content due to the addition of β-carotene, the fatty acids provided by amaranth, and the effects of the treatments applied to the samples.

The color characterization was performed to evaluate the visual effect of adding β-carotene and BC-PE to amaranth milk. The results are presented in Fig. 4. The main parameter affected by the addition of β-carotene was lightness (L^*). The AM_{PE} sample showed the highest lightness value ($L^* \approx 80$), while the fresh amaranth milk showed the lowest ($L^* \approx 70$). Previous studies have reported that the use of fat and type can influence luminosity, with saturated fats tending to decrease this parameter, while pre-emulsified samples have a higher luminosity. This effect is attributed to the fact that the fat globules are smaller and reflect more light.[44] In this case, the use of BC-PE contributes to an increase in luminosity due to its intrinsic

Table 2 Proximate composition of fresh amaranth milk (AM), amaranth milk with β-carotene (AM_C) and amaranth milk with BC-PE (AM_{PE}). Values are percentage mean ± standard deviation. Data on the same column with the same capital letter superscript are not significantly different ($p < 0.05$)

	Moisture	Ash	Lipid	Protein	Carbohydrate
AM	95.52 ± 0.03^{A}	0.14 ± 0.01^{A}	0.17 ± 0.01^{C}	1.70 ± 0.09^{A}	2.65 ± 0.06^{AB}
AM_C	95.51 ± 0.03^{A}	0.12 ± 0.01^{A}	0.36 ± 0.02^{A}	1.70 ± 0.04^{A}	2.50 ± 0.04^{C}
AM_{PE} – heat treated	95.53 ± 0.01^{A}	0.12 ± 0.01^{A}	0.20 ± 0.01^{C}	1.62 ± 0.09^{A}	2.71 ± 0.08^{A}
AM_{PE} – stored	95.50 ± 0.00^{A}	0.12 ± 0.01^{A}	0.31 ± 0.00^{B}	1.70 ± 0.04^{A}	2.55 ± 0.02^{BC}

optical properties, affecting the overall appearance of the amaranth drink.

Differences were also observed in the a^* and b^* values, with lower values recorded for AM and higher values for AM_{PE}. The a^* parameter is associated with green tones, while b^* represents yellow tones, both of which were more pronounced in the AM_{PE} sample. This is likely related to the presence of β-carotene and its characteristic color attributes.[45] The color parameters (a^* and b^*) are more extreme for AM_{PE} than for the AM_C sample. This could indicate that the β-carotene in BC-PE facilitates better distribution in the beverage, which is reflected in its color.

In terms of chromaticity and hue angle ($h°$), all samples showed clear differences. The chromaticity of the AM sample was low, indicating a less saturated and less intense color, closer to gray tones. In contrast, the AM_C sample showed a moderate chromaticity, indicating a medium saturation, more intense than AM but not vivid. Finally, the AM_{PE} sample had a high chromaticity, characterized by more saturated colors and a more pronounced yellowish hue. In terms of hue angle ($h°$), all samples showed values close to 100°, indicating a green-yellow hue with a yellowish tint. These results are consistent with previous reports on the evaluation of fruits rich in β-carotene, which exhibit yellow shades.[46] As mentioned above, these differences are attributed to the presence of β-carotene in the amaranth milk. Compared to the AM, the samples containing β-carotene showed noticeable color differences (ΔE), with the most pronounced change observed in AM_{PE}, followed by AM_C. The addition of β-carotene, particularly in the BC-PE form, notably affected the colorimetric properties of the sample.

Natural vegetable beverages are complex colloidal systems. These types of systems tend to be unstable, due to the diversity of components involved, the numerous interactions and variability induced by the technological processes they undergo. This often leads to phase separation over time.[45] For this reason, the colloidal stability of the prepared amaranth beverages was evaluated using visual stability (VS), which was used to determine the effect of adding BC-PE. The results are included in Fig. 5. As can be seen in the inset images, there were no visual changes in any of the vegetable beverages up to day 7. However, by day 14, a pronounced phase separation had occurred in both the AM and AM_C samples, with VS values of 91.20 ± 0.61% and 89.50 ± 0.85%, respectively. The AM_{PE} sample showed no visual

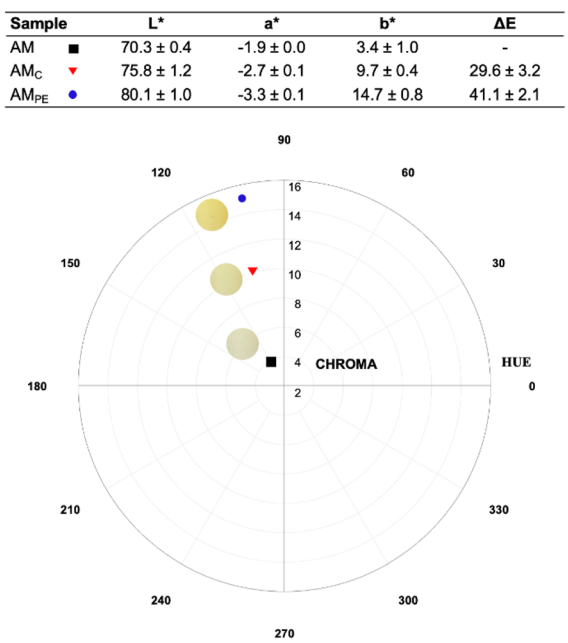

Fig. 4 Color parameters. Values are presented as mean ± standard deviation. Polar plot of °Hue *vs.* Chroma of fresh amaranth milk (AM), amaranth milk with β-carotene (AM_C) and amaranth milk with BC-PE (AM_{PE}).

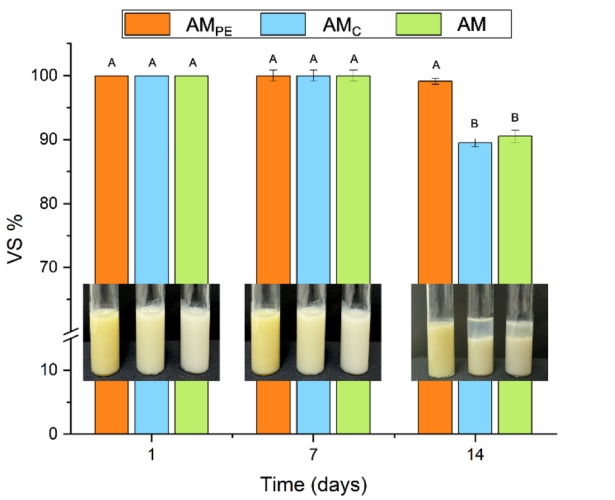

Fig. 5 Visual stability of amaranth milk (AM), amaranth milk with β-carotene (AM_C) and amaranth milk with BC-PE (AM_{PE}). Inset images are photographs of the beverages on days 1, 7 and 14. Samples with different capital letters (Aand B) are significantly different ($p < 0.05$) when compared between AM, AM_C, and AM_{PE} at the same time of measurement.

signs of colloidal instability after 14 days. This suggests that in addition to functioning as a protective system for β-carotene, the addition of PE to the plant-based beverage provides colloidal stability, which was not observed in the other two samples. Therefore, PE could also function as a stabilizer in colloidal beverage formulations.

Conclusions

This study demonstrates that a Pickering emulsion stabilized by chitosan–saponin-rich *Yucca baccata* extract composite nanoparticles can act as an effective system for protecting lipophilic bioactive compounds, as evidenced by the observed protection of β-carotene. Under the experimental conditions, the emulsion achieved a loading efficiency of approximately 100%. The BC-PE system successfully preserved β-carotene under exposure to various stressors, including UV radiation, hydrogen peroxide-induced oxidation, 85 °C heat treatment, and 28 days of storage. In every experiment, the Pickering formulation outperformed the simple Tween-20 emulsion, which exhibited losses ranging from 45% to 65%.

The BC-PE was added to an amaranth milk food matrix, providing protection for the β-carotene through storage and heating treatment. The system also functions as a colloidal stabilizer that prevented the naturally occurring phase separation of AM. A single 200 mL serving of the fortified beverage could provide over 25% of the vitamin A Nutrient Reference Value (NRV) (NOM-051)[39] without relying on synthetic antioxidants or surfactants, thereby highlighting its suitability for "clean-label" and natural-based products. Beyond β-carotene, the Pickering emulsion stabilized by nCsYBE offers a biodegradable and biocompatible foundation that can be modified to stabilize other sensitive, lipophilic, and nutritionally significant nutrients.

This study bridges the gap between formulation science and application, demonstrating that naturally derived Pickering emulsions can fortify plant-based beverages. They can prolong colloidal stability without the use of synthetic surfactants and reliably deliver health-promoting compounds more effectively than conventional emulsifiers. This paves the way for nutritionally superior, clean-label products.

Author contributions

Conceptualization: GJGC, JLM; data curation: GJGC, JLM; formal analysis: GJGC, JLM; funding acquisition: GJGC, JLM; investigation: GJGC, KGMR, OTO, JLM; methodology: GJGC, KGMR, OTO, JLM; project administration: JLM; resources: LQC, YLLF, MALM, JLM; software; supervision: LQC, YLLF, MALM, JLM; validation: LQC, YLLF, MALM, KGMR, OTO, JLM; visualization: GJGC, JLM; writing – original draft: GJGC, JLM; writing – review & editing: GJGC, LQC, YLLF, MALM, KGMR, OTO, JLM.

Conflicts of interest

There are no conflicts to declare.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

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References

1 T. Funami, S. Ishihara, K. Maeda and M. Nakauma, *Food Hydrocolloids*, 2025, **162**, 110901.
2 H. Jiang, Y. Sheng and T. Ngai, *Curr. Opin. Colloid Interface Sci.*, 2020, **49**, 1.
3 C. Albert, M. Beladjine, N. Tsapis, E. Fattal, F. Agnely and N. Huang, *J. Controlled Release*, 2019, **309**, 302.
4 L. Chen, F. Ao, X. Ge and W. Shen, *Molecules*, 2020, **25**, 3202.
5 W. W. Mwangi, K.-W. Ho, B.-T. Tey and E.-S. Chan, *Food Hydrocolloids*, 2016, **60**, 543.
6 L. Yang, X. Cao, A. Gai, X. Qiao, Z. Wei, J. Li, J. Xu and C. Xue, *LWT*, 2022, **165**, 113727.
7 L. Yin, Y. Cao, Y. Deng, F. Li, B. Kong, Q. Liu and H. Wang, *J. Food Eng.*, 2025, **387**, 112305.
8 J. Niu, Q. Lin, X. Li, D. J. McClements, H. Ji, Z. Jin and C. Qiu, *Food Hydrocolloids*, 2024, 110145.
9 S. Wang, J. Wang, J. Zhang, X. Wu, Q. Guo, Y. Wang, L. Tao, X. Shen and Y. Chen, *Int. J. Biol. Macromol.*, 2024, **258**, 128093.
10 R. A. Bhutto, N. ul ain, H. Bhutto, M. Wang, S. Iqbal and J. Yi, *Food Hydrocolloids*, 2024, **147**, 109422.
11 G.-G. Morales-Figueroa, G. D. Pereo-Vega, M. E. Reyna-Murrieta, R. Pérez-Morales, M. A. López-Mata, J. J. Sánchez-Escalante, M. R. Tapia-Rodriguez, J. F. Ayala-Zavala, J. Juárez and L. Quihui-Cota, *Biomed Res. Int.*, 2022, **2022**, e9158836.
12 P. R. Cheeke, in *Saponins in Food, Feedstuffs and Medicinal Plants*, ed. W. Oleszek and A. Marston, Springer Netherlands, Dordrecht, 2000, vol. 45, pp. 241–254.
13 J. Cheon, F. Haji, J. Baek, Q. Wang and K. C. Tam, *J. Agric. Food Res.*, 2023, **11**, 100510.
14 X. Yin, J. Lu, W. Du, Q. Wu, L. Han and S. Su, *Int. J. Biol. Macromol.*, 2024, **277**, 133696.
15 Q. Lin, H. Jiang, X. Li, D. J. McClements, S. Sang, J. Wang, A. Jiao, Z. Jin and C. Qiu, *Food Biosci.*, 2024, **59**, 103845.
16 W. Meng, H. Sun, T.-H. Mu and M. Garcia-Vaquero, *ACS Food Sci. Technol.*, 2024, **4**, 1287–1300.
17 G. J. Góngora-Chi, L. Quihui-Cota, Y. L. López-Franco, W. M. Argüelles-Monal, M. A. López-Mata and J. Lizardi-Mendoza, *Polysaccharides*, 2025, **6**, 56.
18 A. Carazo, K. Macáková, K. Matoušová, L. K. Krčmová, M. Protti and P. Mladěnka, *Nutrients*, 2021, **13**, 1703.

19 S. Sivabalan and S. Sablani, *Food Bioprocess Technol.*, 2022, **15**, 338.
20 A. Basu, S. Basu, S. Bandyopadhyay and R. Chowdhury, *Ind. Crops Prod.*, 2015, **77**, 920.
21 W. Li, Y. Nian, Y. Huang, X. Zeng, Q. Chen and B. Hu, *Food Hydrocolloids*, 2019, **96**, 300.
22 J. Ayala Zavala, T. de J. Castillo Romero, J. I. Méndez Romero, L. Santiago López, A. F. González Córdova, A. Hernández Mendoza, B. Vallejo Cordoba and M. Vargas Ortiz, *Beverages*, 2024, **10**, 96.
23 L. A. Mejia, E. Hudson, E. González de Mejía and F. Vazquez, *J. Food Sci.*, 1988, **53**, 1440.
24 A. Wall-Medrano, G. A. González-Aguilar, G. F. Loarca-Piña, J. A. López-Díaz, M. A. Villegas-Ochoa, O. Tortoledo-Ortiz, F. J. Olivas-Aguirre, A. Ramos-Jiménez and R. Robles-Zepeda, *Plant Foods Hum. Nutr.*, 2016, **71**, 396.
25 AOAC, Official methods of analysis of AOAC International, AOAC International, Gaithersburg, Md, 18th edn, 2005.
26 Y. S. Sidorova, N. A. Petrov, I. B. Perova, A. I. Kolobanov and S. N. Zorin, *Foods*, 2023, **12**, 1728.
27 Y. Li, M. Obadi, J. Shi, J. Sun, Z. Chen and B. Xu, *J. Food Compos. Anal.*, 2020, **87**, 103401.
28 L. R. da Silva, J. I. Velasco and F. M. Fakhouri, *LWT*, 2023, **173**, 114271.
29 A. Zulueta, M. j. Esteve and A. Frígola, *J. Food Sci.*, 2007, **72**, C457.
30 Q. J. Wang, D.-W. Sun, S.-T. Jeong, S.-H. Yeo, J.-H. Choi and H.-S. Choi, *LWT–Food Sci. Technol.*, 2014, **56**, 145.
31 A. Abbou, N. Kadri, A. Servent, J. Ricci, K. Madani, M. Dornier, A. Collignan and N. Achir, *J. Food Process Eng.*, 2022, **45**, e13943.
32 Y. Wei, C. Wang, X. Liu, A. Mackie, M. Zhang, L. Dai, J. Liu, L. Mao, F. Yuan and Y. Gao, *Food Hydrocolloids*, 2022, **122**, 107064.
33 C. Pénicaud, N. Achir, C. Dhuique-Mayer, M. Dornier and P. Bohuon, *Fruits*, 2011, **66**, 417–440.
34 Y.-S. Lee, R. Tarté and N. C. Acevedo, *RSC Adv.*, 2021, **11**, 16275.
35 H. Chen, Z. Wang, X. Guo, S. Yu, T. Zhang, X. Tang, Z. Yang and H. Meng, *J. Agric. Food Chem.*, 2022, **70**, 8052–8063.
36 M. Syamila, M. A. Gedi, R. Briars, C. Ayed and D. A. Gray, *Food Chem.*, 2019, **284**, 188.
37 Q. Sun, Y. Fu and W. Wang, *Chem. Phys.*, 2022, **559**, 111550.
38 National Institute of Health, Vitamin A and Carotenoids, **https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/**, accessed 1 May 2025.
39 Secretaría de Economía, México, NOM-051-SCFI/SSA1-2010, 2010.
40 Y. Tang, X. Li, P. X. Chen, B. Zhang, R. Liu, M. Hernandez, J. Draves, M. F. Marcone and R. Tsao, *J. Agric. Food Chem.*, 2016, **64**, 1103.
41 M. H. Alu'datt, M. Alrosan, S. Gammoh, C. C. Tranchant, M. N. Alhamad, T. Rababah, R. Zghoul, H. Alzoubi, S. Ghatasheh, K. Ghozlan and T.-C. Tan, *Food Biosci.*, 2022, **50**, 101971.
42 L. Geng, K. Liu and H. Zhang, *Front. Nutr.*, 2023, **10**, 1192199.
43 M. T. Resende, T. Osheter, C. Linder and Z. Wiesman, *Foods*, 2021, **10**, 1385.
44 M. O. Vázquez-Meza, H. González-Ríos, G. A. González-Aguilar, M. Viuda-Martos, J. L. Dávila-Ramírez and M. Valenzuela-Melendres, *Int. J. Food Sci.*, 2024, **2024**, 2981134.
45 Y. Yao, W. He, X. Cai, A. E.-D. A. Bekhit and B. Xu, *Int. J. Food Sci. Technol.*, 2022, **57**, 4868–4878.
46 H. S. Lee and W. S. Castle, *J. Agric. Food Chem.*, 2001, **49**, 877.