



Development of Liposomal Systems for the Encapsulation of *Opuntia ficus-indica* var. *Blanca Buenavista* Extracts: Characterization and *in vitro* Digestion

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Abstract

Cactus fruits of *Opuntia ficus-indica* var. *Blanca* from the Canary Islands (Spain) are abundant in phenolic acids, such as piscidic acid, and flavonoids, including isorhamnetin, quercetin, and kaempferol glycosides, with their highest concentrations found in the fruit peel. Typically discarded as waste, the peel represents a valuable, underutilized resource. This study was aimed at repurposing this tissue as a source of bioactive molecules, which can be encapsulated in liposomal systems to enhance their stability and protect them during *in vitro* gastro-intestinal digestion. *Blanca* peel extracts were obtained using environmentally friendly extraction techniques, which were subsequently encapsulated into two liposomal formulations (Liposome A and Liposome B), prepared using ultrasound-assisted methods under different conditions. Liposome A proved to be the most effective system, offering higher encapsulation efficiencies for individual phenolics (82.1–98.4%), smaller particle size distributions (D10: 38–67 nm; D50: 87–128 nm; D90: 306–426 nm), and a more stable zeta potential (–24.2 to –20.8 mV). When subjected to *in vitro* gastrointestinal digestion, Liposome A demonstrated enhanced stability of phenolic compounds, higher recoveries, and significantly improved bioaccessibility, reaching values up to 88.4% compared to the non-encapsulated *Blanca* peel extract. These findings underscore the potential of liposomal encapsulation as a strategy for developing bioactive ingredients from *Opuntia* agricultural waste, offering promising applications in nutraceutical and functional food industries.

Keywords Cactus fruits · Phenolic compounds · Liposomes · Bioactivity · Stability · Bioaccessibility

Introduction

In the pharmaceutical and food industries, encapsulation techniques play a crucial role in enhancing the stability and functionality of bioactive compounds. These systems are designed to protect sensitive ingredients from environmental factors such as light, oxygen, and moisture, thereby extending their shelf life and preserving their nutritional and therapeutic benefits (Garti and McClements, 2012). Various encapsulation methods, including spray drying, extrusion, coacervation, and nanoencapsulation, are employed to integrate bioactives into food products, each offering distinct advantages depending on the nature of the compounds and the intended application (Huang et al., 2023). Among these techniques, liposome-based encapsulation has emerged as a particularly promising strategy in recent years. Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. These systems can encapsulate both hydrophilic and hydrophobic molecules,

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including bioactive compounds, making them highly valuable for various applications in the food industry (Chai & Park, 2024). Liposomes are often utilized to protect nutrients and bioactive compounds from degradation caused by environmental factors and to facilitate a controlled release and targeted delivery, thereby enhancing their nutritional and biological efficacy. Their biocompatibility and capacity to encapsulate a wide range of substances make liposomes a versatile tool for food fortification, functional foods, and the development of novel food formulations (Liu et al., 2020).

As consumer demand for functional foods and plant-derived products increases, liposomes offer innovative solutions to incorporate and enhance the bioactive potential of plant compounds in various food products, nutraceuticals, and dietary supplements. One example of a plant source of molecules with notable bioactive properties is the *Opuntia* genus, more commonly referred to as prickly pear cactus. *Opuntia* belongs to the *Cactaceae* botanical family and is constituted by a large group of succulent plants. Their historical origin is attributed to America, but these cacti are now widespread in arid and semi-arid regions globally due to their resilience to extreme climatic conditions such as high temperatures and droughts (Dubeux et al., 2021). Mexico is the largest producer and consumer of cactus pears, but countries, like Spain, Italy, and South Africa, also represent a significant production. Among the over 300 documented *Opuntia* species, *Opuntia ficus-indica* is the most extensively cultivated (Daniloski et al., 2022).

Opuntia ficus-indica cactus fruits are rich in vitamins, minerals, and bioactive compounds such as phenolics, betalains, and carotenoids, which have been extensively studied for their biological properties (Daniloski et al., 2022). The present research specifically targets the phenolic compounds present in *Opuntia ficus-indica* var. *Blanca Buenavista* fruit peels, which are significantly more abundant than those in the fruit pulp, as described by Gómez-Maqueo et al. (2020) after making the characterization of the complete phenolic profile of peel and pulp of these fruits. Despite their rich composition in phenolic acids (especially p-coumaric acid) and flavonoids (mainly isorhamnetin and quercetin glycosides), the peels are often discarded as waste due to their tough consistency and the presence of spines. This valuable yet underutilized resource was selected as the matrix for study to explore its potential as a by-product, aiming to repurpose it and maximize its value.

Numerous studies highlight the antioxidant, anti-inflammatory, antiobesity, and antiatherogenic properties attributed to the phenolic compounds present in *Opuntia* fruits, assessed by methodologies performed both *in vitro* and *in vivo* (Aragona et al., 2018; Aruwa et al., 2018; Gómez-Maqueo et al., 2021). However, the stability of these compounds can be a challenge, as they are susceptible to degradation due to external factors such as pH, oxygen,

temperature, and light exposure. From a physiological point of view, phenolic compounds can also be affected by digestive processes, where enzymes, digestive fluids, and even other nutrients can interact with their chemical structures, impacting on their stability and bioaccessibility (Gómez-Maqueo et al., 2020), thus reducing the proportion that reaches the bloodstream and so, influencing their biological activities (D'Archivio et al., 2010).

The present study aimed to investigate the impact of ultrasonic processing methods on the development of liposomes to encapsulate biologically active extracts abundant in phenolic compounds derived from the fruit peel waste of *Opuntia ficus-indica* var. *Blanca Buenavista*. Prior to encapsulation, the biological activity of the extracts was evaluated *in vitro* to assess their potential and interest in being encapsulated in liposomal systems. Continuously, two formulations identical in composition were prepared using the thin-film hydration method, differing only in the ultrasonic processing technique: ultrasound probe and ultrasonic bath. These methods were chosen to compare how differences in energy delivery and distribution affect critical liposomal properties, including particle size distribution, zeta potential, encapsulation efficiency, and stability. Previous studies have highlighted the influence of ultrasonic parameters on key characteristics of liposomes, particularly regarding encapsulation efficiency and structural stability, further supporting the relevance of this comparative study (Lu et al., 2011; Chen & Chang, 2020). The liposomal system with the most favorable characteristics, as determined by these evaluations, was subjected to further analysis using the INFOGEST *in vitro* gastrointestinal digestion protocol. This step assessed the impact on liposomal particles and the digestive stability and bioaccessibility of the individual encapsulated phenolic compounds present in *Blanca* peel extracts under simulated digestive conditions. The findings of this study are aimed at providing valuable insights into liposomal encapsulation, contributing to the sustainable utilization of fruit peel waste and the development of advanced delivery systems for bioactive compounds.

Materials and Methods

Plant Material

Opuntia ficus-indica fruits of the variety *Blanca Buenavista* were harvested from Buenavista del Norte (Tenerife, Canary Islands, Spain; 28°2' N, 16°5' W; 127 m above sea level) in 2023 (Supplementary Fig. S1). Fruits were selected by maturity, size and color, washed and separated into pulps and peels, and individually weighed. Physicochemical properties were measured directly on the fruits' fresh tissues to assess maturity (Supplementary Table S1). Titratable acidity

was assessed by neutralizing prickly pear juice with 0.1 N sodium hydroxide until reaching a pH of 8.1. pH, and soluble solid contents were also evaluated from the prickly pear pulp juice. The color of the peels and pulps was recorded using the CIELAB scale color system with a Konica Minolta CM-3500 d (Japan). The peels were then frozen using liquid nitrogen, lyophilized (LyoBeta 15, Azbil Telstar, Barcelona, Spain) and pulverized to a fine particle size (< 2 mm), vacuum packed, and stored at -20°C until use.

Obtention of Phenolic-Rich Green Extracts from *Opuntia ficus-indica* var. *Blanca Buenavista*

Extracts abundant in phenolic compounds from the peels of *Opuntia ficus-indica* var. *Blanca Buenavista* (OBB) were obtained following the methodology reported by Gómez-López et al. (2021). Briefly, 1 g of freeze-dried *Blanca* peel was mixed with 5 mL ethanol (1:1, v/v), homogenized with a vortex for 1 min, and treated in an ultrasonic water bath (3000514, Selecta S.A., Barcelona, Spain) for 4 min. The samples were centrifuged at $10,000 \times g$ for 10 min at 4°C (5804R, Eppendorf, Hamburg, Germany), and the supernatants were collected in rounded flasks. This process was repeated twice with 3 mL of ethanol (1:1, v/v) and once with pure ethanol. The combined supernatants were evaporated using a rotary evaporator (Buchi, Switzerland) at 30°C , then lyophilized, and stored at -20°C until use.

Identification and Quantification of Phenolic Compounds

The phenolic compounds in OBB extracts were analyzed using an Agilent 1200 Series HPLC system with a reverse-phase C18 column (Zorbax SB-C18, 250×4.6 mm i.d., $5\text{ }\mu\text{m}$) at 25°C . Elution phase A was composed of ultrapure water with 1% formic acid and Phase B of methanol with 1% formic acid. A 70-min gradient optimized separation. The injection volume was 20 μL with a flow rate of 0.8 mL/min. Detection was at 370 nm for flavonoids and 280 nm for phenolic acids. The HPLC–DAD was coupled to a mass spectrometer (LCMS SQ 6120) with ESI in positive ion mode. Nitrogen was the drying gas (3 L/min at 137.9 kPa), with a 300°C nebulizer and 3500 V capillary. Spectra from m/z 100–1000 were recorded. Additional mass spectrometry was performed using an LC-QTOF (Bruker Daltonics), with spectra from m/z 50–3000 using the bbCID method. Compounds were identified by retention times, UV/Vis, and mass spectra and quantified using calibration curves for each individual compound.

In Vitro Bioactive Potential

Antihyperglycaemic Activity: α -amylase and α -glucosidase Inhibition

Inhibition of α -amylase was determined following the methodology by Gulati et al. (2012) with some modifications reported by Gómez-Maqueo et al. (2019a). OBB extracts were diluted 1:10 (v/v), mixed with α -amylase solution (0.5 U/mL), and incubated at 25°C for 10 min. After adding 5% starch solution, a secondary 10-min incubation followed. The reaction was stopped by adding 3,5-dinitrosalicylic acid and heating at 95°C for 5 min. Absorbance was measured at 540 nm in a microplate reader, and inhibition of α -amylase was expressed as a percentage.

Inhibition of α -glucosidase was assessed based on the methodology described by Apostolidis et al. (2007) with modifications reported by Gómez-Maqueo et al. (2019a). A volume of 400 μL of α -glucosidase solution (1 U/mL from *Saccharomyces cerevisiae*) was added to 200 μL of OBB and incubated for 10 min at 25°C . Then, 200 μL of 4-nitrophenyl α -D-glucopyranoside substrate was added. After a 5-min incubation, samples were diluted, and absorbance was measured at 405 nm in a microplate reader. Results were expressed as a percentage of α -glucosidase inhibition.

Anti-Inflammatory Activity: Hyaluronidase Inhibition

Hyaluronidase-inhibiting activity was evaluated using the methodology from Bralley et al. (2007) with adaptations reported by Gómez-Maqueo et al. (2019b). To each 15 μL of diluted OBB, 147 μL of acetate buffer (0.2 mol/L sodium acetate, pH 6, with 0.15 mol/L NaCl) was added. Then, 120 μL of 0.5 mg/mL sodium hyaluronate was added and mixed for 30 s. Then, 1 mg/mL hyaluronidase was added, and samples were incubated at 37°C for 15 min. The reaction was finished by 1.2 mL of 2.3% hexadecyltrimethylammonium bromide in 2% NaOH (pH 12). After a 10-min incubation at 25°C , the absorbance was measured at 400 nm. Results were reported as a percentage of hyaluronidase inhibition.

Antioxidant Activity: Oxygen Radical Absorbance Capacity

Oxygen radical absorbance capacity (ORAC) assay was conducted based on the methodology reported by Ou et al. (2001) by measuring fluorescence degradation in a 96-well microplate. Extracts were diluted 1500-fold using a potassium phosphate and sodium phosphate buffer at pH 7.4. In each well, 20 μL of diluted OBB were combined with 120 μL of 11.7 $\mu\text{mol/L}$ fluorescein disodium. The plate was incubated at 37°C for 10 min, after which 60 μL of 153 mmol/L 2,2'-azobis(2-methylpropionamidine) dihydrochloride was

added. Fluorescence readings were taken every min for 55 min at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Data analysis involved calculating the area under the curve (AUC) minus the blank and quantifying antioxidant capacity using a Trolox standard curve ranging from 2 to 10 mmol/L. Results were reported in concentration of μmol Trolox equivalents (eq.) per g of lyophilized OBB.

Liposome Preparation

Two protocols (Liposome A and Liposome B) were developed for the encapsulation of OBB using liposomes, based on the thin film hydration method described by Xiang and Cao (2021) with modifications. Initially, soy phosphatidylcholine (PC) and Tween 20 were combined at a 3:2 (w/w) ratio in pure ethanol to achieve a final concentration of 1.5 mg/mL, stirred magnetically at 300 rpm for 5 min, and subjected to rotary evaporation at 30 °C and 30 mbar for 15 min to form a uniform lipid film. One gram of OBB extract was then dissolved in phosphate-buffered saline (PBS) at pH 7.4 to a concentration of 20 mg OBB/mL and added to the lipid film for hydration. After this, the protocols diverged in the ultrasonic treatments applied: for Liposome A, the mixture underwent 15-min sonication using a Sonifier Digital SFX 550 (Branson, MO, USA) with a 3-mm ultrasound probe at 20% amplitude and 20 s ON/OFF pulses. In contrast, Liposome B was treated with an ultrasonic water bath for 60 min, without probe sonication (3,000,514, 50/60 Hz, 360 W, J.P Selecta S.A., Barcelona, Spain).

Liposome Characterization

Encapsulation Efficiency

The encapsulation efficiency (EE%) of a liposome refers to the percentage of a given substance that is successfully enclosed within the system relative to the total amount of the substance initially used in the preparation process (Brunetti & Felice, 2016). The EE of individual phenolic compounds in OBB was determined indirectly through the separation of the loaded liposome particles from the free phenolic extract by centrifugation at $20,000 \times g$ for 60 min. The free phenolic compounds in the supernatants were identified and quantified by HPLC, as described in the “*In vitro* bioactive potential” section. EE for each compound was expressed as a percentage calculated using the following equation (Eq. (1)):

$$\text{Encapsulation efficiency(\%)} = \frac{(C_i - C_f)}{C_i} * 100$$

where C_i is initial concentration of compound added to the liposome and C_f is concentration of free compound present in the supernatant.

Optical and Confocal Microscopy

Optical and confocal microscopies were employed to examine the structure of the liposomes. Optical microscopy (Leica DM IL, Wetzlar, Germany) was used to confirm their formation by observing unstained samples. Additionally, toluidine blue dye was employed to highlight the phenolic compounds from OBB, which interact with negatively charged functional groups like hydroxyls. Confocal microscopy (TCS SP5, Leica Microsystems) was used to study the liposomal lipid microstructure, with samples stained using 10 μL of Nile red dye to bind the lipid fractions, allowing for clear visualization through fluorescence, with excitation and emission measured between 488 and 650 nm.

Particle Size Distribution, Zeta Potential, and Polydispersity Index

Particle size distribution by intensity (D10, D50, and D90), zeta potential, and polydispersity index of the liposomes were analyzed using a Zetasizer Pro (Malvern Instruments Ltd., Worcestershire, UK) at specific intervals over a 20-day storage period at a controlled temperature of 7 °C in a refrigerator (Liebherr-International AG, Bulle, Switzerland). Measurements were performed on days 0, 1, 3, 6, 10, 15, and 20 to monitor changes in these parameters and assess the stability of the formulations over time. For these analyses, 100 μL of each sample were diluted in 10 mL of ultrapure water, homogenized at 400 rpm on a stirring plate, and allowed to stand for 5 min before analysis. The samples were then placed in folded capillary cells and equilibrated for 2 min at 25 °C. The measurements were conducted with a scattering angle of 174° and a refractive index of 1.47. Results were analyzed using the ZS Xplorer software. Particle size distribution by intensity values were reported in nanometers (nm) and zeta potential in millivolts (mV). The polydispersity index (PDI) was calculated by the equipment using the cumulants method, in accordance with ISO 22412:2017 (International Organization for Standardization, 2017), which applies to the following formula:

$$PDI = \frac{\mu^2}{\Gamma^2}$$

where μ^2 refers to the second cumulant of the size distribution (variance or width of the distribution) and Γ^2 is the first cumulant (average particles hydrodynamic size).

In Vitro Gastrointestinal Digestion Assay

The in vitro gastrointestinal digestion assay was conducted following the standardized INFOGEST® protocol (Brod-korb et al., 2019) with adaptations reported by Parralejo-Sanz et al. (2024). The composition of the digestive simulated solutions is detailed in Supplementary Table S2. Non-encapsulated OBB extracts were used as controls, and freshly prepared liposomes with encapsulated OBB served as test samples. After each phase, samples were filtered (0.22 µm), frozen, and stored at -20°C . Phenolic compound composition was analyzed by HPLC. Digestive stability (µg/g of dry weight) and recovery percentages were calculated by comparing the initial concentration in non-digested samples to those in each digestion phase. Bioaccessibility was determined using the following equation (Eq. (2)):

$$\text{Bioaccessibility (\%)} = \frac{\text{Concentration of compound in the intestinal phase}}{\text{Concentration of compound before digestion}} * 100$$

Statistical Analysis

Results were presented as mean $\pm$ standard deviation from at least three independent measurements ($n = 3$). Statistical significance at $p < 0.05$ was determined using analysis of variance (ANOVA) using the software GraphPad Prism 8.0.2 for Windows (GraphPadSoftware, Inc, Boston, MA, USA). Comparisons between groups or samples were performed using post hoc Tukey's or Student's t -tests.

Results and Discussion

Characterization of *Opuntia ficus-indica* var. *Blanca Buenavista* Green Extracts (OBB)

The characterization data of the main phenolics identified in OBB by HPLC–DAD–MS is shown in Supplementary Table S3, and their individual quantification is shown in Supplementary Table S4, where results are expressed as µg of compound/g of dry weight (d.w.) of OBB extract. Additionally, an example of the chromatograms detected at 280 nm (for the phenolic acids) and at 370 nm (for the flavonoids) can be observed in Supplementary Fig. S2.

A total of 10 individual phenolic compounds were identified, including three phenolic acids (piscidic acid and its derivative and 4-hydroxybenzoic acid (4-HAD)) and seven flavonoids (quercetin glycoside I (QG1), quercetin glycoside II (QG2), isorhamnetin glycosyl-rhamnosyl-rhamnoside (IG1), isorhamnetin glycosyl-rhamnosyl-pentoside (IG2), isorhamnetin hexosyl-rhamnoside (IG7), kaempferol glycosyl-rhamnoside (KG1), and isorhamnetin glycosyl-rhamnoside (IG5)). Among the phenolic acids, piscidic acid was

the most abundant, with a quantity of $76,947 \pm 357$ µg/g of OBB d.w. Among the flavonoids, IG7 and QG2 were the most abundant, with contents of 1058 ± 73 and 1132 ± 21 µg/g of OBB d.w., respectively. Gómez-Maqueo et al. (2020) conducted an extensive characterization of extracts from different varieties and tissues of *Opuntia ficus-indica*, including the peel of *Blanca Buenavista* variety. Their study indicated that, in *Blanca* peels piscidic acid was also the most abundant phenolic acid, with a content of $42,360 \pm 3796$ µg/g of lyophilized peel. Regarding flavonoids, the most abundant one was isorhamnetin hexosyl-rhamnoside (IG7), with 1250 ± 70 µg/g of OBB d.w., which aligns with the results presented in the present study. It should be remarked that the isorhamnetin glycoside IG7 has been detected so far exclusively in the *Blanca* variety of *Opuntia* (Gómez-Maqueo et al., 2020). This observation highlights the distinct biochemical signature of this cactus pear and underscores the potential for identifying novel compounds in less studied plant species. Interestingly, while most *Opuntia* varieties are rich in betalains, these pigments are absent in *Blanca* fruits. This distinctive combination (a different phenolic composition by the presence of IG7 and lack of betalains) suggests that *Blanca* variety may possess unique metabolic or ecological traits worthy of further investigation, especially considering its underutilized potential.

In Vitro Biological Activities

Given the rich composition of OBB extracts in phenolic compounds, a series of in vitro assays were conducted to assess their biological potential. These assays were selected for their efficiency in screening specific bioactivities, cost-effectiveness, and ability to maintain controlled experimental conditions, serving as an essential first step before advancing to more complex in vivo models. The biological activities assessed included antihyperglycaemic effects through the inhibition of α -amylase and α -glucosidase enzymes, as well as anti-inflammatory activity via hyaluronidase inhibition, which, to the best of our knowledge, have not been previously reported on white varieties of *Opuntia ficus-indica*, such as OBB. The antioxidant capacity was evaluated by the ORAC assay. These in vitro tests served as a valuable preliminary step in determining the interest in encapsulating OBB extracts.

The enzyme α -amylase facilitates the breakdown of α -1,4-glucosidic bonds found in starch, glycogen, and various oligosaccharides, while α -glucosidase continues the process by converting disaccharides into simpler sugars that can be easily absorbed in the intestines. Inhibiting the activity of these enzymes in the human digestive system is regarded as an effective approach for managing diabetes, as it reduces the absorption of glucose derived from the breakdown of starch (Bhandari et al., 2008). OBB extract, showed a 32.8

$\pm 5.4\%$ inhibition of the enzyme. Similar inhibitory effects have been observed in various *Opuntia ficus-indica* varieties. For instance, Ydjedd et al. (2021), reported α -amylase inhibition percentages up to 43.5% and 57.3% in extracts from red and yellow varieties, respectively. Similarly, Koss-Mikołajczyk et al. (2019) found inhibition levels up to 32% for extracts from orange and red prickly pears and of 36% for a yellow variety, highlighting the potential of different *Opuntia* extracts in modulating α -amylase activity. With regard to α -glucosidase, OBB extract exhibited $50.1 \pm 1.8\%$ of α -glucosidase inhibition, aligning with the results reported by Gómez-maqueo et al. (2019a), who found inhibitions of 54%, 49%, and 46.8% for the extracts of Morada (purple), Vigor (orange), and Sanguinos (red) varieties. In addition, Rabi et al. (2020), obtained 50–77.5% inhibitions of α -glucosidase in aqueous *O. ficus-indica* juice extracts. It should be remarked that the most desirable combination of activities is a moderate α -amylase inhibition and higher α -glucosidase, as it reduces starch and sucrose digestion and glucose absorption while minimizing bacterial fermentation of undigested carbohydrates in the colon (Gómez-Maqueo et al., 2019a). The findings exposed underscore the promising potential of OBB and other *Opuntia* extracts as natural inhibitors of key digestive enzymes, offering a valuable strategy for controlling postprandial blood glucose levels in diabetes management.

Regarding anti-inflammatory activity, hyaluronidase enzyme contributes to both physiological and pathological processes by breaking down hyaluronic acid in the extracellular matrix during tissue remodeling, triggering inflammation (Zeghibib et al., 2022). Excessive hyaluronidase activity can cause degenerative changes in connective tissue due to increased inflammation. OBB extracts inhibited hyaluronidase by $53.9 \pm 7.2\%$, similarly to the results reported for the Mexican variety *Pelota* by Gómez-Maqueo et al. (2019b), who obtained an inhibition of 53.6%. Montiel-Sánchez et al. (2021) observed over 80% inhibition of hyaluronidase in *Myrtillocactus geometrizans* fruits, another *Cactaceae* species with a comparable phytochemical profile to *Opuntia* cactus fruits. These findings suggest that the inhibitory effects on hyaluronidase observed in OBB extracts and other *Cactaceae* species could be linked to their bioactive compounds, highlighting their potential as natural anti-inflammatory agents.

Antioxidant activity, measured by ORAC, yielded 153 ± 14 $\mu\text{mol TROLOX eq./g}$ of OBB extract. These results can be compared to those reported by Gómez-Maqueo et al. (2021), who obtained 181 ± 14 $\mu\text{mol TROLOX eq./g}$ in OBB methanolic extracts. The slight variations in antioxidant activity could be attributed to differences in harvest seasons and agronomic conditions, which can influence the composition of the fruit, as well as variations in the extraction methods used, such as the choice of solvents. Many

other authors have evaluated the antioxidant potential in other *Opuntia ficus-indica* varieties, obtaining promising results. For example, Fiad et al. (2020) demonstrated robust radical scavenging activity in *Opuntia ficus-indica* peel extracts, with different solvent-based extractions showing over 50% antioxidant activity. Similarly, Galati et al. (2003) observed a decrease of 50% in the absorbance of DPPH in *Opuntia ficus-indica* juice.

These results collectively highlight the versatile biological potential of OBB extracts, emphasizing their relevance promising candidates for their encapsulation and development in nutraceutical and functional food applications.

Liposome Characterization

Encapsulation Efficiency

The encapsulation efficiency (EE%) data of the individual phenolic compounds present in OBB, by their encapsulation on Liposomes A and B, is presented in Fig. 1.

Both Liposomes A and B were prepared using phosphatidylcholine (PC) and Tween 20 as the lipid phase. PC, containing unsaturated fatty acids (linoleic and oleic), provides fluidity to the bilayer and enhances the encapsulation of hydrophilic compounds. Tween 20, a non-ionic surfactant with an amphiphilic structure, partially integrates into the bilayer and forms an external layer that reduces particle aggregation (Chai & Park, 2024). A 3:2 PC:Tween 20 ratio was used, balancing bilayer stability and an adequate incorporation of the target compounds present in OBB.

Attending to the encapsulation efficiency of the individual phenolic compounds, in Liposome A, the most efficiently encapsulated QG1 ($98.4 \pm 0.8\%$), followed by IG1 ($95.5 \pm 1.7\%$) and IG2 ($90.7 \pm 3.2\%$). In Liposome B, QG1 was also the compound with the highest encapsulation efficiency (EE) ($90.8 \pm 1.1\%$), followed by IG7 ($86.5 \pm 2.5\%$) and IG1 ($86.3 \pm 2.1\%$). The higher encapsulation efficiencies observed in Liposome A can be attributed to its preparation method, which involved high-intensity sonication using an ultrasound probe, compared to the gentler bath ultrasound technique used for Liposome B. The probe sonication applies localized, high-energy cavitation, enhancing the incorporation of phenolic compounds into the liposomal bilayer and aqueous core, while the bath ultrasound produces a more uniform but less intense energy distribution, leading to lower encapsulation efficiency (Mehta et al., 2022). Overall, OBB phenolics were more efficiently encapsulated using the Liposome A technique, with encapsulation efficiencies (EEs) ranging from 82.1 ± 4.8 to $98.4 \pm 0.8\%$, compared to the Liposome B technique, which achieved EEs between $55.2 \pm 12\%$ and $90.8 \pm 1.1\%$. These results highlight Liposome A as a more effective method to encapsulate and protect the phenolic compounds present in OBB. Chen et al.

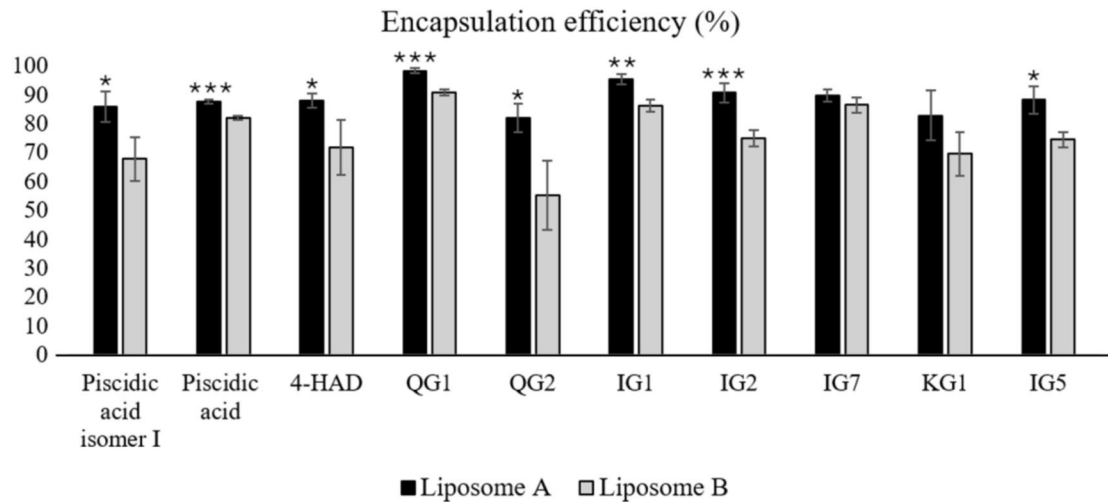


Fig. 1 Encapsulation efficiency of the individual phenolic compounds present in *Opuntia ficus-indica* var. *Blanca Buenavista* peel green extracts (OBB) encapsulated by Liposome A and Liposome B. Results are presented as means $\pm$ standard deviation based on at least three independent measurements ($n = 3$). Statistical analysis was performed using Student's *t*-test where * indicates statistically sig-

nificance of $p < 0.05$, ** of $p < 0.01$, and *** of $p < 0.001$. 4-HAD: 4-hydroxybenzoic acid derivative, QG1: quercetin glycoside I, QG2: quercetin glycoside II, IG1: isorhamnetin glucosyl-rhamnosyl-rhamnoside, IG2: isorhamnetin glucosyl-rhamnosyl-pentoside, IG7: isorhamnetin hexosyl-rhamnoside, KG1: kaempferol glucosyl-rhamnoside, IG5: isorhamnetin glucosyl-rhamnoside

(2021) encapsulated bamboo leaf extracts rich in phenolics using the thin layer dispersion method and obtained EEs above 70%. In contrast, Jahanfar et al. (2021) encapsulated green tea extracts also abundant in phenolics by liposomes with the Mozafari method (an eco-friendly approach that avoids solvents, detergents, and sonication) and showed EEs between 43.5 and 53.7%. Chen and Chang (2020) evaluated the effectiveness of the encapsulation of phenolics from *Clitoria ternatea* extracts, attending to multiple parameters, including sonication time and power. They observed that, moderate sonication generally enhanced EE%, while excessive duration or intensity appeared to reduce it, possibly due to liposome disruption or compound degradation. However,

it is important to note that the previous authors reported EEs for total phenolics, whereas this study analyzed individual phenolic compounds in the OBB green extract. Differences in encapsulation efficiencies can arise due to factors like interactions between the extract and liposomal components, as well as the methods used for liposome preparation (Liu et al., 2020).

Optical and Confocal Microscopy

Figure 2 presents optical and confocal microscopy images of Liposomes A and B encapsulating the OBB green extract. The optical microscopy images (Fig. 2a, b) reveal

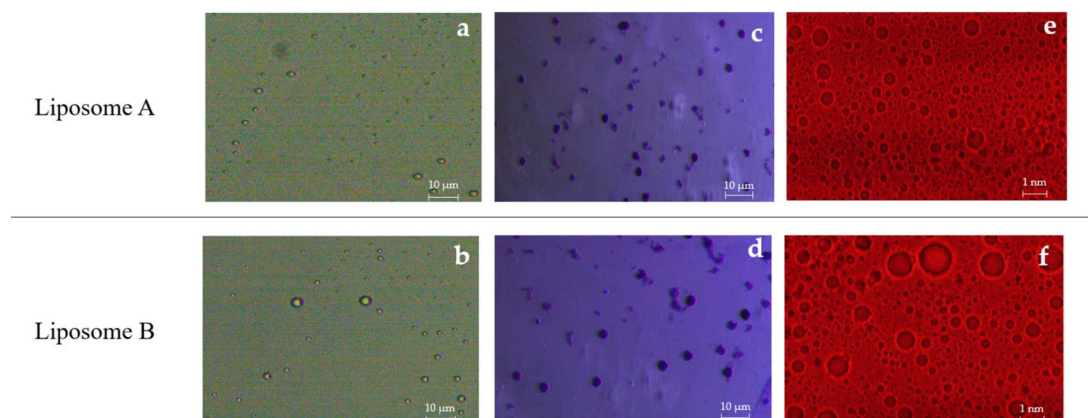


Fig. 2 Optical (a–d) and confocal (e, f) microscopy images of Liposomes A and B with *Opuntia ficus-indica* var. *Blanca Buenavista* peel green extract (OBB) encapsulated

that Liposome A produced smaller and more uniform particles, whereas Liposome B exhibited larger and more variable droplet sizes. Staining with Toluidine Blue (Fig. 2c, d) further highlighted differences in the phenolic compound distribution between the two formulations. Liposome A, prepared using the ultrasound probe method, displayed smaller and more homogeneous particles. In contrast, Liposome B, formulated with the ultrasonic bath method, consisted of larger and less uniform particles. Confocal microscopy with Nile Red staining (Fig. 2e, f) emphasized the lipidic layer of the liposomes, showing that Liposome A formed more compact and well-defined structures. In comparison, Liposome B had larger and less structurally distinct liposomes. Overall, the ultrasound probe method (Liposome A) generated more uniform and stable liposomes, likely enhancing the stability of the encapsulated phenolic compounds compared to the ultrasonic bath method (Liposome B) (Mehta et al., 2022).

Chai and Park (2024) categorized liposomes based on their structure, distinguishing unilamellar liposomes (with a single phospholipid bilayer) from multilamellar liposomes (with multiple bilayers). In this study, microscopy images confirmed that both Liposomes A and B were unilamellar, a structural feature that facilitates efficient and controlled release of encapsulated compounds. This design reduces waste, enhances targeted delivery, and offers cost-effective advantages by requiring less lipid material. Unilamellar liposomes are also easier to characterize, making them suitable for applications in drug delivery, cosmetics, and biotechnology. The findings of this study align with those of Chen et al. (2021), who encapsulated phenolic compounds from bamboo leaf extracts in liposomes also using a thin-layer ultrasound-assisted method. Their fluorescence and

transmission electron microscopy images revealed uniform, homogeneously distributed unilamellar particles, consistent with the results observed here. This parallel validates the effectiveness of the encapsulation methods used and highlights the potential of ultrasound-assisted techniques for creating stable and efficient liposomal systems.

Particle Size Distribution and Polydispersity Index

Particle size distribution is a critical parameter in liposome characterization, as it directly influences stability and interaction with biological membranes (Jahanfar et al., 2021). The polydispersity index (PDI) is a dimensionless parameter that provides a measure of size uniformity, where lower PDI values indicate more homogeneous and stable formulations (Raval et al., 2018). The evolution of particle size distribution by intensity (D10, D50, and D90) for Liposomes A and B, and their respective controls during 20-day storage at 7 °C are presented in Table 1 and represented graphically for day 0 and day 20 in Fig. 3. Polydispersity indexes during 20-day storage are detailed in Table S5.

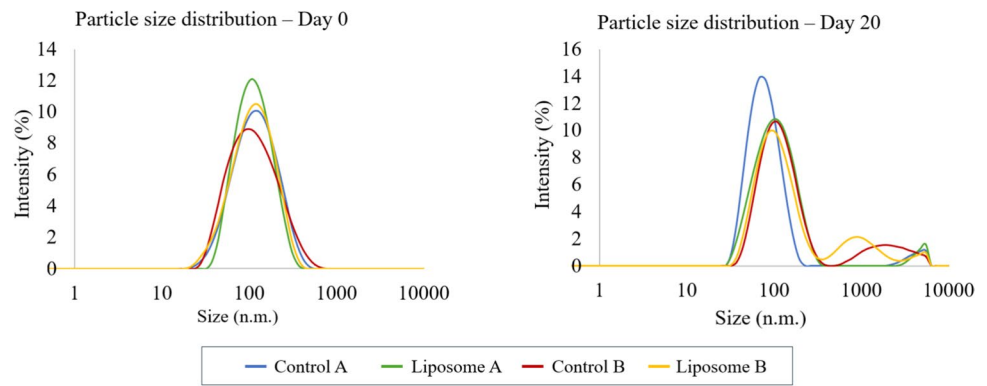
The results revealed distinct stability patterns between the different liposomal systems. For Liposome A, D50 increased moderately from 87.7 ± 5.5 (day 0) to 128 ± 7 nm (day 20), with D90 rising from 306 ± 28 to 359 ± 63 nm. Control A displayed a similar trend, with D50 increasing from 87 ± 4.4 (day 0) to 118 ± 2.5 nm (day 20), and D90 reaching 275 ± 29 nm, indicating slight aggregation but overall good stability. In contrast to Liposome B and its control, Control B showed more substantial size increases, with Liposome B's D50 rose from 117 ± 9.8 (day 0) to 220 ± 11 nm (day 20) and D90 reaching 1036 ± 84 nm, indicating significant

Table 1 Evolution of particle size distribution by intensity (D10, D50, and D90) of Liposomes A and B with *Opuntia ficus-indica* var. *Blanca Buenavista* peel extract (OBB) encapsulated and their respective controls without OBB extract (Control A and Control B) during conservation at 7 °C for 20 days. Values are expressed as

mean $\pm$ standard deviation. Statistical analysis was performed using Tukey's test as a post hoc test. Superscript letters indicate statistically significant differences between conservation days among each system and its three distributions individually

Sample	Distribution	Particle size distribution by intensity (nm)						
		Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 20
Control A	D10	35.1 ± 0.9^d	61.7 ± 3.1^c	43.3 ± 0.8^d	55 ± 6.6^c	70.5 ± 0.4^{ab}	69.3 ± 2.5^b	80.3 ± 6.7^a
	D50	87 ± 4.4^c	95.7 ± 2.9^c	93 ± 2.6^c	92 ± 0^c	110 ± 4^b	110 ± 2^b	118 ± 2.5^a
	D90	272 ± 19^{ab}	246 ± 16^{ab}	213 ± 9.3^b	233 ± 9.1^a	232 ± 2.6^b	223 ± 21^b	275 ± 29^a
Control B	D10	60.7 ± 0.8^b	51.7 ± 1.5^c	71.7 ± 1.5^d	66.3 ± 1.5^e	83.7 ± 2.9^a	51.9 ± 1.6^c	61.0 ± 3^b
	D50	130 ± 8.5^{bc}	119 ± 8.2^c	148.3 ± 3.5^{bc}	143 ± 3^{bc}	155 ± 6.1^b	170 ± 21^{ab}	189 ± 17^a
	D90	335 ± 16^b	383 ± 58^{ab}	376 ± 48^{ab}	586 ± 97^{ab}	750 ± 45^a	712 ± 64^a	737 ± 19^a
Liposome A	D10	38.5 ± 0.8^c	44 ± 1^c	44.3 ± 1.3^c	46.4 ± 5.1^c	42.8 ± 1.7^c	66.7 ± 0.7^a	57.2 ± 4.1^a
	D50	87.7 ± 5.5^c	102 ± 5.2^{abc}	99.7 ± 2.9^{bc}	115 ± 5.7^{ab}	124 ± 5.2^a	125 ± 17^a	128 ± 7^b
	D90	306 ± 28^a	329 ± 24^a	301 ± 34^a	426 ± 94^a	308 ± 1.7^a	351 ± 10^a	359 ± 63^a
Liposome B	D10	57.4 ± 3.3^{ab}	57.4 ± 2^{ab}	60 ± 1.7^a	52.4 ± 1.9^b	53 ± 1.5^{ab}	59 ± 2^a	58.3 ± 0.6^a
	D50	117 ± 9.8^c	126 ± 7.4^c	161 ± 17^{bc}	159 ± 24^b	172 ± 25^{ab}	200 ± 19^{ab}	220 ± 11^a
	D90	757 ± 25.7^b	622 ± 80^b	744 ± 51^{ab}	827 ± 33^{ab}	847 ± 74^{ab}	914 ± 31^{ab}	1036 ± 84^a

Fig. 3 Particle size distribution of Liposomes A and B with *Opuntia ficus-indica* var. *Blanca Buenavista* peel extract (OBB) encapsulated and their respective controls without OBB extract (Control A and Control B) during conservation at 7 °C for 20 days



aggregation. Similarly, Control B exhibited a D50 increase from 130 ± 8.5 (day 0) to 189 ± 17 nm (day 20) and a D90 of 737 ± 19 nm by the end of the storage period. Notably, Liposome A and Control A maintained lower PDI values (0.25 ± 0.01 to 0.35 ± 0.05 for Liposome A and 0.20 ± 0.01 to 0.22 ± 0.02 for Control A), indicating a narrower particle size distribution compared to Liposome B and Control B, which reached PDI values of 0.52 ± 0.02 and 0.41 ± 0.03 , respectively. The observed differences between the systems can be attributed to the preparation methods. Liposome A and Control A, prepared with the ultrasonic probe, showed smaller and more uniform particles with better stability. The high energy and precision of the probe method likely contributed to reduced particle variability and aggregation (Schroeder et al., 2009). Conversely, Liposome B and Control B, prepared with an ultrasonic bath, exhibited less uniformity and stability, consistent with the lower intensity and efficiency of this method. These results align with literature reporting the impact of preparation methods and extract types on liposomal stability. Rafiee et al. (2017) encapsulated phenolics from pistachio using extrusion and achieved average particle sizes below 100 nm, similar to Liposome A. Păvăloiu et al. (2021) reported a decrease in PDI when polyphenols from *Lycium barbarum* were encapsulated using the film hydration method (0.3 to 0.19), contrasting with Machado et al. (2019), who observed PDI increases in *Spirulina*-based liposomes prepared by reverse phase evaporation (0.46 to 0.52). Lu et al. (2011) evaluated the effect of ultrasound application time on liposomes encapsulating tea polyphenols. They observed that, as time progressed, the particle size diminished, which is a favourable factor regarding stability. However, the encapsulation efficiency also decreased. These findings suggest that both encapsulation methods and extract compositions significantly influence liposomal stability. The combination of smaller initial average particle sizes, narrower size distributions, and lower PDI values in Liposome A underscore the importance of using an ultrasonic probe for enhanced stability. Meanwhile, Liposome B's larger particle sizes and higher PDI highlight the limitations of the ultrasonic bath method, particularly

when incorporating OBB extracts. Together, these results demonstrate how preparation techniques and extract interactions are determinants for the physical stability of liposomal systems during storage.

Zeta Potential

Zeta potential is a key indicator of the colloidal stability of the encapsulation systems. Generally, liposomes are considered stable when their zeta potential is greater than ± 30 mV, values between ± 20 mV and ± 30 mV may indicate moderate stability and, a zeta potential below ± 20 mV can show instability, as the repulsive forces are inadequate to prevent the particles from coming close enough to aggregate or flocculate (Chai & Park, 2024). Zeta potential data over the 20-day conservation period at 7 °C are shown in Supplementary Table S6.

Control A and Control B remained relatively stable, with values in a range between -24.3 ± 2.7 mV and -20.4 ± 0.9 mV for Control A and between -18.9 ± 2.3 mV and -16.5 ± 0.8 mV for Control B, suggesting consistent stability throughout the storage period, even though it should be considered that Control B values were under the limit considered stable. Liposome A, consistently maintained a more negative zeta potential, ranging from -24.2 ± 1.5 to -20.8 ± 3.4 mV, which indicated higher stability in comparison with Liposome B, that displayed greater variability, with less negative values observed (from -21.8 ± 0.5 to -15.2 ± 4.7 mV), indicating potential instability or aggregation. These findings highlight the importance of preparation methods on liposome stability, as the use of the ultrasound probe in Liposome A resulted in smaller, more stable particles with minimal zeta potential variation, whereas the ultrasound bath used for Liposome B led to larger, less stable particles with significant variability upon addition of the OBB extract. This aligns with previous research by Machado et al. (2019) where the addition of *Spirulina* extract implied an increase in the liposomes' zeta potential from -18.4 ± 1.13 to -11.63 ± 0.23 mV. Similarly, Dag and Oztop (2017) evaluated the zeta potential of both

unloaded and green tea extract-loaded liposomes (abundant in catechins) over a 14-day storage period at 4 °C. They observed that both the addition of the extracts and the progression of storage time led to an increase in zeta potential values, indicating a trend toward reduced stability. Overall, these results emphasize the critical role of method selection and formulation ingredients and optimization in achieving durable, stable liposomal systems suitable for extended storage (Chai & Park, 2024).

In Vitro Gastrointestinal Digestion of Liposomes

Given that Liposome A demonstrated more favorable characteristics in terms of encapsulation efficiency, composition, and particle stability over the 20-day storage period at 7 °C, it was subjected to *in vitro* digestion using the INFOGEST protocol (Brodkorb et al., 2019). The aim was to evaluate the behavior of the liposomal particles during the digestive process and its impact on the encapsulated individual phenolic compounds, compared to the non-encapsulated OBB extract, to assess the protective potential of Liposome A.

Physicochemical Characterization Through the Gastrointestinal Digestion Phases

Table 2 shows the particle size distribution by intensity (D10, D50, and D90), polydispersity index, and zeta potential data for Liposome A during digestion, comparing it to its Control A (liposome without encapsulated OBB). Before digestion, Control A displayed a D50 of 107 ± 4.4 nm with a narrow size distribution ($PDI = 0.29 \pm 0.01$), while Liposome A showed a slightly higher D50 of 129 ± 1.8 nm and a low PDI (0.25 ± 0.02). During the oral phase, a moderate increase in D50 was observed for Control A (102 ± 8.5 nm) and Liposome A (121 ± 3.7 nm), with corresponding PDI values of 0.37 ± 0.1 and 0.5 ± 0.04 , indicating the onset of particle destabilization. In the gastric phase, both systems exhibited further increases in size, with D50 values reaching 103 ± 4.9 nm for Control A and 141 ± 2.9 nm for Liposome A, alongside slight increases in PDI (0.41 ± 0.1 and 0.57 ± 0.01 , respectively), suggesting mild aggregation. Significant changes occurred in the intestinal phase, where D50 values increased dramatically to 912 ± 24 nm for Control A and 1198 ± 453 nm for Liposome A, with PDI values rising to 0.76 ± 0.06 and 0.73 ± 0.14 , indicating extensive aggregation. Zeta potential values remained relatively consistent throughout all phases (Table 2). This stability can be attributed to the high ionic strength of the digestive media, which compresses the electrical double layer, and the adsorption of proteins and enzymes onto the liposome surfaces, masking structural changes. Additionally, zeta potential reflects the average surface charge of aggregates, which remains consistent despite size growth. Experimental conditions, such as

Table 2 Particle size distribution by intensity (D10, D50, and D90), polydispersity index, and zeta potential data for Control A and Liposome A systems during *in vitro* digestion. Data are presented as means $\pm$ standard deviation. Statistical analysis was performed using Tukey's test as a post hoc test. Superscript letters indicate statistically significant differences among each column ($p < 0.05$)

Phase	Size distribution by intensity (nm)						Polydispersity index		Zeta potential (mV)	
	Control A			Liposome A			Control A	Liposome A	Control A	Liposome A
	D10	D50	D90	D10	D50	D90				
Before digestion	35 ± 0.9^b	107 ± 4.4^b	283 ± 18.8^b	38 ± 0.8^b	129 ± 1.8^b	480 ± 103^b	0.29 ± 0.1^b	0.25 ± 0.02^c	-21.1 ± 2.8^a	-22.5 ± 1.3^a
Oral	57 ± 2.2^b	102 ± 8.5^b	244 ± 36^b	44 ± 0.2^b	121 ± 3.7^b	255 ± 9^b	0.37 ± 0.1^b	0.5 ± 0.04^b	-26.5 ± 3.5^a	-27.9 ± 6.2^a
Gastric	49 ± 2.5^b	103 ± 4.9^b	337 ± 67^b	51 ± 1^b	141 ± 2.9^b	367 ± 74^b	0.41 ± 0.1^b	0.57 ± 0.01^{ab}	-20 ± -1.4^a	-26.5 ± 4.9^a
Intestinal	271 ± 49^a	912 ± 24^a	3289 ± 483^a	268 ± 49^a	1198 ± 453^a	1776 ± 490^a	0.76 ± 0.06^a	0.73 ± 0.14^a	-23.4 ± 0.5^a	-22.5 ± 5.6^a

controlled pH and dilution during measurement, also contribute to this apparent stability, even as particle size and PDI indicate significant structural changes. Overall, the results suggest that Liposome A is more susceptible to changes in the digestive environment but retains comparable characteristics to Control A. Similar results were observed in other studies focused on the digestion of liposomal systems. For instance, Machado et al. (2019) submitted liposomes with encapsulated *Spirulina* extracts to the Minekus et al. (2014) *in vitro* digestion standardized protocol and also observed abrupt increases in particle size, especially after the intestinal phase (from ~260 nm before digestion to ~2400 nm after the intestinal phase). The authors also reported significant changes during the process in the polydispersity indexes and zeta potential of their liposomes. Similarly, Toro-Urbe et al. (2018) digested liposomes loaded with alkaloid, catechin, and cocoa extracts with the same protocol, but differing with what we observed, the highest increases in particle size were observed at the gastric phase (from ~70 nm before digestion to ~2000 nm after the gastric phase).

Liposome A and Control A confocal microscopy images, exposed in Fig. S4, showed that particle sizes remained relatively stable during the oral and gastric phases but increased sharply in the intestinal phase, observing high irregularity and an obviously damaged liposomal membrane. At this digestion stage, bile salts and digestive enzymes interact with the phospholipids on the droplet surfaces, breaking them down into free fatty acids (FFAs) and glycerol. These digestion by-products, which are surface-active, are unable to prevent liposome coalescence, contributing to their instability, as indicated by larger particle sizes (Machado et al., 2019). The disruption of the liposomes upon reaching the intestinal phase suggests that the phenolic compounds from OBB extracts encapsulated could be released and available for absorption. In a previous study of our research group, the INFOGEST protocol was applied to digest double emulsion systems encapsulating *Opuntia dillenii* whole fruit extracts. The most significant changes in the structure and particle size of the encapsulations occurred during the intestinal phase, but in contrast to the obtained results with liposomes, important differences were also observed between the oral and the gastric phases (Parralejo-Sanz et al., 2023).

Effect of Liposome A Encapsulation on Stability and Recovery of Phenolic Compounds from OBB

Liposome A and non-encapsulated OBB extract were subjected to the INFOGEST *in vitro* digestion assay to evaluate whether encapsulation in liposomes enhances the stability of phenolic compounds compared to their non-encapsulated form along the different phases of the digestive process (oral, gastric and intestinal). Data on the digestive stability of non-encapsulated and encapsulated phenolic compounds from

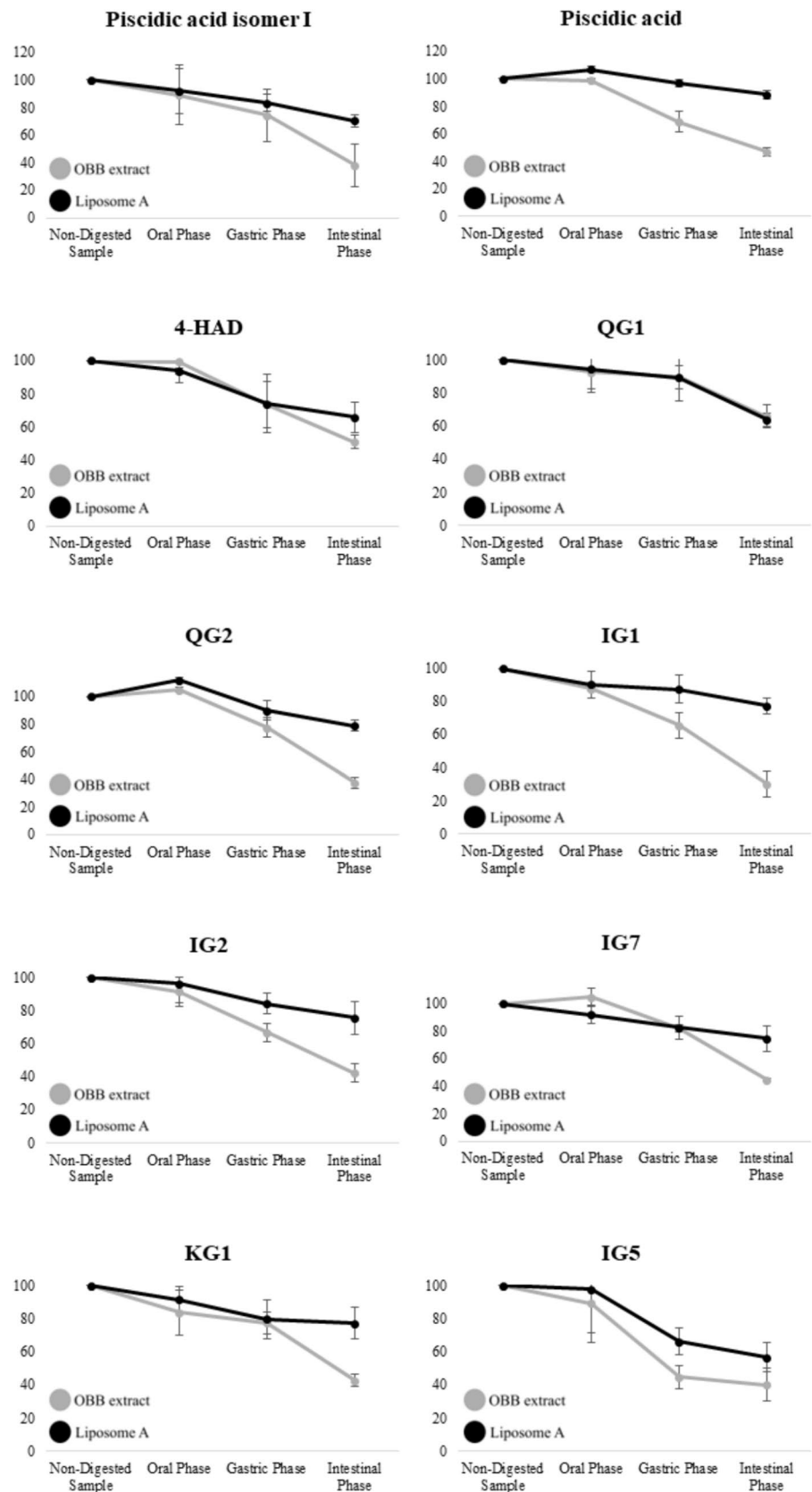
OBB extract are presented in Supplementary Table S7, with recovery percentages of each compound shown graphically in Fig. 4.

Regarding phenolic acids, both piscidic acid and its isomer exhibited improved stabilities when encapsulated in Liposome A. Piscidic acid in the encapsulated form decreased from $67,469 \pm 576$ µg/g d.w. before digestion to $59,674 \pm 2,468$ µg/g d.w. by the end of the intestinal phase, while in non-encapsulated OBB extract it dropped from $76,947 \pm 357$ µg/g d.w. (before digestion) to $36,075 \pm 2,141$ µg/g d.w. (intestinal phase). Piscidic acid isomer I followed a similar pattern, with the encapsulated form decreasing from 4106 ± 282 to 2894 ± 279 µg/g d.w., whereas the non-encapsulated extract showed a sharper reduction from 4793 ± 356 to 1794 ± 650 µg/g d.w. This enhanced stability was also observed in 4-HAD as in OBB extract its concentration decreased from 298 ± 17 µg/g d.w. before digestion to 152 ± 8 µg/g d.w. by the intestinal phase, while the encapsulated form showed a smaller decrease, from 263 ± 14 to 172 ± 16 µg/g d.w. These results suggest that encapsulation in Liposome A generally improved the digestive stability of phenolic acids.

With respect to flavonoids, the quercetin glycosides (QG1 and QG2) followed a similar trend to the phenolic acids, with encapsulation offering a clear advantage, particularly for QG2. In the non-encapsulated OBB extract, the concentration of QG2 decreased significantly from 1132 ± 21 µg/g d.w. before digestion to 426 ± 41 µg/g d.w. at the end of the process. In contrast, the encapsulated form of QG2 in Liposome A showed a much smaller reduction, from 122 ± 3 to 78 ± 6 µg/g d.w. Similarly, the isorhamnetin glycosides (IG1, IG2, IG7, and IG5) demonstrated substantial benefits from encapsulation in Liposome A compared to the non-encapsulated OBB extract.

The most notable result was for IG1, where the concentration in the OBB extract decreased from 203 ± 1 to 61 ± 15 µg/g d.w. after digestion, while the encapsulated form showed only a slight decrease, from 194 ± 3 to 150 ± 7 µg/g d.w. High stability was also observed for IG7, the most abundant isorhamnetin glycoside in OBB extract. Non-encapsulated IG7's concentration dropped from $1,058 \pm 73$ µg/g d.w. before digestion to 474 ± 19 µg/g d.w. at the end of it, while the encapsulated form in Liposome A decreased from 950 ± 75 to 704 ± 43 µg/g d.w. Similarly, kaempferol glycoside (KG1) also benefited from encapsulation, with the concentration in Liposome A decreasing from 168 ± 21 to 129 ± 19 µg/g d.w. after digestion, compared to a larger reduction in the non-encapsulated form, which fell from 202 ± 6 to 86 ± 5 µg/g d.w. Liposome A improved the integrity of the phenolic compounds throughout the simulated gastrointestinal digestive process. Non-encapsulated OBB extract showed a higher decline in the phenolic compounds concentration, especially in the intestinal phase, indicating greater

Fig. 4 Recovery (%) of the individual phenolic compounds present in *Opuntia ficus-indica* var. *Blanca Buenavista* peel green extracts (OBB) and encapsulated by Liposome A along the *in vitro* gastrointestinal digestion phases. 4-HAD: 4-hydroxybenzoic acid derivative, QG1: quercetin glycoside I, QG2: quercetin glycoside II, IG1: isorhamnetin glucosyl-rhamnosyl-rhamnoside, IG2: isorhamnetin glucosyl-rhamnosyl-pentoside, IG7: isorhamnetin hexoxyl-rhamnoside, KG1: kaempferol glucosyl-rhamnoside, IG5: isorhamnetin glucosyl-rhamnoside



susceptibility to breakdown or degradation in the digestive environment. The encapsulation of phenolic compounds from OBB extract in Liposome A likely diminished their degradation, resulting in higher stabilities and recoveries. Guo et al. (2021) used an *in vitro* gastrointestinal digestion model to assess the release behavior of a delivery system for encapsulated curcumin. This system was fabricated using methoxyl pectin, surfactants (rhamnolipid, tea saponin, and ethyl lauroyl arginate hydrochloride), and pea protein isolate. The ternary matrix composed of rhamnolipid showed a low release rate of 17.45% after 60 min in the gastric phase. It also exhibited gradual degradation in the intestinal phase, demonstrating the stability of the matrix under pepsin hydrolysis and controlled release behavior under trypsin activity. These findings are consistent with the results observed for Liposome A encapsulation, where enhanced stability and controlled release were noted for phenolic compounds.

Effect of Liposome A Encapsulation on the Bioaccessibility of Phenolic Compounds

Bioaccessibility refers to the fraction of a consumed compound that is released from the food matrix and made available for absorption in the intestine (Thakur et al., 2020). Figure 5 shows the bioaccessibility percentages of the target phenolic compounds from OBB encapsulated extracts by Liposome A, compared to the

non-encapsulated extract. Piscidic acid isomer I and piscidic acid implied large increases, from 38 ± 15.6 to $70.5 \pm 4.3\%$ and from 46.9 ± 3 to $88.4 \pm 2.9\%$, respectively, indicating that encapsulation by liposomes provided a substantial protection during digestion. Similarly, for 4-HAD, bioaccessibility raised from $50.9 \pm 4.2\%$ in the non-encapsulated extract to $65.7 \pm 9.2\%$ in Liposome A. Isorhamnetin glycosides (IG1, IG2, IG7, and IG5) also show significant improvements, with IG1 increasing from 30.1 ± 7.5 to $77.5 \pm 4.8\%$, and IG7 rising from 44.9 ± 1.3 to $74.6 \pm 9\%$. Quercetin glycosides (QG1 and QG2), while already relatively bioaccessible in the non-encapsulated form, slightly improved bioaccessibility with encapsulation, particularly QG2, which increased from $37.7 \pm 3.8\%$ to $78.8 \pm 8.7\%$. Overall, the results suggest that Liposome A significantly enhanced the bioaccessibility of OBB phenolic compounds, particularly for those with initially low bioaccessibility, such as IG1 and QG2, potentially improving their absorption and bioavailability.

Gómez-Maqueo et al. (2020) studied the bioaccessibility of phenolic compounds in *Opuntia ficus-indica* var. *Blanca Buenavista* peel extracts (OBB), finding isorhamnetin glycosides bioaccessibility between 25.9% and 60.9% and of 79.9% for piscidic acid. In some cases, these values were higher than those of non-encapsulated OBB extract in this study. However, the phenolic compounds encapsulated in Liposome A showed significantly higher bioaccessibility, highlighting the effectiveness of encapsulation in enhancing

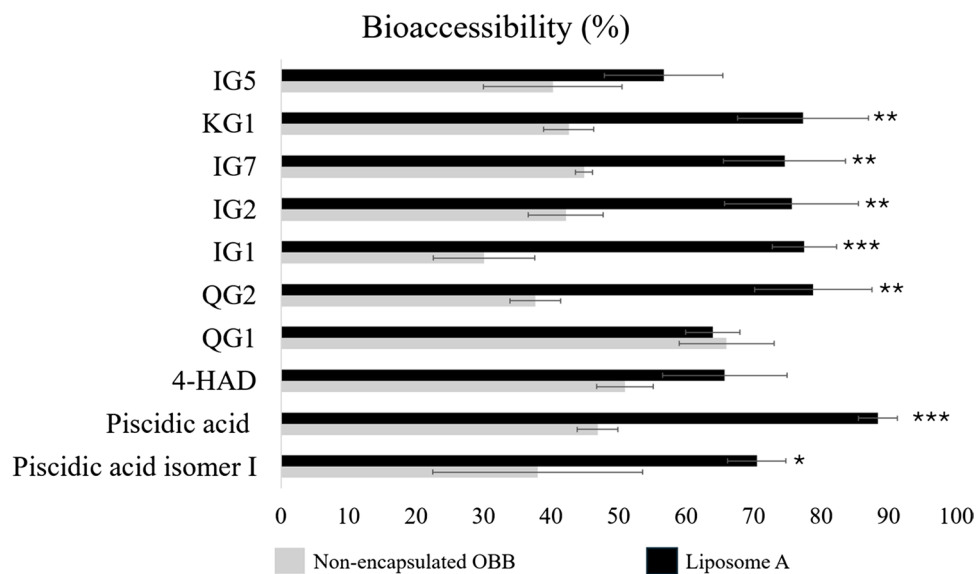


Fig. 5 Bioaccessibility (%) of the main phenolic compounds of *Opuntia ficus-indica* var. *Blanca Buenavista* peel extracts (OBB) non-encapsulated and encapsulated in Liposome A after *in vitro* gastrointestinal digestion. Statistical analysis was performed using t-Student test where * indicates statistical significance of $p < 0.05$, ** of $p < 0.01$, and *** of $p < 0.001$. 4-HAD: 4-hydroxybenzoic acid deriva-

tive, QG1: quercetin glycoside I, QG2: quercetin glycoside II, IG1: isorhamnetin glucosyl-rhamnosyl-rhamnoside, IG2: isorhamnetin glucosyl-rhamnosyl-pentoside, IG7: isorhamnetin hexosyl-rhamnoside, KG1: kaempferol glucosyl-rhamnoside, IG5: isorhamnetin glucosyl-rhamnoside

protection and bioaccessibility. Similarly, Parralejo-Sanz et al. (2024) demonstrated improved bioaccessibility of phenolics from *Opuntia ficus-indica* var. *Colorada* pulp extracts encapsulated in double emulsions, particularly for piscidic acid, 4-HAD, and IG2. Chen et al. (2021) also observed that liposomes encapsulating phenolic compounds from bamboo leaves exhibited a slower release during digestion, particularly at lower extract concentrations. These findings underscore that encapsulation significantly improves bioaccessibility and bioavailability, making it a promising approach for developing nutraceuticals and functional foods in the pharmaceutical and food industries.

Conclusions

Phenolic-rich extracts from the fruit peel waste of *Opuntia ficus-indica* var. *Blanca Buenavista* (OBB), containing piscidic acid, quercetin, isorhamnetin, and kaempferol glycosides, were encapsulated by two liposomal formulations: Liposome A (elaborated with an ultrasound probe) and Liposome B (elaborated with ultrasonic bath). Physicochemical characterization demonstrated that Liposome A exhibited more favorable properties, including higher encapsulation efficiencies for individual phenolic compounds (82.1–98.4%), smaller and more uniform particle size distributions (D10: 38–67 nm; D50: 87–128 nm; D90: 306–426 nm), and a more stable zeta potential (− 24.2 to − 20.8 mV) over a 20-day storage period at 7 °C, in comparison to Liposome B. Consequently, Liposome A was selected for further *in vitro* digestion studies to evaluate the stability, recovery, and bioaccessibility of the encapsulated phenolic compounds. Results indicated that the liposomal structures of both Liposome A and its Control A (without OBB extract encapsulated), underwent a breakage when reaching the intestinal phase, although they remained relatively stable during the oral and gastric phases. On the other hand, Liposome A significantly preserved the integrity of OBB phenolics during digestion, with enhanced bioaccessibility values (56.7–88.4%) compared to the non-encapsulated extract (30.1–66%). These findings highlight the potential of Liposome A as an effective delivery system for OBB as a source of antioxidants, suggesting its suitability for applications in nutraceuticals and functional foods aimed at improving the bioavailability of bioactive molecules.

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Investigation, Methodology, Visualization, Writing – review & editing. M. P.C.: Investigation, Supervision, Project administration, Funding acquisition, Writing – review & editing. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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