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CHARACTERIZATION AND STORAGE STABILITY OF LIPOSOMES AS DELIVERY SYSTEMS FOR CAROTENOIDS

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Abstract

Carotenoids are valuable bioactive compounds with strong antioxidant properties; however, their application in food systems is limited by low stability and reduced bioavailability. Liposomal encapsulation represents an efficient strategy to protect carotenoids and improve their functional performance.

In this study, carotenoid-loaded liposomes were characterized in terms of particle size, antioxidant activity during *in vitro* digestion, and storage stability. The obtained liposomes showed particle sizes ranging between 144.87 ± 3.17 and 205.28 ± 2.48 nm, confirming their nanoscale structure and suitability for food applications. *In vitro* digestion revealed a higher antioxidant activity for encapsulated carotenoids compared to the free extract during both gastric and intestinal phases, demonstrating the protective effect of the liposomal system and improved bioaccessibility.

Storage stability assessment indicated a decrease in the content of encapsulated bioactive compounds after four weeks, from 80.16 ± 0.87 $\mu\text{g/g}$ to 61.48 ± 1.12 $\mu\text{g/g}$, while maintaining significant antioxidant potential. The obtained results confirm that liposomes act as effective delivery systems for carotenoids, enhancing their stability, digestive behavior, and functional properties, and highlighting their potential application in the development of fortified and functional food products.

Keywords: liposomes; carotenoids; food fortification; bioaccessibility.

Carotenoids are a large class of naturally occurring pigments responsible for the yellow, orange, and red coloration of many fruits and vegetables [1–4]. Beyond their role as natural colorants, carotenoids exhibit important biological functions, including antioxidant activity, provitamin A activity, and protective effects against oxidative stress-related diseases such as cardiovascular disorders, certain cancers, and age-related eye diseases. Due to these health-promoting properties, carotenoids are increasingly used as functional ingredients in food product development [5,6].

Natural sources of carotenoids include fruits and vegetables such as sea buckthorn, carrots, tomatoes, pumpkin, sweet peppers, and leafy greens. Among these, sea buckthorn is particularly rich in carotenoids, including β -carotene, lycopene, and zeaxanthin, making it a valuable raw material for the extraction of bioactive compounds intended for food fortification. However, the incorporation of carotenoids into food matrices remains challenging because of their lipophilic nature, low water solubility, sensitivity to light, oxygen, and temperature, as well as their limited bioavailability during digestion [1,7–9].

To overcome these limitations, encapsulation technologies have been widely investigated as effective strategies to protect carotenoids and improve their stability and bioaccessibility. Among these technologies, liposomal encapsulation has attracted significant attention due to its biocompatibility, ability to encapsulate lipophilic compounds, and suitability for food-grade applications. Liposomes are spherical vesicles composed of phospholipid bilayers capable of entrapping bioactive compounds and protecting them from environmental and processing-induced degradation [10–12].

In the food industry, liposomes have been explored as delivery systems for vitamins, antioxidants, polyphenols, and carotenoids, contributing to improved oxidative stability, controlled release, and enhanced nutritional value of fortified products [12–14]. Their application extends to a wide range of food categories, including bakery products, dairy systems, beverages, and plant-based alternatives. Liposomal systems also support clean-label strategies by reducing the need for synthetic additives while maintaining product quality and shelf life [15–17].

In this context, the characterization and storage stability of carotenoid-loaded liposomes are essential for assessing their potential as delivery systems in functional food development. Evaluating their physicochemical properties, behavior during digestion, and stability over storage provides valuable insights into their effectiveness and applicability in real food systems.

The aim of this study was to investigate the physicochemical properties, storage stability, and digestive performance of carotenoid-loaded liposomes, highlighting their potential application in the development of fortified and functional food products.

Sea buckthorn powder was obtained by convective drying (45 ± 1 °C) to a moisture content of 7%, followed by grinding (70 μ m). The powder was mixed with refined sunflower oil (1:10, w/w) and subjected to ultrasonic-assisted extraction (37 kHz, 45 ± 1 °C, 180 min). The lipophilic extract was separated by centrifugation and filtration and stored at 4 ± 1 °C in the dark until further use.

Liposomal formulations were prepared using an adapted heating (Mozafari) method [18,19]. The dispersions were sonicated for 4 min at 37 °C to reduce particle size. The obtained liposomes were stored at 4 ± 1 °C until analysis.

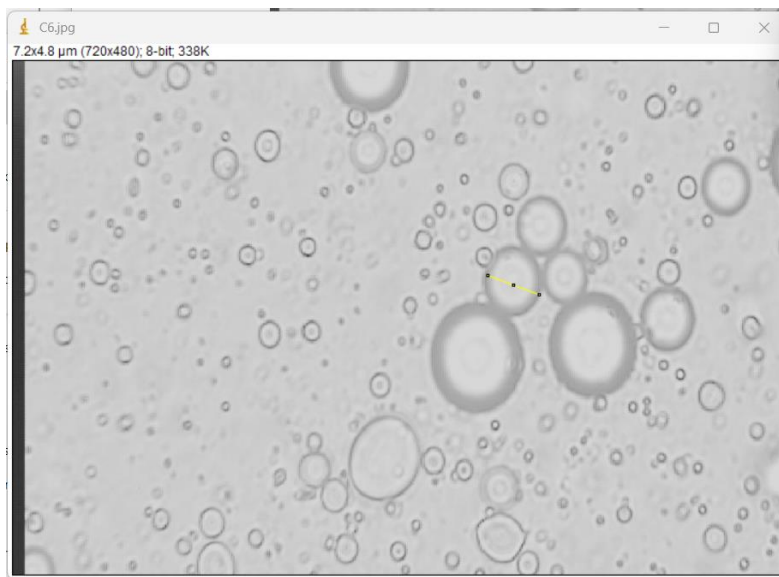


Figure 1. Microscopic image of carotenoid-loaded liposomes

The particle size of the carotenoid-loaded liposomes ranged between 144.87 and 205.28 nm, indicating the successful formation of nanoscale vesicles (Figure 1). This variation in particle size can be attributed to differences in hydration conditions, lipid–bioactive interactions, and sonication efficiency during liposome preparation. Smaller liposomes (≈ 145 – 160 nm) suggest a more uniform bilayer organization, while slightly larger particles (≈ 190 – 205 nm) may result from partial aggregation or increased loading of lipophilic carotenoids within the phospholipid bilayer.

The obtained size range is considered optimal for food applications, as liposomes below 200 nm exhibit improved physical stability, reduced sedimentation, and better dispersion in complex food matrices. Moreover, nanoscale liposomes are less likely to affect the texture, mouthfeel, or visual appearance of fortified foods, which is a critical factor for consumer acceptance.

From a nutritional perspective, particle size plays a key role in the digestive behavior and bioavailability of encapsulated compounds. Smaller liposomes provide a larger surface area, facilitating enzymatic interactions during gastrointestinal digestion and promoting a more efficient release of carotenoids in the intestinal phase, where absorption occurs. This behavior contributes to enhanced bioaccessibility compared to larger delivery systems or non-encapsulated extracts. In addition, liposomes within this size range demonstrate improved resistance to physical destabilization mechanisms such as coalescence and phase separation during processing and storage. Therefore, controlling liposome size is essential not only for technological stability but also for ensuring consistent delivery of carotenoids and maximizing their functional benefits in food products [14,20,21].

Encapsulation efficiency (EE) was determined by extracting free carotenoids with ethanol, followed by centrifugation and spectrophotometric quantification (UV–Vis). The retention rate (RR) during storage was evaluated using the same procedure after storage at 4 ± 1 °C. The carotenoid-loaded liposomes exhibited a high encapsulation efficiency (EE) of $90.8 \pm 1.6\%$, indicating effective incorporation of carotenoids into the phospholipid bilayer. The high EE value confirms the suitability of the applied liposomal formulation method for encapsulating lipophilic bioactive compounds [22,23].

The retention rate (RR) reached $89.4 \pm 2.1\%$, demonstrating good stability of the liposomal system and efficient retention of carotenoids during storage. Such values indicate limited leakage and strong interaction between carotenoids and lipid membranes [19].

The initial content of encapsulated bioactive compounds was 80.16 ± 3.12 μg , which decreased to 61.48 ± 2.85 μg after four weeks of storage (Figure 2). The observed reduction is attributed to gradual oxidative degradation and partial release of carotenoids during storage.

Nevertheless, the relatively low decrease confirms that liposomal encapsulation significantly slows down degradation processes compared to non-encapsulated carotenoids.

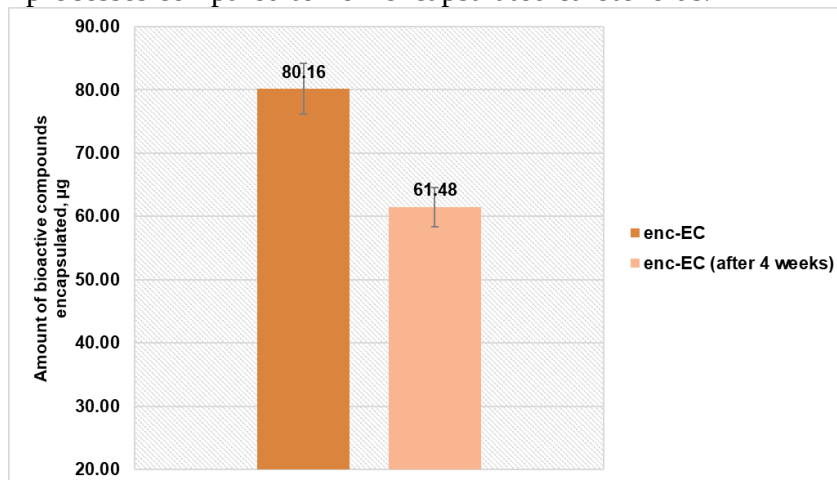


Figure 2. Changes in bioactive compound content of carotenoid-loaded liposomes during 4 weeks of storage

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The retention rate (RR) reached $89.4 \pm 2.1\%$, demonstrating good stability of the liposomal system and efficient retention of carotenoids during storage. Such values indicate limited leakage and strong interaction between carotenoids and lipid membranes.

The initial content of encapsulated bioactive compounds was 82.56 ± 3.12 µg, which decreased to 64.56 ± 2.85 µg after four weeks of storage. The observed reduction is attributed to gradual oxidative degradation and partial release of carotenoids during storage. Nevertheless, the relatively low decrease confirms that liposomal encapsulation significantly slows down degradation processes compared to non-encapsulated carotenoids.

The combination of high encapsulation efficiency, retention rate, and moderate losses during storage highlights the protective effect of liposomes and supports their application as stable delivery systems for carotenoids in food products [21].

In vitro digestion was performed under simulated gastric (pH 1.8, pepsin) and intestinal (pH 8.2, trypsin) conditions at 37 °C for 120 min. Antioxidant activity of the samples was determined at selected time intervals using the DPPH assay, with absorbance measured at 515 nm.

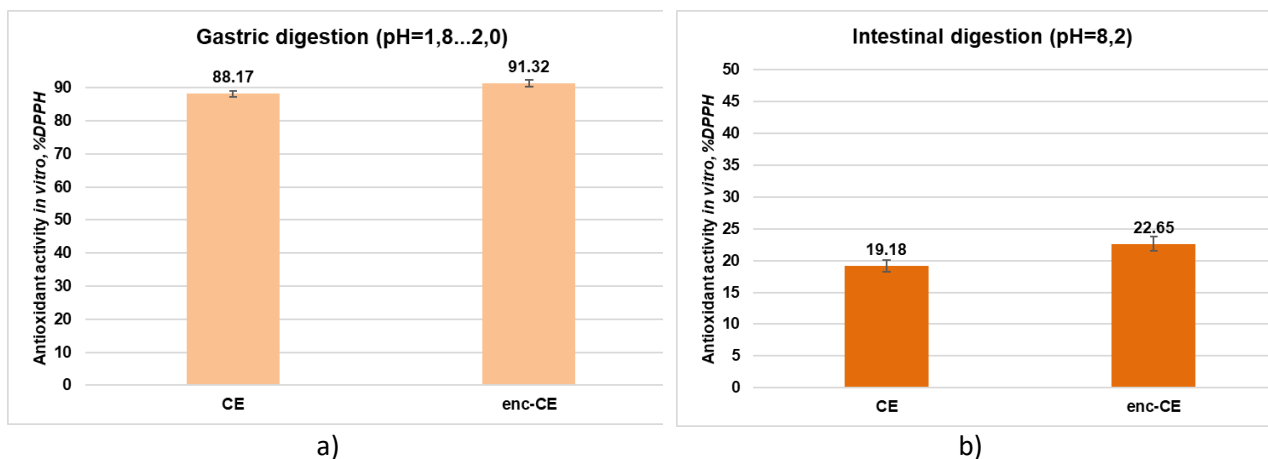


Figure 3. *In vitro* antioxidant activity of free (CE) and encapsulated carotenoids (enc-CE) during simulated digestion: (a) gastric phase (pH 1.8–2.0); (b) intestinal phase (pH 8.2)

The antioxidant activity of free carotenoid extract (CE) and encapsulated carotenoids (enc-CE) was evaluated under simulated gastric and intestinal digestion conditions using the DPPH assay. During the gastric phase (pH 1.8–2.0), both samples exhibited high antioxidant activity; however, the encapsulated extract showed a higher value ($91.32 \pm 1.85\%$) compared to the free extract ($88.17 \pm 2.10\%$). This difference highlights the protective effect of the liposomal membrane against acidic conditions and oxidative stress, preserving the antioxidant capacity of carotenoids (Figure 3a). In the intestinal phase (pH 8.2), a general decrease in antioxidant activity was observed for both samples, which is commonly associated with the lower stability of carotenoids in alkaline environments. Nevertheless, the encapsulated carotenoids maintained significantly higher antioxidant activity ($22.65 \pm 1.42\%$) than the free extract ($19.18 \pm 1.35\%$). This behavior suggests a more controlled release of carotenoids from liposomes and improved bioaccessibility during the intestinal phase, where absorption occurs (Figure 3b).

The enhanced antioxidant activity of encapsulated carotenoids during both digestion stages can be attributed to the structural integrity of liposomes, which protects carotenoids from degradation and facilitates their gradual release. These results confirm that liposomal encapsulation improves the digestive stability and functional performance of carotenoids compared to non-encapsulated extracts, reinforcing the potential of liposomes as efficient delivery systems for food applications.

Conclusions

This study confirmed that liposomal encapsulation is an effective approach for improving the stability and functionality of carotenoids in food applications. The carotenoid-loaded liposomes presented nanoscale particle sizes ranging from 144.87 to 205.28 nm, which are suitable for food systems and ensure good dispersion without negatively affecting product texture or appearance.

High encapsulation efficiency ($90.8 \pm 1.6\%$) and retention rate ($89.4 \pm 2.1\%$) demonstrated the strong ability of liposomes to incorporate and retain carotenoids. During four weeks of storage, the content of encapsulated bioactive compounds decreased from $82.56 \pm 3.12 \mu\text{g}$ to $64.56 \pm 2.85 \mu\text{g}$, indicating moderate losses and effective protection against degradation.

In vitro digestion studies showed that encapsulated carotenoids exhibited higher antioxidant activity compared to free extracts during both gastric ($91.32 \pm 1.85\%$) and intestinal ($22.65 \pm 1.42\%$) phases, confirming improved digestive stability and bioaccessibility.

Overall, these findings highlight the potential of carotenoid-loaded liposomes as efficient delivery systems for the development of functional and fortified food products with enhanced stability, bioavailability, and health-promoting properties.

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