BUCHI Scholar Application Note

Encapsulation of β-carotene in alginate-based hydrogel beads: Impact on physicochemical stability and bioaccessibility

David Julian McClements and Zipei Zhang, University of Massachusetts Amherst
September 2018

Encapsulator B-390
Design of nutraceutical delivery systems for utilization in functional food products.
1. Introduction

- Carotenoids are natural pigments present in certain fruits and vegetables where they play key roles in photosynthesis and photoprotection reactions [1]. β-carotene, one of the major types of carotenoid, is a natural precursor of vitamin A [2]. β-carotene also demonstrates antioxidant and non-antioxidant biological activities, which may account for its ability to reduce the risk of certain chronic diseases including cardiovascular disease, eye disease, and certain cancers [3]. However, the extended hydrocarbon backbone and high degree of unsaturation of β-carotene restrict its incorporation into many foods and beverages because these factors lead to low water-solubility, poor chemical instability and low bioaccessibility [4]. Moreover, β-carotene can be chemically transformed within the gastrointestinal tract (GIT), which may alter its potential health benefits. Consequently, there is a need to develop effective carotenoid delivery systems that can overcome these challenges [5-6]. Hydrogel beads suitable for utilization in foods are usually constructed from food-grade biopolymers such as proteins and/or polysaccharides using a variety of approaches [7-8]. Smaller hydrogel beads (<1 mm) can be fabricated using an extrusion device with a vibrating nozzle (Encapsulator), which may be an advantage for certain applications. The dimensions of hydrogel beads can be further adjusted by altering several factors including biopolymer and cross-linking ion concentration, instrument parameters, and hardening time [9-10].

- In the present study, microfluidization was used to prepare oil-in-water nanoemulsions containing β-carotene. The β-carotene-loaded lipid droplets were then used as delivery systems themselves, or they were incorporated into hydrogel beads by injecting them and an alginate mixture into a gelling solution (Ca²⁺). Calcium alginate beads formed using this approach are widely used in the pharmaceutical industry to encapsulate and protect bioactive agents. Hydrogel beads with different structural, physical, and functional attributes were prepared by using two different alginate concentrations (0.5% and 1%): typically bead hardness increases with increasing alginate, but bead pore size decreases. The chemical stability and simulated GIT fate of β-carotene encapsulated within either nanoemulsions or filled hydrogel beads were compared. The information obtained from this study may facilitate the production of delivery systems that improve the stability and bioavailability of lipophilic bioactives.

2. Equipment

- High-speed blender (M133/1281-0, Bio-spec Products, Inc., ESGC, Switzerland)
- Microfluidizer (Microfluidizer, M110Y, Microfluidics, Newton, MA)
- Encapsulator (B-390, BUCHI, Switzerland)
- Spectrophotometer (Ultraspec 3000 pro, Biochrom Ltd., Cambridge, UK)
- Automatic titration unit (Metrohm, USA Inc.)
- Static light scattering device (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK)
- Confocal scanning laser microscopy (Nikon D- Eclipse C1 80i, Nikon, Melville, NY, U.S.)

3. Chemicals, Materials & Experimental

Whey protein isolate (WPI) was provided by Davisco Foods International Inc. (Le Sueur MN). Corn oil was purchased from a local supermarket. The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): β-carotene; alginic acid (sodium salt); mucin from porcine stomach; pepsin from porcine gastric mucosa; lipase from porcine pancreas; porcine bile extract; and Nile Red. Double distilled water was used to make all solutions.

The β-carotene-loaded lipid droplets were prepared by dispersing β-carotene (0.1%, w/w) in corn oil. A coarse emulsion was prepared by homogenizing 10% (w/w) corn oil with 90% (w/w) aqueous emulsifier solution using a high-speed blender for 2 min with the speed of 10,000 rpm. The droplet
size in this coarse emulsion was further reduced by passing it through a microfluidizer with a 75 mm interaction chamber (F20Y) at an operational pressure of 12,000 psi for 3 passes. The resulting β-carotene-loaded emulsions were stored in a refrigerator at 4 °C before use.

Aqueous solutions containing different amounts of alginate were prepared by dissolving powdered alginic acid (1% or 2%, w/w) in distilled water. Alginate solutions and β-carotene-loaded emulsions were then mixed together (1:1, mass ratio) to form a mixture that contained 5% oil (w/w) and either 0.5% or 1% alginate (w/w).

The β-carotene-loaded hydrogel beads were prepared using Encapsulator B-390 with a 120 mm vibrating nozzle to inject the β-carotene/alginate solution into 10% calcium chloride solution. The encapsulation device was operated under fixed conditions: frequency 800 Hz; electrode 800 V; and pressure 450 mbar. The hydrogel beads were held in the Ca²⁺ solution for 1 h at ambient temperature to promote cross-linking. The formed beads were then stored in a refrigerator so that any residual external water was removed, and then their total weight was determined.

The physicochemical stability of β-carotene encapsulated in the nanoemulsions and in the filled hydrogel particles was tested: 4 mL of nanoemulsions (5% oil) or 4 mL filled hydrogel beads (5% oil) were transferred into glass tubes and stored in the dark at 55 °C for 12 days (pH 7). The β-carotene was isolated from the samples using a solvent extraction method. For the filled hydrogel beads, saturated EDTA solution was used to dissolve the beads and release the encapsulated β-carotene. The transparent lower organic phase containing the β-carotene was collected and transferred to a cuvette, and then its absorbance was measured at 450 nm using a UV-visible spectrometer.

The potential gastrointestinal fate of the delivery systems was monitored using a simulated gastrointestinal tract model. β-carotene delivery systems (free emulsion and filled hydrogel beads) with the same total lipid concentration (2%) and total volume (7.5 mL) were prepared by diluting the original systems with buffer solution (5 mM phosphate buffer, pH 7). The samples were then passed through a static GIT model that simulated the mouth, stomach, and small intestine phases. The particle size, microstructure of hydrogel beads and bioaccessibility of β-carotene was determined after the simulated digestion process.

4. Results and Discussion

The decrease in β-carotene concentration during storage at 55 °C compared to the initial value was measured for the different delivery systems. The relative β-carotene content fell from an initial value of 100% immediately after preparation to around 0.2%, 38% and 55% for the nanoemulsions, 1% beads and 0.5% beads respectively, after storage for 12 days (Figure 1 and 2).

![Figure 1: Degradation rates of β-carotene loaded in different delivery systems (nanoemulsions and hydrogel beads) during storage at 55 °C.](image-url)
These results indicated that the β-carotene in the nanoemulsions was highly unstable to degradation, and that encapsulation of the lipid droplets in the hydrogel beads could significantly improve its stability. Encapsulation of the lipid droplets may have improved β-carotene stability by providing a physical barrier that limited the diffusion of pro-oxidants or free radicals into the hydrogel bead core where they could interact with the carotenoids.

The effect of the different delivery systems on the rate and extent of lipid digestion was evaluated using an automatic titration (pH stat) method. The nanoemulsions were rapidly digested during the first 5 min with over 76% of the fraction of free fatty acids (FFA) being released, followed by a more gradual digestion until a relatively constant final value (96%) was attained. Conversely, the FFA release rate was much slower for the filled hydrogel beads and depended on alginate concentration. After 5 min of digestion, around 22% and 37% of the FFAs were released for the 1% and 0.5% alginate beads, respectively, and around 56% and 83% of the FFAs were released after 120 min of digestion (Figure 3).

These results indicate that encapsulation of the lipid droplets within the hydrogel beads reduced the rate and extent of lipid digestion within the simulated small intestine phase.
The bioaccessibility ($B^*$) of the encapsulated $\beta$-carotene was appreciably higher for the nanoemulsions than for the hydrogel beads (Figure 4). The higher $B^*$ for the nanoemulsions can be attributed to the fact that a greater fraction of the lipid phase was digested, thereby leading to the formation of more mixed micelles capable of solubilizing the $\beta$-carotene molecules. Conversely, the extent of $\beta$-carotene degradation was slightly higher when it was encapsulated in nanoemulsions than when it was encapsulated in filled hydrogel beads (Figure 4).

The general shape of the particle size distribution and the overall microstructure of both bead samples remained fairly similar throughout the entire simulated gastrointestinal tract (Figure 5 and 6), which suggests that they maintained their overall integrity when exposed to upper GIT conditions.

Figure 4: Bioaccessibility (Left) and effective bioavailability (BA) and Chemical Stability (Right) of $\beta$-carotene in different delivery systems measured after exposure to simulated small intestine conditions: (a) nanoemulsions; (b) nanoemulsions in 0.5% alginate beads; and, (c) nanoemulsions in 1% alginate beads.

Figure 5: Particle size distributions of $\beta$-carotene delivery systems after exposure to different regions of the GIT model: nanoemulsions in 0.5% alginate beads (Left); and, (b) nanoemulsions in 1% alginate beads (Right).
5. Conclusion

Filled hydrogel beads were fabricated by injecting a mixture of β-carotene-loaded lipid droplets and alginate molecules into a calcium solution. Storage studies indicated that the hydrogel beads partially protected the β-carotene from chemical degradation, with the extent of the effect depending on the alginate level within the beads. Simulated gastrointestinal studies indicated that free lipid droplets were digested more rapidly and completely than those encapsulated within hydrogel beads.

The rate and extent of lipid digestion was lower when the hydrogel beads contained a higher alginate concentration, which was attributed to an increase in bead diameter and decrease in bead pore size. The bioaccessibility of β-carotene was higher when it was encapsulated within free lipid droplets than within hydrogel beads. This effect was attributed to the fact that some of the β-carotene remained trapped within undigested lipid droplets inside the hydrogel beads, and that fewer mixed micelles were generated to solubilize any β-carotene molecules released from the beads.

Thus, this kind of hydrogel bead may be useful for inhibiting the chemical degradation of β-carotene during food storage or within the gastrointestinal tract, but one must be aware that the bioaccessibility of β-carotene may be compromised.

6. References


