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Characterization and *in vitro* bioavailability of β-carotene: Effects of microencapsulation method and food matrix

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ABSTRACT

The objectives of this study were to determine the effect of microencapsulation method on physical properties and *in vitro* release and bioavailability of three types of β-carotene: a spray-dried powder of β-carotene and maltodextrin, commercially available water-dispersible β-carotene powder, and chitosan-coated β-carotene alginate. *In vitro* digestion trials were conducted with and without food matrices (yogurt, pudding) to elucidate the effect of food matrix on *in vitro* release and bioavailability. Microencapsulation method significantly affected ($p < 0.05$) water activity, moisture content, and particle size. The maltodextrin powder had the lowest moisture content (3.5%) and the smallest volume mean diameter (10.5 μm), whereas the chitosan-alginate beads had the lowest water activity (0.195). The maltodextrin powder had the highest surface β-carotene content (39.5%), while the commercial water-dispersible powder had the highest β-carotene content (10%). The microencapsulation method significantly influenced ($p < 0.05$) release and incorporation into micelles, regardless of food matrix. Water-dispersible β-carotene achieved the highest release (93.3%) and the highest incorporation into micelles (36.4%) in the absence of a food matrix, and the highest release (34.8%) and the highest micelle content (17.0%) with pudding. Food matrix significantly decreased release and micelle incorporation ($p < 0.05$), with yogurt decreasing release and micelle incorporation more than pudding.

1. Introduction

Vitamin A is an important nutrient not only for vision and preventing night blindness, but also for proper immune function, growth, development, and gastrointestinal function (Grune et al., 2010; Haskell, 2012; West & Darnton-Hill, 2001). Humans lack the ability to synthesize vitamin A *de novo* and must get proper amounts of it from diets in the form of dark leafy green vegetables (spinach) and orange and yellow vegetables and fruits (carrots, mangoes) (Haskell, 2012; West & Darnton-Hill, 2001). β-carotene has the highest vitamin A activity among other provitamin A carotenoids (α-carotenes, cryptoxanthins) and also has the most efficient conversion to vitamin A (Grune et al., 2010; Yeum & Russell, 2002). Aside from the primary role of β-carotene as a nutrient is as a source of vitamin A, β-carotene also has antioxidant capabilities and can function as a lipid radical scavenger and a singlet oxygen quencher due to the unique structure of conjugated double bonds and ionone rings (Grune et al., 2010).

The maximum absorption of β-carotene from plant sources is relatively low (~65%) because bioavailability of β-carotene depends primarily on the food matrix in which the β-carotene is located (Grune et al., 2010; Haskell, 2012; Yeum & Russell, 2002). The low bioavailability of β-carotene in plants has created an opportunity for the development of β-carotene forms for supplementation and food fortification. β-carotene supplementation can take many forms, including spray-dried powders, water-dispersible beadlets, polymer-coated microcapsules, and stabilized emulsions (Desobry, Netto, & Labuza, 1998; Haskell, 2012; Qian, Decker, Xiao, & McClements, 2012; Roman, Burri, & Singh, 2012; Thürmann et al., 2002).

Spray drying and gelating β-carotene are two attractive methods for preserving β-carotene, but the use of these methods must be justified by verifying bioavailability in addition to preserving functionality (Desobry et al., 1998). *In vivo*, β-carotene must be incorporated into mixed micelles through interactions with surfactant bile salts during digestion for proper absorption in the small intestine and maximum bioavailability (Yeum & Russell, 2002). *In vitro* digestion models have thus been developed to predict the bioavailability of β-carotene by measuring the β-carotene content of the micelle phase (Garrett, Failla, & Sarama, 1999; Rodriguez-Amaya, 2010). In addition to being cheaper, easier, and more

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reproducible than human trials, *in vitro* models have been validated due to strong correlation to *in vivo* results (Granado-Lorencio et al., 2007; Rodriguez-Amaya, 2010). Preserving the functionality of β-carotene is dependent on the physical properties after microencapsulation; decreasing surface β-carotene content, for example, increases retention by decreasing the amount of β-carotene directly exposed to oxygen and most at risk for oxidation (Desobry et al., 1998).

In addition to spray-dried powders and microcapsules, the development of water-dispersible β-carotene holds promise because it has a significantly higher bioavailability *in vivo* than that of carrot juice (Thürmann et al., 2002). Water-dispersible β-carotene raises serum levels the most when compared to other supplement types (Fuller, Butterfoss, & Failla, 2001). *In vitro* trials using Caco-2 cells to simulate intestinal absorption have been conducted with water-dispersible β-carotene with similar results, confirming it is highly bioavailable (Ferruzzi, Lumpkin, Schwartz, & Failla, 2006).

Spray drying pure β-carotene for preservation and shelf-life stability was achieved by Desobry, Netto, and Labuza (1997) and Loksuwan (2007), but β-carotene bioavailability and release during *in vitro* digestion was not studied. Chitosan-alginate microcapsules of β-carotene developed for enteric release have been thoroughly studied in *in vitro* digestion trials, but the bioavailability of β-carotene from these microcapsules has only recently been investigated (Roman et al., 2012). The objectives of this study were to physically characterize spray-dried β-carotene, water-dispersible β-carotene, and β-carotene chitosan-alginate microcapsules and to study release and bioavailability (amount incorporated into the micelle phase) during *in vitro* digestion as affected by food matrices including yogurt and pudding.

2. Materials and methods

2.1. Materials

Sodium alginate was obtained from Acros Organics (Fair Lane, NJ), and chitosan was obtained from Tokyo Chemical Industry (Tokyo, Japan). Water-dispersible β-carotene beadlets (10%) were obtained from MP Biomedicals (Solon, OH). Porcine mucin, porcine alpha-amylase, porcine pepsin, porcine pancreatin (CAS: 8049-46-6), and porcine bile salts (CAS: 8008-63-7, Lot: 031M0106V) were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO). Dannon Fat-free Plain Yogurt and Jell-O Fat-free Tapioca Pudding were purchased from a supermarket and used as a protein-rich food matrix and a carbohydrate-rich food matrix, respectively. All other chemicals were chemical grade. Deionized water was used throughout the study.

For *in vitro* digestion studies, simulated saliva was prepared according to the method of Kong and Singh (2010), whereas simulated gastric and intestinal juices were prepared according to Hur, Decker, and McClements (2009). Stock solutions for each digestive juice (Table 1) were prepared in bulk and stored at room temperature. To prepare the simulated juices, enzymes were added to the relevant volume of stock solution on the day of *in vitro* digestion trials and adjusted to the appropriate pH using 1 M NaOH or 1 M HCl.

2.2. Spray drying β-carotene with maltodextrin

Spray-dried β-carotene with maltodextrin was produced following the methods of Desobry et al. (1997), Liang, Huang, Ma, Shoemaker, and Zhong (2013), and Rodrigues, Mariutti, Faria, and Mercadante (2012). Given the absence of an emulsifier in these encapsulation protocols, a high ratio of wall material to core material was used in addition to the utilization of homogenization and continuous stirring prior to spray drying to ensure adequate encapsulation (Desobry et al., 1997; Liang et al., 2013; Rodrigues et al., 2012). Briefly, 60 mL of a 0.4 g/mL maltodextrin 15 DE aqueous solution was prepared to which 30 μg β-carotene was added. The mixture was homogenized using a PT-1200 Polytron hand-held homogenizer (Brinkmann Instruments, Westbury, NY) for 5 min at 25,000 rpm prior to spray drying and was continuously stirred during spray drying using a stir-bar. The feed solution was spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Switzerland) with an air inlet temperature of 170 ± 5 °C, an outlet temperature of 95 ± 5 °C, pump speed of 25%, feed rate of 7.5 mL/min, and aspirator rate of 100%. After spray drying, the powder was stored in a refrigerator at 4 °C.

2.3. Microencapsulation with alginate and chitosan

Microspheres of alginate and chitosan were prepared according to the method of Han, Guenier, Salmieri, and Lacroix (2008). The microcapsule core solution was prepared by dissolving sodium alginate (0.02 g/mL) in solution of 10% β-carotene (0.005 g/mL) and deionized water. Microcapsules were formed by extruding the core solution in 30 mL syringes through a 22 gauge needle into a calcium chloride solution (0.1 g/mL) from a height of 5 cm at rate of 30 mL/h using a Harvard Apparatus Dual Syringe pump (Holliston, MA), followed by a hardening time of 30 min. For coating with chitosan, the beads were vacuum filtered using Whatman 42 Ashless filter paper, rinsed with deionized water, and added to a solution of chitosan (0.005 g/mL) and glacial acetic acid (diluted with deionized water to a final concentration of 0.01 mL glacial acetic acid/mL solution) and gently stirred for 1 h, after which they were transferred to Whatman 42 Ashless filter paper to dry overnight. Due to the insolubility of β-carotene in alginate solutions and to achieve a homogenous solution, water-dispersible β-carotene was used.

Table 1
Composition of digestive juices.

	Saliva	Gastric juice	Duodenal juice	Bile juice
Stock solution	0.117 mg/mL NaCl 0.149 mg/mL KCl 2.1 mg/mL $NaHCO_3$ 0.4 mg/mL urea	5.504 mg/mL NaCl 1.648 mg/mL KCl 0.532 mg/mL NaH_2PO_4 0.798 mg/mL $CaCl_2 \cdot 2H_2O$ 0.612 mg/mL NH_4Cl 6.5 mL HCl 0.17 mg/mL urea	14.024 mg/mL NaCl 1.128 mg/mL KCl 6.776 mg/mL $NaHCO_3$ 0.16 mg/mL KH_2PO_4 0.1 mg/mL $MgCl_2$ 180 μL HCl 0.2 mg/mL urea	10.58 mg/mL NaCl 0.752 mg/mL KCl 11.57 mg/mL $NaHCO_3$ 150 μL HCl 0.5 mg/mL urea
Add to stock solution	1 mg/mL mucin 2 mg/mL α-amylase pH: 6.8 ± 0.2	5 mg/mL pepsin 6 mg/mL mucin pH: 1.30 ± 0.02	18 mg/mL pancreatin 3 mg/mL lipase pH: 8.1 ± 0.2	60 mg/mL bile pH: 8.2 ± 0.2