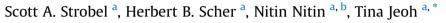
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In situ cross-linking of alginate during spray-drying to microencapsulate lipids in powder



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ABSTRACT

Microencapsulation of emulsified lipophilic bioactive compounds in dry, cross-linked alginate microcapsules (CLAMs) is a promising strategy to facilitate their incorporation into food systems, prolong shelf life, and target delivery within the gastrointestinal tract. However, current technology to produce CLAMs requires multiple time- and energy-intensive unit operations. We developed a novel technology that streamlines CLAM production into a single unit operation by accomplishing particle formation, crosslinking, and drying during spray-drying. Spray-dried CLAMs were prepared using corn oil as the cargo, and dry basis oil loadings up to 35% (w/w) were achieved. Alginate cross-linking was verified by the insolubility of CLAMs in water and ready dissolution in sodium citrate. Volume weighted mean particle size of CLAMs increased with increasing oil content: 8.1 µm, 11.8 µm and 17.9 µm for 15%, 25% and 35% oil, respectively. Spray dried CLAMs were approximately spherical, with oil droplets evenly distributed throughout each microcapsule. The size distribution of oil droplets, with average diameters ranging from approximately 200 to 300 nm, remained unchanged throughout the encapsulation process; spray drying did not induce aggregation or coalescence of oil droplets within CLAMs. CLAMs released 22-35% of oil in simulated gastric fluid (pH 1.5) and 81-93% in simulated intestinal fluid (pH 7) in 2 h, indicating that CLAMs are an enteric system. Coupled with the scalability of this novel CLAM production method, the successful encapsulation of the model lipid suggests that spray-dried CLAMs may be of commercial use for incorporating lipophilic compounds into foods.

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1. Introduction

Incorporating bioactive lipophilic compounds into functional foods is highly desirable, as these extra-nutritional plant and food constituents have been associated with a number of health benefits, including protection against cardiovascular disease and cancer (Kris-Etherton et al., 2002; Sanders, 1996). Microencapsulation, which produces sub-micron to millimeter scale capsules in which a coating material envelops the core material, is regarded as a means of facilitating the incorporation of lipophilic bioactive compounds into foods and beverages. In the food industry, microencapsulation offers a multitude of applications, including protecting bioactives from degradation induced by environmental factors, masking

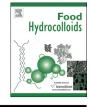
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http://dx.doi.org/10.1016/j.foodhyd.2016.02.031 0268-005X/© 2016 Elsevier Ltd. All rights reserved. undesirable flavors and odors, improving material handling, separating otherwise incompatible components, and controlling the release of bioactives (Desai & Park, 2005; Gibbs, Kermasha, Alli, & Mulligan, 1999).

While numerous food grade coating materials are available for microencapsulation, alginate is the subject of particular interest due to its release applications (George & Abraham, 2006). A polysaccharide derived from algal cell walls, alginate is a polymer of guluronate and mannuronate. Regions of repeating guluronate units in adjacent alginate molecules selectively bind divalent cations such as calcium in egg-box structures; these cross-links induce the spontaneous formation of a gel at room temperature. The crosslinking of alginate may be reversed through the scavenging of calcium ions from the gel matrix, which allows the polymers to separate, thus breaking the gel and releasing the encapsulated material (Draget & Taylor, 2011). This property provides one potential mechanism for triggering the release of an alginate microcapsule's payload in the intestinal environment, which is high in chelators that scavenge calcium. Another mechanism of







physiological release of alginate gel cargo involves the protonation and displacement of calcium ions under low pH conditions in the stomach to form alginic acid gels, which readily dissolve in the intestine due to the displacement of protons by sodium ions (Gombotz & Wee, 1998; Ostberg, Lund, & Graffner, 1994). The disintegration of alginate microcapsules under intestinal conditions makes alginate an attractive choice for nutrient delivery applications.

Dry cross-linked alginate microcapsules (CLAMs) have been used to encapsulate lipophilic bioactives such as carvacrol (Wang, Gong, Huang, Yu, & Xue, 2009), α -tocopherol (Yoo, Song, Chang, & Lee, 2006), and oils containing tocopherols, tocotrienols, carotenoids, and linolenic acid (Chan, Lim, & Heng, 2000; Durante, Lenucci, Laddomada, Mita, & Caretto, 2012). Controlled release studies of CLAMs indicate that while a relatively low fraction of core material is released in gastric conditions, the vast majority of the core material is released in intestinal conditions (Wang et al., 2009; Yoo et al., 2006). In addition to providing a mechanism of targeted and controlled release, encapsulation in CLAMs has been demonstrated to increase the shelf-stability of bioactives, compared to non-encapsulated compounds (Durante et al., 2012).

However, while alginate microcapsules offer advantages in core material protection and release, their utilization in the food industry has been limited by time- and energy-intensive manufacturing methods. A variety of production methods have been developed for preparing CLAMs, which can be broadly classified as external gelation, inverse gelation, and internal gelation methods. External gelation involves dispersing an O/W emulsion (with alginate in the aqueous phase) into a calcium solution by extrusion or spraying. Cross-linking proceeds as calcium ions diffuse inwards, encapsulating the emulsified core material in a cross-linked hydrogel. External gelation methods have been widely used to encapsulate lipophilic compounds (Durante et al., 2012; Sun-Waterhouse, Wang, & Waterhouse, 2014; Sun-Waterhouse, Zhou, Miskelly, Wibisono, & Wadhwa, 2011; Wang et al., 2009; Yoo et al., 2006). Internal gelation methods involve forming an O/ W/O multiple emulsion with alginate and an insoluble calcium salt in the aqueous phase. The core material is contained in the innermost oil phase. The addition of acid solubilizes the calcium salt to make it immediately available for cross-linking the alginate throughout the aqueous phase. As preparing stable multiple emulsions may be challenging, microfluidic techniques have been developed to produce core-shell microcapsules using this technique (Liu et al., 2013). The inverse gelation method forms core-shell microcapsules when oil droplets containing finely divided insoluble calcium chloride are dispersed in an alginate solution. Upon leaving the oil droplet, calcium chloride particles dissolve such that calcium ions are immediately available to form a crosslinked membrane around the droplet (Abang, Chan, & Poncelet, 2012). All of these CLAM preparation technologies require separation of the hydrated alginate beads, followed by a drying step.

To increase the scalability of CLAM production, spray-drying preparations have been suggested. The oldest and most prevalent microencapsulation technology used in the food industry, spray drying involves atomizing a feed solution, suspension, or emulsion in a hot gas current to obtain dry powdered particles (Desai & Park, 2005; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Matrix-style microcapsules are commonly produced using coating materials such as milk proteins, modified starches, maltodextrin, and gums (Gibbs et al., 1999). Alginate has been used as a wall material for spray-dried microparticles containing hydrophilic compounds, but cross-linking was induced after spray-drying by external gelation (Bagheri, Madadlou, Yarmand, & Mousavi, 2014; Schoubben, Blasi, Giovagnoli, Rossi, & Ricci, 2010). A separation step and a second drying step are still required. Others have avoided this complication by weakly cross-linking the alginate with dilute calcium chloride prior to spray-drying (Erdinc & Neufeld, 2011; Estevinho, Damas, Martins, & Rocha, 2014). However, this approach requires a vigorous mixing step prior to spray drying to produce alginate beads of small enough diameter to pass through the nozzle. Recently, we developed a single-step process for preparing cross-linked alginate microparticles by spray-drving (leoh. Scher, Santa-Maria, & Strobel, 2012: Santa-Maria, Scher, & Jeoh, 2012). A suspension consisting of alginate, an acid neutralized by ammonium hydroxide, and insoluble calcium salt is spray-dried. Upon atomization, the volatilization of ammonia decreases the pH in the droplet, which solubilizes the calcium salt, releasing calcium ions to cross-link the alginate. Thus, particle formation, cross-linking, and drying occur in a single, scalable unit operation. However, to our knowledge, none of the aforementioned spraydrying methods have been used to microencapsulate lipophilic compounds in cross-linked alginate.

In the present study, we investigated the potential to encapsulate lipophilic compounds in CLAMs prepared using a novel twostep process consisting of homogenization and spray-drying. CLAMs containing corn oil as a model lipophilic compound were produced at oil loadings up to 35%. We present results on the particle size, morphology, oil content, and oil droplet size of the CLAMs. Additionally, we demonstrate controlled release of oil from CLAMs in simulated digestive fluids.

2. Materials & methods

2.1. Materials

Corn oil (Cal Western Packaging, Memphis, TN) was procured from a local market. Low viscosity sodium alginate (A1112) was purchased from Sigma–Aldrich (St. Louis, MO). Polysorbate 80 (Tween 80), dicalcium phosphate, sodium citrate dihydrate, succinic acid, ammonium hydroxide, n-hexane, isopropanol, methanol, chloroform, potassium chloride, sodium bicarbonate, sodium chloride, ammonium carbonate, potassium phosphate monobasic, magnesium chloride, and hydrochloric acid were purchased from Fisher (Fair Lawn, NJ). MilliQ water with a minimum resistivity of 18 M Ω -cm (Millipore, Billerica, MA) was used for all experiments.

2.2. Methods

2.2.1. Preparation of cross-linked alginate microcapsules (CLAMs)

Spray-dried CLAMs were prepared with target oil loadings of 0%, 15%, 25%, and 35% (w/w). All CLAMs were formulated with a 5:1 corn oil to Tween 80 ratio (w/w), and the target oil loading dictated the quantity of corn oil and Tween 80 in each formulation. An emulsion composed of corn oil, Tween 80, and 2% (w/w) succinic acid adjusted to pH 5.6 with ammonium hydroxide was dispersed at 9500 rpm for 2 min with a hand-held dispersing unit then homogenized using a high pressure homogenizer at 600 bar. The homogenized emulsion was mixed 1:1 (w/w) with a suspension of 4% alginate, 0.2% calcium phosphate dibasic dihydrate, and 0.06% sodium citrate dihydrate to form the spray dryer feed. This feed solution was promptly pumped into a Buchi B290 laboratory spraydryer (New Castle, DE) to produce dry microcapsules. All formulations were prepared under identical operating conditions: inlet air temperature at 150 °C, aspirator airflow rate at maximum $(35 \text{ m}^3/\text{h})$, feed peristaltic pump at 20% of maximum (6 ml/min), and nozzle air flow at two-thirds of maximum (40 mm on Q-flow indicator). Spray dried powders were stored in glass vials in a desiccator until analysis.

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