MICROENCAPSULATION OF BETA-CAROTENE: CHARACTERIZATION, IN VITRO RELEASE, AND BIOAVAILABILITY

by

ERIK GLENN DONHOWE

(Under the Direction of Fanbin Kong)

ABSTRACT

Beta-carotene is the main dietary source of Vitamin A, an essential micronutrient required to maintain proper health. The *in vivo* bioavailability and absorption of beta-carotene, however, is limited due to the inhibition by the natural plant matrices in which it is found. This study focused on microencapsulation methods to improve release and bioavailability *in vitro*. Alginate and chitosan formulations were evaluated for encapsulation efficiency and particle size, and the effect of viscosity on release *in vitro* were determined. Increased viscosity decreased release due to inhibition of diffusion from the microcapsules. Additional research compared the *in vitro* bioavailabilities and physical properties of three types of microencapsulated beta-carotene (spray-dried with maltodextrin, commercial water-dispersible, and chitosan-alginate microcapsules) in terms of morphology, water activity, moisture content, particle size, surface beta-carotene, and encapsulation efficiency. The bioavailability of the commercial water-dispersible beta-carotene was consistently highest, even in the presence of food matrices (yogurt, pudding).

INDEX WORDS: Beta-carotene, microencapsulation, *in vitro* digestion, bioavailability

MICROENCAPSULATION OF BETA-CAROTENE: CHARACTERIZATION, IN VITRO RELEASE, AND BIOAVAILABILITY

by

ERIK GLENN DONHOWE

B.S., Chemical Engineering, Stanford University, 2010

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

MICROENCAPSULATION OF BETA-CAROTENE: CHARACTERIZATION, IN VITRO RELEASE, AND BIOAVAILABILITY

by

ERIK GLENN DONHOWE

Major Professor: Fanbin Kong

Committee: William Kerr
Louise Wicker

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2013

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

LITERATURE REVIEW

Vitamin A is an important nutrient for proper immune function, growth, development, and gastrointestinal function in addition to preventing night blindness (West and Darnton-Hill 2001; Grune and others 2010; Haskell 2012). Humans lack the ability to synthesize vitamin A de novo, so we must get proper amounts of it from our diets in the form of dark leafy green vegetables (i.e. spinach) and orange and yellow vegetables and fruits (i.e. carrots, mangoes) (West and Darnton-Hill 2001; Haskell 2012). Beta-carotene has the the highest vitamin A activity compared to the other provitamin A carotenoids (alpha-carotenes, cryptoxanthins) and the most efficient conversion to vitamin A (Yeum and Russell 2002; Grune and others 2010). Although its primary role as a nutrient is as a source of vitamin A, beta-carotene also has antioxidant capabilities and can function as a lipid radical scavenger and a singlet oxygen quencher due to its unique structure (Grune and others 2010).

Beta-carotene has approximately twice the activity of other provitamin A carotenoids, but its maximum absorption from natural plant sources is low enough (~65%) that the recommended intake (2 -4 mg/day) is not achieved (Grune and others 2010; Haskell 2012). The low bioavailability of beta-carotene in natural sources is due to resistance of carotene-protein complexes and the plant cell walls to digestion and degradation to achieve adequate release (Erdman and others 1993; Rein and others 2013). The low bioavailability from natural sources has led to exciting opportunities to extract and isolate beta-carotene for delivery either as supplements or in fortified food matrices to improve bioavailability and achieve maximum health

benefits (Fernández-García and others 2012). Pure beta-carotene is rapidly degraded in the gastric phase during digestion, which means the delivery vehicle must protect it from degradation without inhibiting release (Cutting 2011). Spray-drying and microencapsulation are two methods of protecting beta-carotene with the aim of improving bioavailability. In order to design a working and practical delivery vehicle for beta-carotene, however, its metabolism during digestion must first be understood.

1. Metabolism of beta-carotene

Release

Release from the food matrix is the first step of the beta-carotene absorption pathway, which begins orally with physical disruption (mastication) of the food matrix (Parker 1996; Yeum and Russell 2002; Yonekura and Nagao 2007). The food matrix has a significant effect on bioavailability because release from the food matrix is a primary factor limiting bioavailability of beta-carotene (Erdman and others 1993; Parker 1996). Beta-carotene can be found in different forms within plants (i.e. chloroplasts, chromoplasts), and it has been shown that the physical form of beta-carotene also has a significant effect on release during digestion (Erdman and others 1993; Schweiggert and others 2012). Release depends on the structural degradation of the food matrix, which may be aided by thermal or mechanical processing prior to digestion (Parker 1996; Yeum and Russell 2002). Mechanical processing (i.e. pureeing) improves bioavailability by reducing particle size, allowing a greater surface area to come into contact with digestive juices to improve digestion and release (Erdman and others 1993; Hedren and others 2002). Thermal processing (i.e. steaming) of raw vegetables has been shown in numerous studies to improve the bioavailability of beta-carotene and contributes to the denaturation of the carotenoprotein

complexes (Erdman and others 1993; Rock and others 1998; Edwards and others 2002; Hornero-Méndez and Mínguez Mosquera 2007; Lemmens and others 2010).

Transfer to oil phase

After release, the hydrophobic beta-carotene dissolves into either the emulsified or bulk oil phase of the digesta (Parker 1996; Rich and others 2003; Yonekura and Nagao 2007). Transfer to the oil phase requires direct contact between the beta-carotene and the oil, which may be hindered if oil is unable to access the beta-carotene due to incomplete digestion of the food matrix (Faulks and Southon 2005). Conversely, reduction in particle size increases the surface area exposed to the oil phase and increases the partitioning of beta-carotene into the oil phase (Parker 1996; Rich and others 2003). Surface-active proteins have been shown to inhibit the incorporation of beta-carotene into the gastric emulsion, indicating that the interfacial characteristics of the gastric emulsion determine the extent of beta-carotene incorporation (Rich and others 2003). Surface charge of the gastric emulsion also determines the extent of transfer to the oil phase, since decreasing surface charge (by lowering pH) increases adherence of oil to the carotene-containing matrix and increases beta-carotene solubilization (Rich and others 1998). The solubility of beta-carotene in oil, which determines the extent of partitioning into the oil phase, depends on the oil and the amount of beta-carotene present (Faulks and Southon 2005). The average dietary fat consumed daily (20-50 g), however, is more than sufficient to completely solubilize the normal amount of beta-carotene ingested daily (~1 mg) (Faulks and Southon 2005).

Micelle phase

Passage into the small intestine stimulates the release of bile salts, which contribute to the formation of mixed micelles from the gastric oil phases (Parker 1996; Yeum and Russell 2002;

Yonekura and Nagao 2007). The bile salts act as surfactants and reduce the size of the gastric emulsion through the formation of micelles with an average diameter of 80Å (Parker 1996; Yonekura and Nagao 2007; Fernández-García and others 2012). The micelles formed contain beta-carotene in addition to free fatty acids, monoglycerides, and phospholipids (Yeum and Russell 2002; Fernández-García and others 2012). Beta-carotene absorption depends on its presence in mixed micelles, so factors affecting the degree of micelle formation affect the bioavailability of beta-carotene during digestion (Tyssandier and others 2003). Many studies extract beta-carotene the micelle phase to determine bioavailability instead of trying to model absorption using Caco-2 cells (Fernández-García and others 2012).

One of the most important factors to influence micelle formation is the presence of dietary fat, since lipids are required not only for incorporation into micelles but also to stimulate release of bile to facilitate micelle formation (Parker 1996; Yonekura and Nagao 2007). An increase in dietary fat increases bioavailability of beta-carotene by increasing the extent of micelle formation, but only up until an optimal level, above which additional fat has no effect (Roodenburg and others 2000; Yonekura and Nagao 2007). The type of fat, in addition to the amount, affects micelle formation, as increasing fatty acyl chain length has been shown to increase micellization and bioavailability of beta-carotene (Huo and others 2007).

Dietary fiber, which is a component of vegetables and fruits, have been shown to inhibit micelle formation and decrease bioavailability of beta-carotene *in vitro* and *in vivo* (Rock and Swendseid 1992; Yeum and Russell 2002; Yonekura and Nagao 2009). Bile salts are able to bind to dietary fibers, which decreases the amount of bile salts available for micelle formation (Kern and others 1978; Yeum and Russell 2002). Decreasing the concentration of bile salts has been shown to significantly decrease micelle formation and bioavailability of beta-carotene *in vitro*

(Wang and others 2012). The increased viscosity during digestion with soluble dietary fibers may also play a role in the inhibition of micelle formation, but more research is necessary to elucidate the mechanism of inhibition (Yonekura and Nagao 2009).

Fed conditions, pH, and beta-carotene concentration, have also been shown to significantly impact micelle formation *in vitro* (Wright and others 2008; Wang and others 2012). Wright and others (2008) tested micelle formation in fasting and fed states by altering the enzyme concentrations during digestion to approximate those before ingestion of a meal (i.e. fasting) and after ingestion of a meal (i.e. fed). Their results show that not only is beta-carotene micelle formation significantly increased under fed conditions, the effect of pH on micelle formation is insignificant between the range of 5 and 9.5 under fed conditions (Wright and others 2008). Under fasting conditions, both Wang and others (2012) and Wright and others (2008) found pH to significantly affect micelle formation, with Wang and others (2012) finding a pH of 6 for optimum micelle formation, whereas Wright and others (2008) finding a pH of 9.5 to be optimum. Wang and others (2012) also found that increasing beta-carotene concentration from 0.025 wt% to 0.05 wt% significantly decreased the amount of beta-carotene found in micelles and then remained stable after increasing past 0.05 wt%.

Absorption

The beta-carotene-containing micelles then come into contact with intestinal epithelial cells, leading to absorption of beta-carotene into the cytosol/plasma membrane (Erdman and others 1993; Yonekura and Nagao 2007). After absorption, beta-carotene is incorporated into chylomicrons before entering the lymphatic system and circulation (Yeum and Russell 2002; Yonekura and Nagao 2007). The primary mechanism for absorption is believed to be passive diffusion governed by the difference in concentration between the micelle and the plasma

membrane of the intestinal cell (Parker 1996). The lipid bilayer of the plasma membrane facilitates the diffusion of lipophilic substances (i.e. beta-carotene) through the membrane into the cytoplasm (Yonekura and Nagao 2007). Viscosity is one factor that affects absorption, since it can inhibit micelle formation in addition to diffusion to intestinal epithelial cells (Rock and Swendseid 1992). Factors that inhibit micelle formation also inhibit absorption, since inhibition of micelle formation decreases the amount of beta-carotene in an absorbable form. As such, there are not many factors that inhibit absorption alone, since inhibition of diffusion would probably inhibit both micelle formation and absorption of beta-carotene.

2. Spray-drying beta-carotene

Spray drying is a popular method for encapsulation because it is cheap, fast, and has high reproducibility (de Vos and others 2010). Within the food industry, it has a wide range of applications including the encapsulation of enzymes, flavors, antioxidants, preservatives, and bioactives (Gibbs and others 1999). For spray-drying functional ingredients, the bioactive compound is first dispersed in a solution of the encapsulating matrix, followed by rapid evaporation to form a shell that encapsulates the bioactive compound (de Vos and others 2010). The selection of the encapsulating matrix is important to ensure that the bioactive compound is adequately protected against degradation during storage (Gharsallaoui and others 2007). In the case of beta-carotene, the encapsulating matrix must also protect against gastric degradation without inhibiting transfer to the oil phase or micelle formation to improve bioavailability.

The specific spray-drying conditions for beta-carotene are based on the wall material selected for encapsulation. Temperature is an important factor during spray drying because there is a risk of melting beta-carotene when operating at temperatures in excess of 193°C, the

approximate melting point of beta-carotene (Desobry and others 1997). The air inlet temperature affects the rate of evaporation during spray drying, so optimization is necessary to avoid powders with high agglomeration, poor fluidity, and high water content (Gharsallaoui and others 2007). Spray-dried beta-carotene with maltodextrin has been performed at an inlet temperature of 175±5°C and an outlet of 95±5°C (Desobry and others 1997; Loksuwan 2007). Air flow rate also affects moisture content of the resulting powder, as Zbicinski and others (2002) showed that increased airflow significantly decreases moisture content.

A review of literature indicates a lack of research on the justification of spray drying functional ingredients by testing bioavailability during *in vitro* digestion. There are multiple forms of beta-carotene spray-dried powders presented in literature, including algae powders, carbohydrate powders, and water-dispersible powders (Ben-Amotz and Levy 1996; Desobry and others 1997; Kowalski and others 2000).

Algae powders

Dunaliella salina and *Dunaliella bardawil* are two naturally-occurring algae notable for producing of high levels of beta-carotene (Ben-Amotz and Levy 1996; Leach and others 1998). Since the beta-carotene is already quasi-encapsulated due to its presence within these unicellular organisms, spray drying may or may not involve the use of a polymer encapsulating matrix (Ben-Amotz and Levy 1996; Leach and others 1998). Leach and others (1998) found that storage stability dramatically increased when *D. salina* was spray dried with maltodextrin and gum Arabic, which decreased exposure to oxygen by providing a barrier to oxygen diffusion. The bioavailability of beta-carotene from spray-dried *Dunaliella bardawil* without an encapsulating matrix proved to be less than that of water-dispersible beta-carotene (Ben-Amotz and Levy 1996). This result may be due to the isomer composition, since water-dispersible beta-carotene

is made from all-*trans* beta-carotene, whereas the beta-carotene from *D. bardawil* and *D. salina* contain isomeric mixtures of all-*trans* and 9-*cis* beta-carotene (Ben-Amotz and Levy 1996; Leach and others 1998).

Carbohydrate powders

Starches, including those modified and hydrolyzed, are popular for spray-drying due to their low viscosities at high concentrations and cost effectiveness (Loksuwan 2007). Tapioca starch, modified food starch, and maltodextrin have all been used to varying success as the wall materials for spray-drying bet-carotene (Desobry and others 1997; Loksuwan 2007; Liang and others 2013). Loksuwan (2007) found that acid-modified tapioca starch had higher microencapsulation efficiency and higher cold water solubility than either native tapioca starch or maltodextrin. Spray-drying, drum-drying, and freeze-drying beta-carotene in maltodextrin solutions were compared by Desobry and others (1997), who found that spray-drying had the highest surface beta-carotene content and the fastest degradation rate during storage. Liang and others (2013) researched the effect of relative humidity on spray-dried emulsions of beta-carotene stabilized by modified food starches, determining that lower film permeability is correlated to higher beta-carotene retention during storage. Although physical characterization of beta-carotene powders is important, since neither Loksuwan (2007), Desobry and others (1997), or Liang and others (2013) studied the bioavailability of their powders *in vitro*, the applicability of these carbohydrate-based powders to food fortification is unknown.

Water-dispersible powders

Spray-drying beta-carotene emulsions with gelatin and antioxidants to protect against oxidative degradation form water-dispersible beta-carotene powders (or "beadlets") (Kowalski and others 2000; Thürmann and others 2002). The bioavailability of water-dispersible powder

has been studied *in vivo*, with results being unanimous in its superiority at increasing plasma concentration of beta-carotene after ingestion (Ben-Amotz and Levy 1996; Fuller and others 2001; Thürmann and others 2002). When compared against other beta-carotene supplement sources, Fuller and others (2001) and Ben-Amotz and Levy (1996) found that the water-dispersible powder was most effective at increasing plasma beta-carotene concentration. Thürmann and others (2002) compared water-dispersible powder fortification of a food matrix (fruit juice-based breakfast drink) to a similar fruit juice-based breakfast drink that contained beta-carotene in the form of carrot juice. Their findings confirm that the water-dispersible powder has superior bioavailability, even when introduced in a food matrix (Thürmann and others 2002). In addition to performance *in vitro*, the physical properties of water-dispersible beta-carotene powders have yet to be studied, so the mechanism by which it achieves higher bioavailability and absorption has yet to be elucidated.

3. Microencapsulating beta-carotene

Microencapsulation of bioactive compounds using a gelation method is widely used and can easily be achieved in a laboratory setting (Champagne and Fustier 2007). Alginate and chitosan are two widely used polymers used for microencapsulation due to their ability to protect against degradation and release enterically, the site of beta-carotene absorption (Champagne and Fustier 2007; de Vos and others 2010). Additionally, the choice of gelation method (ionotropic, external, and internal) has a significant effects on the physical properties of the resulting microcapsules (Champagne and Fustier 2007).

Alginate

Alginate is a naturally occurring polysaccharide extracted from seaweed and brown algae (Chan and others 2006; Han and others 2008). It is composed of M and G monomers that are arranged in alternating M and G blocks that form a linear polymer (Gåserød and others 1998; de Vos and others 2010). The exposure of alginate to polyvalent cations (i.e. Ca^{2+}) induces gelation through the cross-linkage of anionic G-G sequences with the cations (Chan and others 2006; Li and others 2011). The preparation of alginate beads can be performed as easily as extruding an alginate solution into a solution containing polyvalent cations (Gåserød and others 1999).

Its use as an encapsulating matrix for bioactive compounds is desirable because it is not only biocompatible but also easily forms gel beads with chemically inert interiors (Chan and others 2006; Li and others 2011). Additionally, alginate beads can withstand acidic pH while degrading under mild basic conditions, making it an attractive encapsulation material for protection against gastric digestion and directed release into the small intestine (Yoo and others 2006; de Vos and others 2010).

The mechanical strength, porosity, shrinkage, stability, and other physical properties of alginate beads depend on the composition, sequential structure, and molecular size of the alginate polymer (Martinsen and others 1989). One study has shown that beads with a high mechanical strength are produced from alginates with over 70% L-guluronic acid residues and G-blocks with an average length greater than 15 residues long (Martinsen and others 1989). Additionally, high ratios of G to M residues have been shown to increase the porosity of alginate beads (Gåserød and others 1999).

Chitosan

Alginate beads are typically coated or combined with chitosan to guard against gel erosion and instabilities caused by chelators (Gåserød and others 1999; Han and others 2008). Chitosan is a polymer produced from the partial deacetylation of chitin, which can be found in exoskeletons of crustaceans (Han and others 2008). It is attractive for use in microencapsulation since it is non-toxic, improves mechanical strength, and is preferentially degraded in the small intestine (Peng and others 2010; de Vos and others 2010).

As a cationic polysaccharide, chitosan interacts with the anionic alginate gel beads to form an outer membrane through electrostatic binding (Gåserød and others 1999; Han and others 2008). Differing degrees of deacetylated chitosan can be produced, and it has been shown that higher molecular weight chitosan is correlated to reduction in alginate bead permeability and porosity (Gåserød and others 1999). Thus, the type of chitosan used controls alginate bead permeability and porosity, and, by extension, stability and release characteristics (Gåserød and others 1999).

Preparation method

The preparation method used for microencapsulation is an important factor to consider because different methods produce different compositions and structures (McClements and Li 2010). Controlling the physical structure of the alginate beads is important because it is directly related to digestibility, susceptibility to degradation, permeability, and porosity (McClements and Li 2010). Additionally, the preparation method has been shown to affect the release characteristics of the chitosan-alginate beads (Abreu and others 2008).

Ionotropic gelation

The main preparation procedure for chitosan-alginate hydrogels is ionotropic gelation, which involves the cross-linking of alginate chains with cations (Burey and others 2008). The two main types of ionotropic gelation are external (or diffusion) gelation and internal gelation (Burey and others 2008).

External Gelation

External gelation is characterized by the formation of gels after the hydrocolloid solution is introduced to an ionic solution (Burey and others 2008). The diffusion of ions is the primary mechanism through which the gelation occurs and leads to the formation of inhomogeneous gel particles with surface gelation occurring before core gelation (Burey and others 2008). This difference in gelation rates leads to a highly cross-linked surface that is hard with a less cross-linked interior that is soft (Chan and others 2006; Burey and others 2008). One study has shown that external gelation produces beads with higher matrix strengths than internal gelation (Chan and others 2006).

A common technique used in conjunction with external gelation is extrusion, where a solution containing the encapsulation matrix (i.e. alginate) is loaded into a syringe and then injected drop-wise into the ionic solution (i.e. calcium chloride) to induce gelation (McClements and Li 2010). One advantage of extrusion is the size of the syringe needle gauge determines the size of the alginate bead (de Vos and others 2010). Additionally, extrusion can easily be scaled up to large scale production within industry (de Vos and others 2010).

Internal Gelation

A hydrocolloid and inactive ion solution is the basis for internal gelation (Burey and others 2008). The gelation is then induced by ion activation, such as pH change, that causes cross-linking and the formation of a hydrogel matrix (Burey and others 2008). Because the ions

are dispersed prior to gelation and diffusion does not affect the surface and core gelation rates, internal gelation produces more homogeneous beads (Chan and others 2006). The cross-linkages are thus distributed more evenly throughout the beads, which leads to less dense matrices with larger pore sizes, higher permeability, and faster release rates (Chan and others 2006). Internal gelation has proven useful for the encapsulation of lipophilic drugs due to the ability to stabilize the emulsion in alginate before inducing crosslinking and microencapsulation (Ribeiro and others 1999).

4. *In vitro* digestion of beta-carotene supplements

Given the range of factors influencing bioavailability of beta-carotene before absorption, it is vital to study and elucidate the factors that affect beta-carotene bioavailability during digestion. Beta-carotene supplementation and fortification only provides health benefits if the beta-carotene remains bioavailable, which can be determined through digestion. While it would be ideal to use human subjects to study bioavailability, experimentation *in vivo* is impractical due to the large costs, large variations between individuals, and the time consuming nature of the experiments (Ekmekcioglu 2002; Hur and others 2011). The use of *in vitro* digestion models have gained popularity, due to their advantages of reproducibility, rapidity, and the ability to test a large number of samples simultaneously and inexpensively (Rodriguez-Amaya 2010). *In vitro* methods to assess the bioavailability of bioactive compounds (like beta-carotene) have been developed and refined in the last decade, but there is no single standard digestion model currently used (Hur and others 2011). Since the composition of the digesta (i.e. bile salt concentration) affects bioavailability, it is necessary to review the *in vitro* digestion protocol and

determine the important factors to consider during every step of digestion (Yeum and Russell 2002; Wright and others 2008).

Static vs. dynamic

The first step in determining bioavailability is determining whether to use a static or dynamic *in vitro* digestion model. Static digestion models involve simulating the physicochemical changes that occur during digestion (pH, salt concentrations, enzymes) without trying to replicate the peristalsis, fluid flow, or mixing that occurs during digestion (Guerra and others 2012). Dynamic models, on the other hand, aim to simulate the mechanical forces that occur during digestion in addition to mimicking the enzymatic and chemical composition of the GI tract (Kong and Singh 2010; Guerra and others 2012). The basis for selection, then, is on the sample being digested and the scope of measurement. Roman and others (2012) compared the bioavailability of beta-carotene in almond butter using static and dynamic models and found that peristalsis significantly increased release of oil-fortified almond butter in the dynamic model. This suggests that inclusion of a solid or semisolid food matrix requires the use of a dynamic model due to the significant difference peristalsis has on release and bioavailability. Static models, then, are more appropriate for use with simpler samples, like beta-carotene emulsions, since peristalsis likely does not affect digestion or transfer to oil phase as much as pH or enzyme digestion.

Gastric digestion

One of the most cited *in vitro* digestion methods for determining bioavailability of beta-carotene was proposed by Garrett and others (1999). In the study, Garrett and others (1999) found that gastric digestion had no effect on bioavailability of beta-carotene from homogenized

baby food when compared to a trial with intestinal digestion only. Thus, whether or not to include the gastric phase is an important consideration for *in vitro* digestion.

Including the gastric step depends on the sample being digested, as Wright and others (2008) omitted the gastric step in their research on the basis that they were experimenting with beta-carotene already dissolved in oil. The study of stabilized beta-carotene emulsions, however, requires the use of the gastric phase, as Liu and others (2012) determined that the gastric phase significantly affected the microstructure and surface charge of WPI-stabilized emulsions during digestion and led to coalescence.

If the gastric phase is included, the pH must be carefully considered; gastric acidity has been shown *in vivo* to significantly affect blood response, with increased acidity (lower pH) contributing to higher absorption of and response to beta-carotene (Tang and others 1996). Similar results have been confirmed *in vitro* by Rich and others (2003), who found that decreased pH increased beta-carotene transfer to oil during gastric digestion. The pH during gastric digestion must be carefully monitored so as not to overestimate beta-carotene bioavailability by using an artificially low pH for gastric digestion.

Intestinal digestion

Enzyme concentrations of pancreatin and bile during digestion have been found to significantly affect micelle formation and bioavailability of beta-carotene (Hollander and Ruble 1978; Wright and others 2008; Wang and others 2012). Without a standardized model for *in vitro* digestion and intestinal enzyme concentrations, the bioavailability of a given sample could thus vary significantly depending on what model is used. Many *in vitro* studies mimic the fasted state of digestion to study carotenoid bioavailability even though beta-carotene is often ingested as part of a meal (Wright and others 2008; Grune and others 2010). Fed conditions are thus more

physiologically relevant for studying the bioavailability of beta-carotene, especially when testing supplements that may eventually be incorporated into food products for fortification with beta-carotene. Wright and others (2008) used bile and pancreatin concentrations of 20.0 mg/mL and 2.4 mg/mL to represent fed conditions, although found no significant differences between using 10.0 mg/mL of bile and 20.0 mg/mL of bile at a level of 2.4 mg/mL pancreatin. This suggests that a concentration of 10 mg/mL of bile is sufficient to simulate fed conditions, considering the typical bile salt concentrations in the small intestine in fed state is between 10-20 mM (Wright and others 2008).

Modeling absorption

Since absorption of beta-carotene primarily occurs via passive diffusion, some *in vitro* digestion models utilize methods to model absorption after digestion (Liu and others 2004; O'Sullivan and others 2010; Ferruzzi and others 2006; Yonekura and Nagao 2009). Different methods are available to model absorption *in vitro*, including the use of artificial membranes and Caco-2 cells cultures (Fernández-García and others 2012). Thus far, Caco-2 cells have been the primary method of measuring beta-carotene absorption. Although accepted as a physiological valid method of measuring carotenoid absorption, the Caco-2 cell culture method is static by necessity and does not measure absorption under the fluid flow regimes of the small intestine (Fernández-García and others 2012). The procedure for testing uptake by Caco-2 cells involves adding a dilute solution of the micelle phase after digestion to the Caco-2 cell culture followed by extraction of beta-carotene from the cells after a set period of time (Garrett and others 1999; Liu and others 2004). The use of Caco-2 cells to model absorption has been verified due to the correlation between *in vivo* and *in vitro* results of beta-carotene bioavailability (Reboul and others 2006). Considering that absorption of beta-carotene is still not widely understood and

factors impacting micelle formation may also impact absorption, including Caco-2 cells after *in vitro* digestion gives a more complete picture of factors affecting bioavailability of beta-carotene (Yeum and Russell 2002)

5. Quantification of bioavailable beta-carotene after *in vitro* digestion

After digestion, measuring the amount of bioavailable beta-carotene requires isolation of the micelle phase followed by extraction (Rodriguez-Amaya 2010). High-speed centrifugation is used to separate the phases and isolate the aqueous layer that contains micelles of beta-carotene (Rodriguez-Amaya 2010). Given its lipophilic nature, after isolation of the micelle phase, extraction using organic solvents (i.e. mixtures of hexane, acetone, ethanol, etc.) is necessary, but a standard method for extracting beta-carotene has yet to be widely accepted (Rodriguez-Amaya 2010). Quantification of beta-carotene from the extract is primarily done in two ways, either spectrophotometrically or using reverse-phase HPLC (Rodriguez-Amaya 2010; Biswas and others 2011).

Due to its extensive conjugation, beta-carotene is a light-active compound that has a maximum absorption around 450 nm. UV-Vis spectrophotometry is used primarily due to its simplicity and rapidity in achieving results, but also has adequate detection thresholds for beta-carotene and can be used in lieu of HPLC (Roman and others 2012). Quantification is generally simple, as quantification of beta-carotene only requires construction of a calibration curve in the same solvent used for extraction (Liu and others 2012). One downside of analyzing beta-carotene spectrophotometrically is adequate extraction requires a large volume of toxic solvents for measurement (Biswas and others 2011).

HPLC coupled with UV-Vis detection is another widely accepted method for measuring the bioavailability of beta-carotene after *in vitro* digestion. The advantages of HPLC over spectrophotometry are its higher precision and its ability separate beta-carotene from other carotenoids in addition to quantifying the amount of beta-carotene present in a sample (Barba and others 2006; Biswas and others 2011).

6. Conclusions

Beta-carotene is a vital compound for human health as a main dietary source of vitamin A. The limited bioavailability of beta-carotene from fruits, vegetables, and other natural sources is primarily due to the adverse effects of plant material on micelle formation and absorption. Isolation and microencapsulation for food fortification is an attractive method of increasing the bioavailability of beta-carotene for maximum health benefits. Design of microencapsulated beta-carotene must take the absorption pathway into account, as many factors (including the encapsulation material) can negatively affect release, transfer to oil phase, micelle formation, and/or absorption. Spray-drying and microencapsulation via gelation are two straightforward and effective methods for microencapsulating and preserving beta-carotene. The literature on beta-carotene microencapsulation methods, however, fail to study the effect of microencapsulation method on *in vitro* bioavailability. The lack of a standardized method for determining bioavailability *in vitro* means that the digestion conditions used must be thoughtfully chosen and justified, especially considering that the digestion model used affects the estimation of bioavailability. The research on beta-carotene microencapsulation and resulting *in vitro* bioavailability is limited and must be further studied to design effective fortification methodologies to improve health and combat vitamin A deficiency worldwide.

7. References

Abreu FOMS, Bianchini C, Forte MMC, Kist TBL. 2008. Influence of the composition and preparation method on the morphology and swelling behavior of alginate–chitosan hydrogels. Carbohyd Polym 74(2):283-289.

Barba AIO, Hurtado MC, Mata MCS, Ruiz VF, Tejada MLSd. 2006. Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. Food Chem 95(2):328-336.

Ben-Amotz A, Levy Y. 1996. Bioavailability of a natural isomer mixture compared with synthetic all-trans beta-carotene in human serum. Am J Clin Nutr 63(5):729-734.

Biswas AK, Sahoo J, Chatli MK. 2011. A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. LWT - Food Sci Technol 44(8):1809-1813.

Burey P, Bhandari BR, Howes T, Gidley MJ. 2008. Hydrocolloid gel particles: formation, characterization, and application. Crit Rev Food Sci Nutr 48(5):361-377.

Champagne CP, Fustier P. 2007. Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol 18(2):184-190.

Chan LW, Lee HY, Heng PWS. 2006. Mechanisms of external and internal gelation and their impact on the functions of alginate as a coat and delivery system. Carbohyd Polym 63(2):176-187.

Cutting SM. 2011. *Bacillus* probiotics. Food Microbiol 28(2):214-220.

de Vos P, Faas MM, Spasojevic M, Sikkema J. 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20(4):292-302.

Desobry SA, Netto FM, Labuza TP. 1997. Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. J Food Sci 62(6):1158-1162.

Edwards AJ, Nguyen CH, You C-S, Swanson JE, Emenhiser C, Parker RS. 2002. α- and β-carotene from a commercial carrot puree are more bioavailable to humans than from boiled-mashed carrots, as determined using an extrinsic stable isotope reference method. J Nutr 132(2):159-167.

Ekmekcioglu C. 2002. A physiological approach for preparing and conducting intestinal bioavailability studies using experimental systems. Food Chem 76(2):225-230.

Erdman JW, Bierer TL, Gugger ET. 1993. Absorption and Transport of Carotenoids. Ann NY Acad Sci 691(1):76-85.

Faulks RM, Southon S. 2005. Challenges to understanding and measuring carotenoid bioavailability. Biochim Biophys Acta 1740(2):95-100.

Fernández-García E, Carvajal-Lérida I, Jarén-Galán M, Garrido-Fernández J, Pérez-Gálvez A, Hornero-Méndez D. 2012. Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. Food Res Int 46(2):438-450.

Ferruzzi MG, Lumpkin JL, Schwartz SJ, Failla M. 2006. Digestive stability, micellarization, and uptake of β-carotene isomers by Caco-2 human intestinal cells. J Agric Food Chem 54(7):2780-2785.

Fuller CJ, Butterfoss DN, Failla ML. 2001. Relative bioavailability of β-carotene from supplement sources. Nutr Res 21(9):1209-1215.

Garrett DA, Failla ML, Sarama RJ. 1999. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. J Agric Food Chem 47(10):4301-4309.

Gåserød O, Sannes A, Skjåk-Bræk G. 1999. Microcapsules of alginate–chitosan. II. A study of capsule stability and permeability. Biomaterials 20(8):773-783.

Gåserød O, Smidsrød O, Skjåk-Bræk G. 1998. Microcapsules of alginate-chitosan – I: A quantitative study of the interaction between alginate and chitosan. Biomaterials 19(20):1815-1825.

Gharsallaoui a, Roudaut G, Chambin O, Voilley a, Saurel R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Res Int 40(9):1107-1121.

Gibbs BF, Kermasha S, Alli I, Mulligan CN. 1999. Encapsulation in the food industry: a review. Int J Food Sci Nutr 50(3):213-224.

Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, Thurnham D, Yin SA, Biesalski HK. 2010. Beta-carotene is an important vitamin A source for humans. J Nutr 140(12):2268S-2285S.

Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, Alric M. 2012. Relevance and challenges in modeling human gastric and small intestinal digestion. Trends Biotechnol 30(11):591-600.

Han J, Guenier A-S, Salmieri S, Lacroix M. 2008. Alginate and Chitosan Functionalization for Micronutrient Encapsulation. J Agric Food Chem 56(7):2528-2535.

Haskell MJ. 2012. The challenge to reach nutritional adequacy for vitamin A: beta-carotene bioavailability and conversion--evidence in humans. Am J Clin Nutr 96(5):1193S-1203S.

Hedren E, Diaz V, Svanberg U. 2002. Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method. Eur J Clin Nutr 56(5):425.

Hollander D, Ruble P. 1978. Beta-carotene intestinal absorption: bile, fatty acid, pH, and flow rate effects on transport. Am J of Physiol 235(6):G686-G691.

Hornero-Méndez D, Mínguez Mosquera MI. 2007. Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. Innov Food Sci Emerg Technol 8(3):407-412.

Huo T, Ferruzzi MG, Schwartz SJ, Failla ML. 2007. Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. J Agric Food Chem 55(22):8950-8957.

Hur SJ, Lim BO, Decker Ea, McClements DJ. 2011. In vitro human digestion models for food applications. Food Chem 125(1):1-12.

Kern F, Birkner HJ, Ostrower VS. 1978. Binding of bile acids by dietary fiber. Am J Clin Nutr 31(10):S175-S179.

Kong F, Singh RP. 2010. A human gastric simulator (HGS) to study food digestion in human stomach. J Food Sci 75(9):E627-635.

Kowalski RE, Mergens WJ, Scialpi LJ, inventors. 2000. Process for manufacture of carotenoid compositions. U.S. Patent 6093348.

Leach G, Oliveira G, Morais R. 1998. Spray-drying of Dunaliella salina to produce a β-carotene rich powder. J Ind Microbiol Biotechnol 20(2):82-85.

Lemmens L, Van Buggenhout S, Van Loey AM, Hendrickx ME. 2010. Particle size reduction leading to cell wall rupture is more important for the β-carotene bioaccessibility of raw compared to thermally processed carrots. J Agric Food Chem 58(24):12769-12776.

Li Y, Hu M, Du Y, Xiao H, McClements DJ. 2011. Control of lipase digestibility of emulsified lipids by encapsulation within calcium alginate beads. Food Hydrocolloid 25(1):122-130.

Liang R, Huang Q, Ma J, Shoemaker CF, Zhong F. 2013. Effect of relative humidity on the store stability of spray-dried beta-carotene nanoemulsions. Food Hydrocolloid 33(2):225-233.

Liu C-S, Glahn RP, Liu RH. 2004. Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an *in vitro* digestion. J Agric Food Chem 52(13):4330-4337.

Liu Y, Hou Z, Lei F, Chang Y, Gao Y. 2012. Investigation into the bioaccessibility and microstructure changes of β-carotene emulsions during *in vitro* digestion. Innov Food Sci Emerg Technol 15:86-95.

Loksuwan J. 2007. Characteristics of microencapsulated β-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloid 21(5–6):928-935.

Martinsen A, Skjåk-Bræk G, Smidsrød O. 1989. Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. Biotechnol Bioeng 33(1):79-89.

McClements DJ, Li Y. 2010. Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Adv Colloid Interface Sci 159(2):213-228.

O'Sullivan L, Jiwan Ma, Daly T, O'Brien NM, Aherne SA. 2010. Bioaccessibility, uptake, and transport of carotenoids from peppers (Capsicum spp.) using the coupled *in vitro* digestion and human intestinal Caco-2 cell model. J Agric Food Chem 58(9):5374-5379.

Parker RS. 1996. Absorption, metabolism, and transport of carotenoids. FASEB J 10(5):542-551.

Peng H, Xiong H, Li J, Xie M, Liu Y, Bai C, Chen L. 2010. Vanillin cross-linked chitosan microspheres for controlled release of resveratrol. Food Chem 121(1):23-28.

Reboul E, Richelle M, Perrot Es, Desmoulins-Malezet C, Pirisi V, Borel P. 2006. Bioaccessibility of carotenoids and vitamin E from their main dietary sources. J Agric Food Chem 54(23):8749-8755.

Rein MJ, Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar SK, da Silva Pinto M. 2013. Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. Brit J Clin Pharmacol 75(3):588-602.

Rich GT, Bailey AL, Faulks RM, Parker ML, Wickham MS, Fillery-Travis A. 2003. Solubilization of carotenoids from carrot juice and spinach in lipid phases: I. Modeling the gastric lumen. Lipids 38(9):933-945.

Rich GT, Fillery-Travis A, Parker ML. 1998. Low pH enhances the transfer of carotene from carrot juice to olive oil. Lipids 33(10):985-992.

Rock CL, Lovalvo JL, Emenhiser C, Ruffin MT, Flatt SW, Schwartz SJ. 1998. Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. J Nutr 128(5):913-916.

Rock CL, Swendseid ME. 1992. Plasma beta-carotene response in humans after meals supplemented with dietary pectin. Am J Clin Nutr 55(1):96-99.

Rodriguez-Amaya DB. 2010. Quantitative analysis, *in vitro* assessment of bioavailability and antioxidant activity of food carotenoids: A review. J Food Compos Anal 23(7):726-740.

Roman MJ, Burri BJ, Singh RP. 2012. Release and Bioaccessibility of β-Carotene from Fortified Almond Butter during in Vitro Digestion. J Agric Food Chem 60(38):9659-9666.

Roodenburg AJ, Leenen R, van het Hof KH, Weststrate JA, Tijburg LB. 2000. Amount of fat in the diet affects bioavailability of lutein esters but not of α-carotene, β-carotene, and vitamin E in humans. Am J Clin Nutr 71(5):1187-1193.

Schweiggert RM, Mezger D, Schimpf F, Steingass CB, Carle R. 2012. Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. Food Chem 135(4): 2736-42.

Tang G, Serfaty-Lacrosniere C, Camilo ME, Russell RM. 1996. Gastric acidity influences the blood response to a beta-carotene dose in humans. Am J Clin Nutr 64(4):622-626.

Thürmann PA, Steffen J, Zwernemann C, Aebischer C-P, Cohn W, Wendt G, Schalch W. 2002. Plasma concentration response to drinks containing β-carotene as carrot juice or formulated as a water dispersible powder. Eur J Nutr 41(5):228-235.

Tyssandier V, Reboul E, Dumas J-F, Bouteloup-Demange C, Armand M, Marcand J, Sallas M, Borel P. 2003. Processing of vegetable-borne carotenoids in the human stomach and duodenum. Am J Physiol 284(6):G913-G923.

Wang P, Liu H-J, Mei X-Y, Nakajima M, Yin L-J. 2012. Preliminary study into the factors modulating β-carotene micelle formation in dispersions using an *in vitro* digestion model. Food Hydrocolloid 26(2):427-433.

West KP, Darnton-Hill I. 2001. Vitamin A deficiency. In: Semba RD, Bloem MW, editors. Nutrition and Health in Developing Countries. Totowa, New Jersey: Humana Press. p 267-306.

Wright AJ, Pietrangelo C, MacNaughton A. 2008. Influence of simulated upper intestinal parameters on the efficiency of beta carotene micellarisation using an *in vitro* model of digestion. Food Chem 107(3):1253-1260.

Yeum KJ, Russell RM. 2002. Carotenoid bioavailability and bioconversion. Annu Rev Nutr 22:483-504.

Yonekura L, Nagao A. 2007. Intestinal absorption of dietary carotenoids. Mol Nutr Food Res 51(1):107-115.

Yonekura L, Nagao A. 2009. Soluble fibers inhibit carotenoid micellization *in vitro* and uptake by Caco-2 cells. Biosci Biotechnol Biochem 73(1):196-199.

Yoo S-H, Song Y-B, Chang P-S, Lee HG. 2006. Microencapsulation of α-tocopherol using sodium alginate and its controlled release properties. Int J Biol Macromol 38(1):25-30.

CHAPTER 2

MICROENCAPSULATION OF BETA-CAROTENE IN CHITOSAN-ALGINATE AND EFFECT OF VISCOSITY ON IN VITRO RELEASE[1]

[1]Donhowe, E.G., Flores, F.P., and F. Kong. To be submitted to *Journal of Food Science.*

1. Abstract

Microencapsulation is an attractive method for preserving bioactive compounds for selective release during digestion. This study determined the effect of microcapsule formulation on particle size and encapsulation efficiency of beta-carotene by varying the alginate concentration (1, 2%) and the chitosan concentration (0, 0.5, 1%). Both alginate and chitosan significantly ($p<0.05$) affected particle size, which ranged from 852 to 958 μm. Increasing the alginate concentration corresponded to an increase in particle size (from an average of 883 to 940 μm), whereas increasing the chitosan corresponded to a decrease in particle size (from an average of 937 to 912 to 885 μm). Encapsulation efficiency increased with increased alginate concentration (from an average of 36 to 48%), whereas the addition of chitosan significantly increased encapsulation efficiency (from 35 to 46%), with no significant difference with increased concentration of chitosan. Further research was conducted to determine the effect of viscosity and the effect of digestion model on release. Using different guar gum concentrations (0, 0.1, 0.3, 0.5, 1%) to thicken the intestinal digesta, microcapsules that underwent complete digestion (salivary, gastric, and intestinal) were compared to microcapsules that went through intestinal digestion alone. Complete digestion significantly affected release, as complete release was observed only for the microcapsules that underwent all phases of digestion. Viscosity only significantly affected the release of the microcapsules subjected to intestinal digestion alone, ranging from a release of 96 to 2.3%. This suggests that the salivary and gastric phases cannot be eliminated when measuring *in vitro* release due to the facilitation of release during the intestinal phase.

2. Introduction

The food and pharmaceutical industries are actively researching encapsulation as a method of protecting and targeting delivery of lipophilic bioactive compounds during digestion (Burey and others 2008; Han and others 2008; McClements and others 2009; Li and others 2011). Ingestion of lipophilic bioactive compounds (such as beta-carotene) produces many health benefits, but these compounds are easily inactivated or degraded during digestion (de Vos and others 2010; McClements and Li 2010). Encapsulation ensures that these bioactive compounds survive the harsh acidity of the gastric environment to preferentially release in the small intestine, the site of absorption, to produce maximum nutritional efficacy and absorption (Parada and Aguilera 2007; Han and others 2008; McClements and Li 2010).

Alginate and chitosan have been identified as two attractive materials for microencapsulation because in addition to biocompatibility, chitosan-coated alginate beads can withstand acidic pH while degrading under mild basic conditions, making them ideal candidates for directing release in the small intestine (Martinsen and others 1989; de Vos and others 2010). Varying the alginate concentration and the chitosan concentration of the beads significantly affects encapsulation efficiency and particle size, making the composition of the chitosan-alginate beads an important variable to consider (Sezer and Akbuga 1999, Takka and Gürel 2010). Microencapsulation of beta-carotene in hydrogel microcapsules is typically performed using chitosan and alginate, but the effects of formulation parameters have yet to be studied (Han and others 2008, Roman and others 2012).

In vitro digestion models that mimic gastrointestinal conditions during digestion are commonly used to determine the extent of release of encapsulated compounds (Ekmekcioglu 2002). Studies designing microcapsules for enteric release often expose the microcapsules to

simulated gastric and intestinal fluids separately rather than sequentially to determine release properties in the GI tract (Lee and Rosenberg 2000; Yoo and others 2006; Anal and others 2006). Other studies have since shown the importance of gastric digestion prior to intestinal release as it can modify microcapsule release properties (Kosaraju and others 2009; Wang and others 2010; Calija and others 2013). Wang and others (2010) found that sequential digestion (gastric followed by intestinal) lead to almost complete release (98%) after 7 hours of digestion, whereas release was only ~60% after 7.5 hours of digestion in intestinal fluid alone.

Digesta viscosity is another important variable to consider when studying release from microcapsules due to the role of diffusion as a major mechanism of release (Lesmes and McClements 2009). Increasing viscosity decreases mass transfer, and as a result decreases mixing efficiency and convective mass transfer, all of which affect release (Lesmes and McClements 2009; Tharakan and others 2010). Research has shown that using guar gum to increase the viscosity influences starch digestion and reduces the digestibility of starch *in vitro* by reducing the extent of starch hydrolysis (Dartois and others 2010). Despite the demonstrably important role viscosity plays during digestion and release of microcapsules, the effect of digesta viscosity on release of microcapsules has not been adequately studied, especially in regards to beta-carotene.

Microencapsulation of beta-carotene is a burgeoning topic within literature, so the objectives of this study were to determine the effect of alginate and chitosan concentration on encapsulation efficiency of beta-carotene and particle size in addition to determining the effect of digestion method and intestinal viscosity on release of beta-carotene.

3. Materials and Methods

Sodium alginate was obtained by Acros (Fair Lane, NJ), and chitosan was obtained from Tokyo Chemical Industry (Tokyo, Japan). Water-dispersible beta-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), and guar gum (CAS: 9000-30-0, Lot: 041M0058V) were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were chemical grade. Deionized water was used throughout the study.

Simulated saliva was prepared according to the method of Kong and Singh (2010), whereas simulated gastric and intestinal juices were prepared according to Hur and others (2009). Stock solutions for each digestive juice (compositions presented in Table 2.1) 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 with 1 N NaOH or 1 N HCL (Table 2.1).

Preparation of chitosan-alginate beads

Microcapsules were prepared using two levels of alginate (1 and 2%) and three levels of chitosan (0, 0.5, and 1%) following the methods outlined in Han and others (2008). The microcapsule core solution was prepared by dissolving sodium alginate (1 or 2% w/v) in solution of 10% crystalline β-carotene (0.5% w/v) and deionized water. Microcapsules were formed by extruding the core solution through a 22 gauge needle into a calcium chloride solution (10% w/v) from a height of 5 cm at rate of 30 mL/hr using a Harvard Apparatus Dual Syringe pump (Holliston, MA), followed by a hardening time of 30 minutes. 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 (0.5 or 1%) and acetic acid (1%) and gently stirred for 1 hour, after which they were transferred to Whatman Ashless 42 filter paper to dry overnight. For uncoated alginate microcapsules, the beads were hardened in the calcium chloride solution for a total of 1 hour prior to transferring to Whatman Ashless 42 filter paper and drying overnight.

Particle size determination

Particle size analysis was conducted using a Beckman Coulter LS 13 320 Laser Diffraction Particle Size Analyzer with the Universal Liquid Module (Beckman Coulter, Inc., Fullerton, CA). The particle size for each formulation was determined from the mean of at least 3 replications.

Determination of encapsulation efficiency

Microcapsules (10 mg) were dissolved in sodium citrate (2% w/v) and the beta-carotene content was measured spectrophotometrically at 449 nm using a calibration curve. Encapsulation efficiency was calculated using the following equation:

$$Encapsulation\ efficiency\ (\%) = \frac{mass\ of\ \beta\text{-}carotene\ in\ sample}{theoretical\ mass\ of\ \beta\text{-}carotene\ in\ sample} * 100$$

Viscosity

Viscosity of the guar gum solutions was measured using a 20 mm cone and plate geometry with the SR5000 Dynamic Rheometer (Rheometrics Scientific, Piscataway, NJ). For the basic intestinal fluid (without guar gum), a 40 mm parallel plate geometry was used.

In vitro release studies

In vitro digestion was carried out following the protocol of Hur and others (2009) with slight modifications to test the effect of viscosity on release in addition to the effect of complete

Table 2.1: Compositions of simulated digestive juices

Saliva Stock Solution	**Gastric Stock Solution**	**Duodenal Stock Solution**	**Bile Stock Solution**
500 mL DI water	500 mL DI Water	500 mL DI Water	500 mL DI Water
0.0585 g NaCl	2.752 g NaCl	7.012 g NaCl	5.29 g NaCl
0.0745 g KCl	0.824 g KCl	0.564 g KCl	0.376 g KCl
1.05 g $NaHCO_3$	0.266 g NaH_2PO_4	3.388 g $NaHCO_3$	5.785 g $NaHCO_3$
	0.399 g $CaCl_2 \cdot 2H_2O$	0.08 g KH_2PO_4	
	0.306 g NH_4Cl	0.05 g $MgCl_2$	
0.2 g urea	0.085 g urea	0.1 g urea	0.25 g urea
	6.5 mL HCl	180 µL HCl	150 µL HCl
Simulated Saliva	**Simulated Gastric Juice**	**Simulated Duodenal Juice**	**Simulated Bile Juice**
Amount: 100 mL stock	Amount: 100 mL stock	Amount: 100 mL stock	Amount: 100 mL stock
0.1 g mucin	0.5 g pepsin	1.8 g pancreatin	6 g bile
0.2 g α-amylase	0.6 g mucin	0.3 g lipase	
pH: 6.8 +/- 0.2	pH: 1.30 +/- 0.02	pH: 8.1 +/- 0.2	pH: 8.2 +/- 0.2

digestion (salivary, gastric, and intestinal) versus direct digestion (intestinal digestion alone) on release. The effect of viscosity on release was studied by thickening the intestinal digesta with varying amounts of guar gum (final concentrations 0.1, 0.3, 0.5, 1% w/v), with 0% guar as a control. The study was constructed as a two-way design, with two levels for the effect of digestion (complete versus intestinal alone) and four levels for the effect of viscosity (corresponding to the differing guar gum concentrations).

Complete digestion was performed by adding 10 mg of microcapsules to 3 mL of simulated saliva in 125 mL stoppered Erlenmeyer flasks and mixing for 5 minutes in a 37°C orbital shaking water bath at 120 rpm to simulate salivary digestion. Gastric digestion was simulated by then adding 12 mL of simulated gastric juice to the flasks and mixing for an additional 2 hours. Intestinal digestion was simulated after filtering the beads using Whatman Ashless 42 filter paper by adding 12 mL of simulated duodenal juice (thickened with varying amounts of guar gum) and 6 mL of bile juice and mixing for an additional 2 hours. After intestinal digestion, samples were taken by filtering the beads using Whatman Ashless 42 filter paper and dissolving in sodium citrate (2% w/v) and analyzed using a UV-Vis spectrophotometer at 449 nm.

Direct digestion (intestinal digestion alone) was performed by adding 10 mg of microcapsules directly to 12 mL of duodenal juice (thickened with varying amounts of guar gum) and 6 mL of bile juice in 125 mL stoppered Erlenmeyer flasks and mixed for 2 hours using an orbital shaking water bath at 37°C and 120 rpm. After the 2 hours of mixing, samples were taken by filtering the beads and dissolving in sodium citrate (2% w/v) and analyzed using a UV-Vis spectrophotometer at 449 nm.

Statistical analysis

Experiments were done in replicate and analysis of variance (ANOVA) was performed with the method of digestion (complete, direct) and viscosity as fixed effects using SAS 9.3 (Cary, NC).

4. Results and Discussion

Particle size

Microcapsules from 852 to 958 μm diameters were produced through 6 different combinations of alginate and chitosan (Table 2.2). Increasing alginate concentration significantly increased the particle size ($p<0.05$) of the microcapsule, whereas increasing chitosan concentration significantly decreased the particle size ($p<0.05$).

As expected, the higher alginate concentration yields larger particles, since there are more anionic residues available for crosslinking with calcium ions during gelation. Coating with chitosan yielded smaller particles, which may be due to the exposure of alginate beads to an acidic solution of glacial acetic acid for coating with chitosan because chitosan is insoluble in neutral solutions. Alginate shrinks under acidic conditions, so the decrease in particle size after coating with chitosan may be due to the pH sensitivity of alginate (George and Abraham 2006, Pasparakis and Bouropoulos 2006, Ouwerx and others 1998). Electrostatic forces may also explain the decrease in particle size, since as a cationic polysaccharide, chitosan interacts with the anionic alginate gel beads to form an outer membrane through electrostatic binding (Ekmekcioglu 2002; Han and others 2008). The number of cationic residues in chitosan available for binding increases as chitosan concentration increases, yielding a stronger

Table 2.2: Effect of formulation on particle size and encapsulation efficiency

Alginate %	Chitosan %	Particle Size (μm)	Efficiency (%)
1%	0%	916^{a}±2	34^{x}±8
	0.5%	881^{b}±5	37^{x}±6
	1%	852^{c}±14	36^{x}±1
2%	0%	958^{d}±2	36^{x}±9
	0.5%	943^{e}±6	55^{y}±3
	1%	918^{a}±7	54^{y}±6

^{a,b,c,d,e,x,y}: Values followed by different letters in the same column are significant (α=0.05)

electrostatic force that decreases particle size and produces more compact particles (Douglas and Tabrizian 2005; Gazori and others 2009; Takka and Gürel 2010).

Encapsulation Efficiency

Encapsulation efficiencies from 34% to 55% were obtained from the different formulations and are consistent with values found in literature (Sezer and Akbuga 1999). Encapsulation efficiency increased significantly ($p<0.05$) both by increasing alginate concentration and by coating with chitosan. There was no significant difference ($p>0.05$) in encapsulation efficiency between 0.5% and 1% chitosan, but the chitosan-alginate beads had significantly higher encapsulation efficiencies ($p<0.05$) than the uncoated alginate beads. Increased alginate concentration yields more residues for binding with calcium, which increases the extent of cross-linking and may explain the increase in encapsulation efficiency (Joshi and others 2012).

Coating with chitosan has been shown to increase encapsulation efficiency by decreasing porosity of the resulting beads (Vandenberg and others 2001; Li and others 2002). Considering that no leakage of beta-carotene was observed during microencapsulation, the higher encapsulation efficiency may be due to the more compact particles produced after coating with

chitosan. The smaller particle size means that in a 10 mg sample there will be more chitosan-alginate beads than alginate beads, and thus a higher amount of beta-carotene.

In vitro release and complete digestion

The inclusion of salivary and gastric digestion significantly affected release from the microcapsules. Complete release from the microcapsules was achieved only when the microcapsules went through complete digestion, whereas complete release was not achieved when the microcapsules were digested in intestinal juices alone. These results are in accordance with literature, which found significant differences in release from sequential digestion versus exposure to simulated GI fluids separately (Kosaraju and others 2009; Wang and others 2010; Calija and others 2013). Since these microcapsules are designed for eventual incorporation into food products for fortification, complete digestion is more physiologically relevant because they will go through salivary and gastric digestion prior to intestinal digestion. Furthermore, the significant effect salivary and gastric digestion has on release indicates that directly digesting the microcapsules in simulated intestinal juice is not an appropriate method for estimating release during digestion.

Viscosity has a significant effect on release ($p<0.05$), but only when the microcapsules were directly subjected to intestinal digestion alone (Table 2.3). Viscosity had no inhibitory effect on the extent of release as the microcapsules completely released during complete digestion over all levels of viscosity and guar. These differences suggest that salivary and gastric digestion may affect the mechanism of release more significantly than viscosity.

For microencapsulated compounds, there are four major mechanisms of release: diffusion, swelling, fragmentation, and erosion (Matalanis and others 2011). Diffusive release is simply described as the diffusion of the compound through the encapsulating matrix, whereas

Table 2.3: Effect of guar gum concentration and viscosity on release of microcapsules directly added to simulated duodenal and bile juices

Guar (%)	Viscosity (Pa*s)	Release (mg)	Release (%)
0.0%	0.001	0.102^{a}±0.000	96.4^{a}±0.0
0.1%	0.547	0.101^{a}±0.003	95.6^{a}±2.5
0.3%	0.522	0.070^{b}±0.003	65.9^{b}±23.2
0.5%	1.031	0.056^{b}±0.011	53.5^{b}±10.8
1.0%	3.816	0.002^{c}±0.010	2.3^{c}±9.3

^{a,b,c}: Values followed by different letters in the same column are significant (α=0.05)

swelling release occurs via diffusion only after microcapsule absorption of the surrounding medium (Matalanis and others 2011). Fragmentation results in release from the physical degradation of the microcapsules into particle fragments, whereas erosion releases based on physical degradation of the outer layer (Matalanis and others 2011).

Increasing the guar gum concentration (which increases viscosity) decreases mass transfer and mixing, thus inhibiting release from diffusion and swelling (Tharakan and others 2010). Shear forces from the shaking water bath are reduced as viscosity increases, thus delaying or decreasing physical degradation during digestion and inhibiting the release from fragmentation and erosion. The inhibition of both diffusion and physical degradation thus explains the inverse relationship between viscosity and extent of release and why increasing viscosity decreases extent of release.

The complete release of microcapsules after exposure to the salivary and gastric phases suggests that the exposure to saliva and gastric juice promotes physical degradation of the microcapsules. It was observed after complete digestion that the microcapsules completely disintegrated but were not homogenously dispersed, indicating that the major mechanism of release was due to physical degradation rather than diffusion. Guar gum slows down the rate of

absorption in addition to digestion, so although complete release was achieved at high concentrations of guar, the beneficial effects from the absorption of beta-carotene may not be realized due to the inhibitive effect of increased viscosity on diffusion (Dartois and others 2010).

These results have promising implications for food fortification with these microcapsules. Since digesta viscosity increases upon consumption of food, viscosity may be one less factor to consider when selecting the ideal food matrix in which to deliver these microcapsules. Barring food matrix interactions, this allows for a wider range of products in which the microcapsules can be incorporated while still ensuring complete release, rather than having to limit incorporation to low-viscosity products such as juices or milks. Further research on the kinetics of release is necessary to validate these results, as release may be incomplete if the digestion time is shortened.

5. Conclusions

Alginate and chitosan concentration significantly impact size and encapsulation efficiency of beta-carotene microcapsules. Increased alginate concentration yields increased particle sizes and increased encapsulation efficiency, while coating with chitosan yields smaller particle sizes and increased encapsulation efficiency. Comparing direct and sequential digestion over 5 levels of guar gum revealed that gastric digestion significantly affects release. Viscosity was found to significantly affect release during direct digestion but not sequential digestion, as gastric digestion promotes degradation and led to complete release over all levels of guar gum.

6. References

Anal AK, Stevens WF, Remuñán-López C. 2006. Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. Int J Pharm 312(1):166-173.

Burey P, Bhandari BR, Howes T, Gidley MJ. 2008. Hydrocolloid Gel Particles: Formation, Characterization, and Application. Crit Rev Food Sci Nutr 48(5):361-377.

Calija B, Milic J, Cekic N, Krajišnik D, Daniels R, Savic S. 2013. Chitosan oligosaccharide as prospective cross-linking agent for naproxen-loaded Ca-alginate microparticles with improved pH sensitivity. Drug Dev Ind Pharm 39(1):77-88.

Dartois A, Singh J, Kaur L, Singh H. 2010. Influence of guar gum on the in vitro starch digestibility: Rheological and microstructural characteristics. Food Biophys 5(3):149-160.

de Vos P, Faas MM, Spasojevic M, Sikkema J. 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20(4):292-302.

Douglas KL, Tabrizian M. 2005. Effect of experimental parameters on the formation of alginate–chitosan nanoparticles and evaluation of their potential application as DNA carrier. J Biomater Sci, Polym Ed 16(1):43-56.

Ekmekcioglu C. 2002. A physiological approach for preparing and conducting intestinal bioavailability studies using experimental systems. Food Chem 76(2):225-230.

Gazori T, Khoshayand MR, Azizi E, Yazdizade P, Nomani A, Haririan I. 2009. Evaluation of alginate/chitosan nanoparticles as antisense delivery vector: Formulation, optimization and *in vitro* characterization. Carbohyd Polym 77(3):599-606.

George M, Emilia Abraham T. 2006. Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan — a review. J of Control Release 114(1)1-14.

Han J, Guenier A-S, Salmieri S, Lacroix M. 2008. Alginate and Chitosan Functionalization for Micronutrient Encapsulation. J Agric Food Chem 56(7):2528-2535.

Hur SJ, Decker Ea, McClements DJ. 2009. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during *in vitro* digestion. Food Chem 114(1):253-262.

Joshi S, Patel P, Lin S, Madan P. 2012. Development of cross-linked alginate spheres by ionotropic gelation technique for controlled release of naproxen orally. Asian J Pharm Sci 7(2):134-142.

Kong F, Singh RP. 2010. A human gastric simulator (HGS) to study food digestion in human stomach. J Food Sci 75(9):E627-635.

Kosaraju SL, Weerakkody R, Augustin MA. 2009. *In-vitro* evaluation of hydrocolloid–based encapsulated fish oil. Food Hydrocolloid 23(5):1413-1419.

Lee S, Rosenberg M. 2000. Microencapsulation of theophylline in whey proteins: effects of core-to-wall ratio. Int J Pharm 205(1):147-158.

Lesmes U, McClements DJ. 2009. Structure–function relationships to guide rational design and fabrication of particulate food delivery systems. Trends Food Sci Tech 20(10):448-457.

Li S, Wang X-t, Zhang X-b, Yang R-j, Zhang H-z, Zhu L-z, Hou X-p. 2002. Studies on alginate–chitosan microcapsules and renal arterial embolization in rabbits. J Control Release 84(3):87-98.

Li Y, Hu M, Du Y, Xiao H, McClements DJ. 2011. Control of lipase digestibility of emulsified lipids by encapsulation within calcium alginate beads. Food Hydrocolloids 25(1):122-130.

Martinsen A, Skjåk-Bræk G, Smidsrød O. 1989. Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. Biotechnol Bioeng 33(1):79-89.

Matalanis A, Jones OG, McClements DJ. 2011. Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydrocolloid 25(8):1865-1880.

McClements DJ, Decker EA, Park Y, Weiss J. 2009. Structural Design Principles for Delivery of Bioactive Components in Nutraceuticals and Functional Foods. Crit Rev Food Sci Nutr 49(6):577-606.

McClements DJ, Li Y. 2010. Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. Adv Colloid Interface Sci 159(2):213-228.

Ouwerx C, Velings N, Mestdagh MM, Axelos MAV. 1998. Physico-chemical properties and rheology of alginate gel beads formed with various divalent cations. Polym Gel Netw 6(5):393-408.

Parada J, Aguilera JM. 2007. Food Microstructure Affects the Bioavailability of Several Nutrients. J Food Sci 72(2):R21-R32.

Pasparakis G, Bouropoulos N. 2006. Swelling studies and *in vitro* release of verapamil from calcium alginate and calcium alginate–chitosan beads. Int J Pharm 323(1–2):34-42.

Roman MJ, Burri BJ, Singh RP. 2012. Release and Bioaccessibility of β-Carotene from Fortified Almond Butter during in Vitro Digestion. J Agric Food Chem 60(38):9659-9666

Sezer AD, Akbuga J. 1999. Release characteristics of chitosan treated alginate beads: II. Sustained release of a low molecular drug from chitosan treated alginate beads. J Microencapsulation 16(6): 1687-696.

Takka S, Gürel A. 2010. Evaluation of chitosan/alginate beads using experimental design: formulation and *in vitro* characterization. AAPS PharmSciTech 11(1):460-466.

Tharakan A, Norton IT, Fryer PJ, Bakalis S. 2010. Mass transfer and nutrient absorption in a simulated model of small intestine. J Food Sci 75(6):E339-346.

Vandenberg GW, Drolet C, Scott SL,de la Noüe J. 2001. Factors affecting protein release from alginate-chitosan coacervate microcapsules during production and gastric/intestinal simulation. J Control Release 77(3):297-307.

Wang Q, Xie X, Zhang X, Zhang J, Wang A. 2010. Preparation and swelling properties of pH-sensitive composite hydrogel beads based on chitosan-*g*-poly (acrylic acid)/vermiculite and sodium alginate for diclofenac controlled release. Int J Biol Macromol 46(3):356-362.

Yoo S-H, Song Y-B, Chang P-S, Lee HG. 2006. Microencapsulation of α-tocopherol using sodium alginate and its controlled release properties. Int J Biol Macromol 38(1):25-30.

CHAPTER 3

COMPARISON OF MICROENCAPSULATION METHODS AND IN VITRO BIOAVAILABILITY OF BETA-CAROTENE[2]

[2] Donhowe, E.G., Flores, F.P., Kong, F., Kerr, W.L., and L. Wicker. To be submitted to *LWT – Food Science, Technology.*

1. Abstract

This study compared the physical properties of three different types of microencapsulated beta-carotene (spray-dried maltodextrin powder, commercial water-dispersible powder, and chitosan-alginate microcapsules) in addition to performing *in vitro* digestion to determine bioavailability. The method used to microencapsulate beta-carotene had a significant effect on water activity (ranging from 0.195 to 0.279), moisture content (ranging from 3.5 to 16.5%), particle size (ranging from 10.5 to 942.8 μm). Beta-carotene spray-dried with maltodextrin had the highest surface beta-carotene (39.5%), whereas the surface beta-carotene of commercial water-dispersible powder and chitosan-alginate microcapsules were both less than 0. Encapsulation efficiency of the chitosan-alginate microcapsules (54.7%) was higher than the encapsulation efficiency of the spray-dried maltodextrin powder (39.5%). SEM was conducted and revealed unique morphologies based on the material used for microencapsulation. *In vitro* digestion trials revealed that the commercial beta-carotene powder had the highest extent of release (93.3%) and highest incorporation into the micelle phase (36.4%), compared to the spray-dried maltodextrin powder release (62.6%) and incorporation into the micelle phase (25.0%). The chitosan-alginate particles did not release (4.69%) or incorporate beta-carotene into the micelle phase (1.70%) to a large extent. Microencapsulation of beta-carotene must be tested *in vitro* to ensure that adequate bioavailability is achieved to effectively produce the intended health benefits.

2. 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 (West and others 2001; Grune and others 2010; Haskell 2012). Humans lack the ability to synthesize vitamin A *de novo*, so we must get proper amounts of it from our diets in the form of dark leafy green vegetables (spinach) and orange and yellow vegetables and fruits (carrots, mangoes) (West and others 2001; Haskell 2012). Beta-carotene has the the highest vitamin A activity compared to the other provitamin A carotenoids (alpha-carotenes, cryptoxanthins) and also the most efficient conversion to vitamin A (Yeum and Russell 2002; Grune and others 2010). Although its primary role as a nutrient is as a source of vitamin A, beta-carotene also has antioxidant capabilities and can function as a lipid radical scavenger and a singlet oxygen quencher due to its unique structure (Grune and others 2010).

The maximum absorption of beta-carotene from plant sources has been shown to be relatively low (~65%) and its bioavailability depends primarily on the food matrix in which the beta-carotene is located (Yeum and Russell 2002; Grune and others 2010; Haskell 2012). The low bioavailability of beta-carotene in plants has created an opportunity for the development of beta-carotene forms for supplementation and food fortification. There is no evidence from animal studies that beta-carotene is toxic or has harmful effects on health, indicating that reasonable beta-carotene supplementation and fortification is safe (Mathews-Roth 1988; Grune and others 2010). Furthermore, beta-carotene fortified foods are growing in number, as they contribute up to 30% of the daily vitamin A intake, depending on the region (Grune and others 2010). Beta-carotene supplementation can take many forms, including spray-dried powders, water-dispersible beadlets, polymer-coated microcapsules, and stabilized emulsions (Desobry

and others 1998; Thürmann and others 2002; Haskell 2012; Roman and others 2012; Qian and others 2012).

Spray drying and microencapsulating beta-carotene are two attractive methods for preserving beta-carotene, but their use must be justified by verifying bioavailability in addition to preserving functionality (Desobry and others 1998). In vitro digestion models have been developed to determine the bioavailability of beta-carotene by measuring the beta-carotene content of the micelle phase (Garrett and others 1999; Rodriguez-Amaya 2010). Beta-carotene must be incorporated into mixed micelles after release from the food or encapsulating matrix for proper absorption in the small intestine and maximum health benefits (Yeum and Russell 2002). Thus, the beta-carotene content of the micelle phase is an appropriate determinant of bioavailability. In addition to being cheaper, easier, and more reproducible than human trials, *in vitro* models have been validated due to their good correlation to *in vivo* results (Granado-Lorencio and others 2007; Rodriguez-Amaya 2010).

In addition to spray-dried powders and microcapsules, the development of water-dispersible beta-carotene holds promise because its bioavailability *in vivo* is significantly higher than that of carrot juice (Thürmann and others 2002). Additionally, water-dispersible beta-carotene has been shown to raise serum levels the most when compared against other supplement types (Fuller and others 2001). *In vitro* trials using Caco2 cells to simulate intestinal absorption have been conducted with water-dispersible beta-carotene with similar results, confirming its high bioavailability (Ferruzzi and others 2006).

Spray-drying pure beta-carotene for preservation and shelf-life stability was achieved by Desobry and others (1997) and Loksuwan (2007), but beta-carotene bioavailability and release during *in vitro* digestion has not yet been studied. Chitosan-alginate microcapsules of beta-

carotene developed for enteric release have been thoroughly studied in *in vitro* digestion trials, but bioavailability of beta-carotene from chitosan-alginate microcapsules has only recently been investigated (Roman and others 2012). The objectives of this study were to physically characterize spray-dried beta-carotene, water-dispersible beta-carotene, and beta-carotene chitosan-alginate microcapsules and to study release and incorporation into the micelle phase during *in vitro* digestion.

3. Materials and Methods

Sodium alginate was obtained by Acros (Fair Lane, NJ), and chitosan was obtained from Tokyo Chemical Industry (Tokyo, Japaan). Water-dispersible beta-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), and were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of chemical grade. Deionized water was used throughout the study.

Simulated saliva was prepared according to the method of Kong and Singh (2010), whereas simulated gastric and intestinal juices were prepared according to Hur and others (2009). Stock solutions for each digestive juice (compositions presented in Table 2.1) 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 with 1 N NaOH or 1 N HCl (Table 2.1).

Spray-drying

Spray-dried beta-carotene was produced following the methods of Desobry and others (1997). Briefly, 60 mL of a 40% maltodextrin 15 DE solution was prepared to which 0.03 g

beta-carotene was added. The mixture was homogenized using a PT-1200 Polytron hand-held homogenizer (Brinkmann Instruments, Westbury, NY) for 5 minutes at 25,000 rpm prior to spray drying and was continuously stirred using a stir-bar during spray drying. The feed solution was spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Switzerland) with an air inlet of 170±5°C and an outlet of 95±5°C and a pump speed of 25%.

Microencapsulation with alginate and chitosan

Microcapsules of 2% alginate (w/v) and 0.5% chitosan (w/v) were prepared as described previously. Due to the insolubility of beta-carotene in alginate solutions and to achieve a homogenous solution, water-dispersible beta-carotene was used following the methods of Han and others (2008).

Water activity and moisture content

Water activity of the samples (spray-dried maltodextrin powder, commercial water-dispersible powder, chitosan-alginate microcapsules) was measured using a Rotronic HygroLab C1 Water Activity indicator (Rotronic Instrument Corp., Hauppauge, NY). Moisture content of the samples was measured using a vacuum oven at 105°C.

Particle size

Particle size analysis was conducted using a Beckmann-Coulter LS 13 320 Laser Diffraction Particle Size Analyzer with the Universal Liquid Module (Beckman Coulter, Inc., Fullerton, CA). The particle size for each type of beta-carotene was determined from the mean of at least 3 replications.

Scanning electron microscopy

A Zeiss 1450EP scanning electron microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) was used to elucidate particle morphologies after samples were sputter coated with gold (Loksuwan 2007).

Surface beta-carotene, total beta-carotene

Surface beta-carotene and total beta-carotene were determined following the methods of Desobry and others (1997). For the total beta-carotene content of the chitosan-alginate beads, the methods used were the same as previously described.

Surface: 50 mg of sample was added to 25 mL of hexane in 50 mL centrifuge tubes and agitated for 30 seconds at 100 rpm. The supernatant was then measured spectrophotemetrically at 451 nm and carotene content was determined using a calibration curve.

Total: 50 mg of powder was mixed with 2.5 mL of deionized water in 50 mL centrifuge tubes. 25 mL of hexane was added, and agitated for 30 minutes at 500 rpm. The supernatant was then measured spectrophotometrically at 451 nm and carotene content was determined using a calibration curve.

Encapsulation efficiency

Encapsulation efficiency was determined using the following equation:

$$Encapsulation\ efficiency\ (\%) = \frac{total\ \beta\text{-}carotene\ -\ surface\ \beta\text{-}carotene}{theoretical\ \beta\text{-}carotene\ content}$$

In vitro digestion

In vitro digestion was performed using samples containing 0.528 mg beta-carotene (50 mg chitosan-alginate capsules, 686 mg maltodextrin powder, 5.28 mg water-dispersible powder) with the addition 1.2 mL of soybean oil following the digestion protocol of Hur and others (2009) using the simulated juices as mentioned previously. Salivary digestion was simulated by

adding 3 mL of simulated saliva to each sample and mixing for 5 minutes in stoppered 125 mL Erlenmeyer flasks in a 37°C orbital shaking water bath at 120 rpm. Gastric digestion was then initiated by adding 12 mL of simulated gastric juice to the flasks and mixing for an additional 2 hours. Intestinal digestion was simulated by adding 12 mL of simulated duodenal juice and 6 mL of simulated bile juice to the flasks, followed by mixing for an additional 2 hours. After intestinal digestion was complete, samples were taken and analyzed as described below.

Release

Release was measured following the methods of Roman and others (2012) and Biehler and others (2011). Aliquots of 2.5 mL filtered digesta were extracted with 5 mL of acetone/ethanol/hexane (1:1:2) at room temperature. The top layer was collected and 1 mL of hexane was used for an additional extraction. After extraction, beta-carotene content was measured using a UV-Vis spectrophotometer at 451 nm and a calibration curve.

Micelle

Micelle content was measured following the method of Roman and others (2012). Digesta samples were centrifuged for 40 minutes at 9,000 rpm at 4°C, after which 2.5 mL samples of the aqueous (middle) phase were collected. The extraction protocol previously described was followed to determine beta-carotene content.

Statistical analysis

Analysis of variance (ANOVA) was performed with the method of encapsulation as the fixed effect using SAS 9.3 (Cary, NC).

4. Results and Discussion

Water activity, moisture content

The moisture contents and water activities resulting from the different encapsulation methods can be found in Table 3.1. The method of encapsulation significantly affected ($p<0.05$) both the moisture content and water activities of the resulting products. Maltodextrin powder had the lowest moisture content, whereas the chitosan-alginate microcapsules had the lowest water activity. The incongruity of the chitosan-alginate beads having the lowest water activity but the highest moisture content could be due to the kinetics of evaporation. The surface moisture evaporates first, and since surface-area-to-volume ratio decreases as size increases, there is more moisture within the beads than on the surface. Porosity decreases as the beads lose moisture, in effect trapping the remaining moisture within the bead, thus explaining the high moisture content but low water activity. Since the particle size was not significantly different ($p>0.05$) between the two powders, the differences in moisture content and water activity of the powders can be attributed to differences in materials used and spray-drying operating conditions.

Particle size

Encapsulation method significantly affected ($p<0.05$) particle sizes (Table 3.1). There was no significant difference ($p>0.05$) between the spray-dried maltodextrin powder and the

Table 3.1: Effect of microencapsulation method on physical properties

Type	Water activity	Moisture content (%)	Mean diameter (µm)
Maltodextrin powder	$0.265^{a} \pm 0.010$	$3.5^{a} \pm 0.7$	$10.5^{a} \pm 0.2$
Water-dispersible powder	$0.279^{b} \pm 0.008$	$5.5^{b} \pm 0.1$	$14.7^{a} \pm 0.5$
Beads	$0.195^{c} \pm 0.006$	$16.5^{c} \pm 0.2$	$942.8^{b} \pm 6.5$

^{a,b,c}: Values followed by different letters in the same column are significantly different ($p<0.05$)

water-dispersible powder, whereas the chitosan alginate beads were significantly ($p<0.05$) larger. Given that the beads were produced using a 22 gauge needle (with an internal diameter of 413 μm), it was expected that the beads would be significantly larger than the spray-dried powders.

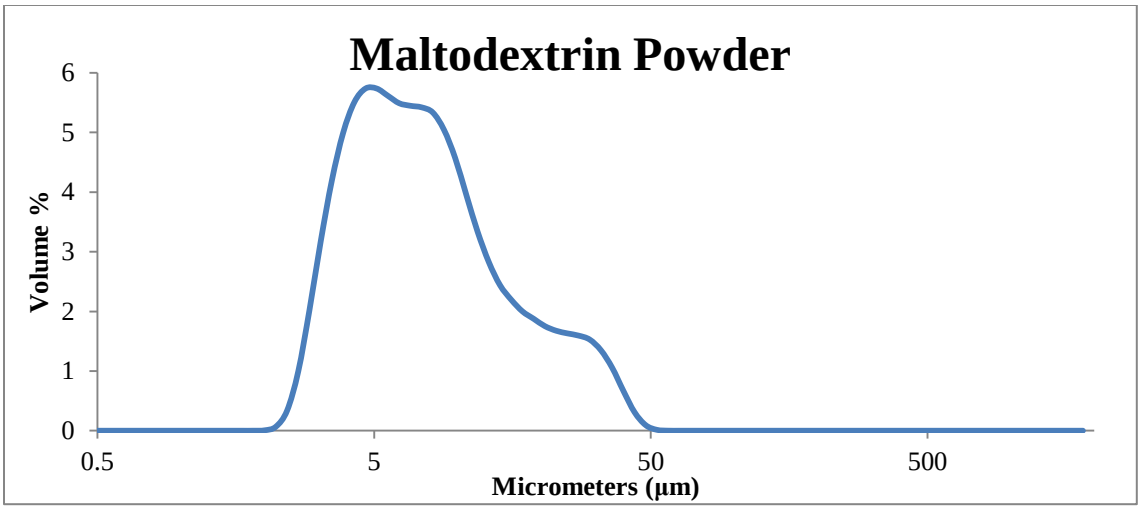
The particle size distributions can be seen in Figures 3.1 to 3.3. The gelation method produced the most uniformly sized beads, whereas the commercial powder had the widest range in sizes. The bimodal distribution of the maltodextrin powder and the high ratio between maltodextrin and beta-carotene concentration could suggest that the larger particle size represents the encapsulated beta-carotene, whereas the smaller peak could represent unencapsulated maltodextrin.

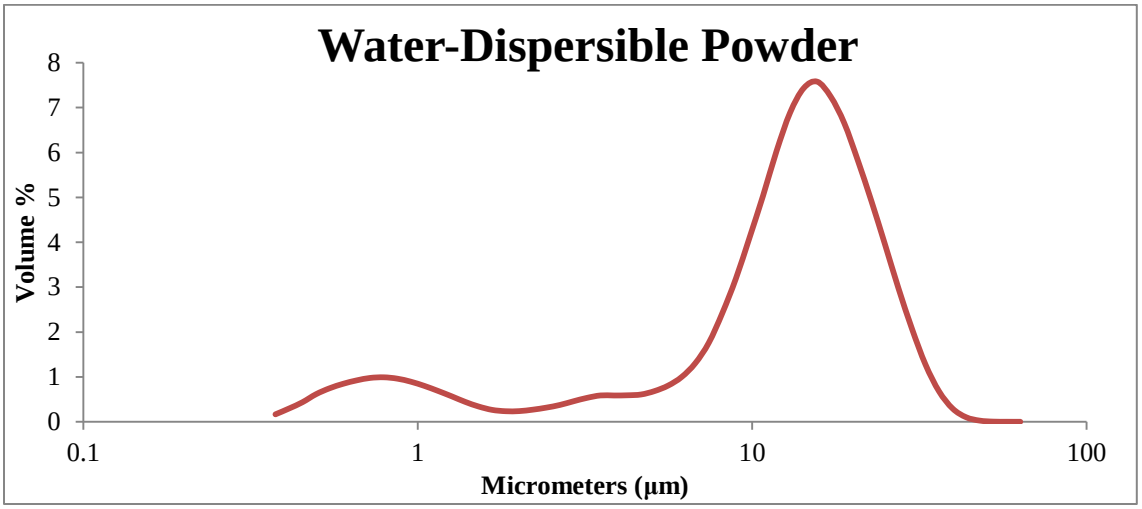
Surface beta-carotene, encapsulation efficiency

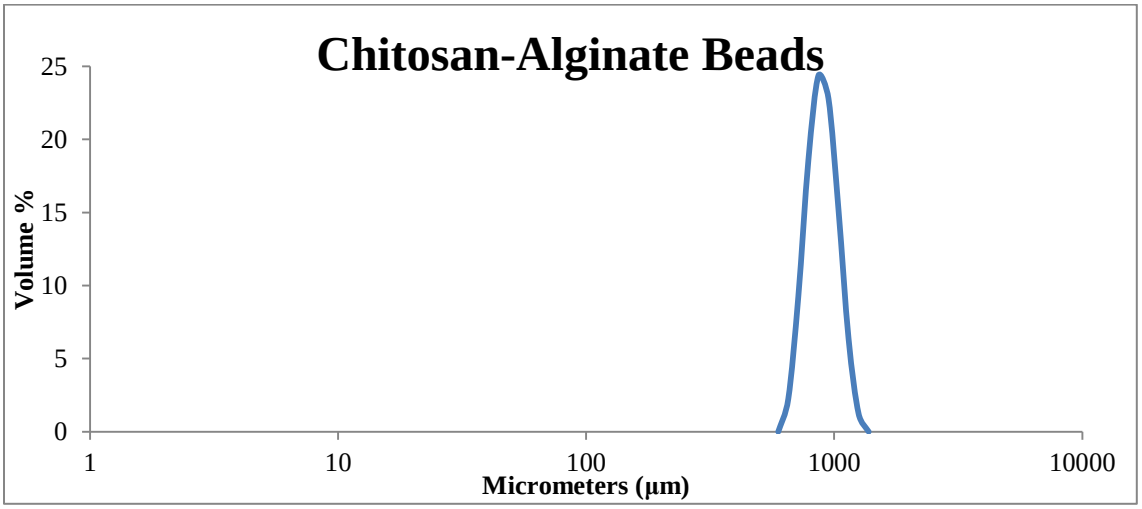
The surface beta-carotene and encapsulation efficiencies can be found in Table 3.2. The surface beta-carotene of the spray-dried beta-carotene powder was significantly higher ($p<0.05$) than both the chitosan-alginate beads and the commercial water-dispersible beta-carotene powder, but is within the range of expected values found in literature (Desobry and others 1997). The high surface content could be due to fact that the feed solution for spray drying was not emulsified, which would increase its homogeneity. Given the insolubility of beta-carotene in water, spray drying a beta-carotene emulsion would presumably increase the amount encapsulated and decrease amount of surface beta-carotene.

Table 3.2: Effect of microencapsulation method on surface *β*-carotene, total *β*-encapsulation efficiency

Type	Surface *β*-carotene	Total *β* -carotene	Encapsulation Efficiency
Maltodextrin powder	39.5%	0.08%	37.7%
Water-dispersible powder	0.004%	10%	-
Beads	0.0004%	1.06%	54.7%







Figures 3.1 (top), 3.2 (middle), 3.3 (bottom): Particle size distributions of β-carotene types

The encapsulation efficiency was also significantly lower ($p<0.05$) for the spray-dried beta-carotene than the chitosan-alginate capsules. This is expected given the maltodextrin powder has the highest amount of unencapsulated beta-carotene, which negatively affects its encapsulation efficiency.

Scanning electron microscopy

The morphologies of the different particles can be seen in Figures 3.4 to 3.6. Spray drying produced spherical particles with noticeable dents, which can be attributed to shrinkage during spray drying and the dextrose equivalence (DE) of the maltodextrin used (Loksuwan 2007). The higher the DE, the more smooth and spherical the resulting particle, since shorter glucose chains may be able to act as plasticizers and prevent shrinkage during spray drying (Loksuwan 2007). Chitosan-alginate beads have a relatively smooth and non-porous surface, which was expected given the cross-linking that occurs during gelation. The cracks may be due to dehydration, which can cause the collapse of polymer networks (Pasparakis and Bouropoulos 2006). The dents noticeable in water-dispersible beta-carotene may be due to the fact that gelatin is a major ingredient in its formulation and spray-dried gelatin forms dented microparticles without the addition of a suitable plasticizer (Kowalski and others 2000; Bruschi and others 2003).

In vitro digestion

The results of release and incorporation into the micelle phase are presented in Table 3.3. The type of beta-carotene (maltodextrin powder, water-dispersible powder, chitosan-alginate bead) has a significant effect ($p<0.05$) on both release and incorporation into the micelle phase.

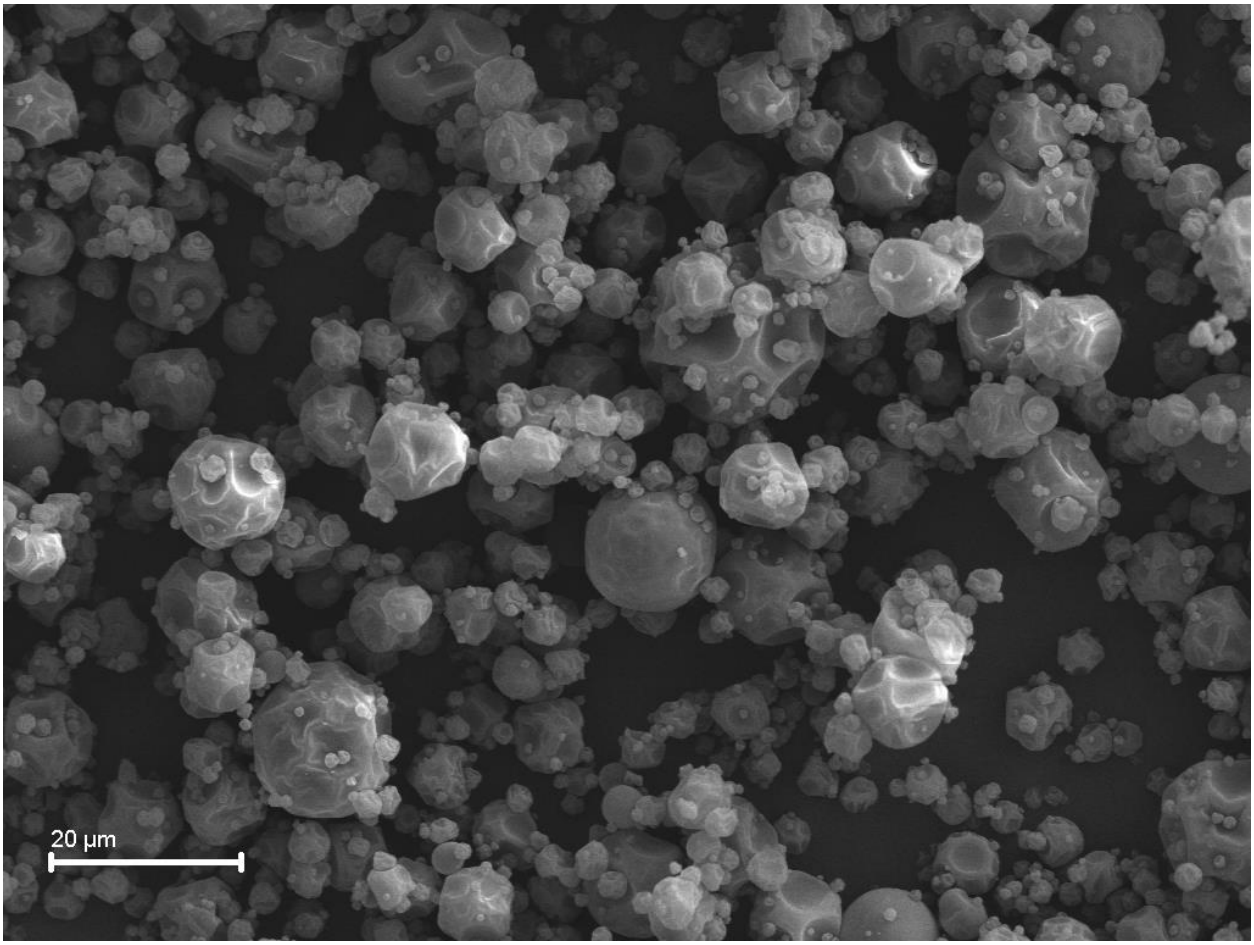


Figure 3.4: SEM of maltodextrin powder

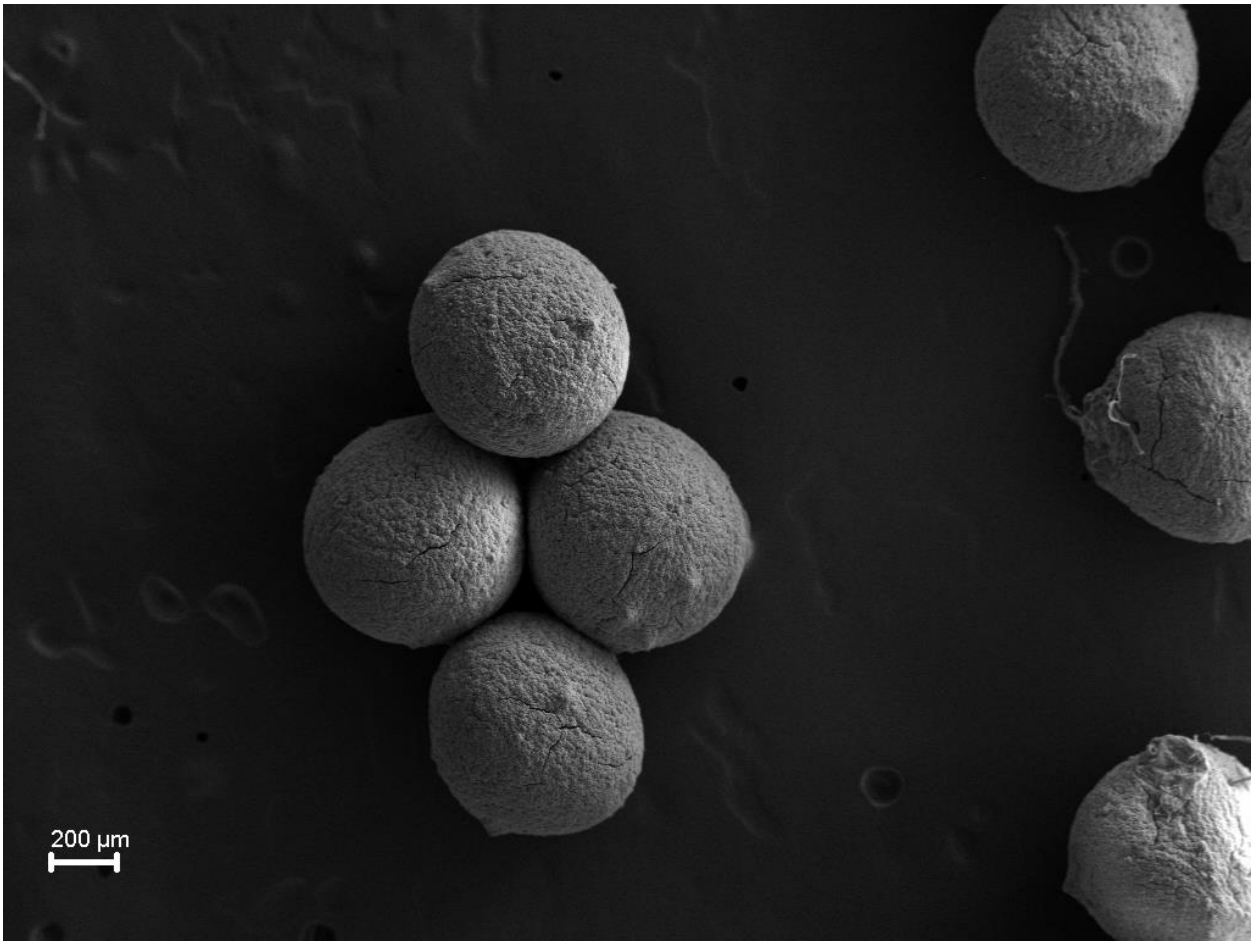


Figure 3.5: SEM of chitosan-alginate microspheres

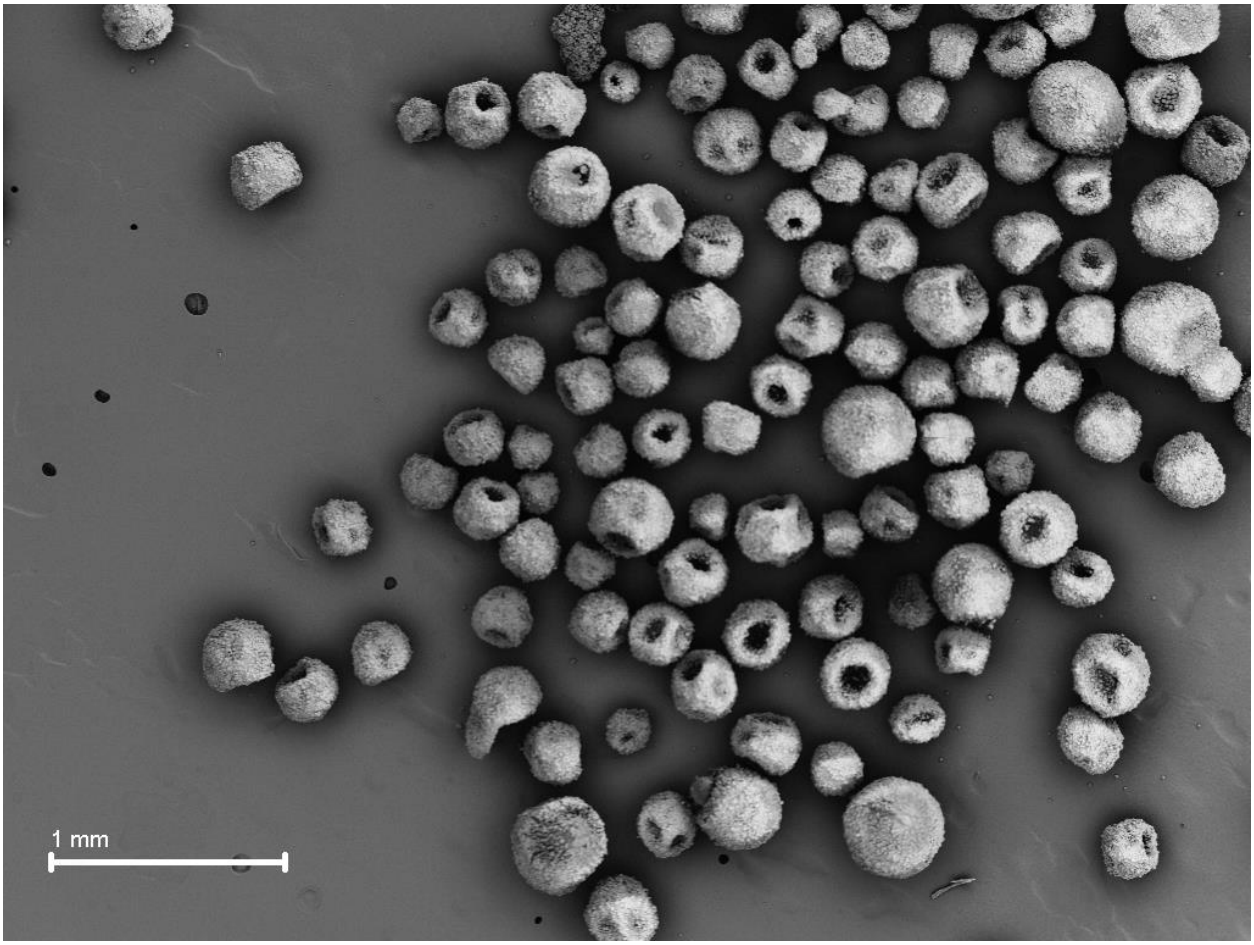


Figure 3.6: SEM of water-dispersible beta-carotene

Table 3.3: Effect of microencapsulation method on release and micelle incorporation

Type	Release		Micelle phase	
Maltodextrin powder	$0.314^{a} \pm 0.043$ mg	$62.6^{a} \pm 9.1\%$	$0.132^{a} \pm 0.018$ mg	$25.0^{a} \pm 2.3\%$
Water-dispersible powder	$0.494^{b} \pm 0.091$ mg	$93.3^{b} \pm 17.2\%$	$0.192^{b} \pm 0.025$ mg	$36.4^{b} \pm 4.7\%$
Chitosan-alginate beads	$0.025^{c} \pm 0.009$ mg	$4.7^{c} \pm 1.7\%$	$0.009^{c} \pm 0.008$ mg	$1.7^{c} \pm 1.5\%$

^{a,b,c}: Values followed by different letters in the same column are significantly different ($p<0.05$)

Release

Commercial water-dispersible beta-carotene had significantly higher release than either the maltodextrin powder or the chitosan-alginate beads. This could be due to the amount required for beta-carotene equivalency, since the commercial powder required the least amount to reach a value of 0.528 mg beta-carotene (the amount used in the digestion trial). The lower amount suggests that it takes less time to disperse during digestion, an important factor for water-dispersible forms of beta-carotene. This will likely be mitigated once incorporated into a food matrix due to the additional inhibition to dispersion and release. Additionally, since the commercial powder is a spray-dried emulsion, the beta-carotene is protected from degradation after release due to its presence in an emulsion (Wang and others 2012).

The maltodextrin powder requires the largest amount to reach 0.528 mg beta-carotene, and it was observed that it took longer than the commercial powder to fully disperse during gastric digestion. Since the maltodextrin powder is simply a spray-dried powder of maltodextrin and beta-carotene and considering the concentration of surface beta-carotene, the beta-carotene has a higher risk for gastric degradation during digestion (Cutting 2011). Research has also verified a linear relationship between concentration and rate of transfer to the oil phase, with a low concentration of beta-carotene leading to a slower rate of transfer to oil (Rich and others 1998). Since the maltodextrin powder is less concentrated than the commercial powder, the rate

of transfer to the oil phase is lower and leads to more opportunities for degradation of beta-carotene. Thus, the lower release compared to the commercial powder is probably primarily due to degradation prior to incorporation into the lipid phase.

Beta-carotene from the chitosan-alginate microcapsules failed to completely release during digestion, which could be due to type of alginate or type of chitosan selected for microencapsulation. Additionally, the chitosan-alginate microcapsules are encapsulating water-dispersible beta-carotene, which is a powder of gelatin and beta-carotene microcapsules. To achieve complete release, the water-dispersible powder must first release from the chitosan-alginate shell, followed by release of beta-carotene from the gelatin. Thus, the dual microencapsulation matrices inhibit release from the chitosan-alginate microcapsules significantly and adversely.

Micelle

Incorporation into the micelle phase is dependent on beta-carotene concentration (Wang and others 2012), so it makes sense that the differences in release translate into differences in micelle content. There is more beta-carotene released for water-dispersible beta-carotene, so there is more available for incorporation into mixed micelles. The difference between release and micelle incorporation for the water-dispersible beta-carotene can be attributed to the use of gelatin for encapsulation because soluble dietary fibers have been shown to reduce micelle formation (Kowalski and others 2000; Yonekura and Nagao 2009). The maltodextrin powder had significantly lower ($p<0.05$) beta-carotene content in the micelle phase than the water-dispersible formulation, which may be due to the lack of emulsifiers present in the powder. Soluble fibers (i.e. alginate) can bind to bile acids and can disrupt micelle structures, which explains the

significantly lower ($p<0.05$) micelle content from the chitosan-alginate beads compared to release (Yonekura and Nagao 2009).

5. Conclusions

Encapsulation method significantly affects water activity, moisture content, particle size, surface beta-carotene, encapsulation efficiency, and morphology of the resulting microcapsules. Ionotropic gelation of alginate and chitosan to microencapsulate beta-carotene yields significantly larger particles with a higher moisture content, whereas spray drying yields smaller particles with lower moisture content. Spray drying beta-carotene without an emulsifier yields a product with significantly higher surface beta-carotene and lower encapsulation efficiency than gelation. *In vitro* digestion revealed that the bioavailability is significantly affected by encapsulation method. The commercial water-dispersible beta-carotene had both the highest extent of release and the highest extent of beta-carotene incorporated into the micelle phase. The spray-dried maltodextrin powder had a lower extent of release and incorporation into the micelle phase, while the chitosan-alginate microcapsules had the lowest release and micelle content. Spray drying beta-carotene is thus validated as an acceptable method to preserve beta-carotene while also maintaining bioavailability, whereas microencapsulation with alginate and chitosan is not recommended due to the low amount incorporated into micelle phase during digestion.

6. References

Biehler E, Kaulmann A, Hoffmann L, Krause E, Bohn T. 2011. Dietary and host-related factors influencing carotenoid bioaccessibility from spinach (Spinacia oleracea). Food Chem 125(4):1328-1334.

Bruschi ML, Cardoso MLC, Lucchesi MB, Gremião MPD. 2003. Gelatin microparticles containing propolis obtained by spray-drying technique: preparation and characterization. Int J Pharm 264(1–2):45-55.

Cutting SM. 2011. *Bacillus* probiotics. Food Microbiol 28(2):214-220.

Desobry SA, Netto FM, Labuza TP. 1997. Comparison of Spray-drying, Drum-drying and Freeze-drying for β-Carotene Encapsulation and Preservation. J Food Sci 62(6):1158-1162.

Desobry SA, Netto FM, Labuza TP. 1998. Preservation of β-carotene from carrots. Crit Rev Food Sci Nutr 38(5):381-396.

Ferruzzi MG, Lumpkin JL, Schwartz SJ, Failla M. 2006. Digestive stability, micellarization, and uptake of β-carotene isomers by Caco-2 human intestinal cells. J Agric Food Chem 54(7):2780-2785.

Fuller CJ, Butterfoss DN, Failla ML. 2001. Relative bioavailability of β-carotene from supplement sources. Nutr Res 21(9):1209-1215.

Garrett DA, Failla ML, Sarama RJ. 1999. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. J Agric Food Chem 47(10):4301-4309.

Granado-Lorencio F, Olmedilla-Alonso B, Herrero-Barbudo C, Pérez-Sacristan B, Blanco-Navarro I, Blazquez-García S. 2007. Comparative *in vitro* bioaccessibility of carotenoids from relevant contributors to carotenoid intake. J Agric Food Chem 55(15):6387-6394.

Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, Thurnham D, Yin SA, Biesalski HK. 2010. Beta-carotene is an important vitamin A source for humans. J Nutr 140(12):2268S-2285S.

Haskell MJ. 2012. The challenge to reach nutritional adequacy for vitamin A: beta-carotene bioavailability and conversion--evidence in humans. Am J Clin Nutr 96(5):1193S-1203S.

Hur SJ, Decker EA, McClements DJ. 2009. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during *in vitro* digestion. Food Chem 114(1):253-262.

Kong F, Singh RP. 2010. A human gastric simulator (HGS) to study food digestion in human stomach. J Food Sci 75(9):E627-635.

Kowalski RE, Mergens WJ, Scialpi LJ, inventors. 2000. Process for manufacture of carotenoid compositions. U.S. Patent 6093348.

Loksuwan J. 2007. Characteristics of microencapsulated β-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloids 21(5–6):928-935.

Mathews-Roth MM. 1988. Lack of genotoxicity with beta-carotene. Toxicol Lett 41(3):185-191.

Pasparakis G, Bouropoulos N. 2006. Swelling studies and *in vitro* release of verapamil from calcium alginate and calcium alginate–chitosan beads. Int J Pharm 323(1–2):34-42.

Qian C, Decker EA, Xiao H, McClements DJ. 2012. Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. Food Chem 132(3):1221-1229.

Rich GT, Fillery-Travis A, Parker ML. 1998. Low pH enhances the transfer of carotene from carrot juice to olive oil. Lipids 33(10):985-992.

Rodriguez-Amaya DB. 2010. Quantitative analysis, *in vitro* assessment of bioavailability and antioxidant activity of food carotenoid: A review. J Food Compos Anal 23(7):726-740.

Roman MJ, Burri BJ, Singh RP. 2012. Release and Bioaccessibility of β-Carotene from Fortified Almond Butter during in Vitro Digestion. J Agric Food Chem 60(38):9659-9666.

Thürmann PA, Steffen J, Zwernemann C, Aebischer C-P, Cohn W, Wendt G, Schalch W. 2002. Plasma concentration response to drinks containing β-carotene as carrot juice or formulated as a water dispersible powder. Eur J Nutr 41(5):228-235.

Wang P, Liu H-J, Mei X-Y, Nakajima M, Yin L-J. 2012. Preliminary study into the factors modulating β-carotene micelle formation in dispersions using an *in vitro* digestion model. Food Hydrocolloids 26(2):427-433.

West KP, Darnton-Hill I. 2001. Vitamin A deficiency. In: Semba RD, Bloem MW, editors. Nutrition and Health in Developing Countries. Totowa, New Jersey: Humana Press. p 267-306.

Yeum KJ, Russell RM. 2002. Carotenoid bioavailability and bioconversion. Annu Rev Nutr 22:483-504.

Yonekura L, Nagao A. 2009. Soluble fibers inhibit carotenoid micellization *in vitro* and uptake by Caco-2 cells. Biosci Biotechnol Biochem 73(1):196-199.

CHAPTER 4

COMPARISON OF MICROENCAPSULATION METHOD AND IN VITRO BIOAVAILABILITY WITH FOOD MATRICES[3]

[3] Donhowe, E.G., Flores, F.P., Kong, F., Kerr, W.L., and L. Wicker. To be submitted to *LWT – Food Science, Technology*.

1. Abstract

Fortifying foods with beta-carotene is an emerging method to combat vitamin A deficiency and improve health worldwide. The bioavailability of beta-carotene, however, is significantly impacted by the food matrix used for delivery. This study researched the effect of yogurt and pudding on the release and bioavailability of beta-carotene using three different types of microecncapsulated beta-carotene: spray-dried maltodextrin powder, commercial water-dispersible powder, and chitosan-alginate microcapsules. The results showed that both microencapsulation method and food matrix significantly affect release and incorporation into the micelle phase. Commercial water-dispersible beta-carotene had the highest release (34.8% in pudding, 27.4% in yogurt) and incorporation into the micelle phase (17.0% in pudding, 5.5% in yogurt) regardless of food matrix. The pudding had significantly higher overall amounts incorporated into the micelle phase (0.24 to 17.0%), regardless of beta-carotene type. These results suggest that the composition of the food matrix may have a significant inhibitory effect on release and bioavailability during digestion.

2. Introduction

Beta-carotene is an important source of dietary vitamin A due to its efficient conversion to Vitamin A (Grune and others 2010; Haskell 2012). Beta-carotene is a lipophilic carotenoid that is not easily absorbed from natural plant sources, because one of the primary factors affecting beta-carotene bioavailability is the food matrix in which the beta-carotene is located (Yeum and Russell 2002; Reboul and others 2006; Grune and others 2010; Haskell 2012). Beta-carotene from plant sources is often found in protein complexes, which studies have shown may inhibit digestion and absorption (Yeum and Russell 2002). The complexity of the matrix has been shown to affect bioavailability, as beta-carotene in vegetables is less bioavailable than in rice or algae (Grune and others 2010).

Isolating and microencapsulating beta-carotene is one way to improve its bioavailability. Studies have shown that water-dispersible beta-carotene is more effective at increasing serum levels when compared to carrot juice and other beta-carotene supplements (Fuller and others 2001; Thürmann and others 2002). After isolation, beta-carotene is often used within the food industry either as a food colorant or to fortify foods to increase provitamin A content (Grune and others 2010).

Fortification of foods with beta-carotene could play an important role in decreasing vitamin A deficiency and is increasing in popularity, as currently ~10% of vitamin A intake in Germany comes from beta-carotene fortified foods (Grune and others 2010). The research on beta-carotene bioavailability from fortified foods is limited, though, and there is growing need to determine the effects of food matrix on bioavailability of beta-carotene (Roman and others 2012). Roman and others (2012) used almond butter as the food matrix to test the release and micelle incorporation of encapsulated and nonencapsulated beta-carotene and found that micelle

incorporation was significantly inhibited by the almond butter. Ranhotra and others (1995) determined the efficacy of incorporation of beta-carotene into whole wheat bread and crackers, but did not investigate the bioavailability of incorporated beta-carotene. Bioavailability is an important metric to justify fortification because fortified foods can only provide health benefits if the beta-carotene incorporated is bioavailable.

In vitro digestion has been validated as an acceptable method to predict absorption and bioavailability *in vivo* (Reboul and others 2006; Alminger and others 2012). Due to the metabolic pathway of beta-carotene during digestion, bioavailability of beta-carotene is measured from the concentration of beta-carotene in the micelle phase. After release from the food matrix, an important step that occurs during salivary and gastric digestion, beta-carotene is incorporated into the lipid phase of a gastric emulsion. Passage to the small intestine and excretion of bile salts leads to the formation of mixed micelles of beta-carotene. Intestinal epithelial cells then absorb beta-carotene from the micelles primarily through passive diffusion. Since the concentration in the micelle phase is correlated to the amount absorbed, *in vitro* studies can mimic GI digestion and get physiologically relevant results (Wang and others 2012; Alminger and others 2012).

The objective of this study was to determine the effect of *in vitro* digestion of three different types of beta-carotene (spray-dried maltodextrin powder, commercial water-dispersible powder, chitosan-alginate beads) with two food matrices (fat-free yogurt, fat-free tapioca pudding) on release and incorporation into the micelle phase.

3. Materials and Methods

Dannon Fat-free Plain Yogurt and Jell-O Fat-free Tapioca Pudding were purchased from the supermarket and used as a protein-rich food matrix and a carbohydrate-rich food matrix. Sodium alginate was obtained by Acros (Fair Lane, NJ), and chitosan was obtained from Tokyo Chemical Industry (Tokyo, Japan). Water-dispersible beta-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), and were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were chemical grade. Deionized water was used throughout the study.

Simulated saliva was prepared according to the method of Kong and Singh (2010), whereas simulated gastric and intestinal juices were prepared according to Hur and others (2009) as described in previous chapters.

pH

pH of the food matrices was tested using a metal pH-piercing probe (Model pH 77-SS, IQ Scientific, HACH, Loveland, CO) that was calibrated with pH 4.0 and 7.0 standard buffer solutions (SB101-500, SB107-500, Fisher Scientific, Fairlawn, NJ) before testing the samples.

In vitro digestion

In vitro digestion was performed using an adapted test meal from Ferruzzi and others (2006). 0.528 mg beta-carotene (686 mg maltodextrin powder, 5.28 mg water-dispersible powder, 50 mg chitosan-alginate beads), 1.2 mL soybean oil, and 8 g of food matrix (yogurt or pudding) were digested following the protocol of Hur and others (2009) and as described previously in Chapter 3. Release and micelle contents were measured following the same

protocol as described previously in Chapter 3.

Statistical analysis

Analysis of variance (ANOVA) was performed with the method of encapsulation and food matrix as fixed effects using SAS 9.3 (Cary, NC). Experiments were done in replicate.

4. Results and Discussion

The results from *in vitro* digestion can be found in Tables 4.1 and 4.2. The food matrix and the type of beta-carotene significantly affected release and incorporation into the micelle phase ($p<0.05$).

Beta-carotene release during digestion

The food matrix significantly affected release ($p<0.05$) during digestion. The addition of a food matrix presents competition for digestion, which may decrease the extent of digestion of the beta-carotene type. This is supported by the finding that digestion with a food matrix significantly decreased release ($p<0.05$) when compared to digestion without a food matrix. Additionally, yogurt and pudding are viscous foods that presumably delay digestion of the beta-carotene type by delaying contact with the gastric juices until structural degradation occurs. The digestion of food matrices produces solids and semi-solids that may inhibit transfer of beta-carotene to the oil phase due to steric hindrances at the oil-water interface. More research is

Table 4.1: Effect of microencapsulation method and food matrix on extent of release

Type	Pudding		Yogurt	
Maltodextrin powder	0.035^{a}±0.001 mg	6.6^{a}±0.1%	0.160^{a}±0.010 mg	30.3^{a}±1.9%
Water-dispersible powder	0.184^{b}±0.001 mg	34.8^{b}±0.2%	0.145^{b}±0.007 mg	27.4^{b}±1.3%
Chitosan-alginate beads	0.004^{c}±0.001 mg	0.7^{c}±0.3%	0.002^{c}±0.001 mg	0.5^{c}±0.1%

^{a,b,c} Values followed by different letters in the same column are significantly different ($p<0.05$)

needed to elucidate what factors significantly affect the transfer of beta-carotene to the oil phase during gastric digestion (Rich and others 1998; Rich and others 2003).

Release of beta-carotene with yogurt was significantly lower ($p<0.05$) than the release of beta-carotene with pudding, excluding the maltodextrin powder. One study has shown that digestion with yogurt significantly decreases lipid digestion in the gastric phase due to coagulation (Shen and others 2011). This would explain the effect of yogurt on release, since coagulation of the food matrix would impede digestion of both the yogurt and the beta-carotene type. Additionally, research has shown that soluble proteins inhibit beta-carotene transfer to oil, thus inhibiting release from the food matrix (Rich and others 2003). Since the protein content of yogurt was higher than the the pudding, there was more protein-based inhibition of transfer of beta-carotene to the oil phase leading to lower release.

The type of beta-carotene significantly affected release ($p<0.05$), with results comparable to the digestion without food matrices. This indicates that the form of beta-carotene is an important factor to consider regardless of food matrix. Water-dispersible beta-carotene is still the best for release, followed by the maltodextrin powder and the beads releasing the least. The significant differences between the types of beta-carotene can be attributed to the differences in release found during digestion without a food matrix.

The pH of the pudding was 6.57 and the pH of the yogurt was 4.34, which may have impacted release. Transfer of beta-carotene to the oil phase is facilitated by lowering the pH during gastric digestion, but given the release of yogurt versus pudding, suggests that composition more significantly affected the release than a difference in pH (Rich and others 1998).

Table 4.2: Effect of microencapsulation method and food matrix on micelle incorporation

Type	Pudding		Yogurt	
Maltodextrin powder	0.069^{a}±0.015 mg	13.1^{a}±2.8%	0.004^{a}±0.001 mg	0.8^{a}±0.1%
Water-dispersible powder	0.090^{b}±0.018 mg	17.0^{b}±3.5%	0.029^{b}±0.007 mg	5.5^{b}±1.4%
Chitosan-alginate beads	0.001^{c}±0.001 mg	0.2^{c}±0.15%	0.001^{c}±0.000 mg	0.2^{c}±0.07%

^{a,b,c}: Values followed by different letters in the same column are significantly different ($p<0.05$)

Beta-carotene micelle incorporation during digestion

The food matrix and type of beta-carotene also both significantly affected micelle incorporation ($p<0.05$) during digestion. This was expected given the significant effect of food matrix and beta-carotene type on release, as the amount released contributes to the amount available for incorporation into the micelle phase.

Beta-carotene content of the micelle phase was significantly lower ($p<0.05$) when yogurt was the food matrix. This could be due to the protein composition of yogurt, since casein binds to bile salts during *in vitro* and *in vivo* studies (Sklan 1980; Miller and others 1990). Bile acids play a crucial role in stabilizing the emulsion from the gastric phase and in the formation of mixed micelles (Yeum and Russell 2002; Yonekura and Nagao 2007; Wang and others 2012). Binding to bile acids reduces the amount available for stabilization of the gastric emulsion and micelle formation for intestinal absorption. This decreases the amount of micelles formed, which leads to a decrease in beta-carotene content of the micelle phase. Pectin was present in the yogurt, which has also been shown to inhibit micelle formation by binding to bile salts and destroying micelle structures (Yonekura and Nagao).

The pH of the food matrices likely did not affect micelle formation, as Wright and others (2008) found that pH did not significantly affect micelle formation under fed conditions when the pH ranged from 5.0 to 9.5. Given that the pH of the *in vitro* digestive system used equilibrates to a pH of ~6.5 after 2 hours of gastric digestion and 2 hours of intestinal digestion

in the absence of a food matrix, adding a food matrix would not skew the pH of the intestinal digesta outside of the pH range 5.0 to 9.5.

The type of beta-carotene significantly affected incorporation into the micelle phase ($p<0.05$), with results comparable to digestion without food matrices. Water-dispersible beta-carotene, regardless of food matrix, is the most bioavailable type of beta-carotene, followed by spray-dried beta-carotene with maltodextrin, and then chitosan-alginate beads.

5. Conclusions

Digestion of beta-carotene with a food matrix significantly decreased both the release and incorporation into the micelle phase. Between the food matrices, yogurt significantly decreased both release and micelle content as compared to pudding. The type of beta-carotene significantly affected release during digestion with food matrices, with water-dispersible beta-carotene being the most desirable for its effective release and incorporation into the micelle phase. Thus, both the type of beta-carotene and the food matrix are important variables to consider during the formulation of fortified foods. Judging by the amount in the micelle phase, beta-carotene was most bioavailable in pudding and should be used instead of yogurt as the food matrix for beta-carotene fortification.

6. References

Alminger M, Svelander C, Wellner A, Martinez-Tomas R, Bialek L, Larque E, Perez-Llamas F. 2012. Applicability of in Vitro Models in Predicting the in Vivo Bioavailability of Lycopene and β-Carotene from Differently Processed Soups. Food Nutr 3:477-489.

Ferruzzi MG, Lumpkin JL, Schwartz SJ, Failla M. 2006. Digestive stability, micellarization, and uptake of β-carotene isomers by Caco-2 human intestinal cells. J Agric Food Chem 54(7):2780-2785.

Fuller CJ, Butterfoss DN, Failla ML. 2001. Relative bioavailability of β-carotene from supplement sources. Nutr Res 21(9):1209-1215.

Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, Thurnham D, Yin SA, Biesalski HK. 2010. Beta-carotene is an important vitamin A source for humans. J Nutr 140(12):2268S-2285S.

Haskell MJ. 2012. The challenge to reach nutritional adequacy for vitamin A: beta-carotene bioavailability and conversion--evidence in humans. Am J Clin Nutr 96(5):1193S-1203S.

Hur SJ, Decker EA, McClements DJ. 2009. Influence of initial emulsifier type on microstructural changes occurring in emulsified lipids during in vitro digestion. Food Chem 114(1):253-262.

Kong F, Singh RP. 2010. A human gastric simulator (HGS) to study food digestion in human stomach. J Food Sci 75(9):E627-635.

Miller MJS, Witherly SA, Clark DA. 1990. Casein: A Milk Protein with Diverse Biologic Consequences. Exp Biol and Med 195(2):143-159.

Ranhotra G, Gelroth J, Langemeier J, Rogers D. 1995. Stability and contribution of beta carotene added to whole wheat bread and crackers. Cereal Chem 72(2):139-141.

Reboul E, Richelle M, Perrot ES, Desmoulins-Malezet C, Pirisi V, Borel P. 2006. Bioaccessibility of carotenoids and vitamin E from their main dietary sources. J Agric Food Chem 54(23):8749-8755.

Rich GT, Bailey AL, Faulks RM, Parker ML, Wickham MS, Fillery-Travis A. 2003. Solubilization of carotenoids from carrot juice and spinach in lipid phases: I. Modeling the gastric lumen. Lipids 38(9):933-945.

Rich GT, Fillery-Travis A, Parker ML. 1998. Low pH enhances the transfer of carotene from carrot juice to olive oil. Lipids 33(10):985-992.

Roman MJ, Burri BJ, Singh RP. 2012. Release and bioaccessibility of β-carotene from Fortified Almond Butter during in vitro digestion. J Agric Food Chem 60(38):9659-9666.

Shen Z, Apriani C, Weerakkody R, Sanguansri L, Augustin MA. 2011. Food matrix effects on in vitro digestion of microencapsulated tuna oil powder. J Agric Food Chem 59(15):8442-8449.

Sklan D. 1980. Digestion and absorption of casein at different dietary levels in the chick: effect on fatty acid and bile acid absorption. J Nutr 110(5):989-994.

Thürmann PA, Steffen J, Zwernemann C, Aebischer C-P, Cohn W, Wendt G, Schalch W. 2002. Plasma concentration response to drinks containing β-carotene as carrot juice or formulated as a water dispersible powder. Eur J Nutr 41(5):228-235.

Wang P, Liu H-J, Mei X-Y, Nakajima M, Yin L-J. 2012. Preliminary study into the factors modulating β-carotene micelle formation in dispersions using an in vitro digestion model. Food Hydrocolloids 26(2):427-433.

Yeum KJ, Russell RM. 2002. Carotenoid bioavailability and bioconversion. Annu Rev Nutr 22:483-504.

Yonekura L, Nagao A. 2007. Intestinal absorption of dietary carotenoids. Mol Nutr Food Res 51(1):107-115.

Yonekura L, Nagao A. 2009. Soluble fibers inhibit carotenoid micellization in vitro and uptake by Caco-2 cells. Biosci Biotechnol Biochem 73(1):196-199.