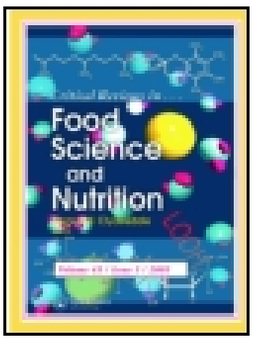

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Emulsion design for the delivery of β-carotene in complex food systems

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ABSTRACT

β-Carotene has been widely investigated both in the industry and academia, due to its unique bioactive attributes as an antioxidant and pro-vitamin A. Many attempts were made to design delivery systems for β-carotene to improve its dispersant state and chemical stability, and finally to enhance the functionality. Different types of oil-in-water emulsions were proved to be effective delivery systems for lipophilic bioactive ingredients, and intensive studies were performed on β-carotene emulsions in the last decade. Emulsions are thermodynamically unstable, and emulsions with intact structures are preferable in delivering β-carotene during processing and storage. β-Carotene in emulsions with smaller particle size has poor stability, and protein-type emulsifiers and additional antioxidants are effective in protecting β-carotene from degradation. Recent development in the design of protein-polyphenol conjugates has provided a novel approach to improve the stability of β-carotene emulsions. When β-carotene is consumed, its bioaccessibility is highly influenced by the digestion of lipids, and β-carotene in smaller oil droplets containing long-chain fatty acids has a higher bioaccessibility. In order to better deliver β-carotene in complex food products, some novel emulsions with tailor-made structures have been developed, e.g., multilayer emulsions, solid lipid particles, Pickering emulsions. This review summarizes the updated understanding of emulsion-based delivery systems for β-carotene, and how emulsions can be better designed to fulfill the benefits of β-carotene in functional foods.

KEYWORDS

β-carotene; emulsion; stability; delivery system; bioaccessibility

1. Introduction

Carotenoids are a group of natural colorants widely distributed in fruits and vegetables, and have been used in food, cosmetic, and pharmaceutical products for a long history. Along with their coloring functions, carotenoids are well known for their health benefits as pro-vitamin A, and as antioxidants to prevent many chronic diseases, e.g., cancer, cardiovascular disease, macular degeneration (Rodriguez-Amaya, 2015). Among different carotenoids, β-carotene has gained particular attention because of its wide distribution in nature and strong bioactivities (Weber and Grune, 2012; Johnson, 2002). Intensive studies have thus been performed in recent years to incorporate β-carotene into functional foods, and to fulfill its health potentials.

β-Carotene is lipophilic with a highly unsaturated structure (Figure 1), which makes it insoluble in water and liable to degradation. Furthermore, naturally occurring β-carotene is usually involved in protein complexes, which restrains its adsorption by human body. Therefore, β-carotene is difficult to be added into food systems, and has low bioavailability (Donhowe and Kong, 2014). To overcome these disadvantages, a lot of studies have been carried out to encapsulate β-carotene into different delivery systems (e.g., gels, emulsions, powder) to improve its dispersant state, chemical stability in foods and bioavailability upon ingestion (Loksuwan, 2007; Mao et al., 2009; Belščak-Cvitanović et al., 2016).

It is known that β-carotene can gain improved bioavailability when co-ingested with lipids, as the digested products of lipids form mixed micelles, which are capable to solubilize and transport β-carotene to epithelium cells (van Het Hof et al., 2000; Borel 2003). This finding implies that food emulsions may be ideal delivery systems for β-carotene for its addition into functional foods (McClements, 2010; McClements and Li, 2010). Lipophilic functional food ingredients (e.g., β-carotene) can be incorporated into the oil droplets of an O/W emulsion, and thus be protected by the water phase and oil-water interface against external attacks. Many studies taking emulsions as delivery systems showed that functional ingredients incorporated in emulsions had improved stability against degradation and were more easily dispersed in water phase. More importantly, it is possible to control the release of the ingredients through adjusting emulsion structures, which would be critical to improve the bioavailability of the ingredients (Velikow and Pelan, 2008; McClements and Li, 2010; Mao et al., 2015). In this context, intensive researches on β-carotene emulsions have been performed in the last 10–15 years, and emulsions with different formulations and structures have thus been designed.

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[1]The authors contributed equally to this work.

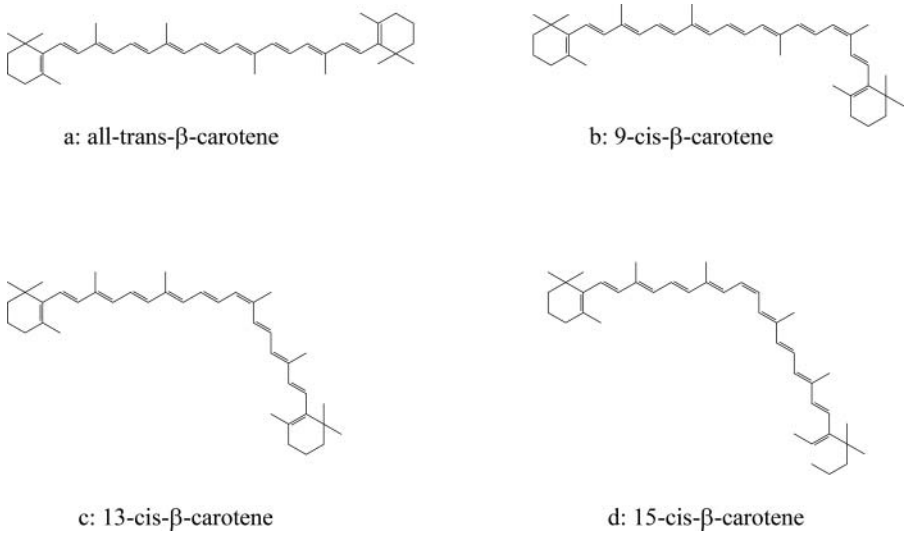

Figure 1. Chemical structures of different β-carotene isomers.

Although several nice reviews have already been published focusing on factors influencing the chemical stability of carotenoids (Boon et al., 2010), encapsulation techniques to adjust the stability or bioavailability of carotenoids (Soukoulis and Bohn, 2015), no systematic review is currently available regarding emulsion-based delivery systems for β-carotene, which have shown increasing importance in the designing of functional foods.

This article is aiming to present a comprehensive review of the knowledge in designing food emulsions as delivery systems for β-carotene, and the chemical and biochemical characteristics of β-carotene emulsions under different circumstances. In this review, a brief introduction about β-carotene and food emulsions is made. More emphasis are then turned to factors determining the physicochemical properties of β-carotene emulsions, particularly β-carotene stability in emulsions, and the release properties and bioaccessibility of β-carotene upon in vivo or in vitro digestion. In the last part of the review, several novel structured emulsions which are able to deliver β-carotene in more complex food systems are discussed.

2. Structures and functions of β-carotene

Figure 1 (a) illustrates the molecular structure of all-trans-β-carotene, which is characterized by a polyene structure with 11 conjugated double bonds and two β-rings. Under certain conditions, it can be transformed into different isomers, e.g., 9-cis- b-carotene, 13-cis- b-carotene, 15-cis- b-carotene" corrected as "9-cis-b-carotene, 13-cis-b-carotene, 15-cis-b-carotene (Figure 1 b, c, d) (Imsic et al., 2010; Knockaert et al., 2012). All-trans-β-carotene is a linear and rigid molecule, and is more likely to crystallize and aggregate than the cis-isomers. Cis-isomers are of bent structures, and are regarded to be more readily solubilized and adsorbed (Schieber and Carle, 2005; Rodriguez-Amaya, 2015).

β-Carotene has found wide applications in the foods and pharmaceuticals, as a colorant, but also as a nutraceutical ingredient. Dietary carotenoids are main sources of vitamin A, which is important for healthy skin and mucus membranes, a good immune system, and good eye health (Johnson, 2002; Grune et al., 2010). Moreover, β-carotene is a strong antioxidant and acquires protective effects against many cancers and cardiovascular diseases (Krinsky and Johnson, 2005). The antioxidant properties of β-carotene are due to its exceptional capability to scavenge free-radicals and to quench singlet oxygen ($^1O_2$). β-Carotene quenches $^1O_2$ mostly through a physical mechanism (Stahl and Sies, 1993), where excitation energy of $^1O_2$ is transferred to β-carotene, and the excited triplet state β-carotene dissipates the energy through rotation and vibrational interactions with surrounding solvents and then returns to the ground state. β-Carotene is also able to quench $^1O_2$ chemically to initiate oxidation and produce several oxidized products (degradation). β-Carotene scavenges free radicals mainly through three mechanisms (Edge et al., 1997; Rodriguez-Amaya, 2015):

$$\mathrm{CAR} + \mathrm{ROO}^* \rightarrow \mathrm{CAR}^{*+} + \mathrm{ROO}^- \,(\text{electron transfer})$$
$$\mathrm{CAR} + \mathrm{ROO}^* \rightarrow \mathrm{CAR}^* + \mathrm{ROOH} \,(\text{hydrogen abstraction})$$
$$\mathrm{CAR} + \mathrm{ROO}^* \rightarrow (\mathrm{ROO} - \mathrm{CAR})^* \,(\text{addition})$$

Some studies reported that the rates and mechanisms of the reactions were dependent on the properties of free radicals and also the environment (aqueous or lipid phase) (Everett et al., 1996; El-Agamey et al., 2004). In fact, β-carotene can also behave as a prooxidant when the oxygen tension is sufficiently high (Burton and Ingold, 1984; Palozza, 1998; Palozza et al., 2003). However, under biological conditions β-carotene is less likely to promote oxidation (Young and Lowe, 2001).

With a highly unsaturated structure, β-carotene is liable to oxidation, leading to the loss of its color and bioactive functions. Moreover, β-carotene is easily isomerized when exposed to acids, light, heat, etc. during food processing and storage (Rodriguez-Amaya, 2002; Borsarelli and Mercadante, 2010). Oxidation can occur enzymatically and nonenzymatically, depending on the accessibility of O_2, and the reaction is favored with the presence of heat, light, metal ions, and peroxides. Carotenoid degradation generally follows a first-order kinetics. Initially, part of the all-trans-carotenoids is isomerized to the

cis-forms, and later both the trans- and cis-isomers are oxidized. Carotenoid oxidation starts with the epoxidation and cleavage to apocarotenals, and hydroxylation is also involved as epoxycarotenoids and apocarotenals can be detected during the reactions. The reactions lead to the fragmentation of carotenoids, and low molecular weight compounds are generated, of which aldehydes, alcohols, ketones and other aromatic compounds contribute to the desirable flavors of processed foods, and are also responsible for some off-flavors (Boon et al., 2010; Rodriguez-Amaya, 2015).

3. Fundamental of food emulsions

3.1 Emulsion stability

Emulsions generally consist of an aqueous phase and an oil phase, one as dispersed phase and one as continuous phase (McClements, 2005). There are two major types of food emulsions: water-in-oil (W/O) emulsions and oil-in-water (O/W) emulsions. Food emulsions are usually produced through a homogenization process, in which the dispersed phase is broken into small droplets and dispersed in the continuous phase under intensive mechanical forces. Due to the large interfacial area between the two immiscible phases, emulsions have poor thermodynamic stability, and flocculation, coalescence, phase separation (e.g., creaming) are frequently observed during processing or storage (Figure 2) (McClements, 2005). Flocculation takes place when neighboring droplets are in close contact with each other, but still keeping their own characteristics. During coalescence, droplets are associated to form bigger droplets after the breaking down of individual interfacial film. As a consequence, the contents of the original droplets are mixed in the coalesced droplets. Flocculation and coalescence are typical destabilization processes in temperature-treated emulsions, either upon heating or freeze-thawing (Tcholakova et al., 2006; Ghosh and Coupland, 2008). Due to the difference in density, the oil phase and water phase of an emulsion are inherent to separate after a certain time of storage, behaving as creaming or sedimentation (Robins, 2000). Severe flocculation and coalescence can result in phase separation, as bigger droplets are more easily moving to the top (creaming) or the bottom (sedimentation). Nevertheless, emulsions can obtain certain kinetic stability because of the strong Brownian motions of droplets, and the repulsion forces (e.g., electrostatic repulsion, steric hindrance) between neighboring droplets in the systems. In order to design food emulsions with desirable appearance and functionality, it is essential to obtain emulsions with high kinetic stability. In the food industry, stabilizers, e.g., emulsifiers, texture modifiers, and weighting agents are usually involved in the formulations to assure stable emulsions.

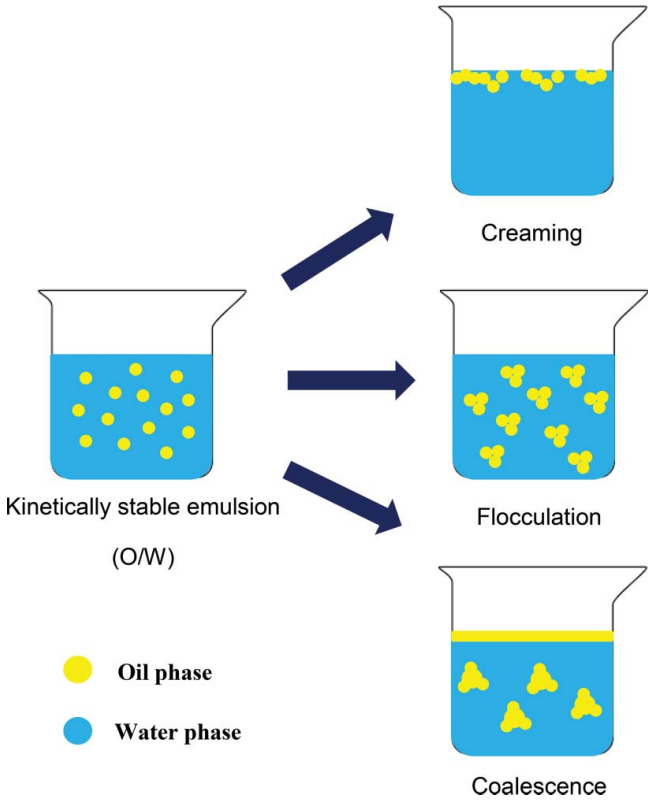

Figure 2. Schematic diagram of common destabilization mechanisms of emulsions.

Emulsifiers: an emulsifier is an amphiphilic ingredient, which consists of both hydrophilic heads and hydrophobic tails. The emulsifier molecule positions itself at the oil/water interface to reduce the interfacial tension, and to facilitate droplet disruption during homogenization. Moreover, the emulsifier at the interface creates a stabilizing film which works to slow flocculation and coalescence. Typical food emulsifiers include small molecular weight surfactants, e.g., tweens, spans, monoglycerides, and surface active biopolymers (e.g., proteins, gum Arabic).

Texture modifiers: texture modifiers are usually added to modify the rheological properties of an emulsion, either by increasing the viscosity of the continuous phase or forming a gel network. The presence of texture modifiers can help to improve the emulsion stability by slowing or preventing droplet movement. Pectin, carrageenan, gelatine, xanthan, and carboxymethyl cellulose (CMC), are typical texture modifiers used in the food industry.

Weighting agents: weighting agents are mostly oil-soluble with high density, and they are added into the oil phase to match its density to the surrounding water phase. Sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO), and ester gum (EG) are the three most widely used weighting agents (Chanamai and McClements, 2000).

3.2 Emulsions as delivery systems

Chronic diseases, such as diabetes, cardiovascular diseases and some cancers, developed in the modern life style have been a big concern of human being. People have turned to diet-based approaches to prevent or ease these diseases. Incorporation of bioactive ingredients, e.g., carotenoids, polyphenols, vitamins, functional lipids into food systems allows an easier design of novel functional foods with desirable health benefits. However, the poor water solubility and stability of many functional food ingredients restrain their wide application. To overcome these disadvantages, different types of delivery systems suitable for lipophilic bioactives have been developed, e.g., emulsions, liposomes, ethosomes, organogels, biopolymer coacervates. Among these systems,

emulsions are particularly suitable for the delivery of functional ingredients into food systems. It is because emulsions are common food types (e.g., milk, ice cream, butter), and the production of many food involves emulsification (e.g., chocolate, juice drinks, some soups and sauces), which allow the easy addition of emulsions into different foods. Different researches on various functional ingredients have proved that emulsions can be indeed potential delivery systems for the designing of novel functional foods (Velikow and Pelan, 2008; McClements, 2010; Mao and Miao, 2015).

4. Physicochemical stability of β-carotene emulsions

Emulsions as delivery systems for β-carotene have been extensively investigated (Table 1), and studies were performed to investigate the preparation methods, the physicochemical properties, and also the digestion behaviors of β-carotene emulsions. Objectives of most studies were to design β-carotene emulsions with reasonable physical and chemical stabilities, and to achieve controlled release and adsorption of β-carotene *in vivo* or *in vitro* (Table 1).

Conventional homogenization techniques, including high-speed shearing, ultrasonic treatment, high pressure valve homogenization, etc. are widely applied to make β-carotene emulsions (McClements, 2005; Mao et al., 2009; Hou et al., 2012). In recent years, some novel techniques, such as ultra-high pressure homogenization, microfluidization, membrane/microchannel emulsification, phase inversion, and solvent displacement have also been developed to produce β-carotene emulsions with desired properties (Ribeiroe t al., 2008; Neves et al., 2008; Mao et al., 2010; Xu et al., 2012; Zhang et al., 2013). Among all the techniques, high pressure homogenization is the most used technique both in laboratories and industries, and emulsions with different particle size ranges can be produced depending on the pressures that the homogenizers can generate. It is observed that either increasing homogenization pressure or cycles under certain thresholds can result in β-carotene emulsions with smaller mean particle size and narrower droplet distribution, but further increase might lead to unchanged particle size or wider distribution, due to the emulsifier shortage in covering the interface (Tan and Nakajima, 2005a; Mao et al., 2010). Another approach to make emulsions with smaller oil droplets is utilizing small molecular weight emulsifiers, e.g., tweens, spans, sugar esters, polyglycerol esters of fatty acids, instead of biopolymers, e.g., milk proteins (Mao et al., 2009; Yin et al., 2009). β-Carotene emulsions with smaller particle size are usually preferable, not only because that these emulsions have better physical stability (Tan and Nakajima, 2005a), and it is possible to make transparent food systems (e.g., β-carotene nanoemulsions for beverages), but that β-carotene in smaller particles is easily adsorbed (Wang et al., 2012; Yi et al., 2014a). However, small particles are not favorable for the stabilization of β-carotene in emulsions upon storage, because of the large interfacial area covering the oil-water interface, which allows more exposure of the oil droplets and β-carotene to the outer environment (Tan and Nakajima, 2005a; Yi et al., 2014a). To overcome this dilemma, a lot of investigations have been performed to improve β-carotene stability in emulsions during preparation, transportation, storage and consuming. From the literatures, the stability of β-carotene can be largely adjusted by altering emulsion compositions (Yuan et al., 2008; Mao et al., 2009; Xu et al., 2013a, b, c) (Table 1).

Emulsifiers. Oil droplets in emulsions are covered by membranes of emulsifier molecules, which work to stabilize the droplets from aggregation. It was also reported that the membranes could protect the oil phase, as well as the bioactive compounds incorporated from oxidation, because the membranes worked as barriers against the attack of oxidation inducers, e.g., metal ions,

Table 1. Examples of formulations of different β-carotene emulsions.

Oil type and content	β-Carotene content	Emulsifier type and content	Other Ingredients	Preparation Method	Reference
Corn oil (10%)	0.01%	β-Lactoglobulin (0.1%), β-Lactoglobulin-catechin conjugate (0.1%)		Microfluidization	Yi et al., 2015a
MCT (3%)	0.03%	Tween 20 (10%), tween 80 (10%), WPI (1%), SPI (1%), SC (1%)		Microfluidization	Jo and Kwon, 2014
Hexane (10%)	0.03%	Polyglycerol esters (0.9%)		Solvent replacement	Tan and Nakajima, 2005a
Soy bean oil (1.8%)	0.03%	WPI (1%) + lecithin (2%)	Eugenol	High shearing	Guan et al., 2016
Triolein (10%)	0.01%	SC (1%)		Microfluidization	Kanafusa et al., 2007
Corn oil (10%)	0.05%	β-Lactoglobulin (1.5%), tween 20 (2%)	Vitamin E acetate, Coenzyme Q10, EDTA, ascorbic acid	Microfluidization	Qian et al., 2012a
Acetone (10%)	0.0015%	SC (0.9%), tween 20 (0.9%), decaglycerol monolaurate (0.9%), sucrose fatty acid ester (0.9%)		Solvent replacement	Yin et al., 2009
Sunflower oil (10%)	0.03%	Tween 20 (2%), BSA (1%), WPC (1%)		Membrane emulsification	Trentin et al., 2011
Sunflower oil (10%), Hydrogenated palm kernel oil (10%)	0.005%	WPI (0.8%), SC (0.8%)	Sucrose	Valve homogenization	Cornacchia and Roos, 2012
MCT (15%)	0.005%	HPMC (2%)+gum arabic (21.25%)		Valve homogenization	Akinosho and Wicker, 2015
Canola oil (1%)	0.02%	OSA starch (1%)		Valve homogenization	Sweedman et al., 2014
MCT (10%), corn oil (10%)	0.01%	SC (2%)	α-Tocopherol, ascorbic acid	High shearing	Yi et al., 2016

free radicals (Coupland and McClements, 1996). Small molecular weight emulsifiers (surfactants) are usually more effective in producing oil droplets with smaller particle size, while surface active biopolymers are more capable to protect β-carotene from degradation in emulsions. Yin et al. (2009) studied the effect of different emulsifiers, i.e., sodium caseinate, tween20, decaglycerol monolaurate, sucrose ester on the physicochemical properties of β-carotene emulsions. They reported that the emulsion stabilized by sodium caseinate had the largest particle size, but β-carotene in the emulsion had the best stability against oxidation during a storage test at 4°C for 8 weeks. When a blend of sodium caseinate and decaglycerol monolaurate was used, the stability of β-carotene was improved compared to that in the emulsion stabilized by decaglycerol monolaurate alone. The authors attributed the improved stability of β-carotene to the thicker interfacial membrane formed by sodium caseinate, and the antioxidative effect of casein (Laakso, 1984). Similar findings were reported in a latter study, where the roles of whey protein isolate, decaglycerol monolaurate, octenyl succinate starch, and tween 20 in stabilizing β-carotene were compared, and β-carotene in whey protein isolate stabilized emulsion was least degraded during storage (55°C, 12 days) (Mao et al., 2010). A different study found that the emulsion stabilized by β-lactoglobulin was more stable to color fading than that stabilized by tween 20 (Qian et al., 2012a). When a blend of tween 20 and WPI was applied, both the physical stability of the emulsion and the chemical stability of β-carotene were higher than those in a tween 20 stabilized emulsion (Mao et al., 2010). When different proteins were tested, sodium caseinate provided better protection than whey protein isolate against β-carotene degradation (Cornacchia and Roos, 2012), and a mixture of whey protein concentrate and tween 20 was capable to retain more β-carotene than a mixture of bovine serum albumin and tween 20 (Trentin et al., 2011). Yi et al. (2014a)suggested that WPI acted more effectively in retarding β-carotene degradation in emulsions than soy protein isolate. The different antioxidative capacities of proteins could be associated with the different amino acid compositions and the interfacial structures of the proteins in the emulsions (Cornacchia and Roos, 2012). β-Lactoglobulin and α-lactalbumin are the two major proteins of whey protein, and they contain cysteyl residues, disulphide bonds and thiol functional groups, which can behave as antioxidants to scavenge free radicals (Sun et al., 2007). Xu et al. (2013a) confirmed a good correlation between the degree of protein oxidation (loss of tryptophan) and the level of β-carotene loss. Starches can be structurally modified to be surface active, and they would also favor the protection of β-carotene in emulsions during storage (Liang et al., 2013).

Small molecular weight emulsifiers generally play a rather weak role in inhibiting β-carotene oxidation, probably because of the thin membrane formed at the oil-water interface. Yuan et al. (2008) failed to find any difference in the retention of β-carotene in emulsions stabilized by a series of polyoxythylenesorbitan esters of fatty acids (i.e., tween 20, 40, 60, 80), and Tan and Nakajima (2005b) found same degradation level of β-carotene in the emulsions stabilized by three glycerol monolaurates (tetraglycerol, hexaglycerol, decaglycerol) or three glycerol monooleates (tetraglycerol, hexaglycerol, decaglycerol). When a polyoxythylenesorbitan ester (tween 20) and a polyglycerol ester (decaglycerol monolaurate) were tested, only slight higher content of β-carotene was retained in the polyglycerol ester stabilized emulsion (Mao et al., 2009; Yin et al., 2009).

Antioxidants. In order to retain more β-carotene in the emulsions, different types of antioxidants are usually added. Prooxidants (e.g., metal ions) and free radicals present in the emulsion matrix or brought in from contaminations are the main inducers of β-carotene oxidation (Boon et al., 2010). Therefore, ingredients that can bind prooxidants or scavenge free radicals are able to improve β-carotene stability. Qian et al. (2012a) showed that both water-soluble (ascorbic acid, EDTA) and oil-soluble (vitamin E acetate, coenzyme Q10) antioxidants were effective in inhibiting β-carotene degradation during storage at an elevated temperature (55°C). They also found that EDTA was working better than ascorbic acid, and Coenzyme Q10 was better than vitamin E acetate. However, a blend of EDTA and vitamin E acetate was not as effective as the individual compound. Yi et al. (2016) found that α-tocopherol had stronger antioxidant activity than ascorbic acid in inhibiting β-carotene degradation in O/W nanoemulsions when stored at 25 or 50°C. α-Tocopherol was also tested to be superior to tertiary butyl hydroquinone (TBHQ) and ascorbylpalmitate when the emulsions were exposed to light (Liu et al., 2015). Xu et al. (2013a) revealed that the addition of EDTA or α-tocopherol could increase the stability of β-carotene in WPI stabilized emulsions when stored at 55°C, and the improvement was more significant at pH 7.0 over pH 4.0. In the water phase of an O/W emulsion, EDTA is able to chelate metal ions. In the oil phase, α-tocopherol scavenges peroxyl radicals and prevents the propagation of free radicals in the lipids. As α-tocopherol was more effective than EDTA, the authors proposed that free radicals played a greater role in inducing β-carotene degradation than metal ions in the tested emulsions (Xu et al., 2013a). Other tested antioxidants, including eugenol and rosemary extracts, were also effective in protecting β-carotene from degradation when exposed to heat and light (Mesnier et al., 2014; Guan et al., 2016).

Protein Conjugates. As widely investigated, proteins are good emulsifiers for β-carotene emulsions due to their amphiphilic properties and antioxidative function. However, proteins are unstable under extreme environments, such as high temperatures, high ionic strength and certain pH ranges, etc. An innovative way to enlarge potential applications of proteins in delivery systems is to covalently conjugate polysaccharides or polyphenols with proteins. Protein-polysaccharide conjugates can be easily formed through the Maillard reaction based on the Amadori rearrangement. It is known that protein–polysaccharide conjugates may have improved antioxidative, emulsifying, and steric stabilizing properties (Oliver et al.,2006). Xu et al. (2011) prepared WPI-dextran conjugates by heat treatment, and the emulsions stabilized by the conjugates showed much better stability against freeze-thawing than that stabilized by WPI alone or a WPI-dextran mixture (unheated), probably because of the thicker and denser interfacial layers formed by the conjugates at droplet surfaces. The same team further designed WPI-pectin conjugates as emulsifiers for β-carotene emulsions, and they observed that the emulsions had smaller particle size with improved physical stability (subjected to centrifugation or freeze-thawing) in comparison to the emulsion stabilized by WPI alone. Moreover, the degradation of β-carotene in the emulsion (pH 7) during storage (dark, 70°C, 5 days) was more obviously retarded by WPI-beet pectin conjugates (25% loss) than WPI (60% loss) and a unheated WPI-pectin mixture

(30% loss) (Xu et al., 2012). In a following study, the emulsions were washed to remove the conjugate not absorbed at the droplet interface, and the authors observed significant higher loss of β-carotene, indicating that the unabsorbed conjugate also protected β-carotene (Xu et al., 2013c). In the same study, it was revealed that the conjugate did not significantly improve the oxidative stability of β-carotene in the emulsion at pH 4 in comparison with the unconjugated mixture, as β-carotene was rapidly degraded at this pH (about 80% loss after 5 days at 55°C). When desferoxamine (a metal chelator) was added into the emulsions to protect β-carotene, it was found that desferoxamine performed more effectively at pH 4 than it did at pH 7, particularly in the emulsion with the conjugate (Xu et al., 2013c).

Polyphenols can serve as antioxidants to protect β-carotene from degradation in emulsions (Liu et al., 2016a). Recent innovative work in our team revealed that polyphenols could be conjugated with proteins, and the conjugates were able to stabilize β-carotene emulsions. Wang et al. (2014b) designed a covalent complex using α-lactalbumin and (−)-epigallocatechin gallate (α-La-EGCG) at alkaline pH through heating, and the complex presented higher antioxidative and emulsifying properties than α-lactalbumin. When the stability of β-carotene was concerned, about 6% loss was observed in the emulsion stabilized by α-La-EGCG after 20 days (25°C), in comparison to about 13% loss in the system with native α-lactalbumin (Wang et al., 2015). A more systematic study on the EGCG complexes formed with different milk proteins (i.e., α-lactalbumin, β-lactoglobulin, lactoferrin, sodium caseinate) suggested that all the tested protein-EGCG complexes worked better than individual proteins in protecting β-carotene from degradation at different temperatures (25, 37, 55°C) or when exposed to UV light. Comparatively, the complexes formed with EGCG and sodium caseinate or β-lactoglobulin behaved more effectively in stabilizing β-carotene emulsions (Wei et al., 2015). Other studies using catechin or chlorogenic acid to form protein-polyphenol conjugates also showed improved stability of β-carotene in the emulsions stabilized by the conjugates (Wang et al., 2015; Yi et al., 2015a). The on-going work in our team found that polyphenol-protein-polysaccharide ternary complex may have enhanced capacity to stabilize β-carotene emulsions than the binary conjugates, particularly the light stability of β-carotene (Liu et al., 2016c).

5. Bioaccessibility of β-carotene in emulsions

The bioavailability of β-carotene in food products is influenced by many factors, including food compositions and structures, the methods of food processing, the co-ingested food, and also the physiological differences among individuals (e.g., eating behavior, compositions of digestive juices, health status) (Rodriguez-Amaya, 2015). Generally, when β-carotene is released from food matrix, it has to be incorporated into oil droplets, either formed during lipid digestion or present in the original food (e.g., emulsions). The attachment of lipases from digestive juices at the oil droplet surface initiates lipid digestion. The digested lipid products, particularly some free fatty acids and monoacylglycerols take part in the formation of mixed micelles (also contain bile salts and phospholipids), which behave as carriers to solubilize β-carotene and transport it to the epithelium cells before adsorption (Yonekura and Nagao, 2007). Therefore, the ingestion and hydrolysis of lipids have been regarded as essential steps in the bioavailability of β-carotene (Rao et al., 2013; Qian et al., 2012b). Technically, any factors that influence lipid digestion would affect the bioavailability of β-carotene.

The bioavailability of the ingested nutrient is partially determined by its bioaccessibility, which is generally defined as the fraction of the ingested nutrient that is incorporated into the mixed micelles and thus becomes available for absorption in the body (Castenmiller and West, 1998; Hedren et al., 2002). Different studies have revealed that the structures and compositions of emulsions play significant roles in the bioaccessibility of β-carotene during digestion. It is believed that smaller droplet size with higher surface area would facilitate higher transfer of lipophilic bioactive compounds into micelles. Wang et al. (2012) observed that the transfer rate of β-carotene into mixed micelles increased from 5 to 10% when droplet size of the emulsions (stabilized by decaglycerol monolaurate) decreased from 18 μm to 0.7 μm. When droplet size of the emulsions was reduced to nano-meter range, the transfer rate was greatly increased, and over 50% of the β-carotene was transferred into the aqueous phase (containing mixed micelles) in the nanoemulsion with an average droplet size of 65 nm (Wang et al., 2012). Higher bioaccessibility (>50%) of β-carotene was also observed in sodium caseinate stabilized emulsions when the droplet size was smaller than 360 nm. The authors also reported a linear inverse relationship between bioaccessibility of β-carotene and emulsion droplet size (130–360 nm) (Yi et al., 2014b), which was in agreement with findings in an earlier study on sucrose monopalmitate stabilized emulsions (Rao et al., 2013). Salvia-Trujillo et al. (2013) reported a ~ 2-fold increase (from 34% to 59%) in the bioaccessibility of β-carotene when reducing the droplet size of emulsions (stabilized by tween 20) from 23 μm to 0.21 μm, which was attributed to the higher content of free fatty acids released after digestion in the emulsion with smaller particle size. In the emulsions with bigger particles, there was more undigested oil and less mixed micelles, which would retain more β-carotene in the oil droplets and solubilize less in the micelles (Malaki Nik et al., 2010). These findings highlighted the important roles of lipid digestion in the bioaccessibility of β-carotene. It was also hypothesized that small molecular weight emulsifiers could form micelles during digestion, which could contribute to a higher rate of β-carotene micellarization (Wang et al., 2012).

Emulsifiers may also influence the bioaccessibility of β-carotene by modifying the interfacial properties of oil droplets in emulsions. Several studies have showed that β-carotene in protein stabilized emulsions exhibited higher bioaccessibility, compared to that in soybean polysaccharide or polyglycerol esters stabilized emulsions (Liu et al., 2012; Hou et al., 2014), as the interfacial proteins were more easily to be replaced by bile salts. When different milk proteins (i.e., WPI, β-lactoglobulin, sodium caseinate, α-lactalbumin, lactoferrin) were tested, WPI and β-lactoglobulin presented higher capability to promote β-carotene release and micellarization during digestion. It was proposed that oil droplets covered by β-lactoglobulin were more easily digested because of the disruption of bile salts into the protein film and a higher rate of proteolysis by pepsin (Liu et al., 2014). Tokle et al. (2013) reported that when lactoferrin was used to stabilize emulsions, either in the presence of β-lactoglobulin or not, lactoferrin could dramatically reduce the micellarization level of β-carotene (<3%). The authors suggested that there were interactions between

β-carotene and lactoferrin or the digestive products of lactoferrin, and the resulting products had poor solubility in the mixed micelles. Compared to milk proteins, soy proteins were less effective in promoting β-carotene adsorption, probably because of their large molecular weight and lower emulsifying capacity (Yi et al., 2014a). The addition of phosphatidylcholine, a major membrane phospholipid in human cells, could increase the bioaccessibility of β-carotene, because phosphatidylcholine has a high emulsifying capacity (to form small particles) and it participates in the formation of mixed micelles (Verrijssen et al., 2015). These findings were in agreement with the results in a study on lycopene, whose bioaccessibility was increased when phosphatidylcholine was added (Nishimukai and Hara, 2004).

As emphasized earlier, the digestion of lipids plays an essential role in the bioaccessibility of β-carotene. Therefore, β-carotene dissolved in indigestible oils (e.g., lemon oil, orange oil) usually had very poor bioaccessibility, as only minor mixed micelles can be formed after the ingestion of these oils(no free fatty acids released) (Qian et al., 2012b; Rao et al., 2013). The bioaccessibility can be significantly improved when digestive oil (e.g., corn oil) was mixed with indigestible oil (Rao et al., 2013). However, the presence of a large quantity of free fatty acids cannot ensure a high bioaccessibility of lipophilic bioactive compounds (e.g., β-carotene), because different free fatty acids had different capacities to form mixed micelles and the micelles formed could dissolve different amount of bioactives (Kossena et al., 2003;Qian et al., 2012b). Long chain fatty acids (e.g., C16, C18) were more likely to form micelles with larger hydrophobic domains, which can well accommodate the lipophilic bioactives, in comparison to medium chain fatty acids (e.g., C8, C10) (Christensen et al., 2004; Qian et al., 2012b). These findings were supported by human feeding studies on the bioavailability of β-carotene (Borel et al.,1998). Yi et al. (2015b) suggested that the saturation level of fatty acids in emulsions may also affect the bioaccessibility of carotenoids, and unsaturated fatty acids (e.g., olive oil, canola oil) were more effective inmicellarizing β-carotene than saturated fatty acids (e.g., coconut oil, palm oil). However, Gleize et al. (2013) found that lutein and zeaxanthin in the meal containing butter had higher bioaccessibility and were better adsorbed in the rat plasma than that in the meal containing olive oil or fish oil. The authors interpreted that the saturation level of fatty acids might affect the size of the mixed micelles and thus alter carotenoid micellarization, but the inconsistent conclusions made in aforementioned studies implied more complicated roles of different lipids in the adsorption of carotenoids.

In many food emulsions, thickening agents (e.g., polysaccharide polymers) are usually added to improve emulsion stability. It is consented that emulsions without droplet aggregation is helpful for lipid digestion (Wang et al., 2012; Yi et al., 2014b). However, the inclusion of modified starch or pectin may reduce the bioaccessibility of β-carotene due to the higher viscosity of the systems and thicker layer formed around droplets (Liang et al., 2013; Verrijssen et al., 2014). Moreover, polymers might inhibit lipid digestion by preventing the anchoring of lipase at droplet surface (Beysseriat et al., 2006). It was reported that soluble fibers might reduce the level of β-carotene micellarization and cellular uptake in comparison to the fiber-free control (Yonekura and Nagao, 2009).

6. Novel structured emulsions for the delivery of β-carotene

With simple structures, conventional emulsions are prone to flocculation, coalescence, or creaming when exposed to unfavorable conditions, and most of the emulsions are not effective in delivering bioactive ingredients during food processing/storage and consumption (McClements, 2012). Some novel emulsions with tailor-made structures in the oil phase, water phase or interface have been developed, in order to better deliver bioactive compounds and enhance their functionality.

Multilayer Emulsion. A multilayer emulsion is characterized in two or more interfacial films covering oil droplets formed by emulsifiers and/or biopolymers. The interfacial films are

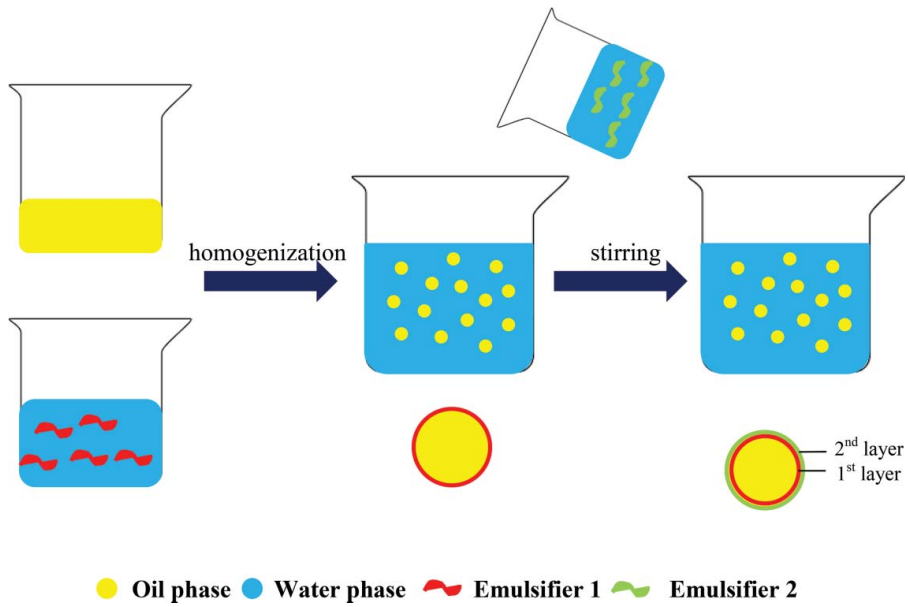

Figure 3. Schematic diagram of the formation of a multilayered emulsion (double layers) (emulsifier 1 and emulsifier 2 are oppositely charged).

deposited onto the droplets layer-by-layer, and the neighboring films are electrostatically attracted. Therefore, the ingredients forming the interface must be charged in dispersions and at the interface. Ionic emulsifiers, like SDS, lecithin and different types of proteins, are usually chosen to form the first layer (inner layer), and biopolymers (either surface active or non-surface active) (e.g., proteins, pectin, chitosan) are used to form the subsequent layers. As the formation of multi-layers is driven by electrostatic forces, pH and ionic strength of the emulsions play critical roles in the properties of the multi-layered interfaces (Guzey and McClements, 2006). It is possible to design multilayer emulsions with desired interfacial structures by choosing the proper interfacial ingredients and the pH or salt concentrations of the systems. Subsequently, the multilayer emulsions can be designed to have different responsiveness to the change of environmental stresses (Hou et al., 2010; Mao et al., 2013). Multilayer emulsions have been reported to have improved stability at acidic pH, upon heating or cooling (or freeze-thawing), or against high ionic strength, etc. (Guzey and McClements, 2006), and they can be used to protect and control the release of functional ingredients (Djordjevic et al.,2007). Hou et al.(2010, 2012) prepared β-carotene emulsions stabilized by soybean polysaccharide single layer or soybean polysaccharide-chitosan double layers, and they found that the adsorption of a chitosan layer onto the pre-adsorbed polysaccharide layer could significantly inhibitβ-carotene degradation during storage at different temperatures. The percentage of β-carotene remained after storage was dependent on the concentration and molecular weight of the chitosan used, and the emulsion stabilized by chitosan of medium molecular weight at a concentration over 0.25% had the best β-carotene stability (Hou et al., 2010, 2012). The same authors also observed an increased light stability of β-carotene in a soybean polysaccharide-chitosan (medium molecular weight) multilayered emulsion over a soybean polysaccharide emulsion (Hou et al., 2012). Tokle et al. (2013) designed lactoferrin-β-lactoglobulin double-layer interface to improve the stability of β-carotene emulsions. They reported that the inclusion of lactoferrin at the interface, either located at the inner layer or outer layer, could significantly inhibit droplet aggregation in a wide range of NaCl concentrations and improve β-carotene stability during storage (37°C). When the emulsions were freeze-dried (trehalos as wall material), the multilayered emulsion (WPI-gum arabic) was capable to retain more β-carotene after storage (25, 37, and 45°C) over WPI single layered emulsion, though higher level of isomerization was observed in the multilayered system (Lim et al., 2014).

Recent work in our team utilized polysaccharide/protein-polyphenol conjugates to form β-carotene multilayered emulsions. With a thicker interfacial layer containing antioxidative polyphenols covering the oil droplets, the stability of β-carotene inside droplets was greatly improved. Liu et al. (2016b) tested the effect of beet pectin or soybean soluble polysaccharides (outer layer) on the stability of β-carotene emulsions coated by lactoferrin-polyphenol (EGCG or chlorogenic acid-CA) conjugates (inner layer), and they found that β-carotene in the multilayered emulsions had very minor loss (about 22–24%) when exposed to UV for 8h, in comparison to a much higher loss of β-carotene in the single layered emulsions stabilized by lactoferrin-polyphenol conjugates (>70%) or lactoferrin alone (100%). Similar improvement in the stability of β-carotene was also observed when the multilayered emulsions were heat-treated (55°C for 12 days). Comparatively, EGCG was more effective in protecting β-carotene from degradation than CA in the multilayered emulsions. When the conjugates were located at the outer layer of the multilayered interface, they were also effective in stabilizing β-carotene in the emulsions (Wei and Gao, 2016).

Solid Lipid Particle. Solid lipid particles refer to the fully or partly solidified lipid phase of an O/W emulsion, and they are better known as solid lipid nanoparticles (SLN) when the lipid particle size is at nano-meter range. Solid lipid particles are usually produced through a two-step thermal process: (1) conventional emulsions are made at elevated temperatures; (2) emulsions are cooled to trigger lipid solidification (crystallization) (Figure 4). By proper control of the thermal process (temperature, heating rate, duration, etc.), the lipid crystals may have different morphology and packing styles, leading to different characteristics of the emulsions with desired functionality. The solid lipid particles can restrain mass transfer and diffusion of the ingredients incorporated, and can behave as shelters to protect the ingredients against the attack of external stresses. Furthermore, solid lipid particles can be used to design low-calorie foods, as they are usually difficult to be digested by lipase (McClements and Li, 2010). Uniquely, the solid particles can be designed to melt at desired temperatures, and thermally controlled release of the incorporated ingredients is then obtained. Further studies indicated that conventional solid lipid particles had poor encapsulation efficiency during storage, due to the higher degree of order of the lipid crystal lattice (from α or β' order to β order) formed by a single type of lipid, which can promote encapsulant expulsion. To overcome this weakness, nanostructured lipid carriers (NLCs) have been developed by using lipid blends containing both solid and liquid lipids to form imperfect crystals (Müller et al., 2002).

Different types of solid lipid particles have been developed to deliver β-carotene, and they have shown evidences to slow down β-carotene oxidation and improve its bioaccessibility. Trombino et al. (2009) prepared stearylferulate-based solid lipid nanoparticles dispersed in a microemulsion, as vehicles for β-carotene. After a storage test at room temperature (28–30°C) with sunlight exposure for 3 months, the lipid nanoparticles underwent a quite small change in the particle size (from 169.8 to 188.4 nm), and the entrapment efficiency of β-carotene was hardly altered (from 49.2% to 48.4%). A similar finding was reported in a milkfat-based nanoemulsion system prepared using phase inversion-temperature technique, and the lipid nanoparticles were dilution-and dialysis-stable and maintained the particle size ($\sim$ 25 nm) for about 90 days, and higher stability of β-carotene was also confirmed (Zhang et al., 2013). In most cases, the role of lipid particles in protecting β-carotene from degradation is dependent on the emulsifiers used, which not only influence the interfacial properties of the particles, but also has impact on the crystalline structures of the lipids. Malaki Nik et al. (2012a) reported that only β type lipid crystals were formed in Poloxamer 188 stabilized SLNs, while both β' and β types were observed in tween 20 stabilized ones. This study also discovered that the incorporation of β-carotene at a

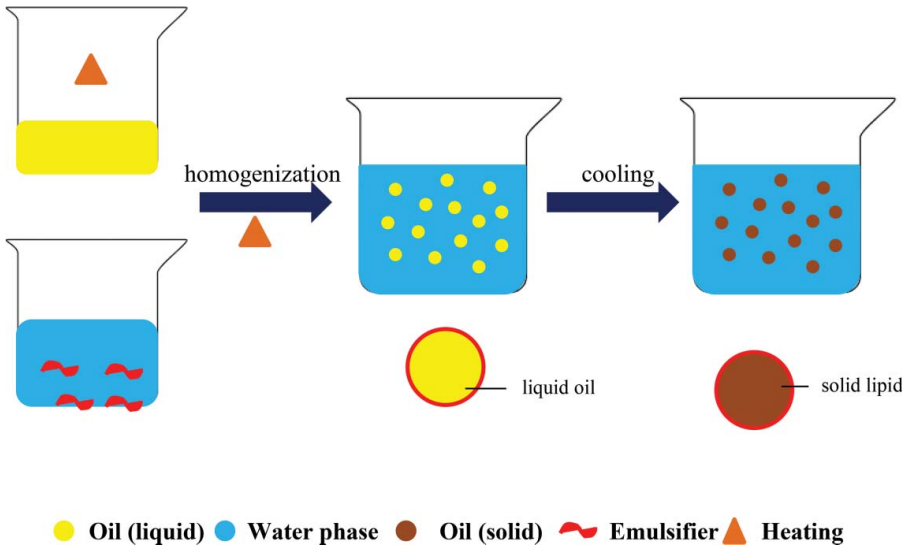

Figure 4. Schematic diagram of the formation of an emulsion containing solid lipid particles.

level of 0.1 wt% did not affect the polymorphism and melting behaviors of the lipid crystals. Helgason et al. (2009) suggested that lecithin was more capable to inhibit β-carotene degradation in solid particle systems than tween type emulsifiers. Under simulated digestive conditions, solid lipid particles were also able to maintain their structures, and to slow lipolysis and β-carotene transferring to the aqueous phase (Malaki Nik et al., 2012b). Yi et al. (2014a) prepared solid lipid nanoparticles stabilized by different proteins, and they reported that the uptake of β-carotene by Caco-2 cells was significantly higher from the lipid nanoparticles than that from free β-carotene. More importantly, the nanosized particles could be directly transported across the epithelium cell layer, facilitating β-carotene adsorption. As the permeation of particles into the cells (negatively charged) was modulated by surface charge of the nanoparticles, the use of different emulsifiers could work to modulate the levels of β-carotene uptake (Yi et al., 2014a).

Microemulsion. Microemulsions have droplet sizes in the nano-meter range (20–100 nm), and they are transparent systems, as the light scattered from the oil droplets is relatively weak (McClements, 2010). More importantly, microemulsions are thermodynamically stable. Differently from other types of emulsions, microemulsions are formed spontaneously and no or very little external force is required (Figure 5). During the formation of an O/W microemulsion, high content of emulsifiers (usually with co-surfactants) are dispersed in the water phase, and the emulsifier molecules self-assemble into micelles with large interfacial film covering lipophilic domains (Garti et al., 2003). Compared with other emulsions, microemulsions have much larger surface area to volume ratios, and can therefore solubilize higher amount of functional ingredients in their inner phase and at the interface. Chen and Zhong (2015) prepared β-carotene microemulsions (0.1% w/v β-carotene, <10 nm particle size) stabilized by a mixture of lecithin and tween 20. They reported that about 75% of the β-carotene was retained in the microemulsion system (3% lecithin, 20% tween 20) after 15 days of storage (ambient temperature), while β-carotene dissolved in ethyl acetate was almost completely degraded. After 65 days of storage, about 20% of the β-carotene was still retained in the system containing lecithin. As the authors stated, lecithin played a significant role in protecting β-carotene from degradation in the microemulsion system.

Currently, applications of microemulsions as delivery systems in the food industry are quite limited, mainly because that there are limited types of food grade emulsifiers (and co-surfactants) and oils suitable to produce these emulsions (Rao and McClements, 2011). Second, the preparation of microemulsions generally requires large amount of emulsifiers, which may exceed the maximum concentration allowed. Third, current techniques cannot guarantee sufficient encapsulation of nutrients in the nano-sized droplets, while maintaining the physicochemical properties of the microemulsions (Coupland et al., 1996).

Pickering Emulsion. In a Pickering emulsion, droplets are stabilized by solid particles (Figure 6), and the system can be formed without the addition of emulsifiers (Aveyard et al., 2003). Therefore, Pickering emulsions are particularly favored in "emulsifier-free" food products. The solid particles forming Pickering emulsions are not necessarily amphiphilic, and partial wetting of the particles by the water and oil phase allows strong anchoring of the particles at the oil-water interface (Chevalier and Bolzinger, 2013).The interface of a Pickering emulsion is mechanically stronger, and it can provide sufficient protection for the ingredients incorporated. Studies revealed that smaller particles are more effective in stabilizing Pickering emulsions, and it is essential to use particles with an average size < 1/10 of emulsion droplet size (Dickinson, 2012a). A lot of solid particles have been developed to form food grade Pickering emulsions, e.g., cellulose nanocrystals (Kalashnikova et al., 2011), chitin nanocrystals (Tzoumaki et al., 2011), starch particles (Yusoff and Murray,2011), flavonoid particles (Luo et al., 2011), kafirin (Xiao et al., 2016).These solid particles are not able to quickly reduce interfacial tension during emulsion

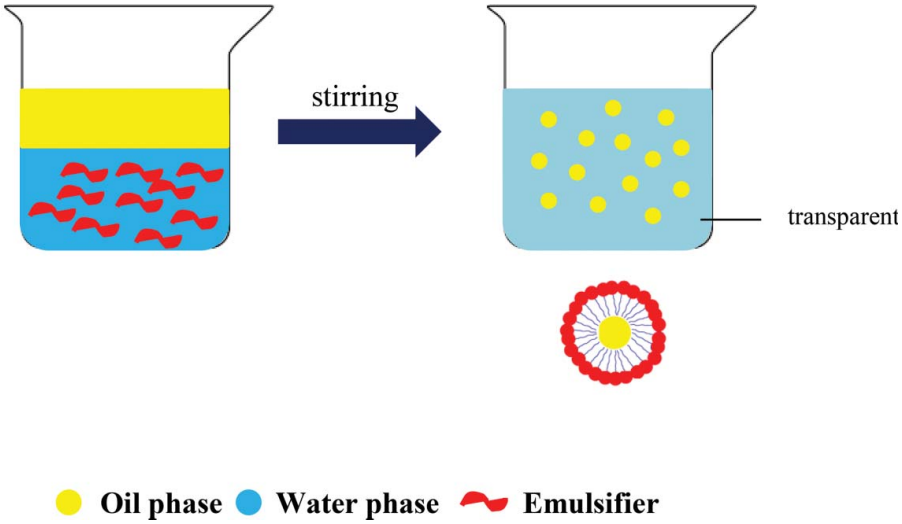

Figure 5. Schematic diagram of the formation of a microemulsion.

preparation, and surface active ingredients are usually added to accelerate the process and allow a better adsorption of the solid particles at the interface (Dickinson, 2012a).

Pickering emulsions generally have good physical stability because of the strong steric repulsion originated from the interfacial particles, and they have shown advantages in delivering functional food ingredients. Liu and Tang (2016) designed an O/W emulsion stabilized by soy glycinin particles as a delivery system for β-carotene, and they tested the release behavior of β-carotene under simulated intestinal conditions. The findings suggested that β-carotene in the Pickering emulsion was released at a much lower rate than that in a conventional emulsion, and β-carotene was rather stable during the digestion process. The same team also used pea protein isolate to make Pickering emulsions, and they showed that the release rate of incorporated β-carotene could be modulated by modifying oil fractions in the emulsions. With a higher concentration of oil, the emulsion presented gel-like properties, and the release of β-carotene was well slowed down (Shao and Tang, 2016). Many studies using Pickering emulsions as delivery systems for functional ingredients have showed that the release of the ingredients was highly related to the structure of the solid particles, and it can be effectively controlled by tailoring the structures of the adsorbed articles or using suitable triggers (e.g., pH, enzyme, heat) (Ghouchi Eskandar et al., 2009; Ruiz-Rodriguez et al., 2014; Wang et al., 2014a).

Multiple Emulsion. Multiple emulsions include water-in-oil-in-water (W/O/W) emulsions and oil-in-water-in-oil (O/W/O) emulsions, which are characterized in the two dispersed phases, with one being incorporated in the other (Garti, 1998; McClements, 2010). A multiple emulsion has oil-water interface and water-oil interface in one system, which requires both

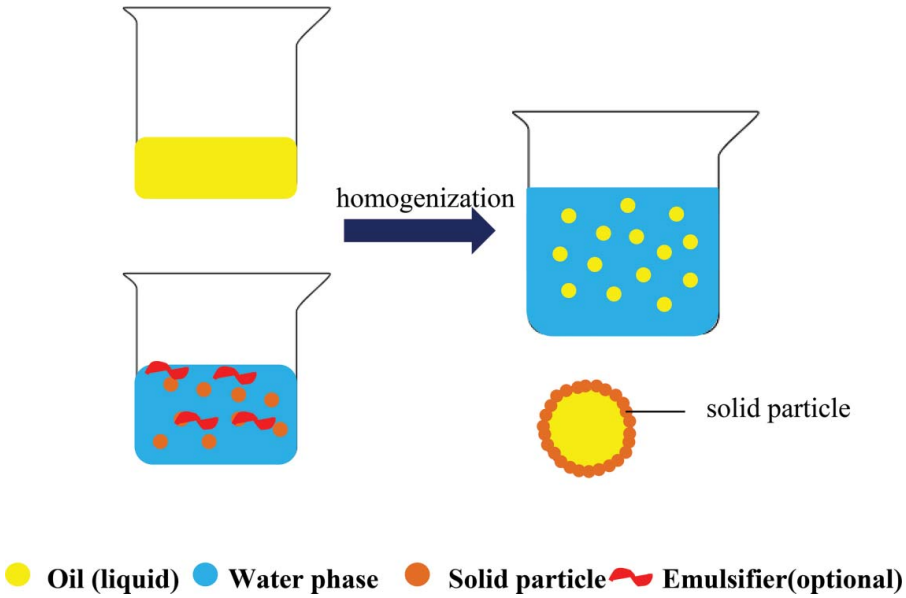

Figure 6. Schematic diagram of the formation of a Pickering emulsion.

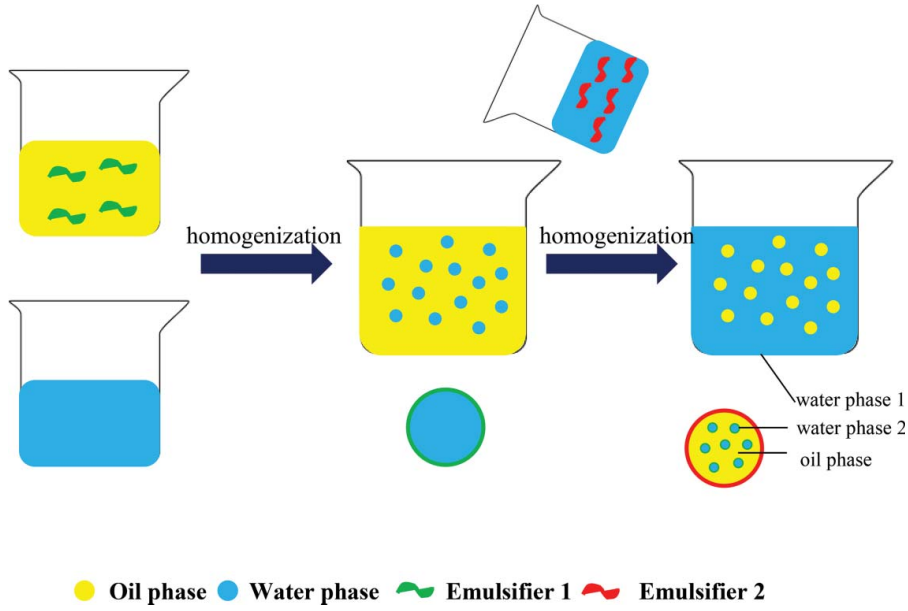

Figure 7. Schematic diagram of the formation of a multiple emulsion ($W_2/O/W_1$).

hydrophilic and lipophilic emulsifiers to stabilize the emulsion. To prepare multiple emulsions, conventional emulsions are first made, followed by a second homogenization process to disperse the emulsions into the continuous (outer) phase. It is recommended to apply mild forces to conduct the second homogenization, in order to prevent the breakdown of the primary emulsions.

Multiple emulsions have advantages in delivering functional ingredients, as they have two oil (or water) compartments in one system, which is helpful in slowing the diffusion of the ingredients dissolved in the inner compartment. Moreover, a multiple emulsion is able to accommodate both water-soluble and oil-soluble functional ingredients. For example, Vernon-Carter et al. (2001) designed W/O/W multiple emulsions to protect water soluble carmine and oil soluble carotenoids from degradation, and they reported that the stability of the encapsulated colorants can be greatly improved by using mesquite gum as a wall material when the emulsions were spray dried. Rodríguez-Huezo et al. (2004) prepared W/O/W multiple emulsions containing β-carotene and spray dried them into powder, and they observed that higher encapsulation efficiency could be obtained when higher volume of biopolymers was involved in the primary emulsion, but a lower biopolymer ratio was favored when slower carotenoid degradation kinetics was required. W/O/W emulsions containing functional ingredients are particularly suitable for the development of fat-reduced food, as part of the oil can be displace by water (from oil to W/O droplets), but without losing their rheological properties and flavor profile (Lobato-Calleros et al., 2006).

Gelled Emulsion (filled hydrogel). Gelled emulsions generally contain gelling agents, which form gel network in the emulsions, and turn the liquid emulsions into soft solids. In gelled emulsions, oil droplets are immobilized in the gel networks (Figure 8), leading to slowed mass transfer and diffusion of the compounds incorporated (Dickinson, 2012b; McClements, 2010). To prepare gelled emulsions, gelling agents are usually added after homogenization, followed by gelation initiated by heat, acid, enzyme, salt or mechanical forces. Gelling agents can also be added into the aqueous phase of an emulsion before homogenization, but the high viscosity of the system may hinder the efficiency of emulsification. Typically, there are two types of gelled emulsions: (1) oil droplets are not interacting with gel network, for example, emulsions containing calcium-alginate, pectin, gelatine, starch (Malone and Appelqvist, 2003); (2) oil droplets behave as active fillers, which means that the droplets are involved in the development of gel networks, for example, protein-stabilized emulsions with a protein gel network in the water phase (Mao et al., 2014). To facilitate application, the gelled emulsions can be further broken down to form small gel particles, known as filled hydrogel particles (McClements, 2010).

Gelled emulsions are effective in protecting nutrients against external stresses, and they have been used to deliver food flavors, fish oil, flavor oil, etc. (Mao et al., 2015; Lamprecht et al., 2001; Weinbreck et al., 2004). As the gel structures can be adjusted by changing the concentrations of gelling agents, oil fractions, duration of gelation, magnitude of triggering forces, it is possible to produce gels with desired dimensions, hardness, permeability, making the gels have different responsiveness to environmental stresses (pH, temperature, enzyme, mechanical forces, etc.). Therefore, gelled emulsions can be used to control the release of functional compounds. Mun et al. (2015a) designed rice starch gels containing protein stabilized fat droplets (filled hydrogel), which were used to deliver β-carotene in simulated digestive conditions. The study indicated that β-carotene in the gels had higher bioaccessibility than that in ungelled emulsions or hydrogels without fat droplets, and this finding was attributed to the higher aggregation stability of the fat droplets in the gelled network. A following study by the same group reported that emulsifier type (WPI or tween 20) or starch type (rice starch or Mung bean starch) did

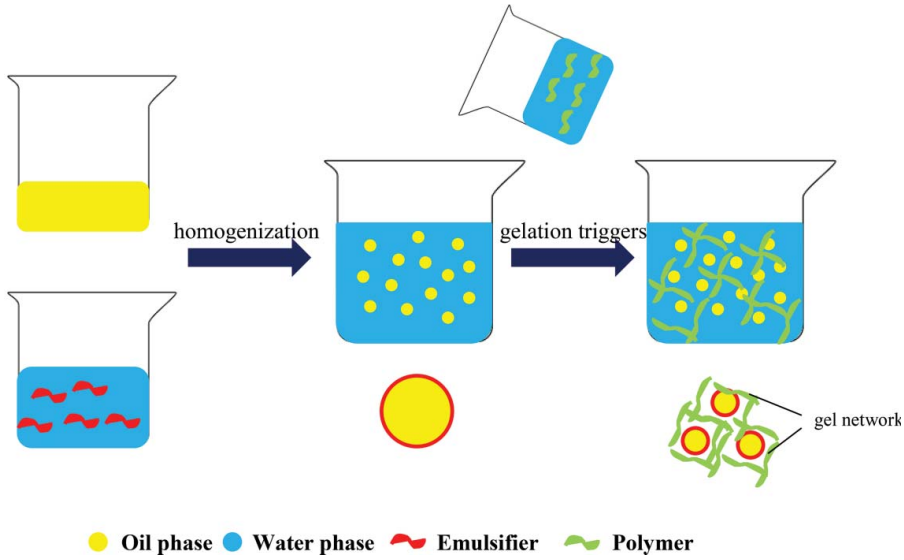

Figure 8. Schematic diagram of the formation of a gelled emulsion (gelation triggers could be heat, enzyme, pH change, etc.).

not affect the bioaccessibility of β-carotene in the gelled emulsions (Mun et al., 2015b).

Other Delivery Systems. Some other emulsion-based systems for the delivery of β-carotene were also developed in the literatures, though not intensively investigated for food application purposes. These systems include micelles (Ge et al., 2015), liposomes (Toniazzo et al., 2014), polymer gels (Belščak-Cvitanović et al., 2016), protein-polysaccharide coacervates (Jain et al., 2016).

7. Conclusion

O/W emulsions have been proved as effective delivery systems for β-carotene, and emulsions containing β-carotene are widely used in the food industry. The knowledge accumulated in the last decade has showed that the stability of β-carotene in emulsions is highly associated with its structures and the compositions. Although emulsions with small particle size are of better kinetic stability and are preferable for a higher bioaccessibility of β-carotene, the large interfacial area covering the oil droplets would accelerate β-carotene degradation. Therefore, ways to build up protecting structures (e.g., multilayer interface, solid interface, gelled matrix), or the addition of extra antioxidative compounds (e.g., antioxidants, milk proteins, polyphenols), are essential to ensure the functionality of β-carotene. It is preferable to use structure design techniques to modify the functionality of β-carotene emulsions to avoid the excessive use of food additives. In reality, loss of β-carotene in emulsion based foods is still difficult to control because of the various mechanisms of β-carotene degradation. On the other hand, the isomerization of β-carotene in emulsions was less investigated, which could alter the functionality of β-carotene. When emulsions are ingested, both emulsion structures and ingredients experience complicated changes in the digestive tract, and they would largely affect the bioavailability of β-carotene. A lot of studies suggested that the lipolysis of oils plays an important role in β-carotene digestion. However, food with higher content of fat is not favored in the current food market, and consumers are more interested in fat-reduced food enriched with nutrients. Therefore, knowledge about the release and micellarization of β-carotene in emulsions with lower content of fat is meaningful. Furthermore, current understanding of β-carotene bioaccessibility is mostly obtained from simplified in vitro models with no or less emphasis on the dynamic process of digestion, and it is not able to fully reflect the digestion of β-carotene emulsions. This suggested that more complicated digestion models are required for a better understanding of the biochemical and physiological changes of β-carotene emulsions, and how they affect the bioavailability of β-carotene.

Funding

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References

Akinosho, H. and Wicker, L. (2015). Stability of b-carotene loaded emulsions vary by viscosity of hydroxypropyl methylcellulose dispersions. *LWT-Food Sci. Technol.* **63**:582–589.

Aveyard, R., Binks, B. P. and Clint, J. H. (2003). Emulsions stabilized solely by solid colloidal particles. *Adv. Coll. Interf. Sci.* **100–102**:503–546.

Belščak-Cvitanović, A., Bušić, A., Barišić, L., Vrsaljko, D., Karlović, S., Špoljarić, I., Vojvodić, A., Mršić, G. and Komes, D. (2016). Emulsion templated microencapsulation of dandelion (*Taraxacumofficinale* L.) polyphenols and β-carotene by ionotropic gelation of alginate and pectin. *Food Hydrocoll.* **57**:139–152.

Beysseriat, M., Decker, E. A. and McClements, D. J. (2006). Preliminary study of the influence of dietary fiber on the properties of oil-in-water emulsions passing through an in vitro human digestion model. *Food Hydrocoll.* **20**:800–809.

Boon, C. S., McClements, D. J., Weiss, J. and Decker, E. A. (2010). Factors influencing the chemical stability of carotenoids in foods. *Crit. Rev. Food Sci. Nutr.* **50**:515–532.

Borel, P. (2003). Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin. Chem. Lab Med.* **4**:979–994.

Borel, P., Tyssandier, V., Mekki, N., Grolier, P., Rochette, Y., Alexandre-Gouabau, M.C., Lairon, D. and Azaïs-Braesco, V. (1998). Chylomicron

β-carotene and retinyl palmitate responses are dramatically diminished when men ingest β-carotene with medium-chain rather than long-chain triglycerides. *J. Nutr.* **128**:1361–1367.

Borsarelli, C. D. and Mercadante, A. Z. (2010). Thermal and photochemical degradation of carotenoids. **In**: Carotenoids: physical, chemical and biological functions and properties, pp. 229–253. Landrum, J. T., Eds., CRC Press, Boca Raton.

Burton, G. W. and Ingold, K. U. (1984). Beta-carotene: An unusual type of lipid antioxidant. *Science* **224**:569–573.

Castenmiller, J. J. M. and West, C. E. (1998). Bioavailability and bioconversion of carotenoids. *Annu. Rev. Nutr.* **18**:19–38.

Chevalier, Y. and Bolzinger, M.-A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Coll. Surf. A: Physicochem Eng. Asp.* **439**:23–34.

Chen, H. and Zhong, Q. (2015). Thermal and UV stability of β-carotene dissolved in peppermint oil microemulsified by sunflower lecithin and tween 20 blend. *Food Chem.* **174**:630–636.

Chanamai, R. and McClements, D. J. (2000). Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J. Agric. Food Chem.* **48**:5561–5565.

Christensen, J. O., Schultz, K., Mollgaard, B., Kristensen, H. G. and Mullertz, A. (2004). Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols. *Eur. J. Pharm. Sci.* **23**:287–296.

Cornacchia, L. and Roos, Y. H. (2012). Stability of β-carotene in protein-stabilized oil-in-water delivery systems. *J. Agric. Food Chem.* **59**:7013–7020.

Coupland, J. N. and McClements, D. J. (1996). Lipid oxidation in food emulsions. *Trends Food Sci. Tech.* **7**:83–91.

Coupland, J. N., Weiss, J., Lovy, A. and McClements, D. J. (1996). Solubilization kinetics of triacylglycerol and hydrocarbon emulsion droplets in a micellar solution. *J. Food Sci.* **61**:1114–1117.

Dickinson, E. (2012a). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. *Trends Food Sci. Tech.* **24**:4–12.

Dickinson, E. (2012b). Emulsion gels: the structuring of soft solid with protein-stabilized oil droplets. *Food Hydrocoll.* **28**:224–241.

Donhowe, E. G. and Kong, F. (2014). Beta-carotene: digestion, microencapsulation, and in vitro bioavailability. *Food Bioprocess Tech.* **7**:338–354.

Djordjevic, D., Cercaci, L., Alamed, J., McClements, D. J. and Decker, E. A. (2007). Chemical and physical stability of citral and limonene in sodium dodecyl sulfate-chitosan and gum arabic-stabilized oil-in-water emulsions. *J. Agric. Food Chem.* **55**:3585–3591.

Edge, R., McGarvey, D. J. and Truscott, T. G. (1997). The carotenoids as antioxidants—a review. *J. Photochem. Photobiol. B* **41**:189–200.

El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G. and Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Arch. Biochem. Biophys.* **430**:37–48.

Everett, S. A., Dennis, M. F., Patel, K. B., Maddix, S., Kundu, S. C. and Willson, R. L. (1996). Scavenging of nitrogen dioxide, thiyl, and sulfonyl free radicals by the nutritional antioxidant β-carotene. *J. Biol. Chem.* **271**:3988–3994.

Garti, N. and Bisperink, C. (1998). Double emulsions: progress and applications. *Curr. Opin. Coll. Interf. Sci.* **3**:657–667.

Garti, N., Yaghmur, A., Aserin, A., Spernath, A., Elfakess, R. and Ezrahi, S. (2003). Solubilization of active molecules in microemulsions for improved environmental protection. *Coll. Surf. A Physicochem Eng. Asp.* **230**:183–190.

Ge, W., Li, D., Chen, M., Wang, X., Liu, S. and Sun, R. (2015). Characterization and antioxidant activity of β-carotene loaded chitosan-graft-poly (lactide) nanomicelles. *Carbohydr. Polym.* **117**:169–176.

Ghosh, S. and Coupland, J. N. (2008). Factors affecting the freeze-thaw stability of emulsions. *Food Hydrocoll.* **22**:105–111.

GhouchiEskandar, N., Simovic, S. and Prestidge, C. A. (2009). Chemical stability and phase distribution of all-trans-retinol in nanoparticle coated emulsions. *Int. J. Pharm.* **376**:186–194.

Gleize, B., Tourniaire, F., Depezay, L., Bott, R., Nowicki, M., Albino, L., Lairon, D., Kesse-Guyot, E., Galan, P. and Hercberg, S. (2013). Effect of type of TAG fatty acids on lutein and zeaxanthin bioavailability. *Brit. J. Nutr.* **110**:1–10.

Grune, T., Lietz, G., Palou, A., Ross, A. C., Stahl, W., Tang, G., Thurnham, D., Yin, S. and Biesalski, H. K. (2010). β-Carotene is an important vitamin A source for humans. *J. Nutr.* **140**:2268S–2285S.

Guan, Y., Wu, J. and Zhong, Q. (2016). Eugenol improves physical and chemical stabilities of nanoemulsions loaded with β-carotene. *Food Chem.* **194**:787–796.

Guzey, D. and McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Adv. Coll. Interf. Sci.* **128–130**:227–248.

Hedren, E., Diaz, V. and Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *Eur. J. Clin. Nutr.* **56**:425–430.

Helgason, T., Awad, T. S., Kristbergsson, K., Decker, E. A., McClements, D. J. and Weiss, J. (2009). Impact of surfactant properties on oxidative stability of β-carotene encapsulated within solid lipid nanoparticles. *J. Agric. Food Chem.* **57**:8033–8040.

Hou, Z., Gao, Y., Yuan, F., Liu, Y., Li, C. and Xu, D. (2010). Investigation into the physicochemical stability and rheological properties of β-carotene emulsion stabilized by soybean soluble polysaccharides and chitosan. *J. Agric. Food Chem.* **58**:8604–8611.

Hou, Z., Liu, Y., Lei, F. and Gao, Y. (2014). Investigation into the in vitro release properties of β-carotene in emulsions stabilized by different emulsifiers. *LWT-Food Sci. Technol.* **59**:867–873.

Hou, Z., Zhang, M., Liu, B., Yan, Q., Yuan, F., Xu, D. and Gao, Y. (2012). Effect of chitosan molecular weight on the stability and rheological properties of β-carotene emulsions stabilized by soybean soluble polysaccharides. *Food Hydrocoll.* **26**:205–211.

Imsic, M., Winkler, S., Tomkins, B. and Jones, R. (2010). Effect of storage and cooking on β-carotene isomers in carrots (*Daucuscarota* L. cv. '*Stefano*'). *J. Agric. Food Chem.* **58**:5109–5113.

Jain, A., Thakur, D., Ghoshal, G., Katare, O. P. and Shivhare, U. S. (2016). Characterization of microcapsulated β-carotene formed by complex coacervation using casein & gum tragacanth. *Int. J. Biol. Macromol.* **87**:101–113.

Johnson, E. J. (2002). The role of carotenoids in human health. *Nutr. Clin. Care* **5**:56–65.

Jo, Y. J. and Kwon, Y. J. (2014). Characterization of β-carotene nanoemulsions prepared by microfluidization technique. *Food Sci. Biotechnol.* **23**:107–113.

Kalashnikova, I., Bizot, H., Cathala, B. and Capron, I. (2011). New Pickering emulsions stabilized by bacterial cellulose nanocrystals. *Langmuir* **27**:7471–7479.

Kanafusa, S., Chu, B.-S. and Nakajima, M. (2007). Factors affecting droplet size of sodium caseinate-stabilized emulsions containing β-carotene. *Eur. J. Lipid Sci. Tech.* **109**:1038–1041.

Knockaert, G., Pulissery, S. K., Lemmens, L., Van Buggenhout, S., Hendrickx, M. and van Loey, A. (2012). Carrot β-carotene degradation and isomerization kinetics during thermal processing in the presence of oil. *J. Agric. Food Chem.* **60**:10312–10319.

Kossena, G. A., Boyd, B. J., Porter, C. J. H. and Charman, W. N. (2003). Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. *J. Pharm. Sci.* **92**:634–648.

Krinsky, N. I. and Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Mol. Aspects Med.* **26**:459–516.

Laakso, S. (1984). Inhibition of lipid peroxidation by caseins: evidence of molecular encapsulation of 4, 4-pentadiene fatty acids. *BBA-Lipid Metab.* **792**:11–15.

Lamprecht, A., Schäfer, U. and Lehr, C. M. (2001). Influences of process parameters on preparation of microparticle used as a carrier system for omega—3 unsaturated fatty acid ethyl esters used in supplementary nutrition. *J. Microencapsul.* **18**:347–357.

Liang, R., Shoemaker, C. F., Yang, X., Zhong, F. and Huang, Q. (2013). Stability and bioaccessibility of β-carotene in nanoemulsions stabilized by modified starches. *J. Agric. Food Chem.* **61**:1249–1257.

Lim, A., Griffin, C. and Roos, Y. H. (2014). Stability and loss kinetics of lutein and β-carotene encapsulated in freeze-dried emulsions with layered interface and trehalose as glass former. *Food Res. Int.* **62**:403–409.

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

Liu, Y., Lei, F., Yuan, F. and Gao, Y. (2014). Effects of milk proteins on release properties and particle morphology of β-carotene emulsions during in vitro digestion. *Food Funct.* **5**:2940–2947.

Liu, Y., Hou, Z., Yang, J. and Gao, Y. (2015). Effects of antioxidants on the stability of β-carotene in O/W emulsions stabilized by gum Arabic. *J. Food Sci. Tech.* **52**:3300–3311.

Liu, F., Wang, D., Sun, C. and Gao, Y. (2016a). Influence of polysaccharides on the physicochemical properties of lactoferrine-polyphenol conjugates coated β-carotene emulsions. *Food Hydrocoll.* **52**:661–669.

Liu, L., Gao, Y., McClements, D. J. and Decker, E. A. (2016b). Role of continuous phase protein, (−)-epigallocatechin-3-gallate and carrier oil on β-carotene degradation in oil-in-water emulsions. *Food Chem.* **210**:242–248.

Liu, F., Ma, C., McClements, D. J. and Gao, Y. (2016c). Development of polyphenol-protein-polysaccharide ternary complexes as emulsifiers for nutraceutical emulsions: Impact on formation, stability, and bioaccessibility of β-carotene emulsions. *Food Hydrocoll.* **61**:578–588.

Liu, F. and Tang, C. (2016). Soy glycinin as food-grade Pickering stabilizers: Part. III. Fabrication of gel-like emulsions and their potential as sustained-release delivery systems for β-carotene. *Food Hydrocoll.* **56**:434–444.

Lobato-Calleros, C., Rodriguez, E., Sandoval-Castilla, O., Vernon-Carter, E. J. and Alvarez-Ramirez, J. (2006). Reduced-fat white fresh cheese-like products obtained from W1/O/W2 multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Res. Int.* **39**:678–685.

Luo, Z., Murray, B. S., Yusoff, A., Morgan, M. R. A., Povey, M. J. W. and Day, A. J. (2011). Particle-stabilizing effects of flavonoids at the oil-water interface. *J. Agric. Food Chem.* **59**:2636–2645.

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

Malaki Nik, A., Corredig, M. and Wright, A. J. (2010). Changes in WPI-stabilized emulsion interfacial properties in relation to lipolysis and β-carotene transfer during exposure to simulated gastric-duodenal fluids of variable composition. *Food Digest.* **1**:14–27.

MalakiNik, A., Langmaid, S. and Wright, A. J. (2012a). Digestibility and β-carotene release from lipid nanodispersions depend on dispersed phase crystallinity and interfacial properties. *Food Funct.* **3**:234–245.

MalakiNik, A., Langmaid, S. and Wright, A. J. (2012b). Nonionic surfactant and interfacial structure impact crystallinity and stability of β-carotene loaded lipid nanodispersions. *J. Agric. Food Chem.* **60**:4126–4135.

Malone, M. and Appelqvist, I. A. M. (2003). Gelled emulsion particles for the controlled release of lipophilic volatiles during eating. *J. Control Rel.* **90**:227–241.

Mao, L., Xu, D., Yang, J., Yuan, F., Gao, Y. and Zhao, J. (2009). Effects of small and large molecule emulsifiers on the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization. *Food Technol. Biotech.* **47**:336–342.

Mao, L., Yang, J., Xu, D., Yuan, F. and Gao, Y. (2010). Effects of homogenization models and emulsifiers on the physicochemical properties of β-carotene nanoemulsions. *J. Disper. Sci. Technol.* **31**:986–993.

Mao, L., Roos, Y. H., O'Callaghan, D. J. and Miao, S. (2013). Volatile Release from whey protein isolate-pectin multilayer stabilized emulsions: effect of pH, salt and artificial salivas. *J. Agric. Food Chem.* **61**:6231–6239.

Mao, L., Roos, Y. H. and Miao, S. (2014). Study on the rheological properties and volatile release of cold-set emulsion-filled protein gels. *J. Agric. Food Chem.* **62**:11420–11428.

Mao, L. and Miao, S. (2015). Structuring food emulsions to improve nutrient delivery during digestion. *Food Eng. Rev.* **7**:439–451.

Mao, L., Roos, Y. H., Biliaderis, C. G. and Miao, S. (2015). Food emulsions as delivery systems for flavor compounds—A review. *Crit. Rev. Food Sci. Nutr.* DOI: 10.1080/10408398.2015.1098586.

McClements, D. J. (2005). Food Emulsions: Principles, Practices and Techniques (2nd ed.). CRC Press, Boca Raton, FL.

McClements, D. J. (2010). Emulsion design to improve the delivery of functional lipophilic components. *Annu. Rev. Food Sci. Tech.* **1**:241–269.

McClements, D. J. (2012). Edible delivery systems for nutraceuticals: designing functional foods for improved health. *Therapeut. Deliv.* **3**:801–803.

McClements, D. J. and Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Adv. Coll. Interf. Sci.* **159**:213–228.

Mesnier, X., Gregory, C., Fança-Berthon, P., Boukobza, F. and Bily, A. (2014). Heat and light colour stability of beverages coloured with a natural carotene emulsion: Effect of synthetic versus natural water soluble antioxidants. *Food Res. Int.* **65**:149–155.

Müller, R. H., Radtke, M. and Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv. Drug Deliv. Rev.* **54**:S131–S155.

Mun, S., Kim, Y. and McClements, D. J. (2015a). Control of β-carotene bioaccessibility using starch-based filled hydrogels. *Food Chem.* **173**:454–461.

Mun, S., Kim, Y., Shin, M. and McClements, D. J. (2015b). Control of lipid digestion and nutraceutical bioaccessibility using starch-based filled hydrogels: influence of starch and surfactant type. *Food Hydrocoll.* **44**:380–389.

Neves, M. A., Ribeiro, H. S., Kobayashi, I. and Nakajima, M. (2008). Encapsulation of lipophilic bioactive molecules by microchannel emulsification. *Food Biophys.* **3**:126–131.

Nishimukai, M. and Hara, H. (2004). Enteral administration of soybean phosphatidylcholine enhances the lymphatic absorption of lycopene, but reduces that of alpha-tocopherol in rats. *J. Nutr.* **134**:1862–1866.

Oliver, C. M., Melton, L. D. and Stanley, R. (2006). Functional properties of caseinate glycoconjugates prepared by controlled heating in the dry state. *J. Sci. Food Agric* **86**:732–740.

Palozza, P. (1998). Prooxidant actions of carotenoids in biologic systems. *Nutr. Rev.* **56**:257–265.

Palozza, P., Serini, S., Nicuolo, F. D., Piccioni, E. and Calviello, G. (2003). Prooxidant effects of β-carotene in cultured cells. *Mol. Aspects Med.* **24**:353–362.

Qian, C., Decker, E. A., Xiao, H. and McClements, D. J. (2012a). Inhibition of β-carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. *Food Chem.* **135**:1036–1043.

Qian, C., Decker, E. A., Xiao, H. and McClements, D. J. (2012b). Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. *Food Chem.* **135**:1440–1447.

Rao, J. and McClements, D. J. (2011). Food-grade microemulsions, nanoemulsions and emulsions: fabrication from sucrose monopalmitate and lemon oil. *Food Hydrocoll.* **25**:1413–1423.

Rao, J., Decker, E. A., Xiao, H. and McClements, D. J. (2013). Nutraceutical nanoemulsions: influence of carrier oil composition (digestible versus indigestible oil) on β-carotene bioavailability. *J. Sci. Food Agric.* **93**:3175–3183.

Ribeiro, H. S., Chu, B., Ichikawa, S. and Nakajima, M. (2008). Preparation of nanodispersions containing β-carotene by solvent displacement method. *Food Hydrocoll.* **22**:12–17.

Robins, M. M. (2000). Emulsions—creaming phenomena. *Curr. Opin. Coll. Interf. Sci.* **5**:265–272.

Rodriguez-Amaya, D. B. (2002). Effects of processing and storage on food carotenoids. *Sight Life Newsl.* **3**:25–35.

Rodriguez-Amaya, D. B. (2015). Food carotenoids: chemistry, biology and technology. Wiley Blackwell, West Sussex, UK.

Rodríguez-Huezo, M. E., Pedroza-Islas, R., Prado-Barragan, L. A., Beristain, C. I. and Vernon-Carter, E. J. (2004). Microencapsulation by spray drying of multiple emulsions containing carotenoids. *J. Food Sci.* **69**:E351–E359.

Ruiz-Rodriguez, P., Meshulam, D. and Lesmes, U. (2014). Characterization of Pickering O/W emulsions stabilized by silica nanoparticles and their responsiveness to in vitro digestion conditions. *Food Biophys.* **9**:406–415.

Salvia-Trujillo, L., Qian, C., Martín-Belloso, Q. and McClements, D. J. (2013). Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chem.* **141**:1472–1480.

Schieber, A. and Carle, R. (2005). Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. *Trends Food Sci. Technol.* **16**:416–422.

Shao, Y. and Tang, C. (2016). Gel-like pea protein Pickering emulsions at pH 3.0 as a potential intestine-targeted and sustained-release delivery system for β-carotene. *Food Res. Int.* **79**:64–72.

Soukoulis, C. and Bohn, T. (2015). A comprehensive overview on the micro- and nano-technological encapsulation advances for enhancing the chemical stability and bioavailability of carotenoids. *Crit. Rev. Food Sci. Nutr.* DOI: 10.1080/10408398.2014.971353.

Stahl, W. and Sies, H. (1993). Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. *Ann. NY Acad. Sci.* **691**:10–19.

Sun, C., Gunasekaran, S. and Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocoll.* **21**:555–564.

Sweedman, M. C., Hasjim, J., Schäfer, C. and Gilbert, R. G. (2014). Structures of octenylsuccinylated starches: Effects on emulsions containing β-carotene. *Carbohydr. Polym.* **112**:85–93.

Tan, C. P. and Nakajima, M. (2005a). β-carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chem.* **92**:661–671.

Tan, C. P. and Nakajima, M. (2005b). Effect of polyglycerol esters of fatty acids on physicochemical properties and stability of β-carotene nanodispersions prepared by emulsification/evaporation method. *J. Sci. Food Agric.* **85**:121–126.

Tcholakova, S., Denkov, N. D., Ivanov, I. B. and Campbell, B. (2006). Coalescence stability of emulsions containing globular milk proteins. *Adv. Coll. Interf. Sci.* **123–126**:259–293.

Tokle, T., Mao, Y. and McClements, D. J. (2013). Potential biological fate of emulsion-based delivery systems: lipid particles nanolaminated with lactoferrin and β-lactoglobulin coatings. *Pharm. Res.* **30**:3200–3213.

Toniazzo, T., Berbel, I. F., Cho, S., Fávaro-Trindade, C. S, Moraes, I. C. F. and Pinho, S. C. (2014). β-carotene-loaded liposome dispersions stabilized with xanthan and guar gums: Physico-chemical stability and feasibility of application in yogurt. *LWT—Food Sci. Technol.* **59**:1265–1273.

Trentin, A., De Lamo, S., Güell, C., López, F. and Ferrando, M. (2011). Protein-stabilized emulsions containing beta-carotene produced by premix membrane emulsification. *J. Food Eng.* **106**:267–274.

Trombino, S., Cassano, R., Muzzalupo, R., Pingitore, A., Cione, E. and Picci, N. (2009). Stearylferulate-based solid lipid nanoparticles for the encapsulation and stabilization of β-carotene and α-tocopherol. *Coll. Surf. B* **72**:181–187.

Tzoumaki, M. V., Moschakis, T., Kiosseoglou, V. and Biliaderis, C. G. (2011). Oil-in-water emulsions stabilized by chitin nanocrystal particles. *Food Hydrocoll.* **25**:1521–1529.

van Het Hof, K. H., West, C. E., Weststrate, J. A. and Hautvast, J. G. (2000). Dietary factors that affect the bioavailability of carotenoids. *J. Nutr.* **130**:503–506.

Velikow, K. P. and Pelan, E. (2008). Colloidal delivery systems for micronutrients and nutraceuticals. *Soft Matter* **4**:1964–1980.

Vernon-Carter, E. J. and Ponce-Palafox, J. T., Arredondo-Figueroa, J. L. and Pedroza-Islas, R. (2001). Development of microcapsules containing water and lipid soluble natural colorants for trout pigmentation. *J. Aquat. Food Prod. Tech.* **10**:59–74.

Verrijssen, T. A. J., Balduyck, L. G., Christiaens, S., van Loey, A. M., van Buggenhout, S. and Hendrickx, M. E. (2014). The effect of pectin concentration and degree of methyl-esterification on the in vitro bioaccessibility of β-carotene-enriched emulsions. *Food Res. Int.* **57**:71–78.

Verrijssen, T. A. J., Smeets, K. H. G., Christiaens, S., Palmers, S., van Loey, A. M. and Hendrickx, M. E. (2015). Relation between in vitro lipid digestion and β-carotene bioaccessibility in β-carotene-enriched emulsions with different concentrations of L-α-phosphatidylcholine. *Food Res. Int.* **67**:60–66.

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

Wang, M. S., Chaudhari, A., Pan, Y., Young, S. and Nitin, N. (2014a). Controlled release of natural polyphenols in oral cavity using starch Pickering emulsion. *MRS Proceedings 1688, mrss14-1688-y08-11* doi:10.1557/opl.2014.482.

Wang, X., Zhang, J., Lei, F., Liang, C., Yuan, F. and Gao, Y. (2014b). Covalent complexation and functional evaluation of (−)-epigallocatechingallate and α-lactalbumin. *Food Chem.* **150**:341–347.

Wang, X., Liu, F., Liu, L., Wei, Z., Yuan, F. and Gao, Y. (2015). Physicochemical characterisation of β-carotene emulsion stabilised by covalent complexes of α-lactalbumin with (−)-epigallocatechingallate or chlorogenic acid. *Food Chem.* **173**:564–568.

Weber, D. and Grune, T. (2012). The contribution of beta-carotene to vitamin A supply of humans. *Mol. Nutr. Food Res.* **56**:251–258.

Wei, Z. and Gao, Y. (2016). Physicochemical properties of β-carotene bilayer emulsions coated by milk proteins and chitosan-EGCG conjugates. *Food Hydrocoll.* **52**:590–599.

Wei, Z., Yang, W., Fan, R., Yuan, F. and Gao, Y. (2015). Evaluation of structural and functional properties of protein–EGCG complexes and their ability of stabilizing a model β-carotene emulsion. *Food Hydrocoll.* **45**:337–350.

Weinbreck, F., Minor, M. and de Kruif, C. G. (2004). Microencapsulation of oils using whey protein/gum Arabic coacervates. *J. Microencapsul.* **21**:667–379.

Xiao, J., Wang, X, Gonzalez, A. J. P. and Huang, Q. (2016). Kafirin nanoparticles-stabilized Pickering emulsions: Microstructure and rheological behaviour. *Food Hydrocoll.* **54**:30–39.

Xu, D., Yuan, F., Wang, X., Li, X., Hou, Z. and Gao, Y. (2011). The effect of whey protein isolate-dextran conjugates on the free-thaw stability of oil-in-water emulsion. *J. Disper. Sci. Technol.* **32**:77–83.

Xu, D., Wang, X., Jiang, J., Yuan, F. and Gao, Y. (2012). Impact of whey protein-beet pectin conjugation on the physicochemical stability of β-carotene emulsions. *Food Hydrocoll.* **28**:258–266.

Xu, D., Wang, X., Jiang, J., Yuan, F., Decker, E. A. and Gao, Y. (2013a). Influence of pH, EDTA, α-tocopherol, and WPI oxidation on the degradation of β-carotene in WPI-stabilized oil-in-water emulsions. *LWT—Food Sci. Technol.* **54**:236–241.

Xu, D., Wang, X., Yuan, F., Hou, Z. and Gao, Y. (2013b). Stability of β-carotene in oil-in-water emulsions prepared by mixed layer and bilayer of whey protein isolate and beet pectin. *J. Disper. Sci. Technol.* **34**:785–792.

Xu, D., Yuan, F., Gao, Y., McClements, D. J. and Decker, E. A. (2013c). Influence of pH, metal chelator, free radical scavenger and interfacial characteristics on the oxidative stability of β-carotene in conjugated whey protein–pectin stabilised emulsion. *Food Chem.* **139**:1098–1104.

Yi, J., Lam, T. I., Yokoyama, W., Cheng, L. W. and Zhong, F. (2014a). Cellular uptake of β-carotene from protein stabilized solid lipid nanoparticles prepared by homogenization-evaporation method. *J. Agric. Food Chem.* **62**:1096–1104.

Yi, J., Li, Y., Zhong, F. and Yokoyama, W. (2014b). The physicochemical stability and in vitro bioaccessibility of beta-carotene in oil-in-water sodium caseinate emulsions. *Food Hydrocoll.* **35**:19–27.

Yi, J., Zhang, Y., Liang, R., Zhong, F. and Ma, J. (2015a). Beta-carotene chemical stability in nanoemulsions was improved by stabilized with beta-lactoglobulin-catechin conjugates through free radical method. *J. Agric. Food Chem.* **63**:297–303.

Yi, J., Zhong, F., Zhang, Y., Yokoyama, W. and Zhao, L. (2015b). Effects of lipids on in vitro release and cellular uptake of β-carotene in nanoemulsion-based delivery systems. *J. Agric. Food Chem.* **63**:10831–10837.

Yi, J., Fan, Y., Yokoyama, W., Zhang, Y. and Zhao, L. (2016). Thermal degradation and isomerization of β-carotene in oil-in-water nanoemulsions supplemented with natural antioxidants. *J. Agric. Food Chem.* **64**:1970–1976.

Yin, L. J., Chu, B. S., Kobayashi, I. and Nakajima, M. (2009). Performance of selected emulsifiers and their combinations in the preparation of β-carotene nanodispersions. *Food Hydrocolloids* **23**:1617–1622.

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

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

Young, A. J. and Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Arch Biochem. Biophys.* **385**:20–27.

Yuan, Y., Gao, Y., Zhao, J. and Mao, L. (2008). Characterization and stability evaluation of β-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Res. Int.* **41**:61–68.

Yusoff, A. and Murray, B. S. (2011). Modified starch granules as particle stabilizers of oil-in-water emulsions. *Food Hydrocoll.* **25**:42–55.

Zhang, L., Hayes, D. G., Chen, G. and Zhong, Q. (2013). Transparent dispersions of milk-fat-based nanostructured lipid carriers for delivery of β-carotene. *J. Agric. Food Chem.* **61**:9435–9443.