Boosting the bioavailability of hydrophobic nutrients, vitamins, and nutraceuticals in natural products using excipient emulsions

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Abstract

Many highly hydrophobic bioactives, such as non-polar nutrients, nutraceuticals, and vitamins, have a relatively low or variable oral bioavailability. The poor bioavailability profile of these bioactives may be due to limited bioaccessibility, poor absorption, and/or chemical transformation within the gastrointestinal tract (GIT). The bioavailability of hydrophobic bioactives can be improved using specially designed oil-in-water emulsions consisting of lipid droplets dispersed within an aqueous phase. The bioactives may be isolated from their natural environment and then incorporated into the lipid phase of *emulsion-based delivery systems*. Alternatively, the bioactives may be left in their natural environment (*e.g.*, fruits or vegetables), and then ingested with *emulsion-based excipient systems*. An excipient emulsion may have no inherent health benefits itself, but it boosts the biological activity of bioactive ingredients co-ingested with it by altering their bioaccessibility, absorption, and/or chemical transformation. This review discusses the design and fabrication of excipient emulsions, and gives some examples of recent research that demonstrates their potential efficacy for improving the bioavailability of hydrophobic bioactives. **The concept of excipient emulsions could be used to formulate emulsion-based food products (such as excipient sauces, dressings, dips, creams, or yogurts) specifically designed to increase the bioavailability of bioactive agents in natural foods, such as fruits and vegetables.**

Keywords: food effects; nutraceuticals; vitamins; excipient emulsions; delivery systems; functional foods.

1. Introduction

Many natural and processed foods contain hydrophobic bioactive agents that may have beneficial effects on human health when consumed as part of a regular diet, including nutrients (such as certain triacylglycerols), vitamins (such as vitamin A, D, and E), and nutraceuticals (such as carotenoids, curcumin, flavonoids, and coenzyme Q10) (Aluko, 2012; Huang, Yu, & Ru, 2010; McClements, 2010). However, the potential health benefits of these dietary bioactives are often not fully realized because of their low or variable oral bioavailability (Fernandez-Garcia et al., 2012; Rein et al., 2013; Zou, Liu, Liu, Xiao, & McClements, 2015a). Numerous factors may contribute to the poor bioavailability profiles of hydrophobic bioactives, but these can often be divided into three major categories: poor bioaccessibility; low absorption; transformation into an inactive form. The dominant factors that limit the bioavailability of a bioactive agent in a specific food have to be established before a successful strategy can be developed to improve its bioavailability profile (McClements & Xiao, 2014; Zou et al., 2015a).

In general, there are two major approaches that can be used to improve the bioavailability of hydrophobic bioactives in foods (**Figure 1**). The first approach is to isolate the bioactive components from their natural environment (such as a fruit or vegetable) and then to incorporate them into a suitable delivery system (McClements, 2015b). A wide range of colloidal delivery systems have been developed to encapsulate bioactive agents and have been reviewed in detail elsewhere, such as molecular complexes, microemulsions, emulsions, liposomes, solid lipid nanoparticles, biopolymer particles, and microgels (Huang et al., 2010; Marze, 2013; McClements, 2010; Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015; Ting, Jiang, Ho, & Huang, 2014; Velikov & Pelan, 2008). **Emulsion-based delivery systems are particularly suitable for this purpose because they are already widely used in the food industry, and because of their ease of fabrication from widely used food ingredients and processing operation.** Delivery systems must be carefully designed to ensure they are compatible with the food they are to be incorporated into, cost-effective, safe, robust, and efficacious. Colloidal delivery systems are particularly suitable for applications where the hydrophobic bioactive is initially present within an underutilized waste-stream, such as the peel of a fruit or vegetable that would normally be discarded. The second approach for increasing bioavailability is to leave the bioactive components within their natural environment (such as fruits or vegetables), but consume them with specially designed “excipient foods” (McClements & Xiao, 2014). An excipient food may

have no bioactivity itself, but it boosts the bioavailability of bioactive agents that are consumed with it by altering their bioaccessibility, absorption, or transformation within the gastrointestinal tract (GIT). Oil-in-water emulsions are particularly effective vehicles for developing excipient foods because of the great flexibility in designing their compositions and structures. Thus, an “excipient emulsion” can be defined as an emulsion that increases the bioactivity of bioactive agents in foods that are co-ingested with it, without necessarily having any intrinsic bioactivity itself. In summary, oil-in-water emulsions can either be used as delivery systems or as excipient systems. For delivery systems, the hydrophobic bioactive agents are encapsulated within the oil phase of the emulsion, but for excipient systems a bioactive-free emulsion is consumed with a bioactive-rich food.

The purpose of this article is to highlight the major factors that limit the oral bioavailability of hydrophobic bioactive agents from fruits and vegetables, and then to describe how excipient emulsions can be developed to overcome these hurdles. This knowledge could be used to develop a new range of excipient food products specifically designed to increase the bioavailability of bioactive agents from natural products (such as fruits, vegetables, cereals, meats, or fish), e.g., excipient sauces, dressings, dips, creams, or beverages.

2. Characterizing Factors Limiting Oral Bioavailability

The major factors that limit the oral bioavailability of bioactives in fruits and vegetables can be characterized using the recently developed *Nutraceutical Bioavailability Classification Scheme* (NuBACS), which has recently been discussed in detail elsewhere (Zou et al., 2015a). Briefly, this scheme divides the limiting factors into three categories based on the physiochemical or physiological mechanisms limiting bioavailability (**Figure 2**): Bioaccessibility (B^*); Absorption (A^*); Transformation (T^*). Each of these categories is given the symbol “(+)” when it is a non-limiting factor and “(-)” when it is a limiting factor. The precise mechanism involved for limiting the bioavailability is described by utilizing sub-categories within each major category. For bioaccessibility, the sub-categories are poor liberation from the food matrix (L), poor solubility in the gastrointestinal fluids (S), and interactions that promote insolubility (I). For absorption, the sub-categories are poor transport through the mucus layer (ML), through the tight junctions (TJ), or through the bilayer membrane (MP), the inhibition of active transporters (AT), or the presence of efflux transporters (ET) in the

epithelium cells. For transformation, the sub-categories indicate whether the change in bioactivity is due to chemical (C) or metabolic (M) mechanisms, such as lipid oxidation, acid hydrolysis, isomerization, or Phase 1/Phase 2 metabolisms. As an example, the NuBACS characterization of carotenoids present within carrots would be $B^{*}(-)_{L,S} A^{*}(+) T^{*}(-)_{C,M}$, since the bioavailability is mainly determined by limited liberation from the plant cell tissues, poor solubility in gastrointestinal fluids, and chemical/metabolic transformation within the GIT.

The NuBACS is particularly useful for placing bioactives into groups whose bioavailability may be improved using a common strategy. For example, strongly hydrophobic bioactives (such as carotenoids, curcumin, and Coenzyme Q10) can be placed within the $B^{*}(-)_S$ group to indicate that their bioavailability is normally limited by their low solubility in aqueous gastrointestinal fluids. The bioavailability of bioactives in this group can be improved by ingesting them with lipids that are digested within the GIT and form mixed micelles with a high solubilizing capacity. A more detailed discussion of some of the major factors influencing the bioaccessibility, absorption, or transformation of bioactive agents is given later in this article.

3. Excipient Emulsions

The composition and structure of excipient emulsions are specifically designed to increase the bioavailability of bioactives by altering their bioaccessibility, absorption, or transformation in the GIT. In this section, we highlight how excipient emulsions with different properties can be fabricated, and then highlight how they can be designed to improve bioavailability.

3.1. Fabrication

Oil-in-water emulsions ($d > 100$ nm) or nanoemulsions ($d < 100$ nm) consist of emulsifier-coated lipid droplets dispersed within an aqueous medium (McClements, 2015a). For the sake of convenience, the term “emulsion” will be used to refer to both conventional emulsions and nanoemulsions in the remainder of this article since the only major difference between these two systems is their particle dimensions. Emulsions can be fabricated using many numerous different approaches in the laboratory and commercially (McClements, 2015a). Nevertheless, these approaches can be divided into two major categories: high- and low-energy methods. High-energy methods utilize mechanical devices specially designed to thoroughly mix the oil and water phases and/or to break up the oil droplets. Some of the most commonly used mechanical devices for this purpose are high shear mixers, colloid mills, high-pressure valve

homogenizers, microfluidizers, and sonicators. Low-energy methods utilize changes in system composition or surfactant properties to spontaneously generate small oil droplets, and include spontaneous emulsification, emulsion inversion point, and phase inversion temperature methods. The major advantage of high-energy methods is that they can be applied to a wide variety of oil and emulsifier types, and can produce small droplets using relatively low emulsifier-to-oil levels. The major limitations of these methods are that specialized mechanical devices (homogenizers) are required that must be purchased and maintained. The major advantage of low-energy methods is that no specialized or costly equipment is needed to fabricate them, but the major limitation is that they are typically only suitable for forming emulsions from a narrow range of oils and surfactants, and require relatively high emulsifier-to-oil ratios. Nevertheless, the availability of numerous different emulsion fabrication methods gives great scope for designing excipient emulsions with different compositions and structures.

3.2. Design Parameters

Oil-in-water emulsions have considerable flexibility for forming excipient foods since both the composition and structure can easily be altered (**Figure 3**) (McClements, 2015a):

Droplet concentration: The level of lipid droplets within an excipient emulsion can simply be varied by using a different ratio of oil phase to water phase during emulsion formation, or by diluting or concentrating an emulsion after it has been formed. Lipid droplet concentrations ranging from less than 0.1% to greater than 80% can be produced, which allows one to tailor the lipid content for particular applications.

Droplet size distribution: The size of the droplets in an emulsion can be varied by changing the type of homogenization method used to fabricate it, by altering homogenization conditions (such as pressure and number of passes), or by altering system composition (such as emulsifier type and concentration). The droplet size distribution will influence the rate and extent of lipid droplet digestion in the GIT, and therefore alter the gastrointestinal fate of bioactives.

Droplet composition: The lipid phase composition of oil-in-water emulsions can be varied by including different lipophilic component within it, such as triacylglycerol oils, flavor oils, essential oils, mineral oils, waxes, and antioxidants. The nature of the lipophilic materials will determine the rate and extent of lipid digestion within the gastrointestinal tract. In addition, the chemical structure of the lipid molecules can be varied, such as the chain length, unsaturation, and position of the fatty acids in triacylglycerol molecules. This factor has important

consequences for determining the solubilization capacity of the mixed micelle phase formed within the small intestine (see later).

Droplet location: The location of the lipid droplets within an emulsion may alter their gastrointestinal fate, and therefore their ability to promote the bioavailability of co-ingested hydrophobic bioactives. For example, the lipid droplets may be freely dispersed throughout the aqueous phase, or they may be embedded within larger structures, such as microgels or biopolymer particles (McClements, 2015b). These larger structures can be designed to prevent the lipid droplets from being released and digested in certain regions of the GIT (such as mouth and stomach), but then be released and digested in other regions (such as small intestine). This may impact the rate and extent of mixed micelle formation or antioxidant release, which may be a useful tool to alter the bioavailability of some bioactives.

Interfacial composition: The composition, thickness, structure, and charge of the interfacial layer surrounding the lipid droplets can be varied using different types of emulsifiers to prepare the emulsion, or by post-homogenization methods (such as layer-by-layer deposition) (McClements & Rao, 2011). The interfacial composition determines the aggregation state of the lipid droplets in the different regions of the GIT (*e.g.*, whether they are stable, flocculated, or coalesced), which will alter the exposed lipid surface area and therefore digestibility. In addition, the nature of the interface will influence the ability of bile salts and digestive enzymes to adsorb to the lipid droplet surfaces and initiate lipolysis (Couedelo et al., 2015).

Aqueous phase composition: A variety of hydrophilic ingredients can be added to the aqueous phase of excipient emulsions either before or after homogenization, including chelating agents, antioxidants, surfactants, minerals, and biopolymers (McClements & Xiao, 2014). Some of these components can act as excipient ingredients by modulating the bioaccessibility, absorption, or transformation of hydrophobic bioactives in the GIT. **This is a potential advantage of using emulsions as excipient systems, since hydrophilic, hydrophobic, and amphiphilic excipient ingredients can be incorporated into a single food.**

3.3. Mechanisms of Action

Excipient emulsions can be designed to improve the bioavailability of hydrophobic bioactives through a variety of different mechanisms (**Figure 2**). In this section, we highlight some of the major ways that excipient emulsions may alter the bioaccessibility, absorption, or transformation of hydrophobic bioactives within the GIT.

3.3.1. Bioaccessibility

The bioaccessibility can be defined as the amount of a bioactive agent that is present within the GIT in a form suitable for absorption (m_B) compared to the total amount ingested (m_{Total}): $B^* = 100 \times m_B/m_{Total}$. For a hydrophobic bioactive agent, the amount in a bioaccessible form is usually taken to be the amount solubilized within the mixed micelle phase. There are a number of ways that excipient emulsions may be designed to increase this amount.

Liberation: The lipid droplets in excipient emulsions may serve as an organic solvent that can dissolve hydrophobic bioactives and therefore facilitate their liberation from the original plant tissue. For example, carotenoids are usually present as crystals or liquid crystals in specialized structures (chromoplasts) in plant cells (Jeffery, Holzenburg, & King, 2012; Schweiggert, Mezger, Schimpf, Steingass, & Carle, 2012). The nature of these chromoplasts varies between plant species, and has a major impact on their liberation within the GIT and therefore on bioaccessibility. An excipient emulsion may contain ingredients that can help dissociate the plant tissue and release the bioactives (such as chelating agents or surfactants), as well as ingredients that can help solubilize the bioactives once they are released (such as lipid droplets). In this respect, excipient emulsions are different from emulsion-based delivery systems, since in this latter case the hydrophobic bioactives are already solubilized within their lipid phase.

Solubilization: After the hydrophobic bioactives have been liberated from their original location in the plant cells, an excipient emulsion may further improve their bioaccessibility by altering their solubility in the aqueous intestinal fluids. Digestible lipid droplets are hydrolyzed by lipases within the small intestine and form surface-active digestion products (free fatty acids and monoacylglycerols) that associate with endogenous surface-active species (bile salts and phospholipids) to form mixed micelles (Porter & Wasan, 2008; Yao, Xiao, & McClements, 2014). The mixed micelle phase is actually a complex colloidal dispersion consisting of micelles, vesicles, and other structures that can solubilize hydrophobic bioactives. Nevertheless, the hydrophobic domains in the mixed micelle phase must be large enough to accommodate the hydrophobic bioactives released (Qian, Decker, Xiao, & McClements, 2012).

Interactions: The bioavailability of some hydrophobic bioactives may be limited because they interact with other components within the GIT and form insoluble complexes that are not absorbed. For example, long chain fatty acids can form insoluble salts with calcium in the small

intestine that are not easily absorbed. In principle, ingredients could be added to an excipient emulsion to modulate any interactions that may reduce bioaccessibility through this mechanism. For instance, a calcium-chelating agent (such as EDTA) could be added to prevent the formation of insoluble calcium salts with long chain free fatty acids (LC-FFAs). However, it is important to consider that an excipient ingredient added for one purpose may also influence other processes within the GIT that may alter bioavailability. For example, some digestive enzymes require a certain amount of calcium to operate properly, and therefore the chelation of calcium ions may impact their proper functioning. In addition, the accumulation of LC-FFAs around lipid droplet surfaces during digestion may inhibit further lipid digestion by preventing the lipase from accessing the triacylglycerols within the droplet interiors (Devraj et al., 2013). In this case, the presence of free calcium ions may facilitate lipid digestion by forming insoluble soaps with the LC-FFAs, thereby removing them from the lipid droplet surfaces. Thus, chelation of the calcium ions may again have an adverse effect on the extent of lipid digestion by preventing the LC-FFAs from being removed. It is therefore important to be aware of any potential unintended consequences when utilizing excipient ingredients.

3.3.2. Absorption

Once the bioactives have been liberated from the plant tissue and solubilized within the intestinal fluids they must be transported through the lumen, across the mucus layer, and through the layer of epithelium cells lining the GIT (Porter, Trevaskis, & Charman, 2007). The uptake of certain types of bioactives is limited because their transport across one or more of these barriers is inhibited. The absorption can therefore be defined as the amount of bioaccessible bioactive that actually gets transferred across the epithelium cells (m_{BA}) compared to the total amount in the GIT (m_B): $B^* = 100 \times m_A/m_B$. Excipient emulsions can be designed to contain one or more ingredients that may promote the absorption of hydrophobic bioactives.

Mucus layer: Bioactives must be transported through the mucus layer, a porous hydrogel that coats the GIT, before they can be adsorbed by enterocytes (Cone, 2009). The transport of a bioactive may be inhibited if it is present in a form that is too large to penetrate through the pores (≈ 400 nm) or that is strongly attracted to the hydrogel network (e.g., through electrostatic or hydrophobic attraction) (Ensign, Cone, & Hanes, 2012). The transport of hydrophobic bioactives across the mucus layer may therefore be facilitated by using excipient emulsions that generate small mixed micelles in the GIT that easily penetrate through this hydrogel (Lafitte,

Thuresson, & Soderman, 2007).

Epithelium Cell membranes: Once a bioactive reaches the apical side of the epithelial cells it may be absorbed through passive and/or active transport mechanisms (Fasinu, Pillay, Ndesendo, du Toit, & Choonara, 2011) (**Figure 2**). Typically, hydrophobic bioactives are easily transported through the phospholipid bilayer by passive diffusion because of their relatively high solubility in the non-polar regions formed by the phospholipid tails. However, in certain cases there are active transporters embedded within the phospholipid bilayers that facilitate uptake. Excipient emulsions may be formulated to include ingredients that enhance cell membrane permeability (Bohdanowicz & Grinstein, 2013). For example, a number of food-grade ingredients are capable of promoting the absorption of bioactives by altering passive or active transport mechanisms, such as piperine (Dudhatra et al., 2012), sucrose monoesters (Yamamoto, Katsumi, Kusamori, & Sakane, 2014), and rhamnolipids (Jiang, Long, & Meng, 2013). **Some of these components may become incorporated into the phospholipid cell membrane and alter its structure, flexibility, and permeability, whereas others may bind to protein-based transporter systems embedded in the cell membrane and alter their activity.**

Tight Junctions: Sufficiently small molecules or particles may be absorbed by travelling through narrow channels (“tight junctions”) that separate adjacent epithelial cells (Groschwitz & Hogan, 2009). Typically, substances must have dimensions below a few nanometers to pass through the tight junctions. Nevertheless, certain food components can increase tight junction dimensions, and thereby facilitate absorption by this route (Maher, Brayden, Feighery, & McClean, 2008). For example, surfactants such as Tween 80 (Buyukozturk, Benneyan, & Carrier, 2010; Gupta, Hwang, Doshi, & Mitragotri, 2013); biopolymers such as chitosan (Chen et al., 2013; Pillay et al., 2012), mineral ions such as zinc (Wang, Valenzano, Mercado, Zurbach, & Mullin, 2013), and chelating agents such as EDTA (Lemmer & Hamman, 2013) have all been shown to increase tight junctions.

Efflux Mechanisms: The bioavailability of certain types of bioactive molecule is relatively low because of the presence of efflux transporters in the phospholipid bilayers that pump them back out of the epithelial cells (Constantinides & Wasan, 2007; Fasinu et al., 2011). For instance, P-glycoprotein (P-gp) and multidrug resistant protein (MRP) are capable of pumping out various kinds of bioactives from epithelial cells (Constantinides & Wasan, 2007; Fasinu et al., 2011). These efflux mechanisms reduce bioavailability by decreasing the total amount of

bioactive absorbed, as well as by increasing the total amount of bioactive that is metabolized as its moves in and out of the cells. Certain types of food components are capable of interfering with efflux transporters, and thus promoting the absorption of bioactives (Fasinu et al., 2011; Jin & Han, 2010; Rein et al., 2013). For example, quercetin, resveratrol, and piperine are all food-grade components that can block efflux mechanisms (Challa, Babu, Challa, Johnson, & Maheswari, 2013; Choi, Choi, & Kang, 2009; Jin & Han, 2010; Kang, Cho, Shim, Kim, & Lee, 2009).

Excipient emulsions may therefore be designed to contain food-grade ingredients that increase cell membrane permeability, increase tight junctions, or inhibit efflux inhibitors, which could promote the absorption of bioactives liberated from fruits and vegetables. Nevertheless, careful consideration of any potential adverse affects would have to be taken into account, and it may be necessary to carry out appropriate safety tests.

3.3.3. Transformation

The bioavailability of many bioactives in fruits and vegetables is reduced due to their tendency to be chemically or biochemically transformed within the GIT. For example, curcumin is highly unstable to alkali-induced degradation and enzyme-induced metabolism (Heger, van Golen, Broekgaarden, & Michel, 2014), whereas ω -3 fatty acids and carotenoids are susceptible to oxidation (Kenmogne-Domguia, Moisan, Viau, Genot, & Meynier, 2014; Sy, Dangles, Borel, & Caris-Veyrat, 2013). Excipient emulsions can be designed to retard or modulate these transformations through different strategies.

Alteration of Physical Environment: The rate, extent, and pathway of degradation reactions often depends on the local molecular environment of a bioactive molecule, *e.g.*, interfacial, oil, or water phases (McClements & Decker, 2000). Many chemical degradation reactions occur more rapidly when the molecules are in an aqueous environment than in an organic environment, *e.g.*, citral (Choi, Decker, Henson, Popplewell, & McClements, 2009) or curcumin (Zou et al., 2015c). In this case, it is an advantage to ensure that the bioactives are rapidly transferred from the plant tissue to the lipid phase so that they do not spend too long in the aqueous phase, and that the lipid droplets are not so small that the bioactive is in close proximity to the aqueous phase. Indeed, studies have shown that the extent of curcumin degradation under simulated GIT conditions increased with decreasing lipid droplet size (**Figure 4**), which was attributed to the fact that more of the curcumin molecules were in close proximity in the aqueous phase in small

droplets and therefore susceptible to alkaline-induced degradation (Zou et al., 2015c). In addition, lipid digestion occurs more rapidly for smaller droplets and therefore the curcumin molecules may have been released more quickly, and exposed to aqueous phase components for longer.

Presence of Protectants: Some food components are able to inhibit the transformation of bioactives in the GIT by interfering with chemical or biochemical reactions (Dudhatra et al., 2012). For example, antioxidants can inhibit the oxidation of bioactive agents such as carotenoids and polyunsaturated fatty acids (Tarvainen, Phuphusit, Suomela, Kuksis, & Kallio, 2012). These antioxidants may operate through numerous physicochemical mechanisms, including free radical scavenging, singlet oxygen quenching, and chelation (Decker, Chen, Panya, & Elias, 2011). Food-grade antioxidants may be natural or synthetic molecules, such as tocopherols, carotenoids, flavonoids, rosemary extract, green tea extract, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). Certain food components are able to inhibit the activity of digestive or metabolic enzymes, and therefore alter the digestion or metabolism of bioactives in the GIT (Dudhatra et al., 2012; Shimizu, 2010). For example, piperine (from black pepper) has been shown to inhibit the metabolism of vitamins, curcumin, resveratrol, and carotenoids (Dudhatra et al., 2012). Certain types of sugar molecules have been shown to inhibit the chemical degradation of polyphenols (EGCG) in aqueous solutions, which was attributed to their ability to reduce oxygen solubility, chelate transition metal ions, and scavenge reactive oxygen species (Shpigelman, Zisapel, Cohen, & Livney, 2013). Once the degradation mechanism of a bioactive molecule liberated from a fruit or vegetable has been identified, and the major factors influencing the rate and extent of degradation have been determined, it may be possible to incorporate specific protectant ingredients into an excipient emulsion to inhibit or control degradation. In addition, it may be possible to design excipient emulsions so that they solubilize the released bioactives in one region of the GIT, protect them in other regions of the GIT, and then release them in the location where they will be absorbed.

3.3.4. Other Effects

Some other possible factors that should be considered when designing excipient emulsions to control the bioavailability of bioactives in fruits and vegetables are briefly highlighted in this section. The composition and structure of food matrices can alter the functional performance of the GIT, e.g., by altering the rate of gastric emptying or the motility of the stomach and small

intestine (Dudhatra et al., 2012; Koziolk, Garbac, Neumann, & Weitschies, 2013; Varum, Hatton, & Basit, 2013). The time a bioactive or food spends within the stomach can be increased by incorporating phytochemicals that retard gastric emptying, such as piperine (Bajad, Bedi, Singla, & Johri, 2001). An increase in the residence time of a food in the stomach may lead to greater disruption of those plant tissue structures in fruits and vegetables that normally hinder bioactive release, such as chromoplasts (Jeffery et al., 2012). In addition, an increased gastric residence time may also alter the extent of transformation of a bioactive within the stomach, which could decrease or increase its bioavailability and/or bioactivity depending on the nature of the changes. Consumption of digestible fats usually stimulates hormonal changes in the body that promote the release of bile salts, phospholipids, and digestive enzymes (Erlanson-Albertsson, 2005), which may facilitate the breakdown of plant tissues and the solubilization of any bioactives released. Certain types of food components (such as spices) have also been shown to stimulate the release of bile salts, which may also lead to an increase in bioavailability (Prakash & Srinivasan, 2012).

4. Applications of Excipient Emulsions

In this section, we highlight the potential application of excipient emulsions for improving the bioavailability of bioactives in natural products using some examples from our laboratories as well as others.

4.1. Enhanced Bioaccessibility

One of the most widely used applications of excipient emulsions has been to increase the bioaccessibility of hydrophobic bioactives in gastrointestinal fluids. The lipid droplets may act as a non-polar solvent that facilitates the liberation of the hydrophobic bioactives from their original location in plant tissues (Degrou, George, Renard, & Page, 2013; Zou, Liu, Liu, Xiao, & McClements, 2015b). In addition, excipient emulsions containing digestible lipids (such as triacylglycerols) that are rapidly digested within the small intestine will lead to the generation of free fatty acids (FFAs) and monoacylglycerols (MAGs) (**Figure 5**). These lipid digestion products form “mixed micelles” with phospholipids, bile salts, and cholesterol from the intestinal secretions (Williams et al., 2013). The mixed micelle phase is actually a complex colloidal dispersion that contains micelles, vesicles, and liquid crystals that contain hydrophobic domains capable of solubilizing non-polar bioactives (Yao et al., 2014). The ability of excipient emulsions

to increase the bioaccessibility of hydrophobic bioactives has been shown to depend on the amount, composition, and size of the lipid droplets they contain, which can be attributed to the impact of these factors on bioactive liberation and solubilization.

Oil Concentration: It is well established that the bioaccessibility of hydrophobic bioactives increases as the concentration of digestible lipids ingested with them increases. This effect is demonstrated in **Figure 6**, which shows that the bioaccessibility of carotenoids (α - and β -carotene) from carrots is increased when the concentration of digestible lipids (LCT) in excipient emulsions is increased from 0 to 8%. In the presence of higher lipid levels, more mixed micelles are formed in the small intestine after lipid digestion, which leads to a higher solubilization of the carotenoids. The bioaccessibility of genistein incorporated into milk fat was found to increase as the fat content was increased in model custards, which was also attributed to increased solubilization in mixed micelles (Sanz & Luyten, 2007). The bioaccessibility of carotenoids from salads has been shown to increase as the amount of fat co-ingested with them increases, which suggests that emulsion-based salad dressings could be specifically designed to improve their potential health benefits (Huo, Ferruzzi, Belury, Schwartz, & Failla, 2007a; Huo, Ferruzzi, Schwartz, & Failla, 2007b; Moelants et al., 2012; Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011).

Oil Type: The nature of the oil phase has an important impact on the bioaccessibility of hydrophobic bioactive molecules because it influences the solubilization capacity of the mixed micelle phase (Porter et al., 2007; Porter & Wasan, 2008). In this section, we review experiments carried out with both emulsion-based delivery systems and with excipient emulsions that have highlighted the importance of this factor. The data from emulsion-based delivery systems is relevant for understanding excipient emulsions because hydrophobic bioactives are often transferred into the lipid phase of excipient emulsions prior to their digestion. **This transfer may occur during food preparation or within the GIT, and depends on the food preparation method, the nature of the bioactive-rich food being consumed, and the properties of the excipient emulsion.** The bioaccessibility of a hydrophobic bioactive may be reduced considerably when the lipid phase in an emulsion is indigestible, such as a mineral, essential, or flavor oil (Cho et al., 2014; Ozturk, Argin, Ozilgen, & McClements, 2015; Qian et al., 2012; Rao, Decker, Xiao, & McClements, 2013; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013b). There are two main reasons for this phenomenon: (i) the hydrophobic bioactives may not be released from

the undigested lipid phase; (ii) there are no digestion products (FFAs and MAGs) generated that can increase the solubilization capacity of the mixed micelle phase.

The bioaccessibility also depends on the dimensions of the non-polar domains formed within the mixed micelle phase compared to the molecular dimensions of the hydrophobic bioactive agent (**Figure 5**). These non-polar domains may be the hydrophobic core of micelles or the hydrophobic bilayers in vesicles formed by the hydrocarbon tails of phospholipids, fatty acids, and monoacylglycerols. For saturated hydrocarbons, the chain length is around 1.17, 1.42, 1.67, 1.93, 2.18, 2.43, and 2.68 nm for 8, 10, 12, 14, 16, 18, and 20 carbon atoms, respectively (Israelachvili, 2011), and so the dimensions of the non-polar regions in the micelles or vesicles formed from saturated fatty acids would be expected to be about twice this length. For unsaturated hydrocarbons, these distances would be expected to be shorter because the tails are kinked due to the presence of the double bonds. The non-polar domains in the mixed micelle phase must be large enough to easily accommodate the hydrophobic bioactives, otherwise there will be a thermodynamically unfavorable contact between the non-polar and polar regions in the system due to the hydrophobic effect. This phenomenon has been clearly demonstrated for carotenoids, which are relatively long linear hydrophobic molecules (Qian et al., 2012; Salvia-Trujillo et al., 2013b). The bioaccessibility of carotenoids is much greater when corn oil is used as a lipid phase than when MCT or fish oil is used (**Figure 6**). The long chain FFAs released from LCT form non-polar domains that are longer than the molecular dimensions of a carotenoid molecule and can therefore accommodate it. Conversely, the medium chain FFAs released from MCT do not form big enough non-polar domains. Fish oil contains long chain FFAs, but they are highly kinked, which may reduce the dimensions of the non-polar domains compared to saturated FFAs. Oil phase composition has also been shown to influence the bioaccessibility of vitamin D (Ozturk et al., 2015), vitamin E (Yang, Decker, Xiao, & McClements, 2015; Yang & McClements, 2013), Coenzyme Q10 (Cho et al., 2014), and curcumin (Ahmed, Li, McClements, & Xiao, 2012). Carotenoid bioaccessibility from salad vegetables has also been shown to depend on the nature of the lipid co-ingested with them, suggesting that excipient salad dressings could be developed to enhance the uptake of these potentially beneficial nutraceuticals (Huo et al., 2007a; Huo et al., 2007b).

Droplet Size: The size of the lipid droplets in an excipient emulsion may also impact its

ability to solubilize hydrophobic bioactive molecules. Typically, smaller lipid droplets are hydrolyzed more rapidly than larger ones because they have a greater surface area available for the lipase molecules to adsorb to (Li & McClements, 2010). Nevertheless, it is important to highlight that it is the size of the lipid droplets in the small intestine, where the majority of lipid digestion occurs, that is important, rather than their initial size. Some droplets may become highly flocculated or coalesced as they pass through the mouth, stomach, and small intestine, which will restrict the ability of the lipase to adsorb to the lipid droplet surfaces and initiate digestion (**Figure 8a**).

The impact of particle size of excipient emulsions containing LCT on the bioaccessibility of carotenoids from carrots is shown in **Figure 8b** (Zhang et al 2015). The bioaccessibility was much higher for fine (170 nm) or medium (460 nm) sized droplets than for large ones (10,000 nm) or for bulk oils. This effect may be attributed to the fact that the lipid droplets in the fine and medium emulsions were digested more rapidly and fully than those in the other systems (**Figure 8c**), thereby generating a mixed micelle phase capable of rapidly solubilizing any released carotenoids. It is possible that carotenoids liberated from the plant tissues will form insoluble crystals if there are not enough mixed micelles present in their immediate vicinity when they are first released. Recent studies have shown that excipient nanoemulsions containing LCT are effective at increasing the bioaccessibility of crystalline carotenoids, but that solubilized carotenoids have a higher bioaccessibility than crystalline ones (Xia, McClements, & Xiao, 2015). Studies using emulsion-based delivery systems have also shown that the bioaccessibility of highly hydrophobic molecules increases as the droplet size decreases, which was again attributed to the more rapid and complete digestion of the lipid phase for smaller droplets (Cho et al., 2014; Liu, Hou, Lei, Chang, & Gao, 2012; Salvia-Trujillo, Qian, Martin-Belloso, & McClements, 2013a).

Interfacial Properties: The nature of the interfacial layer coating the lipid droplets in an excipient emulsion may also have an impact on the bioaccessibility of hydrophobic bioactives. The interfacial layer has a major influence on the aggregation state of the lipid droplets in different regions of the GIT (*e.g.*, individual, flocculated, or coalesced droplets), which will influence the surface area of the lipid phase exposed to the digestive enzymes. It may therefore be possible to control the region where the lipid phase is digested, which will impact how

quickly the bioactives liberated from the natural products are solubilized. For example, if an emulsifier was used that caused the lipid droplets to be highly aggregated in the small intestine, then the ability of lipase to adsorb to the surfaces of the droplets in the interior of the flocs would be inhibited thereby retarding digestion. The nature of the interfacial layer will also impact the ability of digestive enzymes to adsorb to the droplet surfaces and access the lipids below. For example, some strongly adsorbed small molecule surfactants may inhibit the adsorption of lipase molecules to the droplet surfaces. Thus, by altering the interfacial structure or composition, it may be possible to alter lipid digestion and bioactive bioaccessibility. Similar effects may be achieved by encapsulating lipid droplets within larger structures, such as hydrogel beads or biopolymer particles (Figure 3). Differences in initial interfacial properties of emulsions may account for differences in the bioaccessibility of carotenoids reported in some studies (Liu et al., 2012).

4.2. Modulated Transformation

Excipient emulsions have also been shown to be capable of modulating the chemical or biochemical transformation of bioactives under GIT conditions. In some cases, it may be desirable to inhibit the transformation of a parent molecule because it is the most bioavailable or bioactive form, but in other cases it may be desirable to promote the transformation of a parent molecule to a particular metabolite because it is more bioavailable or bioactive.

Studies have shown that the chemical degradation of a carotenoid (astaxanthin) trapped within lipid nanoparticles can be retarded by adding chelating agents (EDTA) or antioxidants (α -tocopherol) to slow down its chemical degradation (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2014). Certain types of food-grade emulsifiers (whey protein and lecithin) have also been shown to inhibit the oxidation of a carotenoid (lutein) trapped in lipid droplets (Frede et al., 2014). The composition of the oil phase in nanoemulsions has been shown to influence the transformation of pterostilbene (Sun et al., 2015). Pterostilbene is a methoxylated analog of resveratrol that is naturally found in some fruits (*e.g.*, blueberries and grapes) at relatively high levels, which has been shown to have a number of beneficial biological activities, including anticancer and anti-inflammatory effects. The type and amount of metabolites generated within a cell culture model were appreciably different when olive oil or flaxseed oil was used as the lipid phase. It is possible that the location of the bioactive molecules within the lipid droplets depends on the nature of the lipid phase, which would alter their accessibility to any promoters of degradation

reactions in the aqueous phase. In addition, some of the lipid digestion products (such as FFAs or MAGs) may be able to interact with metabolic enzymes and alter their activity. Clearly, further work is needed in this area to identify the mechanisms involved.

Curcumin is known to be a highly susceptible to metabolism in the GIT due to the action of various metabolic enzymes. Studies have shown that piperine (from black pepper) can be used to reduce curcumin degradation, which was attributed to its ability to inhibit metabolic enzymes, such as β -glucuronidase (Gulseren, Guri, & Corredig, 2014). As a result, the amount of curcumin absorbed by the model epithelium cells was increased in the presence of piperine. This phytochemical may therefore be suitable for utilization as an excipient ingredient in certain formulations. Studies with powdered curcumin have shown that the nature of emulsifier used to stabilize the droplets had an impact on curcumin transformation (Zou, Liu, Liu, Xiao, & McClements, 2015d). The total amount of curcumin transferred into the mixed micelle phase from the powdered curcumin was higher for protein-stabilized excipient emulsions than surfactant-stabilized ones, which was attributed to the ability of the proteins to stabilize the curcumin against chemical degradation.

The metabolism of bioactive compounds may also be altered depending on the route by which they are transported into the systemic circulation, *i.e.*, portal vein *versus* lymphatic system (Porter & Wasan, 2008; Pouton & Porter, 2008). More hydrophilic compounds tend to be transported into the systemic circulation by the portal vein route, which means that they must first pass through the liver where they undergo first-pass metabolism. Conversely, more hydrophobic compounds tend to be assembled into lipoproteins (such as chylomicrons) in the enterocytes and then transported *via* the lymphatic system. In this case, the bioactive agents do not undergo first-pass metabolism, which may alter their bioactivity.

These results suggest that it may be possible to direct the metabolism of bioactives within the GIT by careful selection of appropriate ingredients used to formulate an excipient emulsion. Consequently, a pathway could be promoted that leads to the highest concentration of the most bioavailable and bioactive metabolites being produced.

4.3. Increased Absorption

It is also possible to improve the absorption characteristics of bioactives by controlling the properties of excipient emulsions. As mentioned earlier, a number of food-grade ingredients have been identified that may increase the absorption of bioactives by altering active or passive

transport mechanisms, tight junction dimensions, or efflux mechanisms. For example, certain surfactants, biopolymers, fatty acids, chelating agents, minerals, and phytochemicals have been shown to alter absorption mechanisms (Aungst, 2012; Jin & Han, 2010; Whitehead & Mitragotri, 2008), and could therefore be added to excipient emulsions. To date, there has been much less research on designing excipient emulsions to modulate the absorption characteristics of bioactive agents, and this is certainly an area that would benefit from future research.

The type of lipid phase used has been shown to influence the nature of the mixed micelles formed under simulated small intestinal conditions, as well as the nature of the oil bodies and chylomicrons formed in epithelium cells (**Figure 9**). Consequently, lipid type may influence the absorption of bioactive components, and their subsequent GIT fate (Yao et al., 2013; Yao et al., 2014). The nature of the lipid phase in nanoemulsions has been shown to influence the absorption of pterostilbene using a cell culture model (Sun et al., 2015). In these systems, olive oil led to higher trans-enterocyte transport of pterostilbene than flaxseed oil. Olive oil contains a relatively high fraction of monounsaturated fatty acids (such as oleic and linoleic acids), which are known to be effective at increasing cell membrane permeability (Whitehead, Karr, & Mitragotri, 2008). Oleic acid has also been shown to be an effective inhibitor of efflux mechanisms (Aspenstrom-Fagerlund, Tallkvist, Ilback, & Glynn, 2012), which may help increase the absorption of certain bioactive agents. Consequently, selecting an appropriate lipid phase may be a viable means of enhancing absorption of those bioactive agents whose bioavailability is normally limited by this mechanism. Finally, piperine has been shown to increase the intestinal absorption of hydrophobic bioactive agents by altering epithelium cell transport mechanisms (Han, 2011), although studies still need to be carried out to determine its impact on absorption in systems containing excipient emulsions.

5. Potential Limitations of Excipient Emulsions

The previous discussion has highlighted the potential benefits of using excipient emulsions to increase the oral bioavailability of bioactive food components. Nevertheless, it is also important to highlight the fact that there may also be some potential challenges that should be addressed before their widespread application.

One of the most important concerns is that there may be some potentially adverse effects associated with using excipient emulsions. First, an excipient emulsion designed to increase the

bioavailability of a beneficial bioactive agent may also increase the bioavailability of any toxic agents present in a food. Second, an excipient emulsion may boost the bioavailability of a bioactive agent so much that it changes its toxicity profile. Many substances have beneficial health effects when consumed at low levels, but adverse effects when consumed at higher levels. This effect should be considered on a case-by-case basis for specific bioactive agents. Third, excipient ingredients that alter the barrier properties of cell membranes may promote dysfunction of the intestinal barrier (Csaki, 2011). Fourth, designing emulsions to alter the gastrointestinal fate of bioactive components may alter the type and concentration that reaches the colon, which could alter the nature of the gut microbiome. Consequently, it will be important to use this new technology wisely, and to carry out appropriate safety tests, prior to adopting this approach.

6. Conclusions

This review article has highlighted the potential of excipient emulsions for enhancing the bioavailability of hydrophobic bioactive agents, such as nutrients, nutraceuticals, and vitamins, present in natural products such as fruits and vegetables. The composition and structure of excipient emulsions can be designed to modulate the bioaccessibility, absorption, or transformation profile of bioactive agents, and therefore increase their bioavailability and bioactivity. This technology could be used to create a new category of food products specifically designed to boost the bioavailability of bioactives in natural products, such as excipient dressings, sauces, creams, dips, desserts, yogurts, and beverages. Nevertheless, it will also be important to establish the safety of these products if excipient ingredients are added that could have potentially adverse health effects.

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Figure 1: Two emulsion-based approaches can be used to improve the bioavailability of hydrophobic bioactives in foods: (i) *emulsion-based delivery systems* - isolated bioactives are encapsulated within lipid droplets; (ii) *excipient emulsions* – bioactives present within fruits or vegetables are consumed with a specially designed emulsion.

Bioaccessibility
Liberation & Solubilization

Absorption
Transport across Mucus
Layer & Epithelium Cells

Transformation
Chemical or Biochemical Changes

$$BA = B^* \times A^* \times T^*$$

B^* = Bioaccessibility

A^* = Absorption

T^* = Transformation

Figure 2: The overall bioaccessibility of a hydrophobic bioactive may be limited because of its poor bioaccessibility, absorption or transformation characteristics.

Figure 3: The composition or structure of excipient emulsions can be designed to alter the gastrointestinal fate of bioactives from fruits or vegetables consumed with them.

Figure 4: Curcumin may be more protected in large droplets than in small ones because it is better protected from alkaline degradation induced by the surrounding aqueous phase (Zou et al 2015): large ($d_{32} \approx 14 \mu\text{m}$); medium ($d_{32} \approx 0.52 \mu\text{m}$); small ($d_{32} \approx 0.18 \mu\text{m}$).

Figure 5: The ability of the mixed micelle phase to solubilize hydrophobic bioactive components depends on the dimensions of the non-polar domains in the mixed micelle phase.

Figure 6: The bioaccessibility of carotenoids from carrots increases as the concentration of digestible lipids in the excipient emulsions increases (Zhang et al 2015).

Figure 7: The bioaccessibility of carotenoids from carrots increases as the concentration of digestible lipids in the excipient emulsions increases (Zhang et al 2015).

Figure 8a: Influence of passage through different regions of the GIT on microstructure of excipient emulsions that initially had different droplet sizes: $d_{32} = 0.17 \mu\text{m}$ (fine), $0.46 \mu\text{m}$ (medium), and, $10 \mu\text{m}$ (large). The excipient emulsions were initially mixed with carrots, but then separated before analysis (Zhang et al 2015).

Large droplets
enter intestine

Figure 8b: The bioaccessibility of carotenoids from carrots increases as the size of the digestible lipids in excipient emulsions decreases: $d_{32} = 0.17 \mu\text{m}$ (fine), $0.46 \mu\text{m}$ (medium), and, $10 \mu\text{m}$ (large). (Zhang et al 2015).

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Figure 8c: The rate of lipid digestion increases as the size of the digestible lipids in excipient emulsions decreases: $d_{32} = 0.17 \mu\text{m}$ (fine), $0.46 \mu\text{m}$ (medium), and, $10 \mu\text{m}$ (large). (Zhang et al 2015).

Figure 9a: The degree of unsaturation of fatty acids influences the nature of the mixed micelles formed, as well as the nature of the oil bodies and chylomicrons formed by epithelium cells (Data provided by Olivia Yao, 2015).

Figure 9b: The degree of unsaturation of fatty acids influences the concentration of chylomicrons formed by epithelium cells (Data provided by Olivia Yao, 2015).

**Lipid phase composition
influences chylomicron
morphology and
concentration**

Table 1: Summary of the main factors limiting the bioavailability of bioactive agents as characterized by the Nutraceutical Bioavailability Classification System (NuBACs).

Major Classes	Sub-Classes				
Bioaccessibility (B*)	Liberation (L)	Solubilization (S)	Interactions (I)		
Absorption (A*)	Mucus Layer (ML)	Tight Junction (TJ)	Membrane Permeability (MP)	Active Transporters (AT)	Efflux Transporters (ET)
Transformation (T*)	Chemical (C)	Metabolism (M)			