Review
Designing emulsion droplets of foods and beverages to enhance delivery of lipophilic bioactive components – a review of recent advances

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Summary
Lipophilic bioactive compounds such as lipids, vitamins and phytochemicals serve important antioxidant, functional, nutritional and structural roles in the human body. Colloidal systems such as emulsions are particularly suitable matrices for the protection and delivery of these compounds. This article summarises the principal lipophilic bioactives important for human health and challenges associated with their delivery. It discusses the compositional and physical characteristics of emulsions in relation to bioactive delivery, and chemical stability aspects to consider when engineering efficient emulsion delivery systems. The literature shows that aspects such as oil type, droplet size, interfacial composition and solubilisation capacity impact bioactive availability and that their effects are bioactive specific. Therefore, emulsions must be tailored to the bioactives delivered. Much of the present knowledge is based on in vitro studies, and more data from animal and human models are required to better understand the relationship between emulsion characteristics and bioavailability of lipophilic bioactives.

Keywords
Emulsifier, emulsion, interface, lipophilic bioactive, oil type.

Introduction
In the 19th century, one of the main challenges for the food and drink industry was to manufacture safe and nutritious food products. Market research indicates that the perception of consumers on food has evolved during the last few years and is diverted towards products that promote human health and wellbeing. The main driving force for this trend is the accumulation of vast scientific evidence linking diet and chronic diseases in humans such as cardiovascular disease, type-2 diabetes and obesity (Magrone et al., 2013; Braithwaite et al., 2014). In addition, technological advances in the field of food processing present the opportunity of new product development with desirable biofunctional activities which can be released in a precise and controlled manner.

The term dietary bioactives is commonly used to describe food components which although are not essential, they may exert a positive effect on one or more physiological processes and hence may be beneficial to health. A substantial number of these bioactives are highly lipophilic such as polyunsaturated lipids, oil-soluble vitamins, phytosterols, curcuminoids, carotenoids and flavonoids (McClements, 2015). Thus, although from a sensory perspective the inclusion of hydrophobic bioactive molecules into an aqueous medium would be desirable, their nonpolar nature is often a limiting factor for their incorporation into commercial food products due to incompatibility with many food matrices (McClements et al., 2007). Furthermore, many hydrophobic bioactive components in foods are sensitive to food processing and storage and are poorly bioaccessible, which results in low or variable bioavailability profiles (Augustin & Sanguansri, 2012; Rein et al., 2013; McClements, 2014).

A common approach to overcome the technical limitations associated with the inclusion of hydrophobic compounds in aqueous foods is to isolate the bioactives from their original matrix and to incorporate them into a suitable delivery system. Colloidal delivery systems, such as oil-in-water emulsions, are particularly suitable food matrices for the encapsulation, protection and delivery of lipophilic components (McClements & Li, 2010). This is because emulsions, if formulated and processed accordingly, they can deliver the bioactive ingredient in a precise and controlled manner. In practice, this means that ideally the
loading efficiency of the bioactive ingredient should be relatively high and should be retained during processing, transport and storage. Thus, the product should be able to withstand changes during the manufacturing stages and maintain its physical and chemical stability for a prolonged period of time. Furthermore, the delivery system should protect the compound from chemical or enzymatic degradation during its passage through the gastrointestinal tract so it can be released and absorbed in its active form at the site of action.

Emulsions typically consist of two immiscible liquids (usually oil and water), with one of the liquids being dispersed in the form of small spherical droplets in the other. Biofunctional oil-in-water emulsions can be manufactured by solubilising the hydrophobic component within the oil phase, which is then dispersed in the aqueous phase in the form of emulsifier-coated droplets by the process of homogenisation. The size of the droplets is critical for the stability of the system and is commonly used to distinguish conventional emulsions \((r > 100\, \text{nm})\) from nanoemulsions \((r < 100\, \text{nm})\) (Ahmed et al., 2012). Both types of emulsions are thermodynamically unstable and as a result tend to separate in their constituent phases over time through different instability mechanisms, such as gravitational separation, flocculation, coalescence and Ostwald ripening (Dickinson, 1992; Solans et al., 2005). The lifespan of each type of emulsion largely depends on the particle size and the bulk physico-chemical properties (i.e. viscosity) and varies from a few hours to months (Fig. 1).

The bulk physico-chemical properties of emulsions including stability and release characteristics are determined to a great extent by the properties of the droplets that they contain (McClements, 2005). Droplet composition in emulsions is highly variable from common triacylglycerol (short, medium or long chain) to flavour and mineral oils and waxes. This variability in lipid phase has a major impact on the degree of lipid digestibility within the gastrointestinal tract, which in turn has important implications for the absorption and metabolism of fat or fat-soluble compounds (McClements et al., 2016). In addition, the type and concentration of the surface-active species present during homogenisation are important determinants of the functional performance of the delivery system matrices (McClements et al., 2007). This happens because the interfacial layer contributes significantly towards the chemical stability of the dispersed phase and is also linked to droplet digestibility.

The aim of this review was to present and critically discuss recent findings on emulsion systems aiming to deliver lipophilic bioactive compounds. Emphasis is given on the influence of carrier oil composition and emulsifier type for enhancing the functionality of the delivery system. The overall objective was to provide useful insights for the design and formulation of emulsion-based products with increased bioavailability of lipophilic bioactive agents. This information can be of interest to the food and beverage industry specialising in the manufacture of functional foods and drinks.

![Figure 1](image_url)

**Figure 1** Schematic representation of the structure and main components of an oil-in-water (O/W) emulsion. The relationship between shelf life of O/W emulsions and average droplet diameter (not to scale) is highlighted.

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Major lipophilic bioactive components and associated challenges for the food industry

Lipophilic bioactives represent all lipid-soluble compounds that exert biological effects in humans. They include natural bioactives, such as lipid fractions, phytochemicals and vitamins, and synthesised bioactives such as drugs that are poorly soluble in water (Humsterone & Charman, 1997). They fulfil important antioxidant, functional, nutritional and structural functions in the human body. The salient lipophilic bioactives that are important for human nutrition are summarised in Table 1 and briefly discussed below.

Vitamins

Dietary fat is essential for delivery of the fat-soluble vitamins A, D, E and K which are pivotal for human health. Preformed vitamin A (retinol) is usually found in animal foods and prevails as its fatty acid ester (retinyl esters). Vitamin A plays a central role in vision, cell differentiation and epithelial integrity (Johnson & Mohn, 2015) (Table 2).

Similarly, vitamin D has now been shown to be critical throughout life for Ca and P metabolism (bone function) as well as reducing the risk of chronic diseases such as cardiovascular disease, autoimmune conditions and cancer (Chowdhury et al., 2014). Vitamin D exists as two major forms, D2 (ergocalciferol) found in plants and produced by UVB irradiation of ergosterol, and D3 (cholecalciferol) which is the predominant form found naturally in foods and produced by the UVB irradiation of 7-dehydrocholesterol (Holick, 2007). The bioavailability of these two forms appear to be dissimilar with D3 being more efficient at improving vitamin D status in humans (Itkonen et al., 2016). Vitamin E in comparison is more abundant and occurs in at least eight forms in nature (α, β, δ and γ forms of tocopherol and tocotrienol) (Dutta & Dutta, 2003). α-Tocopherol is the biologically most active form (Johnson & Mohn, 2015), and therefore, most used in supplements although γ-tocopherol is the predominant form in dietary sources (Dutta & Dutta, 2003). The primary role of vitamin E in the human body is as an antioxidant and protecting lipids against

Table 1 Major lipophilic bioactives, their nutritional significance and intake guidelines

<table>
<thead>
<tr>
<th>Bioactive group</th>
<th>Types</th>
<th>Principle molecular species relevant to humans</th>
<th>Nutritional/metabolic role</th>
<th>Dietary recommendations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td>A</td>
<td>Retinol</td>
<td>Vision, cell differentiation, epithelial integrity</td>
<td>RNI: 700 μg d⁻¹ men, 600 μg d⁻¹ women</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>Ergocalciferol and Cholecalciferol</td>
<td>Ca and P metabolism, infant development, reducing chronic disease risk</td>
<td>RNI: Infants and young children: 7–8.5 μg d⁻¹, Pregnant and breastfeeding women: 10 μg d⁻¹, Elderly: 10 μg d⁻¹</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>Tocopherols and Tocotrienols</td>
<td>Antioxidant role: protecting lipid-based components and reducing the risk of diseases with oxidative stress</td>
<td>Safe Intake: Men &gt;4 mg d⁻¹, Women ≥3 mg d⁻¹</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>Phyloquinone and Menaphyllolide</td>
<td>Blood coagulation, skeletal health, glucose tolerance</td>
<td>Safe Intake: Adults: 1 μg kg⁻¹ d⁻¹; Infants: 10 μg d⁻¹</td>
</tr>
<tr>
<td>Phytochemicals</td>
<td>Carotenoids</td>
<td>α-Carotene, β-Carotene, β-Cryptoxanthin, Lutein, Lycopene, Zeaxanthin</td>
<td>Pro-vitamin A activity, biological antioxidants, protective role in macula, protection against diseases associated with oxidative stress, immunity, gap junction communication</td>
<td>No guidelines at present. Consume at least 400 g of fruits and vegetables per d</td>
</tr>
<tr>
<td></td>
<td>Phytosterols and phytostanols</td>
<td>Campesterol, Sitosterol, Campestanol, Sitostanol</td>
<td>Reduction in blood total cholesterol and LDL</td>
<td>No guidelines at present. 2 g d⁻¹ shown to reduce blood total cholesterol and LDL by 7–10% (AbuMweis et al., 2008; Weingartner et al., 2014), 0.3–0.5% of daily energy requirement (Buttriss, 1999)</td>
</tr>
</tbody>
</table>

*For UK: vitamin guidelines as recommended by the UK Department of Health (Buttriss, 2000); RNI, reference nutrient intake, the amount that will meet the needs of 97.5% of the population; safe intake, intake adequate to meet the needs of nearly all individuals, but not high enough to cause undesirable effects; LDL, low-density lipoprotein; PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain PUFA.
Table 2 Compilation of recent studies on the delivery of lipophilic compounds using emulsion-based delivery systems

<table>
<thead>
<tr>
<th>Lipophilic compound</th>
<th>Carrier oil</th>
<th>Emulsifier</th>
<th>Study model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td>MCT, LCT</td>
<td>Tween 20</td>
<td>In vitro (GID)</td>
<td>Salvia-Trujillo et al. (2013a)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>MCT, LCT, orange oil</td>
<td>Tween 20</td>
<td>In vitro (GID)</td>
<td>Qian et al. (2012a)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>MCT, LCT, SCT</td>
<td>β-Lactoglobulin</td>
<td>In vitro (GID)</td>
<td>Ahmed et al. (2012)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>LCT, MCT</td>
<td>OSA modified PGU</td>
<td>In vitro (GID)</td>
<td>Majeed et al. (2016)</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>MCT, LCT, orange/mineral oil</td>
<td>Tween 80</td>
<td>In vitro (GID)- in vivo (rats)</td>
<td>Salvia-Trujillo et al. (2015)</td>
</tr>
<tr>
<td>Pterostilbene</td>
<td>Flaxseed, olive oil</td>
<td>Tween 20</td>
<td>In vitro (GID)- in vivo (Caco-2)</td>
<td>Sun et al. (2015)</td>
</tr>
<tr>
<td>vitamin D₃</td>
<td>MCT, corn, fish, mineral, orange oil</td>
<td>Quillaja saponin</td>
<td>In vitro (GID)</td>
<td>Ozturk et al. (2015a)</td>
</tr>
<tr>
<td>vitamin E</td>
<td>MCT, LCT</td>
<td>Quillaja saponin</td>
<td>In vitro (GID)</td>
<td>Yang &amp; McClements (2013b)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Orange oil</td>
<td>β-Lactoglobulin</td>
<td>In vitro (IGD)</td>
<td>Qian et al. (2012b)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>MCT</td>
<td>LF, LF-CA, LF-EGCG</td>
<td>β-Carotene degradation</td>
<td>Liu et al. (2016)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Corn oil</td>
<td>Tween 20</td>
<td>In vitro (IGD)</td>
<td>Salvia-Trujillo et al. (2013b)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Corn oil</td>
<td>Sodium caseinate</td>
<td>In vitro (IGD)</td>
<td>Yi et al. (2014)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Olive oil</td>
<td>L-a-phosphatidylcholine</td>
<td>β-Carotene degradation</td>
<td>Verrijssen et al. (2015)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Kernel oil</td>
<td>WPI</td>
<td>In vitro (IGD)</td>
<td>Cornacchia &amp; Roos (2011)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>MCT</td>
<td>Tween 80</td>
<td>In vitro (IGD)</td>
<td>Li et al. (2016)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Corn oil</td>
<td>LF, LF-alginate</td>
<td>In vitro (IGD)</td>
<td>Pinheiro et al. (2016)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>MCT</td>
<td>Tween 20</td>
<td>In vitro (IGD)</td>
<td>Joung et al. (2016)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Soya oil</td>
<td>Tween 20, Poloxamer 407</td>
<td>In vivo (caco-2)</td>
<td>Guiser et al. (2014)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Corn oil</td>
<td>WPI, sodium caseinate, Tween 80</td>
<td>In vitro (GID)</td>
<td>Zou et al. (2015)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Soya bean oil</td>
<td>SPI-GA</td>
<td>Lycopene degradation</td>
<td>Li et al. (2015)</td>
</tr>
<tr>
<td>Omega-3 PUFA</td>
<td>MCT/fish oil</td>
<td>Tween 80, Citrem, sodium caseinate, lecithin</td>
<td>Oxidative stability</td>
<td>Haahr &amp; Jacobsen (2008)</td>
</tr>
<tr>
<td>Omega-3/6 PUFA</td>
<td>Algal oil</td>
<td>ZH-TA</td>
<td>Oxidative stability</td>
<td>Wang et al. (2016)</td>
</tr>
<tr>
<td>ALA</td>
<td>Flaxseed oil</td>
<td>Sodium caseinate, Tween 80, soya lecithin</td>
<td>In vitro (GID)- in vivo (rats)</td>
<td>Couedelo et al. (2015)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>MCT</td>
<td>Tween 20, 40, 60, 80, 85</td>
<td>Particle size</td>
<td>Saberi et al. (2013)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>MCT</td>
<td>Tween 80, Quillaja saponin</td>
<td>Particle size</td>
<td>Yang &amp; McClements (2013a)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Orange oil</td>
<td>WPI, GA</td>
<td>Particle size</td>
<td>Ozturk et al. (2015b)</td>
</tr>
</tbody>
</table>

OSA, octenyl succinic anhydride; PGU, purity gum ultra; LF, lactoferrin; CA, chlorogenic acid; EGCG, Epigallocatechin-3-gallate; WPI, whey protein isolate; SPI, soy protein isolate; GA, gum arabic; ZH, zein hydrolysate; TA, tannic acid; ALA, alpha-linolenic acid.

Oxidative damage. Vitamin E has also been shown to protect edible oils and other oil-based systems against oxidation (Huang et al., 1996; Choe & Min, 2006), which suggests that its inclusion in foods can have both nutritional and physical benefits.

Although these vitamins are relatively stable in food systems, their incorporation presents some challenges to the food industry. Similar to unsaturated fatty acids, they are prone to autocatalytic processes and provoked by light, air and transition metals (Reddy & Love, 1999). Incorporation into oil-in-water emulsions has been proposed as an effective method of improving the stability of these vitamins (Gibbs et al., 1999; Yoshida et al., 1999). These vitamins are temperature sensitive, and therefore, processing techniques using excessive time–temperature combinations (such as extrusion and prolonged high temperature cooking methods) can destroy them (Ryley & Kajda, 1994; Riaz et al., 2009). The critical time and temperature combinations are vitamin specific and should be taken into consideration when developing functional foods.

Phytochemicals

This section will focus on carotenoids and sterols as they constitute the nutritionally most relevant lipophilic phytochemicals.

Carotenoids are a family of pigments that are ubiquitous in nature and often responsible for the colour of organisms. Structurally, carotenoids are isoprenoid compounds that are biosynthesised by the tail-to-tail coupling of two C₂₀ geranylgeranyl diphasphate molecules (Lu & Li, 2008).

Of the large number of carotenoids found in nature, six specific types (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin) predominate in human tissue (Maiani et al., 2009). Carotenoids have been shown to play a central role in key biological processes related to immunity, gap junction
communication, and antioxidant activity, and function as beneficial xenobiotics in the human body (Rao & Rao, 2007). They also serve as antioxidants in quenching pro-oxidant molecules and as precursors for vitamin A (α-Carotene, β-Carotene and β-Cryptoxanthin). Carotenoids are a safer form of vitamin A supplementation than retinol as the latter has been shown to induce toxicity, DNA damage and neoplasms (Klamt et al., 2003).

Although carotenoids are abundant in foods, they are poorly bioavailable and absorption could be as low as 1% (Hedren et al., 2002). As fat-soluble components, the presence of oil improves their bioavailability by up to 50% (Deming & Erdman, 1999). Emulsions have been shown to be particularly effective in improving the bioavailability of carotenoids (Takeda et al., 2009, 2011). Processing conditions could affect structural changes to carotenoids and affect bioavailability. For example, heating has been shown to alter the cis-trans conformation of natural carotenoids and impact availability (Aherne et al., 2010). Processing could also be beneficial for some carotenoids as demonstrated with lycopene (Gartner et al., 1997). Fibre has been shown to reduce carotenoid absorption (Rock & Swendsen, 1992). Exogenous carotenoids are susceptible to both auto-oxidation and oxidation by light, heat and oxygen (McClements et al., 2007), and therefore, maintaining their integrity in food systems is a challenge for the food industry. Carotenoids have a high melting point, and the resulting high crystallinity is another challenge to their usage in functional foods (McClements et al., 2007).

Plant sterols and stanols (PS) are another group of lipophilic bioactives that are important for human health. Evidence shows that their consumption along with meals can help improve lipid profiles by attenuating serum low-density lipoproteins (LDL). Structurally, PS are similar to cholesterol and occur in two forms, β-sitosterol and campesterol (Katan et al., 2003). Stanols differ from sterols in that they are saturated and do not contain double bonds. Both types are widely prevalent in fruits, vegetables, cereals and vegetable oils (Piironen et al., 2000; Ellegard et al., 2007). Fortified foods such as spreads, dairy products, cereals and beverages are now predominant dietary sources of PS (Katan et al., 2003) and are good examples of the effective use of food reformulation for therapeutic ends.

Fortifying foods with PS present several challenges one of which is their poor bioavailability which could be as low as 1% (Nik et al., 2011). The specific bioavailability of PS depends on the type (Piironen et al., 2000) where campesterol is more available than sitosterol. Similarly, the physical form of PS (esterified vs. nonesterified) and the matrix they are present have also been shown to affect their bioavailability (Katan et al., 2003) Emulsification conditions could also impact on bioavailability (Katan et al., 2003). For example, PS emulsified with lecithin or diacylglycerols were shown to be more effective in attenuating serum cholesterol (Ostlund et al., 1999; Meguro et al., 2001). Another impediment to their incorporation into foods is their high melting point and propensity to crystallise (McClements et al., 2007). Their solubility could be improved by esterification into their fatty acid forms. As PS are prone to oxidation, microencapsulation has been suggested to be an effective method for improving their stability in food systems (Chen et al., 2013).

Lipid fractions

The nutritionally most pertinent lipid fractions are the essential fatty acids (EFAs) which cannot be synthesised by the body. These constitute the omega-3 and omega-6 fatty acids both of which are polyunsaturated fatty acids (PUFA). Structurally the omega-3 fatty acids are derived from alpha-Linolenic acid (ALA) and have an 18C backbone with three double bonds (DB), and omega-6 fatty acids are derived from cis-Linolenic acid (CLA) and have an 18C backbone with two DBs.

Omega-6 PUFA is widely found in nature, and its needs are often easily met by most (Simopoulos, 2006). Omega-3 fatty acids are relatively less abundant and therefore are ideal candidate for supplementation.

Many food systems have been used and assessed as functional vehicles for delivering EFAs and include bread, pasta, biscuits and beverages. Developing functional foods containing EFAs is challenging as these lipids are highly susceptible to oxidative degradation (McClements et al., 2007). Factors such as the ingredients, pH, processing and storage conditions impact on the stability of EFAs and reduce bioactivity. Furthermore, the degradation of these oils impairs the sensory properties of the food and generates oxidation products that provoke morbidity (Jacobsen et al., 1999). The incorporation of antioxidants such as tocopherols and polyphenols could help improve the stability of EFAs in functional foods (Arab-Tehrany et al., 2012). Encapsulation of EFAs using microencapsulation and nanoencapsulation methods could also protect these lipids from oxidation (Gökmen et al., 2011; Arab-Tehrany et al., 2012). pH has a direct effect on lipid oxidation and could be modulated in functional foods to improve their oxidative stability (Hu et al., 2003). Oil-in-water emulsions have been suggested to be particularly effective for maximising the oxidative stability of EFAs (Djordjevic et al., 2004).

Involvement of droplet composition on delivery of lipophilic components

Emulsions can be ideal matrices for the encapsulation and delivery of lipophilic bioactive compounds. The
design and fabrication of the carrier matrix is a critical step in achieving this target, and this is related to the physiological process of lipid digestion in the stomach and small intestine. The digestive process of emulsified foods is schematically represented in Fig. 2. In brief, emulsified oil droplets are hydrolysed by gastric lipase during gastric digestion and diacylglycerols, monoacylglycerols and free fatty acids (FFAs) are released. Lipid hydrolysis proceeds in the stomach until 10–30% of FFAs are released (Armand, 2007). The digestion products within the chyme are transferred to the duodenum, where pancreatic lipases continue the process of lipid digestion. In the presence of digestible lipids, the small lipid molecules are mixed with phospholipids, bile salts and cholesterol and form a complex mixture of colloidal nanostructures (mixed micelles, vesicles, liquid crystals) capable to encapsulate nonpolar bioactives (Müllerz et al., 2012). Lipid digestion continues in the small intestine where these complex colloidal dispersions are solubilised in the lumen, are absorbed into the enterocytes by passive diffusion and are packed into chylomicrons before entering the systemic circulation through the lymphatic system.

Droplet composition and the total amount of lipid consumed have a major impact on the bioaccessibility of hydrophobic bioactive compounds because both parameters determine the solubilisation efficiency of the mixed micelle phase (Porter & Wasan, 2008; Xiao & Lewis, 2012). Indigestible oils (i.e. flavour oils) are not ideal ‘vehicles’ for delivering lipophilic agents as compared to digestible ones (Rao et al., 2013). This is mainly because undigested lipids do not generate molecules which are considered essential for the solubilisation of the mixed micelle phase such as free fatty acids (McClements et al., 2016). In this case, the indigestible components pass through the stomach and small intestine and enter the colon. Subsequently, the release and absorption of bioactive ingredients from undigested lipids is limited and bioaccessibility, which is defined as the amount of the bioactive agent solubilised within the intestinal fluids and available for uptake by the epithelium cells, is negligible. Other factors including the size and degree of saturation of the fatty acids

Figure 2 Overview of digestion and absorption of lipids and lipophilic compounds in the human alimentary tract. Bioavailability refers to the fraction of the ingested bioactive component (in an active state) that is released from the food matrix, absorbed by the intestinal epithelial cells, packed in chylomicrons and transported to systemic circulation.

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present in the triacylglycerol molecules may affect the bioaccessibility of hydrophobic bioactive molecules. The nonpolar domains in the mixed micelle phase must be large enough to accommodate long linear hydrophobic molecules such as carotenoids (Qian et al., 2012a). Thus, the long-chain fatty acids of corn oil provide a better environment to host lipophilic compounds compared with the free fatty acids released during digestion of medium-chain triacylglycerols or unsaturated fatty acids released from fish oil (Zhang et al., 2016).

In vitro data on bioaccessibility
The influence of carrier oil type on the bioaccessibility of lipophilic bioactive compounds is demonstrated by numerous studies using an in vitro model which simulates digestion in the gastrointestinal tract. Qian et al. (2012a) investigated the impact of oil composition on the bioaccessibility of β-carotene encapsulated in nanoemulsion delivery systems. The results of this study indicated that both the degree of oil digestibility and the size of the mixed micelles formed during digestion determine the bioaccessibility of β-carotene. Although digestibility and release of free fatty acids (FFA) was similar for LCT (long-chain triglycerides) and MCT (medium-chain triglycerides), the former are better ‘carriers’ than the latter simply because the micelle core of the LCT’s is large enough to accommodate the β-carotene molecules (Fig. 3). On the other hand, orange oil was not a suitable ‘carrier’ as it is a nondigestible type of oil and as a result the solubilisation of β-carotene in mixed micelles is not mediated. Mixed ratios of LCTs and MCTs have also been tested for delivering β-carotene in nanoemulsion systems (Salvia-Trujillo et al., 2013a). For low-fat emulsions, increasing LCT within the lipid phase can increase the solubilisation capacity of the mixed micelle and enhance bioaccessibility of β-carotene. The same effect was not observed in high fat emulsions, possibly because some portion of the oil remains undigested. Similar but not identical results were reported for emulsion-based delivery systems containing encapsulated curcumin as a functional ingredient (Ahmed et al., 2012). In this case, emulsions formed with MCT as carrier oil favoured bioaccessibility of curcumin in comparison with LCT and short-chain triglycerides (SCT). This effect was attributed to the amount of FFA available to from mixed micelles after the digestion process, in which case the digestion extent followed the order MCT > SCT > LCT. Majeed et al. (2016) also reported that the rate and extent of in vitro lipid digestion in a simulated small intestine model was higher for emulsions containing MCT compared to LCT. According to Devraj et al. (2013), during digestion long-chain FFAs may accumulate at the droplet surface and thus inhibit the action of lipases, whereas medium-chain FFAs are transported into the aqueous phase facilitating the breakdown process of lipid molecules. However, bioaccessibility of eugenol was higher for emulsions containing LCT, which was related to the ability of long-chain FFA to form mixed micelles of a size capable to accommodate the large lipophilic eugenol molecule. The same pattern was observed in nanoemulsions with encapsulated vitamin D3 (Ozturk et al., 2015a). In vitro lipid digestion was favoured for MCT nanoemulsions, presumably because gastric and intestinal lipases have a better access to the lipid droplet surface. Nevertheless, nanoemulsions containing LCT (corn and fish oil) showed a better solubilisation capacity and were more efficient in increasing the bioaccessibility of vitamin D3. In agreement with the previous study, the same trend with respect to lipid digestibility and bioaccessibility was reported for MCT- and LCT emulsions loaded with vitamin E (Yang & McClements, 2013b). Furthermore, in this case, the conversion of α-tocopherol acetate to α-tocopherol after in vitro digestion was also higher for LCT- than MCT emulsions, which may affect the bioavailability of vitamin E.

Figure 3 The solubilisation capacity of the mixed micelle and thus the bioaccessibility of lipophilic components depend on the dimensions of the bioactive and the nature of the carrier oil. Large hydrophobic molecules (i.e. carotenoids) are better accommodated in the hydrophobic core of mixed micelles and vesicles formed from long-chain fatty acids.
In vivo data from cell line and animal models
Factors other than the length of fatty acid chain may also determine the rate and extent of oil digestibility. Sun et al. (2015) calculated the percentage of FFAs released from flaxseed and olive nanoemulsions using an in vitro digestion model. At the end of the digestion process, 66% and 100% of the FFAs were released for flaxseed and olive oil, respectively. The lower digestibility shown by flaxseed was attributed to the higher fraction of polyunsaturated fatty acids present, which are known to be more resistant to pancreatic lipases compared to monounsaturated fatty acids. Nevertheless, bioaccessibility of pterostilbene was similar for olive oil and flaxseed (44% and 47%, respectively) nanoemulsions, indicating that the solubilisation capacity of the bioactives into mixed micelles is possibly more important than the degree of oil digestibility. However, trans-enterocyte transport of pterostilbene using a Caco-2 cell model was favoured suggesting that absorption and bioavailability of bioactives is a far more complicated process than the one simulated by the in vitro digestion model. This statement is in agreement with studies combining in vitro and in vivo methods to investigate the process of digestion and absorption of fucoxanthin from emulsion-based delivery systems (Salvia-Trujillo et al., 2015). According to the findings of the study, the solubilisation of the bioactive ingredient in mixed micelles after in vitro and in vivo digestion in the small intestine followed the order LCT > MCT > orange oil. However, the concentration of fucoxanthin in the serum of rats was not significantly affected by the oil type used for formulation. This suggests that the molecular characteristics of the fatty acid side chains may not be the only determinant for the disassembly of the mixed micelles within the gastrointestinal tract and absorption of bioactives. The physicochemical processes involved in the transportation of lipophilic components into the enterocyte cells, packaging into chylomicrons and release into the systemic circulation via the lymphatic system are not fully understood. Evidence exists to support the hypothesis that factors such as the amount of ingested lipids, the molecular characteristics of the bioactives and the interaction with other food components are important for the bioavailability of lipophilic bioactives (Yao et al., 2014). Clearly, more in vivo work is required to elucidate the effect of oil composition on delivery of individual lipophilic bioactive components. Cell culture studies with mixed ratios of different oil carriers would provide useful information on the transfer mechanism of encapsulated components into the enterocytes. Furthermore, human intervention studies can be used to investigate the release of lipophilic bioactives into the systematic circulation using emulsions as delivery systems.

Influence of interfacial composition on delivery of lipophilic bioactives
In an oil-in-water emulsion, the oil droplets encapsulating the hydrophobic bioactive ingredients are coated by a thin layer (1–50 nm) of surface-active chemical compounds formerly known as emulsifiers (Güzey & McClements, 2007). The type and concentration of emulsifiers present during and after the process of homogenisation determine the characteristics of the interfacial layer (Dickinson, 2003). Interfacial properties such as droplet size/charge and layer thickness are critically important for the chemical stability and bioactivity of the delivery system and may be tailored accordingly to enhance functionality. This is because bioactive lipids such as omega-3 fatty acids and carotenoids are particularly susceptible to chemical deterioration through oxidative mechanisms. Depending on the type and concentration of the surface-active species, the average droplet size and particle size distribution of an emulsion may vary. This may have an impact on the oxidative stability of the oil-in-water emulsion as small particle size corresponds to a large interfacial area and the contact area between the oil phase and the pro-oxidants present in the aqueous phase increases (Berton-Carabin et al., 2014). Furthermore, droplets are often electrically charged which is mainly due to the ionic nature of the adsorbed species (McClements, 2005). Droplet charge is considered an important factor governing the process of lipid oxidation in emulsions because it determines the interaction of droplets with other charged species such as pro-oxidant metal ions (Kellerby et al., 2006; Choi et al., 2010). In addition, the electrostatic interactions between charged droplets affect the stability of the latter against aggregation (Piorkowski & McClements, 2014). This affects lipid digestibility as flocculated or coalesced droplets show limited surface area of the lipid phase exposed and as result accessibility to the digestive enzymes is hindered (McClements et al., 2016). Finally, the thickness of the interfacial layer is an important factor with regard to the chemical stability of the dispersed lipids and any lipophilic compounds. Thick interfacial layers formed by some proteins (e.g. caseins) are known to provide better stability against oxidation (Kiokias et al., 2006). Strongly adsorbed small molecule surfactants may also inhibit the action of lipase molecules to the droplet surfaces by limiting their access to the substrates (Couedelo et al., 2015). As a result, it is evident that droplet composition and interfacial characteristics affect to a great extent lipid digestibility and chemical stability of bioactives and should be taken into consideration when products are designed to increase bioaccessibility of lipophilic compounds.
In vitro data on chemical stability
A wide range of surface-active species, which differ in their structure, physicochemical properties and functional performance, are commercially available for encapsulating lipophilic compounds in emulsion-based delivery systems (Saberi et al., 2013; Yang & McClements, 2013a,b; Ozturk et al., 2015b). The interfacial composition affects to a great extent the chemical stability and thus bioaccessibility of the bioactive compound. Therefore, the selection of the molecular species which will form an interfacial layer to cover the oil droplet is a crucial step in emulsion formulation. Qian et al. (2012b) stated that nanoemulsions stabilised with β-lactoglobulin were less susceptible to β-carotene degradation compared to the ones stabilised with Tween 20. Similarly, proteins (caseinate and whey protein isolate) were more effective to confer chemical stability to curcumin during in vitro gastric digestion of excipient emulsions than their synthetic counterpart (Tween 80) (Zou et al., 2015). In agreement with the previous studies, Haahr & Jacobsen (2008) reported that the type of emulsifier significantly affected the rate of lipid oxidation of omega-3 enriched emulsions and decreased in the order Tween 80 > Citrem > lecithin > caseinate. As mentioned earlier in this review, the ability of proteins to control effectively the rate of oxidation of lipophilic compounds is attributed to their ability to (i) scavenge free radicals and chelate transition metals, (ii) form thick interfacial layers that act as physical barriers, (iii) form complexes with the bioactive compounds that may protect them from degradation and (iv) generate droplets with larger size and likewise reduce the surface area. Food products are often subjected to processing.

Emulsions containing lipophilic bioactive compounds are particularly susceptible to chemical degradation during processing and storage, and therefore, the food industry is under pressure to identify new methods or ingredients that can effectively enhance the chemical stability of these products. Engineering new emulsifier molecules with desired functional properties is becoming an increasingly popular method to control the oxidation of the dispersed phase and/or the degradation process of lipophilic compounds in emulsion-based delivery systems. Wang et al. (2016) recently reported that algal oil nanoemulsions stabilised with zein protein hydrolysate complexed with tannic acid (TA) showed an increased oxidative stability. Complex formation between the peptides and tannic acid was mediated through noncovalent interactions, and the effect on the oxidative stability was attributed to the phenolic groups of TA. A similar attempt to enhance chemical stability of β-carotene against heat treatment and ultraviolet exposure of emulsions was published by Liu et al. (2016). In this case, emulsions were coated with lactoferrin–chlorogenic acid and – epigallocatechin-3-gallate (EGCG) conjugates and were subsequently exposed to different environmental stresses. Results demonstrated that the conjugate between the protein and EGCG was particularly effective at delaying the degradation of the encapsulated β-carotene. Similarly, coating of lycopene with soya protein–gum acacia conjugates and curcumin with chitosan proved to be an effective method for protecting the bioactives from chemical degradation when exposed to different environmental and processing conditions (Li et al., 2015, 2016).

In vitro data on bioaccessibility
The molecular structure of the emulsifier is not the only parameter that needs to be taken into consideration when designing an emulsion-based delivery system of a lipophilic compound. Other factors such as processing conditions (i.e. homogenisation pressure) and emulsifier concentration are known to affect the properties of the interfacial layer and the droplet size and as a result can have a major impact on the chemical stability of the bioactive compound (Cornacchia & Roos, 2011; Yi et al., 2014). Furthermore, both processing conditions and emulsifier concentration are important determinants of lipid digestibility and bioaccessibility of the bioactive compound. Yi et al. (2014) stated that bioaccessibility of β-carotene and FFA release in sodium caseinate emulsions increased with decreasing initial droplet diameter. According to the authors, decreasing the droplet diameter (by increasing the homogenisation pressure) led to an increase in surface area, which allowed easier access to lipases to facilitate hydrolysis. These results are in agreement with previous studies which investigated the effect of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions (Salvia-Trujillo et al., 2013a,b). The effect on β-carotene bioaccessibility was attributed to the fact that large droplets may not be digested to the same extent as smaller ones, and as a result, the amount of β-carotene incorporated in micelles decreases with increasing droplet size. Recent studies also highlight the importance of emulsifier concentration on lipid digestibility and bioaccessibility of lipophilic compounds using simulated in vitro gastrointestinal digestion systems. Verrijssen et al. (2015) stated that increasing the concentration of phosphatidylcholine led to an increase in the bioaccessibility of β-carotene. This indicates that there is correlation between the emulsifier concentration and the amount of β-carotene incorporated in mixed micelles. Other studies suggest that high surfactant concentration can retard lipid oxidation of the oil phase in emulsions and hence enhance the chemical stability of the encapsulated bioactive components (Joung et al., 2016).

Limited data from in vivo studies
Although many published studies indicate the significance of interfacial composition on the delivery of
bioactives encapsulated in emulsion-based delivery systems, the vast majority of the data is based on in vitro studies simulating the process of digestion. Gülseren et al. (2014) used a Caco-2 cell culture system to demonstrate that the uptake of curcumin from oil-in-water emulsions by enterocytes is significantly affected by the emulsifiers present at the interface. Poloxamer 407, an emulsifier commonly used in medical nutrition favoured the uptake of curcumin in the presence of piperine, whereas the same effect was not observed for Tween 20. A recently published study based on an animal model investigated the effect of different emulsifiers (sodium caseinate, soya lecithin, Tween 80) on the lipolysis of flaxseed emulsions and the intestinal bioavailability of ALA (Couedelo et al., 2015). Results clearly indicated that soya lecithin increased the rate of gastrointestinal lipolysis and favoured the intestinal uptake of ALA by the enterocyte and its subsequent transport to the lymph system. Thus, evidence from in vivo studies also exists to confirm that the interfacial composition plays an important role on lipolysis by modulating the access and activity of lipolytic enzymes. This in turn is likely to affect the solubilisation of lipophilic compounds in mixed micelles and their subsequent absorption by the enterocytes prior to their transport to the lymphatic route. More studies using in vitro models are required to increase our understanding on how the emulsifier type used to encapsulate lipophilic bioactives affects the release of the latter into the systematic circulation.

Conclusions

Emulsions offer many advantages for the delivery of lipophilic bioactive compounds. Their performance as ‘delivery vehicles’ can improve provided that formulation and processing aspects of product development are taken into consideration. The type of the carrier oil is an important determinant of the rate and extent of lipid digestion and the solubilisation capacity of the mixed micelle phase. MCTs are easily digested but LCTs are more effective for accommodating the bioactives during the formation of this complex colloidal dispersion consisting mainly of mixed micelles and vesicles. The interfacial composition and concentration is equally important for maintaining the structural integrity and thus bioactivity of the lipophilic components. Proteins either in their native form or conjugated with other functional components appear to be advantageous in comparison with their synthetic counterparts.

The vast majority of the data available is based on in vitro studies simulating the process of digestion in the gastrointestinal tract. In addition, the limited studies combining in vitro and in vivo models for testing the efficacy of emulsion systems to deliver lipophilic compounds quite often generate contradictory results. This suggests that there is a knowledge gap on the factors and/or mechanisms involved following the stage of micelle formation and prior to the transportation of the bioactives to systemic circulation. In vivo studies using animal or human models are clearly needed to elucidate the complex relationship between emulsion formulation and bioavailability of encapsulated hydrophobic bioactives.

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References


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