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Conventional Emulsions

Mehrdad Niakousari[1], Maral Seidi Damyeh[1], Hadi Hashemi Gahruie[1,2], Alaa El-Din A. Bekhit[3], Ralf Greiner[4], and Shahin Roohinejad[4]

[1] *Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran*
[2] *Biomolecular Engineering Laboratory, Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran*
[3] *Department of Food Science, University of Otago, Dunedin, New Zealand*
[4] *Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany*

1.1 Introduction

Food active ingredients are widely used in the food industry to improve the nutritional and physicochemical properties and prolong the shelf-life of food products. Incorporation of food active ingredients within foods has its own challenges, such as poor chemical stability, low bioavailability, and low water solubility. Thus, various studies have been conducted to develop effective systems for the delivery of these compounds (Cummings and Overduin, 2007; Maljaars, et al., 2009).

A variety of food active compounds can be incorporated into foods via specifically designed delivery systems with the aim of achieving a certain level of protection and reaching a specific targeted site, i.e. to control their release at specific locations within the gastrointestinal tract (e.g. mouth, stomach, small intestine, or colon) (Kosaraju, 2005). Delivery systems should be able to protect these compounds from physical and/or chemical degradation during processing, handling, and storage, and deliver them to the required site in the gastrointestinal tract without adverse effects on the appearance, stability, texture, or flavor of the food products (McClements, 2010; McClements et al., 2009). Conventional emulsions are considered the most important systems since they are the most widely used in the food industry (Augustin and Sanguansri, 2017).

Conventional emulsions are defined as emulsions having a particle size over 100 nm and are mostly produced using high-energy techniques. Homogenizers are usually used to facilitate the conversion of two immiscible liquids into an emulsion with the aid of an emulsifier. High-speed mixers, microchannel homogenizers, high-pressure valve homogenizers, microfluidizers, colloid mills, membrane and ultrasonic homogenizers are some of the important high-energy systems used at the industrial and research scales.

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This chapter will focus on the formation, characterization, and recent advances in the application of conventional emulsions as a delivery system of valuable food ingredients. The stability of these systems under *in vitro*/*in vivo* conditions is also discussed.

1.2 Conventional Emulsions Formation and Stability

1.2.1 Formation

There are two types of naturally occurring emulsions that are found in food systems: typical low-volume fraction emulsions such as milk and sauces, and a high-volume fraction such as butter and margarine (Moschakis, 2013). Numerous food emulsions are prepared by combining raw materials, some of which are not found conjointly in nature. For example, a salad dressing product is manufactured by combining water, milk protein, soybean oil, apple vinegar, and seaweed polysaccharides. Each component of the formulation will influence the physical (intermolecular or interdroplet forces, phase separations) and chemical (formation of covalent bonds) properties of the product (Friberg et al., 2004).

Depending on the nature of the starting materials and method used to create an emulsion, the process may involve a single or a number of consecutive steps. In order to convert two separate oil and water phases into an emulsion, different functional ingredients should be dispersed first into the phase in which they are most soluble. For instance, lipid-soluble compounds (e.g. oil-soluble vitamins, antioxidants, and pigments) are mixed first in the oil phase, while water-soluble compounds (e.g. proteins, polysaccharides, phenolic compounds, water-soluble pigments, and vitamins) are mixed first in water phase (McClements and Li, 2010). However, sometimes it is more convenient to mix powdered functional ingredients directly into a mixture of oil and water. In order to prevent clumping during subsequent mixing and homogenization processes, the intensity and duration of the mixing process need to be optimized (Kinsella and Whitehead, 1989).

The presence of crystalline materials in the lipid phase prevents the formation of a stable emulsion. Therefore, a preheating step to melt fats prior to homogenization is required. Excessive heating, however, may initiate/promote the oxidation of polyunsaturated lipids, which in turn adversely affects the product quality (Ochomogo and Monsalve-Gonzalez, 2009). Some parameters such as optimum conditions of ingredient mixing, solvent type, and operation temperature are important in the production of a stable functional emulsion.

As mentioned earlier, homogenization is used to convert two immiscible liquids into an emulsion with the aid of an emulsifier. Depending on the initial concentration of the two liquid phases, two different types of emulsions can be obtained in the presence of an emulsifier; at high oil and low oil concentrations, water in oil (w/o) and oil in water (o/w) emulsions will be formed, respectively (Figure 1.1). The balance between two opposing physical processes as well as the droplet disruption and coalescence will have a huge impact on the size of the droplets produced by the homogenization process (Schubert et al., 2003; Walstra, 2003).

Figure 1.1 Cream and butter as oil-in-water and water-in-oil food emulsion.

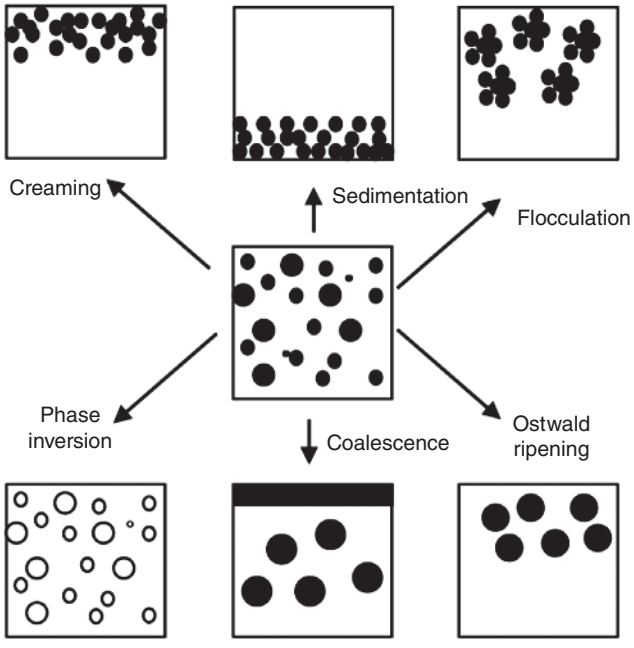

Figure 1.2 Schematic diagram of emulsion destabilization.

1.2.2 Stability

Emulsion stability is defined as the ability of an emulsion to resist changes to its properties over time. Stable emulsions have high resistance against changes in their properties and vice versa. Various physical and chemical processes may cause instability of an emulsion. As shown in Figure 1.2, several mechanisms such as creaming, sedimentation, flocculation, coalescence, partial coalescence, phase inversion, and Ostwald ripening can contribute to physical instability of an emulsion (Walstra, 2003).

Oxidation and hydrolysis are the most common reasons for chemical instability of emulsions (McClements and Decker, 2000). A clear understanding of each mechanism, the interplay of relationships between oxidation and hydrolysis, and the processing and storage factors that influence them should be established in order to achieve more stable products.

Although several studies have tried to elucidate the general principles governing the stability of emulsions in order to predict their behavior under different processing conditions, the compositional and structural complexities of some emulsions do not allow accurate prediction of stability during storage (Goff and Hartel, 2003). Analytical techniques are normally used to monitor changes in emulsion properties over time. Environmental conditions (e.g. temperature and humidity of storage, mechanical agitation, and storage time) as well as composition and microstructure of an emulsion have major effects on the rate of breakdown and the mechanism by which this breakdown occurs.

1.3 Composition of Conventional Emulsions for Food Applications

The aqueous phase (W) of conventional emulsions can solubilize different amounts of water-soluble ingredients such as acids, minerals, bases, preservatives, flavors, vitamins, surfactants, sugars, polysaccharides, and proteins. The partitioning, solubility, conformation, volatility, and chemical reactivity of some of these food components are measured by their interactions with water.

Lipids play an important part in beverage emulsions as flavor carriers or as the source of flavor themselves, such as essential oils (Tan, 2004). The choice of a suitable lipid in a formulation will have a huge impact on the nutritional, physicochemical, and sensory properties of conventional emulsions. In the formulation of a conventional emulsion, lipid acts as a solvent for hydrophobic food active ingredients such as oil-soluble vitamins and antioxidants, preservatives, and essential oils.

The term "emulsifier" describes any surface active component that is able to interact with oil and water phases and create an oil-water interface and protect emulsions from destabilization through aggregation, flocculation, and coalescence. In the food industry, low molecular weight surfactants, amphiphilic biopolymers, and surface active particulates are the emulsifiers most commonly used to form and stabilize emulsions (Table 1.1). An ideal emulsifier must be rapidly adsorbed to the oil-water interface, eliminates the interfacial tension, and prevents the occurrence of droplet coalescence during homogenization through the creation of an interfacial membrane.

Texture modifiers are divided into thickening agents and gelling agents, depending on the molecular origin of their functional characteristics. These ingredients are added to the formulation of conventional emulsions to modify the texture of the continuous phase. In practice, there is often no clear distinction between thickening and gelling agents due to the ability of thickening agents to form gels when they are added at high concentrations as well as the ability of gelling agents to increase the viscosity of aqueous solutions without forming gels when they are used at low concentrations. A particular biopolymer can act as either a thickening or a gelling agent under particular conditions,

Table 1.1 Comparison of emulsifier (protein, polysaccharide, phospholipid, and small molecular surfactant) properties that may be utilized in the food industry (McClements, 2015; McClements and Gumus, 2016).

Emulsifier	Molecular properties	Emulsion properties
Phospholipids		
Lecithin	Surface active because of polar head group (phosphate moiety) and non-polar (two fatty acids) tail group	Can form fairly small droplets at low levels using high-pressure homogenization. Unstable under acidic conditions (pH <3), and at high ionic strength. May break down at high temperatures
Lysolecithin	Surface active because of polar head group (phosphate moiety) and non-polar (one fatty acid) tail group	
Small molecule surfactants		
Quillija saponins	Surface active because they contain both hydrophilic (e.g. sugars) and hydrophobic (e.g. phenolics) regions	Can form small droplets at low levels using high-pressure homogenization. Emulsions unstable at highly acidic conditions (pH <3), and at high ionic strengths. Stable to heating
Proteins		
Whey protein	Mıxture of globular proteins from milk MW ≈ 18 kDa; pI ≈ 5, T_m ≈ 80 °C	Unstable at pH near pI, at high ionic strength, and at temperatures > T_m. Stable at pH below or above pI, at low ionic strength, and at temperatures below T_m
Beta-lactoglobulin	Globular protein from whey protein MW ≈ 18.4 kDa; pI ≈ 5.4; T_m ≈ 83 °C	Unstable at pH near pI, at high ionic strength, and at temperatures > T_m. Stable at pH below or above pI, at low ionic strength, and at temperatures below T_m
Alpha-lactalbumin	Globular protein from whey protein MW ≈ 14.2 kDa; pI ≈ 4.4; T_m ≈ 83 °C	
Bovine serum albumin	Globular protein from whey protein MW ≈ 66.3 kDa; pI ≈ 5.1; T_m ≈ 75 °C	
Lactoferrin	Globular glycoprotein from whey protein MW ≈ 80 kDa; pI ≈ 8; T_m ≈ 60 °C and 85 °C	
Caseinates	Mixtures of flexible proteins from milk MW ≈ 24 kDa; pI ≈ 5	Unstable at pH near pI, and at high ionic strength. Stable at pH below or above pI, at low ionic strength, and to heating
Alpha-s-casein	Flexible protein from milk MW ≈ 23.6 kDa; pI ≈ 5.1	
Beta-casein	Flexible protein from milk MW ≈ 24.0 kDa; pI ≈ 5.5	

(*Continued*)

Table 1.1 (Continued)

Emulsifier	Molecular properties	Emulsion properties
Egg proteins	Mixture of globular proteins from egg white or yolk	Unstable at pH near pI, at high ionic strength, and at temperatures > T_m. Stable at pH below or above pI, at low ionic strength, and at temperatures below T_m
Ovalbumin	Globular protein from egg white MW ≈ 45 kDa; pI ≈ 4.5; T_m ≈ 80 °C	
Lysozyme	Globular protein from egg white MW ≈ 14.3 kDa; pI ≈ 11.3; T_m ≈ 72 °C	
Legume proteins (soy, pea, lentil, chickpea, fava bean, etc.)	Mixture of globular proteins from legumes with variable molecular weights pI ≈ 4.3–5.0; T_m ≈ 82–90 °C	Unstable at pH near pI, at high ionic strength, and at temperatures > T_m. Stable at pH below or above pI, at low ionic strength, and at temperatures below T_m
Gelatin	Fairly hydrophilic flexible protein from animal sources (collagen). Variable molecular weight depending on processing conditions pI ≈ 5 (Type B) or 8 (Type A); T_m ≈ 10–30 °C	Often not very surface active due to high hydrophilic character. Some types of gelatin can be used successfully as emulsifiers
Polysaccharides		
Gum arabic	Branched glycoprotein MW ≈ 1000 kDa; pKa ≈ 3.5	Requires a high surfactant-to-oil ratio, but forms droplets stable to a wide range of pH, ionic strength, and temperature
Beet pectin	Branched anionic hydrophilic polysaccharide with hydrophobic ferulic acid groups	
Citrus pectin	Branched anionic hydrophilic polysaccharide with hydrophobic protein groups attached	

MW, molecular weight; pI, isoelectric point; T_m, melting temperature.
Source: McClements and Gumus (2016). Reproduced with permission of Elsevier.

for example temperatures, pH, or ionic strengths. Texture modifiers in conventional emulsions are used to provide the product with appropriate textural and mouthfeel characteristics, and to enhance emulsion stability by decreasing the rate at which particulates diffuse (McClements, 2005).

1.4 Characterization of Conventional Emulsions

Various analytical techniques are used to determine the physicochemical and sensory properties of conventional emulsions, such as stability, texture, flavor, and appearance. Some of the methods which are used to characterize the conventional emulsions are described below.

1.4.1 Testing Emulsifier Effectiveness

The effectiveness of an emulsifier for a specific food application depends on the minimum concentration needed to prepare a stable emulsion and to prevent droplets from aggregation and consequently destabilization of the emulsion during storage. In order to assess emulsifier efficiency, two simple empirical procedures (e.g. emulsifying capacity (EC) and emulsion stability index (ESI)) and several sophisticated methods (e.g. surface activity, saturation surface pressures, excess surface concentration, critical micelle concentrations (CMC), adsorption kinetics, and interfacial rheology) are used (McClements, 2007).

1.4.2 Dispersed Phase Volume Fraction

The dispersed phase volume fraction of a conventional emulsion can be simply determined by measuring its density (Pal, 1994). Densities of the dispersed (ρ_1) and continuous (ρ_2) phases are used to determine the density of emulsion (ρ_e) using the following equation: $\rho e = \phi\rho 1 + (1 - \phi)\ \rho 2$ ($\phi = (\rho_e - \rho_1)/(\rho_2 - \rho_1)$). With knowledge of the density of oil (~900 kg m^{-3}), aqueous (1000 kg m^{-3}), and dispersed phases, the dispersed phase volume fraction can be calculated. The density of emulsion is simply measured to 0.2 kg m^{-3} and the dispersed phase volume fraction is 0.002 or 0.2% of the volume. Increasing the oil content leads to a reduction in emulsion density (due to the lower density of liquid oil compared to water) (Pal, 1994).

The proximate analysis method is applied to determine the concentration of the dispersed phase in a conventional emulsion (Nielsen, 2003). Solvent extraction techniques such as continuous, semi-continuous, and discontinuous phase of extraction can be used for measuring the fat content in an emulsion (Min and Boff, 2003). If the sample can be dried, then it can be ground and mixed with a solvent to extract fat. After the lipid extraction step, the solvent is separated from the sample, evaporated, and the remaining fat is weighed. A non-solvent extraction technique can also be used to measure the fat content of an o/w emulsion in dairy products using the Babcock and Gerber methods.

Electrical conductivity (ε) of the dispersed phase of a conventional emulsion is used to determine its volume fraction (Asami, 1995). Water has a much higher electrical conductivity than oil, so the greater the oil content of an emulsion, the less the electrical conductivity (Clausse, 1983).

1.4.3 Measurement of Droplet Size Distribution and Microstructure

Several analytical instruments, such as photon correlation spectroscopy (PCS) and Doppler shift spectroscopy (DSS), were primarily designed to measure the particle size of emulsions. Dynamic light scattering (DLS) is one of the most popular techniques used to effectively determine particle size and distribution of emulsions. There is no particle-particle interaction in a diluted emulsion and the size of the droplets can be determined by the Stokes–Einstein equation.

Static light scattering is another rapid and reproducible technique that has been used to measure the particle size of emulsions, varying from 0.05 to 2000 μm. In this method, a beam of light passes through an emulsion and is scattered by the droplets in a well-defined manner (Hiemenz and Rajagopalan, 1997). Some instruments are used only to

measure the light intensity, especially non-scattered light by passing it directly through the emulsion, while others determine the light intensity which has been scattered by the emulsion droplets.

Nuclear magnetic resonance (NMR) investigates the interaction between hydrogen atom nuclei and electromagnetic waves. NMR can be used for the measurement of particle size distribution in conventional emulsions based on limited diffusion of molecules within the droplets (Balinov et al., 2004). The hydrogen atoms in the sample, which is located in a static magnetic field gradient, are excited by exposure to radiofrequency pulses. These hydrogen atoms will emit signals that are detectable by NMR. The amplitude of emitted signals is used to examine molecular movements. Sizes in the range of 0.2–100 μm can be determined by this method (Dickinson and McClements, 1995).

In the ultrasonic spectrometry techniques, the size of droplets with radii between 10 nm and 1000 μm can be measured from the interactions between ultrasonic waves and emulsions (Coupland and McClements, 2004). Compared to other traditional technologies, this method has some advantages due to its ability to analyze concentrated and optically opaque emulsions without any sample preparation.

The neutron scattering method determines the particle size, size distribution of particles, and the thickness of the interfacial layer in a conventional emulsion on the basis of the interaction between emulsion and neutron beam (Reynolds et al., 2000). Two specific features of this method are low scattering of emulsions that make it possible to use concentrated emulsion without dilution, and the ability to manipulate the contrast between different ingredients in heterogeneous foods to differentiate between their neutron scatterings. Thus, particular structural features can be detected in the emulsion. The main limitation of this method is the need for a nuclear reactor to create the neutron beam (Hone et al., 2002).

A combination of electricity and sound waves, known as electroacoustics, is used for the measurements of zeta potential and particle size distribution of emulsions (Dukhin et al., 2000). Particle sizes in the range of 0.1–10 μm can be measured by electroacoustics. Coupled with ultrasound spectrometry, this technique can determine the particle size in a larger range of 10 nm to 1000 μm (Hsu and Nacu, 2003). Particle size of high concentration emulsions and a wide variety of w/o and o/w conventional emulsions can be measured using this technique (Djerdjev et al., 2003; Kong et al., 2003; O'Brien et al., 2003). The main disadvantages of this method are the presence of charged droplets, a significant difference between the density of droplets and continuous phase, and a defined viscosity of continuous phase in each frequency.

In the dielectric spectroscopy technique, the conventional emulsion is exposed to a wide range of electromagnetic frequencies and its electric permittivity measured, which can be converted to particle size distribution and droplet size (Sjoblom et al., 1996). Only emulsions containing charged particles can be analyzed using this method. In addition to particle size distribution, zeta potential can also be determined. Dielectric spectroscopy is suitable to measure particle size distribution of opaque and concentrated emulsions without dilution.

A number of microscopic techniques, such as conventional optical microscopy, laser scanning confocal microscopy, electron microscopy (transmission electron microscopy (TEM), scanning electron microscopy (SEM)), and atomic force microscopy, can be used to study the structure, dimensions, and organization of the components of a conventional emulsion (Kirby et al., 1995; Morris et al., 1999). According to Aguilera

and Stanley (1990), any type of microscopy that is going to be used in examining the structure of small objects must have three qualities: resolution, magnification, and contrast.

1.4.4 Droplet crystallinity

Nuclear magnetic resonance has good potential for measurement of the solid content in conventional emulsions (Dickinson and McClements, 1995). Despite the high initial cost of the NMR apparatus, rapid analysis of emulsions without any preparation of concentrated or optically opaque samples has resulted in it replacing the time-consuming dilatometry method. When an emulsion is exposed to a radiofrequency pulse, the change of some of the hydrogen nuclei into an excited state leads to the generation of a detectable NMR signal. The basic parameters of this signal include frequency, amplitude, and decay time. Analyzing these parameters provides a wide range of information about the solid content of the material.

Dilatometry is a useful technique to detect the crystallinity of dispersed and continuous phases of conventional emulsions based on density changes that occur during melting or crystallization of materials (Phipps, 1964). In principle, the dilatometer measures the decrease and increase in density of a material when it melts or crystallizes, respectively. Compared to the liquid state of a material, the density of solid state is usually greater due to the more efficient packing of the molecules.

Differential scanning calorimetry (DSC) and differential thermal analysis (DTA) can be used to monitor the melting and crystallization behavior of conventional emulsion droplets (Palanuwech and Coupland, 2003). Since the materials have a tendency to release heat during crystallization and absorb heat during melting, these methods have been used to measure the release or absorption of heat by a sample when it is exposed to a controlled temperature change process.

Ultrasound is another method which can be applied to detect phase transitions in a conventional emulsion (Coupland and McClements, 2004). The melting or crystallization process can be altered significantly by the ultrasonic characteristics of a material. Since the velocity of ultrasonic waves in a solid medium is higher than in a liquid one, cooling of an emulsion from initial temperature of liquid droplets to the temperature where droplets start to crystallize causes a sharp increase in sound velocity. Conversely, increasing the emulsion temperature leads to a sharp decrease in sound velocity, due to the droplet melting process. Generally, supercooling effects cause droplet crystallization to occur at much lower temperatures than the melting point of the bulk oil (Dickinson et al., 1992).

1.4.5 Droplet Charge

Different instruments are used based on electroacoustics for determination of the concentration, size, and zeta potential of particles in emulsion systems (Kong et al., 2001a–c). Electroacoustic properties analysis can be conducted in two different ways:

- colloid vibration potential (CVP), in which an acoustic signal is sent to a sample and the resulting electric signal obtained by the oscillating particles is detected
- electrosonic amplitude (ESA), in which an electric signal is applied to a sample and the resulting acoustic signal formed by the oscillating particles is registered.

In the particle electrophoresis technique, the conventional emulsion is located in a cell and a static electrical field is transmitted through it via two electrodes. This results in charged particles being absorbed by the electrode on the side having an opposite charge (Hunter, 1993). The movement of droplets can be detected using different analytical methods. For instance, optical microscopy or static light scattering can be used to monitor the movements of large particles (>1 μm), while for detection of the movement of smaller particles, ultramicroscope, DLS, or static light scattering is normally used.

The relationship between droplet interaction and the physicochemical properties of a conventional emulsion is an interesting area in emulsion research. Unfortunately, there are no commercially available devices to study the charge, magnitude, and range of droplet interactions. However, some experimental techniques have been introduced to predict and control the fundamental interactions and to provide information about how these forces can influence the stability of an emulsion. For instance, in order to investigate the attractive forces between flocculated emulsion particles, plotting apparent viscosity as a function of shear stress by applying a suitable mathematical model can be used (Berli et al., 2002; Quemada and Berli, 2002).

Evaluation of emulsion stability is also a good tool to assess the magnitude of certain types of dispersed particle interactions. For example, it is possible to obtain indirect information about the strength of attractive interactions by determining the minimum salt concentration needed to improve flocculation of droplets in an emulsion stabilized electrostatically (Montagne et al., 2003).

The magnetic chaining approach directly monitors the force-separation distance profile between colloidal particles. When an emulsion-based paramagnetic colloid is exposed to an external magnetic field, monodispersed particles spontaneously align into linear chains due to magnetic dipole induction (Dimitrova and Leal-Calderon, 1999). The equilibrium between repulsive and attractive magnetic forces gives rise to the separation of droplets within chains, which can be measured using light scattering techniques. In this method, changing the strength of the applied external magnetic field can control the magnitude of the magnetic forces.

Atomic force microscopy has permitted the probing of droplet-droplet interactions by directly measuring force versus distance profiles for a particle attached to the end of a cantilever and a particle immobilized on a solid surface (Gunning et al., 2004).

1.5 Conventional Emulsions as Carriers for Delivery of Food Active Compounds

Conventional emulsions have been considered as stable delivery systems especially for lipophilic components that can be solubilized in the hydrophobic area of the oil droplet in an o/w emulsion (Iwamoto et al., 1991; O'Mullane et al., 1987). A hydrophobic or hydrophilic bioactive component is dispersed in the oil or aqueous phase, respectively, prior to homogenization and then a stabilized emulsion system will be created to provide protection to the bioactives in food products (Fustier et al., 2010).

Encapsulation and delivery of lipophilic compounds in o/w conventional emulsions have numerous advantages, including relative ease of preparation and low cost (McClements et al., 2009), high physical and chemical stability of the component by

designing the oil-water interface (Mao et al., 2013), ability to produce different rheological properties (Genovese et al., 2007), and versatility of production in wet state (Ru et al., 2010) or as solid powders, which facilitates their transportation and storage (Kumari et al., 2011).

However, conventional emulsions as delivery systems have some disadvantages, such as susceptibility to environmental stresses (e.g. heating, extreme pH, chilling, and high ionic strength) that may lead to physical and chemical instability under these conditions. Examples of chemical instabilities include oxidation and hydrolysis, and examples of physical instabilities include coalescence, flocculation, Ostwald ripening, and creaming (Dickinson, 2010; McClements and Decker, 2000). Applying chelating agents and antioxidants such as tocopherols results in sequestering heavy metals and consequently reduces the oxidation of lipophilic bioactive components (Hu et al., 2004; Ribeiro and Shubert, 2003). Maillard reaction products created by heat treatment of aqueous protein-carbohydrate mixtures were reported by Augustin et al. (2006) to protect oxidation-sensitive compounds such as fish oil. Protein (e.g. beta-lactoglobulin), carbohydrates (in the amorphous state), and gums (e.g. gum arabic, guar gum) as encapsulating matrices may be able to stabilize emulsions by acting as an oxygen barrier around the emulsion oil droplets. Moreover, milk protein, as an emulsifier system, can be used to protect polyunsaturated lipids from oxidation (Fustier et al., 2010). The protection afforded by the emulsifiers and release of the encapsulated component may result in small droplets (approximately μm) and small interfacial layers (approximately nm) due to very short time scales for molecular diffusion (McClements et al., 2007, 2009).

Using emulsifying agents, such as dairy proteins, gives rise to lipid droplets that carry cationic charge and repelling cationic transition metal ions (e.g. Fe^{2+}). In order to sequester the transition metal ions and avoid contact with the lipid phase, a chelating agent (e.g. EDTA) can be added to the aqueous phase. Antioxidants with the ability to partition into the lipid droplet interfaces can be used to prevent oxidation reactions (McClements et al., 2007). Due to the emulsifying and ligand-binding characteristics of whey protein isolate (WPI), it can be used in an emulsion formulation for simultaneous encapsulation of food active ingredients with various physicochemical properties. Accordingly, binding of bioactive components such as vitamins, polyphenols, and fatty acids to the oil droplets membrane in the o/w emulsion can be stabilized by whey protein (Wang et al., 2016a).

Wang et al. (2016a) demonstrated the possibility of simultaneous encapsulation of alpha-tocopherol and resveratrol in the oil phase and at the oil-water interface of o/w emulsions, in which roughly 94% of alpha-tocopherol and 50% of resveratrol were encapsulated in oil droplets stabilized by 0.01% WPI. Binding to WPI leads to partitioning of amphiphilic resveratrol between the aqueous phase and the oil-water interface (Wang et al., 2016a). Due to a physical barrier created by WPI, alpha-tocopherol was protected from decomposition (Ries et al., 2010).

Different bioactive lipophilic components have been encapsulated by conventional emulsions to prevent their oxidation, such as omega-3 fatty acids (Chee et al., 2007; McClements and Decker, 2000), lycopene (Ribeiro and Shubert, 2003; Ribeiro et al., 2006; Tyssandier et al., 2001), astaxanthin (Ribeiro et al., 2006), lutein (Losso et al., 2005; Santipanichwong and Suphantharika, 2007), beta-carotene (Santipanichwong and Suphantharika, 2007), plant sterols (Sharma, 2005), and conjugated linoleic acids

(Jimenez et al., 2004), and have been incorporated into different food products (e.g. milk, yoghurts, ice cream, and meat patties) (Chee et al., 2007; McClements and Decker, 2000; Sharma, 2005).

Beta-carotene, a natural colorant and antioxidant, is a compound beneficial to human health through its ability to decrease the risk of cancer, cardiovascular disease, and cataracts (Hou et al., 2014). Encapsulating beta-carotene in emulsion-based delivery systems can help in overcoming drawbacks such as poor water solubility and chemical instability (Roohinejad et al., 2014a, 2014b, 2015). Gu et al. (2017) investigated beta-carotene protection in o/w conventional emulsion using conjugates made from egg white protein and catechin as emulsifiers. Applying the egg white protein and prepared conjugate in the emulsion resulted in droplets with a mean diameter of 0.203 and 0.328 μm, respectively. Unexpected large oil droplets were produced by the conjugate due to its impact on lowering the interfacial tension, but a physically stable emulsion was produced (McClements and Gumus, 2016).

The mean droplet diameter of emulsions stabilized by polyphenol-protein conjugates is influenced by the type of proteins and polyphenols used in the emulsion (Gu et al., 2017). For example, Wei et al. (2015) reported that droplet size was reduced in emulsions stabilized by epigallocatechin gallate attached to alpha-lactalbumin or beta-lactoglobulin compared to the protein alone. This was in contradiction to the findings of Gu et al. (2017) who used egg white protein-catechin conjugates that resulted in larger droplet size. Interestingly, in emulsion stabilized by egg white protein-catechin conjugates, the degradation rate of beta-carotene was significantly less than those emulsions prepared with egg white protein alone (Gu et al., 2017). Moreover, better physical and chemical stability was observed for emulsions stabilized by egg white protein-catechin conjugates, due to an increase in the thickness of the interfacial layer and strong antioxidant activity of conjugates.

Crystalline bioactive lipid components such as carotenoids should be melted prior to homogenization in order to prevent fouling of homogenizers as well as to ensure that they are below saturation concentration in the carrier oil to prevent emulsion instability (McClements et al., 2007). Lycopene, for example, is crystalline at ambient temperature (melting point 173 °C); dispersing its crystals into carrier oil (e.g. medium chain fatty acid triacylglycerols (MCT)) and heating it until melted (about 140–210 °C) is critical to reach a stable emulsion as a delivery system. Also, the homogenization condition should be controlled to prevent chemical degradation of highly susceptible lycopene by minimizing homogenization time and reducing the oxygen content in the system (Ribeiro and Schubert, 2003). Investigation of emulsified lycopene incorporated into milk, orange juice, and water demonstrated more chemical stability in orange juice than in milk or water, which can be enhanced by adding alpha-tocopherol as an antioxidant (Ribeiro and Schubert, 2003). Homogenization conditions (i.e. temperature, pressure, and cycles) should be carefully controlled to avoid labile bioactive compounds such as omega-3 fatty acids and carotenoids being subjected to factors enhancing the degradation rate, including high temperatures, oxygen, light, or transition metals (McClements et al., 2007). The homogenization conditions largely affect the properties of emulsions, including droplet size, stability, and viscosity, and consequently must be considered in the design of emulsions for targeted delivery (Lu et al., 2015; Yuan et al., 2008).

The effect of conventional emulsion components (i.e. Arabic gum, xanthan gum, orange oil) on volatile flavor release from a model orange beverage was investigated by Mirhosseini et al. (2008b). Increasing orange oil content resulted in an increase in the average droplet size (Mirhosseini et al., 2008a), which consequently enhanced flavor release due to an increase in the total oil-water interfacial surface area. In fact,the transfer rate of the hydrophobic component from oil phase to water phase may be increased as a result of an increase in interfacial surface area (Mirhosseini et al., 2008b). A high content of xanthan gum enhanced the resistance to transfer hydrophobic components due to its distinctive hydrophobic character, and consequently results in a negative impact on flavor release (Mirhosseini et al., 2008b). This reduction in flavor release was caused by adsorption, entrapment, and binding forces as a result of interactions between flavor components and matrix constituents (Shahidi and Zhong, 2011). This phenomenon was also observed by other researchers using other materials or processing conditions, such as enhanced release of aroma components from o/w emulsions containing Tween 20 (van Ruth et al., 2000), and enhanced release of lemon and citrus aromas (Charles et al., 2000) due to an increase in droplet size.

Vitamin E, an oil-soluble antioxidant, exhibits various health effects, such as reducing cardiovascular disease, diabetes, and cancer, and has consequently gained interest as an ingredient for food products (Sylvester et al., 2011). Partial absorption of vitamin E at intestinal sites reduces its bioavailability. A better absorption was found in the presence of surfactants or emulsions (Julianto et al., 2000; Nacka et al., 2001). Yang and McClements (2013) investigated o/w emulsion delivery of vitamin E using a natural food-grade surfactant (Q-Naturale®) and compared its performance to Tween 80. Q-Naturale was effective at producing emulsions with relatively small droplets, which was found to decrease even more by adding glycerol in the aqueous phase prior to homogenization. Higher percentages of small droplets were produced by the presence of Tween 80 and Q-Naturale in an oil phase containing low levels (<40%) and high levels (60–80%) of vitamin E acetate, respectively. It was previously reported that a reduction in emulsion droplet size could be achieved by either a decrease in vitamin concentration in the oil phase or an increase in glycerol content in the aqueous phase (Yang and McClements, 2013).

Ergocalciferol or vitamin D2 is a lipid-soluble vitamin with important effects on the functioning of the human metabolic system (Khalid et al., 2015). Khalid et al. (2016) encapsulated ergocalciferol and cholecalciferol in an o/w conventional emulsion system using Tween 20 as an emulsifier. Emulsification was carried out using two different processes: rotor-stator homogenizer at 5000–20 000 rpm for 5 min, and high-pressure homogenization at 100 MPa after rotor-stator homogenization at 7000 rpm for 5 min. The oil droplet size depended on the conditions of the homogenization process. Applying high-pressure homogenization resulted in a significant reduction in droplet size with a stable emulsion for a longer period of time. An increase in the rotation speed of the rotor-stator decreased the volume mean diameter of emulsions. The type of homogenization process had little effect on the release profile of these components. After 10 and 30 days of storage at 4 °C, the encapsulation efficiencies for these two components were less than 70% and 10%, respectively. However, low rotation speed of the rotor-stator homogenizer resulted in Ostwald ripening and flocculation, which destabilized the emulsions.

In recent years, the health benefits of essential oils and their application as possible new food preservatives have attracted attention (Nielsen et al., 2016; Saljoughian et al., 2017). However, essential oils have a short shelf-life and are unstable in the presence of light, heat, moisture, and oxygen. Encapsulation of essential oils by emulsion systems has been suggested as one of the most effective techniques to overcome these challenges. Nielsen et al. (2016) evaluated the antibacterial activity of isoeugenol encapsulated in o/w conventional emulsions containing beta-lactoglobulin and n-OSA starch (a modified starch) as emulsifiers. High loading capacity was obtained and the antibacterial efficacy of isoeugenol against bacteria (*Escherichia coli* K12 and *Listeria monocytogenes*) was enhanced in carrot juice (2.5-fold) compared to unencapsulated isoeugenol. However, no significant increase in the antibacterial efficacy of milk as the result of isoeugenol encapsulation was observed, which was suggested to be because the unencapsulated isoeugenol was distributed in the emulsions already present in the milk.

Coenzyme Q10 is a fat-soluble antioxidant with several health-promoting effects (Fedacko et al., 2011). Stratulat et al. (2013) investigated the encapsulation of coenzyme Q10 in a nutraceutical emulsion composed of calcium caseinate, flaxseed oil, and lecithin. This emulsion was applied as a functional cream in cheese production without adverse effect on protein retention and cheese yield, and the retention of this component into the cheese matrix was reported to be 93%. Lower viscosity was observed for the flaxseed oil with coenzyme Q10 compared to the flaxseed oil alone, in which this reduction facilitated the disruption of fat droplets and decrease in size (Stratulat et al., 2013). In another study, coenzyme Q10 was encapsulated in a conventional emulsion system containing organic acid monoglyceride and milk protein to provide sufficient emulsifying power (Ikehara and Ogino, 2004). The resultant emulsion had resistance to coenzyme Q10 separation or creaming for nearly 2 weeks.

A few studies have been conducted on delivery of hydrophilic bioactive components via conventional emulsions. One example is epigallocatechin-3-gallate, a polyphenolic compound, which was encapsulated in a canola oil emulsion (o/w) using high-pressure homogenization, and stabilized by iota-carrageenan and beta-lactoglobulin with a droplet size of about 0.4 μm (Ru et al., 2010). Enhanced *in vitro* anticancer activity was observed for the epigallocatechin-3-gallate encapsulated in emulsion compared to the free epigallocatechin-3-gallate. Another example is L-ascorbic acid that was encapsulated into w/o emulsions, with an average droplet diameter of 2.0–3.0 μm (Khalid et al., 2013). The resulting emulsions were stable for over 30 days, with L-ascorbic acid retention being 50% at 4–8 °C and 30% at 8–25 °C. Moreover, a significant survival rate of probiotics encapsulated in emulsion prepared from sesame oil was obtained (Hou et al., 2003).

1.6 *In Vitro*/*In Vivo* Digestion of Conventional Emulsions

Different methods are used to understand the basic physicochemical and physiological processes occurring in a delivery system passing through the human gastrointestinal tract (Figure 1.3). The pH-stat method is applied to study the impact of simulated gastrointestinal conditions on lipid-based delivery systems. This simple and rapid method has been extensively used in pharmaceutical and food research (Armand et al., 1992; Dahan and Hoffman, 2008; Hu et al., 2010; Li and McClements, 2010). After

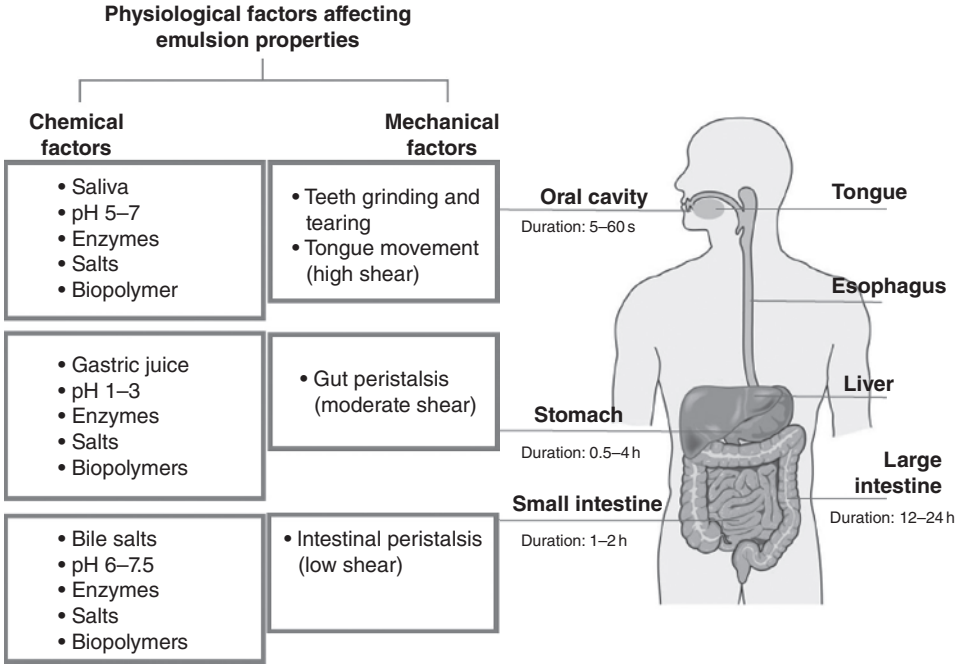

Figure 1.3 Schematic diagram of the physiological conditions in the mouth-GI tract which can cause significant changes in emulsion structures. *Source:* Mao and Miao (2015). Reproduced with permission of Springer.

adding lipase at pH values close to neutral, the amount of free fatty acids released from lipids is measured. The required pH should be selected carefully since fatty acid ionization and solubility and lipase activity are pH dependent in this region (Ali et al., 2010; Bauer et al., 2005). In order to achieve more realistic results, conditions close to those in the whole digestive tract should be simulated by incorporating the appropriate concentrations of the major components, such as bile salts, phospholipids, lipase, colipase, and mineral ions (McClements and Li, 2010).

Generally, an increase in concentration of the encapsulated bioactive component in the continuous phase or at the target sites (e.g. nose, mouth, stomach, gastrointestinal tract) as a function of time is considered as release in an emulsion system (Aguilera, 2006). By selecting appropriate ingredients and specific designs of microstructures of emulsion systems (e.g. droplet size and layer thickness), controlling the release of components is feasible (Lian et al., 2004). The lipid digestibility of conventional emulsions can be controlled by the following approaches.

1.6.1 Droplet Size (Surface Area)

The dimension of emulsion droplets determines the release kinetics of conventional emulsion; that is, a decrease in droplet diameter leads to an increase in release rate (McClements, 2005). For the lipase to come into close proximity with its substrate (mostly triacylglycerols) and digest the lipid, the enzyme molecules should be adsorbed

to the lipid droplet surface, which is generally an interfacial phenomenon. Consequently, an increase in lipid digestion rate (expressed as free fatty acids released per unit of time) would be obtained as a result of an increase in exposed lipid surface area to the lipase due to a reduction in droplet size, which has been confirmed by previous studies (Armand et al., 1992; Bauer et al., 2005; Lundin and Golding, 2009).

McClements and Li (2010) reported that the rate of lipid digestion in emulsions stabilized by beta-lactoglobulin increased with a decrease in lipid droplet diameter. On the other hand, the rate of lipid digestion in a beta-lactoglobulin-stabilized nanoemulsion (d = 60 nm) was found to be slower than in the beta-lactoglobulin-stabilized conventional emulsion (d = 0.2 μm), despite the larger droplet size in the conventional emulsion. This may be attributed to the differences in interfacial structure (McClements and Li, 2010) as well as more available lipase per unit droplet surface area, which results in more lipase molecules to be adsorbed per unit surface area in emulsions with large lipid droplets (Reis et al., 2009). To be more explicit, a thin monolayer of globular protein molecules coats the lipid droplets in conventional emulsions, while droplets are coated in nanoemulsion by aggregated globular protein molecules having a much thicker layer.

1.6.2 Droplet Composition

The rate of lipid digestion in emulsion-based delivery systems is mostly determined by the ability of lipase to convert the lipid substrate into free fatty acids and monoacylglycerols. It is worth mentioning that lipase activity may be decreased due to ingredient interactions in the digestion fluid, the presence of a physical barrier such as a layer of surfactant to sterically limit access of the enzymes to the droplet surface or lipid digestion products remaining on the droplet surface in the absence of mixed micelles or calcium (Chu et al., 2009). To determine the rate of lipid digestion, the amount of released free fatty acids from the lipid phase as a result of lipase activity is measured (Dahan and Hoffman, 2008). According to literature, lipid digestion and release of bioactive compounds are significantly influenced by the oil type used in the preparation of emulsions. For instance, McClements et al. (2008) found a higher digestion rate for o/w emulsions prepared from medium chain triglyceride oils compared to long chain triglyceride oils, both stabilized by beta-lactoglobulin.

1.6.3 Emulsifier Type

The effect of emulsifier type on lipid digestion has been investigated. Phospholipids were found to cause faster lipid digestion compared to proteins (Wickham et al., 1998). In another study, the impact of different emulsifiers on lipid digestion was investigated and protein (caseinate or whey protein isolate) was found to lead to rapid lipid digestion compared to lecithin (phospholipids), followed by Tween 20 (surfactant) (Mun et al., 2007). The same results were found by Reis et al. (2008). However, another study demonstrated that the rate and extent of lipid digestion were not influenced by the type of emulsifier (e.g. beta-lactoglobulin, lecithin, and Tween 20) in emulsions prepared by medium chain triglyceride (d = 0.240–0.300 μm) (McClements and Li, 2010). The impact of o/w macroemulsion parameters on dynamic flavor release was investigated and the results showed that the release of flavor was not affected by emulsion particle size and emulsifier type or concentration (Rabe et al., 2003).

Fernandez-Avila et al. (2016) investigated delivery of conjugated linoleic acids by o/w emulsions (20% oil) using soy or pea protein isolates (4%) as stabilizer. Smaller particle size distribution was obtained using soy protein isolate than pea protein isolate. Conjugated linoleic acid was better protected by emulsions than in non-emulsified controls, with no difference in bioaccessibility and bioavailability of the component in emulsions. The extent of lipid digestion for the two prepared emulsions was almost similar, suggesting that the type of protein present at the interface (soy and pea protein isolates) and the droplet size of the emulsions would not affect the *in vitro* oil digestibility in emulsions. The free fatty acid release percentages for emulsions stabilized by soy protein isolate and pea protein isolate were 23.3% and 25.3%, respectively. Also, soy and pea polypeptides were completely hydrolyzed after digestion of emulsions (Fernandez-Avila et al., 2016). Malaki Nik et al. (2011a,b) reported higher values of lipid hydrolysis (>80%) in emulsions consisting of soybean oil and soy protein isolate. Differences in simulated gastrointestinal conditions and a reduction in lipolysis, due to a physical barrier formed by high amounts of proteins adsorbed at the interface, may be the reasons for smaller free fatty acid values in the former study (Fernandez-Avila et al., 2016).

It has been demonstrated that the presence of appropriate amounts of bile salts would lead to a reduction in droplet size due to the disruption of flocs created in the gastric phase (Malaki Nik et al., 2011a,b). The larger particle size in emulsion investigated by Fernandez-Avila et al. (2016) may be a result of lower concentrations of bile salts. Consequently, a reduction in lipolysis rate was observed by larger droplet size.

1.7 Bioaccessibility and Bioavailability of Food Active Ingredients

Bioaccessibility can be expressed as the amount of released bioactive component within the mixed micelle phase after digestion of lipids. The bioactivity of a component is determined according to the available fraction for absorption. Since *in vitro* digestion investigation is a prerequisite to develop an effective delivery system, a successful model should be designed to predict *in vitro-in vivo* correlations (Fricker et al., 2010). The amount of a bioactive component with therapeutical benefits reaching the systemic circulation is defined as "bioavailability" (Holst and Williamson, 2008; Versantvoort et al., 2004). The bioavailability of an encapsulated bioactive component should be improved or at least not adversely affected by an appropriate delivery system (Versantvoort et al., 2004).

Concentration, composition, size, aggregation state, and interfacial properties of the oil droplets determine the emulsion's ability to increase the bioaccessibility of hydrophobic bioactive components. An increase in the concentration of oil droplets in emulsions leads to the enhancement of bioaccessibility of hydrophobic bioactive components (Zhang and McClements, 2016), which was observed for carotenoids and coenzyme Q10 (Cho et al., 2014; Salvia-Trujillo et al., 2015). The interfacial layer of globular proteins may have some effects on lipid digestion and release by inhibiting either the close contact between lipase and lipids or the release of digested lipids via a two-dimensional network formation of globular proteins by cross-linked molecules at the oil-water interfaces. This coating may collapse and form a thick layer through the

digestion process, which enhances entrapment of the remaining lipid or lipid digestion products (Lee and McClements, 2010). A close correlation between solubilization capacity of lipid digestion products and lipophilic compounds bioaccessibility in lipid-based delivery systems has been found in other studies (Porter et al., 2004; Sek et al., 2006).

An increase in the chain length of the hydrocarbon tails of phospholipids, fatty acids, and monoacylglycerols leads to an increase in dimensions of the non-polar domains in mixed micelles, which influence bioaccessibility (Israelachvili, 2011). Also, increasing unsaturation of the hydrocarbon tails results in a decrease in non-polar domains, which should be large enough to place hydrophobic bioactives within themselves. The importance of this particular phenomenon for carotenoids, considered as long hydrophobic molecules, has been reported (Qian et al., 2012; Salvia-Trujillo et al., 2013).

In order to promote the absorption of hydrophobic bioactive components by epithelium cells, ingredients such as fatty acids, minerals, chelating agents, phytochemicals, surfactants, and certain biopolymers can be used in the emulsion to alter diffusion or transport mechanisms or tight junctions (Jin and Han, 2010; Whitehead and Mitragotri, 2008). Wang et al. (2016b) investigated the effect of BSA-dextran conjugate (a Maillard product) as emulsifier and stabilizer on curcumin-loaded o/w emulsions. The emulsion produced by heat treatment having an integrated and cross-linked interfacial film was chemically and physically stable at different pHs and temperatures. *Ex vivo* fluorescence images of mice after oral administration revealed that the emulsion was mostly in the gastrointestinal tract but it had disappeared 24 hours post administration. The curcumin oral bioavailability in mice was shown to be enhanced 4.8-fold using BSA-dextran emulsion compared to curcumin/Tween 20 suspension and promoted curcumin adsorption in the gastrointestinal tract. Therefore, protein-polysaccharide emulsions were considered as good oral delivery systems for curcumin (Wang et al., 2016b).

The influence of the type of lipid (long, medium, and short chain triacylglycerol) on bioaccessibility of curcumin was investigated by Ahmed et al. (2012) via o/w conventional and nanoemulsions. A low bioaccessibility of curcumin was obtained by an emulsion with short chain triacylglycerols as lipid phase, which is due to not forming mixed micelles to solubilize highly lipophilic components (Fatouros and Mullertz, 2008). Interestingly, a substantial increase in curcumin bioaccessibility was observed using emulsions with medium and long chain triacylglycerols as lipid phase, which was the result of the formation of mixed micelles. Consequently, an increase in total lipid concentration resulted in an increase in curcumin bioaccessibility, except for the long chain triacylglycerols in which a greater fraction of lipid remained non-digested and thus some of the curcumin would not be released.

Lipid digestibility of conventional emulsions with three emulsion delivery systems (small microcluster emulsions, large microcluster emulsions, and filled hydrogel beads) prepared from protein-coated lipid droplets, alginate, and/or calcium was compared (Li et al., 2012). Conventional emulsions exhibited smaller mean diameter of particles (0.36 μm) than the other three systems. In order to study the fate of delivery systems within the gastrointestinal tract, a fluorescent dye (Nile Red in this study) was added to the lipid phase and the emulsion sample was introduced into rat stomachs. According to confocal microscopy results, considerable disruption was observed for conventional emulsions in the stomach, leading to droplet coalescence, and consequently the delivery

system was not intact in the small intestine (Li et al., 2012). Based on lipid absorption measurements, considerable amounts of marker lipid (tridecanoic acid) for conventional emulsion (12–59% in serum, 6–28% in small intestine) were detected in the blood of the rats, which was due to the digestion and adsorption of lipids within the small intestine. An increase in lipid digestion and a decrease in lipid absorption were observed by calcium ions preventing long chain fatty acid accumulation at the droplet surfaces, contributing as a cofactor for pancreatic lipase (Fave et al., 2004), and forming precipitates through interaction with long chain saturated fatty acids (Karupaiah and Sundram, 2007; Lorenzen et al., 2007).

1.8 Conclusion and Future Directions

Conventional emulsions, particularly the o/w type, are widely used to encapsulate bioactive compounds (especially lipophilic ones) and other food active ingredients. Food active ingredient bioaccessibility/bioavailability and absorption can be improved by controlling the composition, structure, and properties of emulsions. For instance, enhancement of bioaccessibility can be obtained by forming a mixed micelle phase containing numerous large non-polar domains to accommodate all the released hydrophobic bioactives. Moreover, applying emulsions with the ability to form mixed micelles, which easily penetrate through mucous layers, can enhance absorption of bioactive components. Controlling the molecular environment of labile bioactive components (oil, water, or interfacial regions) in the gastrointestinal tract, as well as using food components such as antioxidants or chelating agents to modulate degradation reactions, can help in manipulating their chemical or biochemical transformation. Designing novel conventional emulsions to enhance the bioavailability of food active compounds is a growing area which needs further work to achieve a rational design of functional food and beverage products with enhanced health-promoting effects.

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