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Shahin Roohinejad, Ralf Greiner,
Indrawati Oey, Jingyuan Wen

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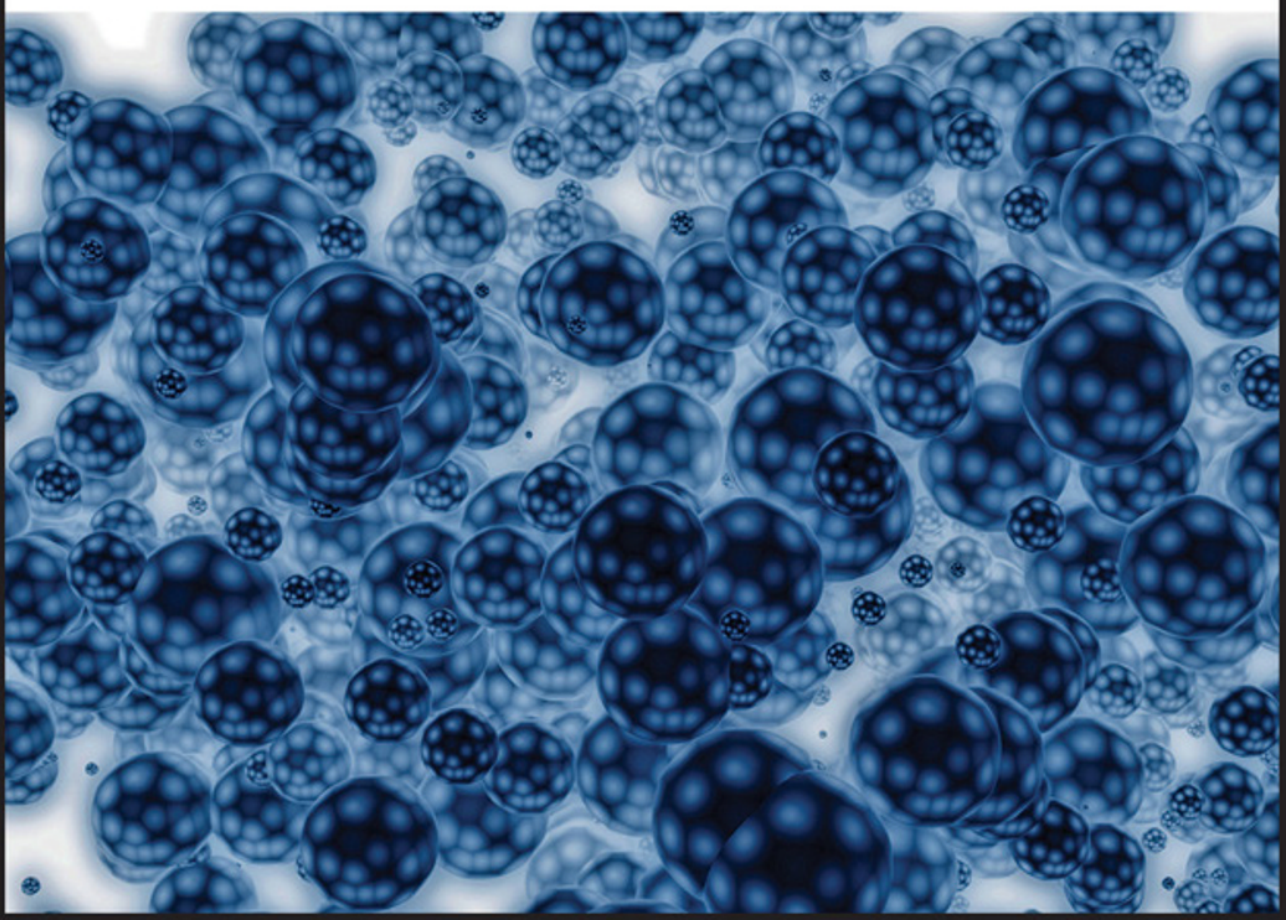
Formation, Application, Health and Safety



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Edited by

Shahin Roohinejad
Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Karlsruhe, Germany

Ralf Greiner
Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Karlsruhe, Germany

Indrawati Oey
Department of Food Science, University of Otago, Dunedin, New Zealand

Riddet Institute, Palmerston North, New Zealand

Jingyuan Wen
School of Pharmacy, University of Auckland, Auckland, New Zealand

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Preface

Emulsion-based delivery systems are mainly designed to encapsulate, deliver, and control the release, digestion, and absorption of hydrophobic and hydrophilic food active ingredients including small (e.g. volatiles), medium (e.g. food bioactives such as omega-3 fatty acids, conjugated linoleic acid, butyrate, phytosterols, carotenoids, antioxidants, vitamins), and large molecular weight compounds (e.g. enzymes) in the food and pharmaceutical industries. Their functionality can be tailored by controlling their chemical composition and physical properties. This has led to the development of different emulsion systems, including conventional emulsions, Pickering emulsions, multiple emulsions, multilayered emulsions, solid lipid nanoparticles, nanostructured lipid carriers, filled hydrogel particles, nanoemulsions, microemulsions, liposomal emulsions, and niosomes. Each of these systems has its own advantages and disadvantages for controlling the absorption and release of food active compounds.

This book covers the principles of emulsion-based systems formation, their characterization and application as carriers for delivery of food active ingredients as well as their digestibility and health and safety challenges for use in food systems. In each chapter, the formation of a specific emulsion-based system and its application for delivery of food active compounds used in food systems are discussed. Additionally, the biological fate, bioavailability, and health and safety challenges of using emulsion-based systems as carriers for delivery of food active compounds in food systems are reviewed.

This book is designed to assist food scientists as well as those working in the food, nutraceutical, pharmaceutical, and beverage industries. The topics covered in this book are suitable for teaching in courses such as food chemistry, food biochemistry, sensory science, new product development, and food processing.

We gratefully acknowledge the contribution of all colleagues from around the world and the professional assistance provided by the staff of Wiley.

Shahin Roohinejad, Ralf Greiner, Indrawati Oey, and Jingyuan Wen

About the Editors

Shahin Roohinejad

Dr Roohinejad obtained his BSc in 2000 from the Islamic Azad University, Iran, in the field of food science and technology. He completed his MSc in 2009 in food biotechnology at the University of Putra Malaysia (UPM). In July 2011, he received a full doctoral scholarship award from the Department of Food Science at the University of Otago in New Zealand, and he graduated in December 2014. In June 2015, Dr Roohinejad received a Georg Forster Research Fellowship award granted by the Alexander von Humboldt Foundation to pursue his postdoctoral research at the Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut (MRI), The German Federal Research Institut of Nutrition and Food. Currently, he is a postdoctoral research associate at the Food Science and Nutrition Department, University of Minnesota, USA. He is a professional member of the Institute of Food Technologists (IFT), graduate member of the New Zealand Institute of Food Science and Technology (NZIFST), associate newsletter editor of the IFT Non-thermal Processing Division (NPD), a member of the IFT Press Advisory Group and GHI (Global Harmonization Initiative) Ambassador in Germany. In the last 10 years, he has worked on different food areas such as emulsion-based systems, emerging food processing, nanotechnology, and functional foods. Dr Roohinejad's research activities have resulted in more than 70 original papers in peer-reviewed journals, book chapters, abstracts, and short papers in congress proceedings.

Ralf Greiner

Dr Greiner joined the Federal Research Centre for Nutrition, Karlsruhe, Germany, in 1990 as a PhD student after graduating in chemistry at the University of Stuttgart. In the early stages of his career as Deputy Head of the Centre for Molecular Biology, he was mainly engaged in research relating to genetically modified food and enzymes for food processing, with phytases being the center of his interests. In 2007, he held a position as Visiting Professor for Biochemistry and Molecular Biology, Department of Bioprocess Engineering, Federal University of Paraná, Curitiba, Brazil, working on solid-state fermentation and fungal enzyme production. In 2008, he returned to Karlsruhe where he became Head of the Department of Food Technology and Bioprocess Engineering of

the Max Rubner-Institut (MRI). His research covers the studying and modeling of conventional and new processing technologies, as well as food nanotechnology, but phytases are still the main focus of his interests. Dr Greiner is a representative of MRI in several international and national associations on food technology, food control, and food nanotechnology. In 2012, he accepted a position as an Honorary Assistant Professor in the School of Biological Sciences of the University of Hong Kong. His research activities have resulted in approximately 120 original papers in peer-reviewed journals, 37 book chapters and 260 abstracts or short papers in congress proceedings. In addition, Dr Greiner is Editor of *Food Control*.

Indrawati Oey

Professor Oey is Head of the Food Science Department at the University of Otago, Dunedin, New Zealand, and Principal Investigator at the Riddet Institute, Palmerston North, New Zealand. The University of Otago awarded her a full professorial chair as Professor of Food Science in 2009. She graduated with a BSc in Agricultural Technology (Food Science and Nutrition) from Bogor Agricultural University, Indonesia, and earned her MSc in Postharvest Food Processing and Preservation and PhD in Applied Biological Sciences from Katholieke Universiteit Leuven (KU Leuven), Belgium. She was a postdoctoral fellow at the Research Foundation-Flanders at KU Leuven, Belgium (2000–2009). She was the chair of Training and Career Development for the European Union-funded NovelQ Integrated Project (2006–2008). She is a Fellow of the New Zealand Institute of Food Science and Technology (NZIFST), a professional member of the Institute of Food Technologists (IFT) and NZIFST, served as Member-at-Large for the Executive Committee Board of Non-thermal Processing Division – Institute of Food Technologists (2012–2015), and as secretary of the NZIFST Otago/Southland branch (2010–2015). She received the George Stewart Award from the Institute of Food Technologists (United States) in 2006. In the last decade, she has been actively involved in building international collaboration in the area of novel food processing, nanotechnology, functional foods, and food innovation. Professor Oey's research activities have resulted in more than 200 original papers in peer-reviewed journals, book chapters, abstracts, and short papers in congress proceedings.

Jingyuan Wen

Professor Wen has over 20 years' research experience in pharmacology, nutraceuticals, pharmaceuticals, drug discovery from natural products, novel drug formulation, and delivery system design. She received her Bachelor of Medicine in 1986 and her Master's degree in pharmacology in 1991 at the School of Pharmacy, Fudan University, China. She was awarded a PhD in Pharmaceutical Science in 2003 from the School of Pharmacy, University of Otago, New Zealand. She worked as a post-doc researcher at Neuren Pharmaceuticals Ltd, New Zealand. She was appointed as a lecturer/senior lecturer at the School of Pharmacy, University of Auckland, in 2005. In 2015, she was promoted to Associate Professor, recognizing excellence in teaching and research. She was also appointed as a research theme leader in drug delivery in 2013. Under her supervision,

12 PhD students, 12 Master's students, and 160 research dissertation and international exchange students have completed their degree. To date, she has published over 200 peer-reviewed articles, patents, book chapters, and conference abstracts in drug delivery, natural products, and biological science. Professor Wen is a committee member of the Controlled Release Society (CRS), NZ local chapter. She is also an abstracts reviewer for the CRS (from 2007 to present) and was chair of the Oral Drug Delivery Award Committee of the CRS (2007 to 2012). She has established contacts with national and international commercial companies and has been involved in joint projects and consultations for national and international pharmaceutical and healthcare companies since 2005.

List of Contributors

Alireza Abbaspourrad
Department of Food Science
Cornell University
Ithaca, USA

Murad Al Gailani
School of Pharmacy
University of Auckland
Auckland, New Zealand

Alaa El-Din A. Bekhit
Department of Food Science
University of Otago
Dunedin, New Zealand

Claire C. Berton-Carabin
Laboratory of Food Process Engineering
Wageningen University and Research
Wageningen, The Netherlands

Guanyu Chen
School of Pharmacy
University of Auckland
Auckland, New Zealand

Shuo Chen
School of Pharmacy
University of Auckland
Auckland, New Zealand

Meinou N. Corstens
Laboratory of Food Process Engineering
Wageningen University and Research
Wageningen, The Netherlands

Maral Seidi Damyeh
Department of Food Science and Technology
School of Agriculture, Shiraz University
Shiraz, Iran

David W. Everett
Riddet Institute, Palmerston North
New Zealand
Dairy Innovation Institute
California Polytechnic State University
San Luis Obispo, California, USA

Hadi Hashemi Gahruie
Department of Food Science and Technology
School of Agriculture
Shiraz University
Shiraz, Iran

Kelvin K.T. Goh
Massey Institute of Food Science and Technology, College of Sciences
Massey University
Auckland, New Zealand

Ralf Greiner
Department of Food Technology and Bioprocess Engineering
Max Rubner-Institut
Federal Research Institute of Nutrition and Food
Karlsruhe, Germany

Seyedeh Sara Hashemi
Burn and Wound Healing Research Center
Shiraz University of Medical Sciences
Shiraz, Iran

Kacie K.H.Y. Ho
Laboratory of Food Process Engineering
Wageningen University and Research
Wageningen, The Netherlands
Plants for Human Health Institute
North Carolina State University
Kannapolis, NC, USA

Mohamed Koubaa
Ecole Supérieure de Chimie Organique et Minérale, Compiègne, France

Sung Je Lee
Massey Institute of Food Science and Technology, College of Sciences
Massey University
Auckland, New Zealand

Mehrdad Niakousari
Department of Food Science and Technology
School of Agriculture, Shiraz University
Shiraz, Iran

Nooshin Nikmaram
Department of Food Science and Technology
Islamic Azad University of Sabzevar
Sabzevar, Iran

Indrawati Oey
Department of Food Science
University of Otago, Dunedin, New Zealand
Riddet Institute, Palmerston North
New Zealand

Alireza Rafati
Division of Pharmacology & Pharmaceutical Chemistry
Sarvestan Branch, Islamic Azad University
Sarvestan, Iran

Ali Rashidinejad
Riddet Institute, Centre of Research Excellence
Massey University
Palmerston North, New Zealand

Shahin Roohinejad
Department of Food Technology and Bioprocess Engineering
Max Rubner-Institut
Federal Research Institute of Nutrition and Food
Karlsruhe, Germany

Anja Schröder
Laboratory of Food Process Engineering
Wageningen University and Research
Wageningen, The Netherlands

Karin Schroën
Laboratory of Food Process Engineering
Wageningen University and Research
Wageningen, The Netherlands

Pankaj Sharma
Fonterra Co-operative Group Limited
Palmerston North, New Zealand

Anges Teo
Massey Institute of Food Science and Technology, College of Sciences
Massey University
Auckland, New Zealand

Jingyuan Wen
School of Pharmacy
University of Auckland
Auckland, New Zealand

Marie Wong
Massey Institute of Food Science and Technology, College of Sciences
Massey University
Auckland, New Zealand

Naibo Yin
School of Pharmacy
University of Auckland
Auckland, New Zealand

Quan Yuan
Massey Institute of Food Science and Technology, College of Sciences
Massey University
Auckland, New Zealand

1

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).

Cream: Oil-in-water Butter: Water-in-oil

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

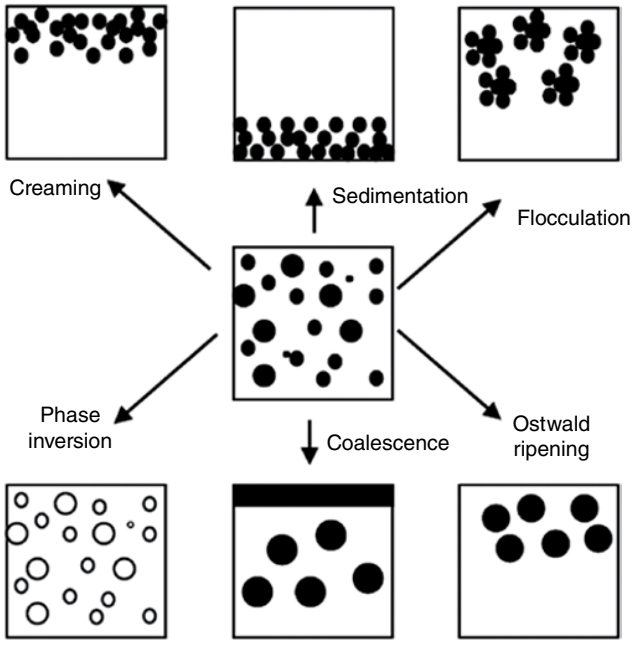

Figure 1.2 Schematic diagram of emulsion destabilization.

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