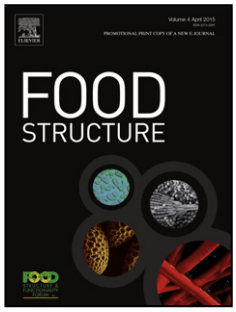
CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems
Authors	Drapala, Kamil P.;Mulvihill, Daniel M.;O'Mahony, James A.
Publication date	2018
Original Citation	Drapala, Kamil P.; Mulvihill, Daniel M.; O'Mahony, James A. (2018) 'A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems'. Food Structure, 16, pp. 27-42. doi: 10.1016/j.foostr.2018.01.004
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S2213329118300078 - 10.1016/j.foostr.2018.01.004
Rights	© 2018, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2026-10-30 01:55:35
Item downloaded from	https://hdl.handle.net/10468/5585

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Accepted Manuscript



Title: A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems

Authors: Kamil P. Drapala, Daniel M. Mulvihill, James A. O'Mahony

PII: S2213-3291(18)30007-8
DOI: https://doi.org/10.1016/j.foostr.2018.01.004
Reference: FOOSTR 91

To appear in:

Received date: 3-6-2017
Revised date: 18-12-2017
Accepted date: 29-1-2018

Please cite this article as: Drapala, Kamil P., Mulvihill, Daniel M., & O'Mahony, James A., A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems.*Food Structure* https://doi.org/10.1016/j.foostr.2018.01.004

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems

Kamil P. Drapala, Daniel M. Mulvihill and James A. O'Mahony

School of Food and Nutritional Sciences, University College Cork, Cork, Ireland

Abstract

Nutritional beverage emulsions contain water and oil, stabilised by surfactants, and are both diverse and complex. Their susceptibility to changes induced by manufacturing processes and on storage, results in challenges with their stability, quality and shelf-life. An understanding of the relationship between structure and stability of an emulsion is essential to designing and competently formulating food products with the desired nutritional functionality and sensory properties, while achieving the required shelf-life. This article critically reviews a selection of commonly-used analytical approaches focused on characterisation of emulsion structure in the context of emulsion formation, techno-functional properties and stability to intrinsic and environmental factors.

Keywords: emulsion structure; emulsion stability; rheology; CLSM; interfacial properties; emulsifiers

ACCEPTED MANUSCRIPT

1. Introduction

Many food products are emulsion-based systems (e.g., protein-based beverages, ready-to-feed infant formulae, ice cream and mayonnaise) and in some food products the emulsion is immobilised by subsequent processing (e.g., spray drying, gelation or freezing). Among emulsion-based foods, nutritional beverage emulsions represent a high value product category (available in either ready-to-drink liquid or instant powder formats and in some cases, both formats) tailored to deliver a specific nutritional content to a particular consumer group. Nutritional beverage emulsions encompass products such as infant formulae, clinical, medical, elderly and sports nutrition products, and a variety of milk-based beverages developed to meet consumer health, wellness and sensory satisfaction needs. Owing to the immiscible nature of the two major components of an emulsion (i.e., water and oil), these systems are inherently thermodynamically unstable, hence, efforts to improve their stability are an ongoing focus of food research. Stability of a food emulsion can be divided into stability against time-induced changes resulting from the differences in the density of the two immiscible components of the emulsion (i.e., floatation and phase separation; Fig. 1 B) as described by Stokes' law (see below) and stability of the system against undesirable interactions between its individual components (i.e., flocculation, aggregation and coalescence of oil globules) (Fig. 1 C and D). Based on Stokes' law, a relationship between the velocity of separation (i.e., creaming) and particle size, phase density and viscosity can be established. This relationship helps to explain how changes to these parameters affect its stability to gravity-governed changes under laminar flow:

$$v_{creaming} = \frac{2r^2(\rho_{oil\ globule} - \rho_{serum})g}{9\eta}$$

ACCEPTED MANUSCRIPT

Where $v_{creaming}$ describes the velocity of upward movement (i.e., creaming) of an oil globule, r is the radius of the oil globule, ρ is the density of the corresponding dispersed phase (i.e., the oil globule) and the dispersant (i.e., continuous phase), g is the acceleration due to gravity, and η is the viscosity of the continuous phase. Conversely, when the dispersed phase has a higher density than the continuous phase, a downwards movement is observed for the dispersed particles (i.e., sedimentation). In regards to Stokes' law, it should be noted that it assumes that the separating particles are spherical, which is not always true for oil globules with structured interfacial layers. In addition, the equation does not take into account charge-based interactions between particles (i.e., electroviscous effects) nor the hydrodynamic interactions between the particles and the solvent (McClements, 2015).

Processing- and shelf life-stability are other terms commonly used when discussing emulsion stability; this terminology is especially relevant from an industrial perspective as it represents the stability of a formulation (i.e., mixture of ingredients prepared according to a formula) during different stages of product manufacture and stability of the final product during its intended storage time. Processing stability refers to the ability of emulsions to resist changes incurred in response to adverse processing conditions during a relatively short time (i.e., heat treatment, high shear forces, turbulent flow and short time storage); these generally include changes due to the interactions between the components of the system as detailed in Fig. 1 C. These undesirable changes include flocculation and coalescence of oil globules, aggregation of protein and formation of complexes between different component classes (i.e., bridging flocculation, surfactant/protein/polysaccharide complexes) (Antipova, Semenova, Belyakova,

ACCEPTED MANUSCRIPT

and Il'in, 2001; Drapala, Auty, Mulvihill, and O'Mahony, 2015; Drapala, Auty, Mulvihill, and O`Mahony, 2016a,b; Regost, 2016; Ye and Singh, 2006). On the other hand, shelf life stability represents a holistic view of the stability of the formed emulsion structure and it addresses changes that can take place gradually over a relatively long storage time (i.e., weeks-months); these incorporate both changes in the system based on Stokes' law (i.e., creaming, sedimentation; Fig. 1 B), changes due to undesirable interactions between components (Fig. 1 D) as well as chemical reactions that can take place in an emulsion (e.g., lipid oxidation).

The area of food emulsion science is not new and a number of comprehensive reviews and books on the topic are available (Chung & McClements, 2014; McClements 2007; McClements 2015; Piorkowski & McClements 2014; Wilde, Mackie, Husband, Gunning, & Morris, 2004) and the reader is referred to these publications for broad, fundamental information on emulsion science. The scope of this review is to emphasize the relationship between the structures of emulsion building blocks (i.e., emulsifiers), the structure and role of emulsion interfacial layer and the interactions of emulsion components in these food matrices in the context of developing and stabilising nutritional beverage emulsions. Numerous approaches have been developed and employed to test emulsion stability, studying the prevalence of the undesirable changes during processing (Guzey and McClements, 2006; Liu, Sun, Xue, and Gao, 2016; McCarthy et al., 2012; McSweeney, Healy, and Mulvihill, 2008; Mustapha, Ruttarattanamongkol, and Rizvi, 2012) and during their shelf life (Pan, Tikekar, and Nitin, 2013; Sarkar, Arfsten, Golay, Acquistapace, and Heinrich, 2016; Tcholakova, Denkov, Ivanov, and Campbell, 2006; Xu, Wang, Jiang, Yuan, and Gao, 2012). In line with Stokes' law, many of the analytical approaches have been directed at measuring and controlling fat globule size distribution, viscosity, flow- and phase separation-behaviour, with selected methods being discussed in detail in this review. Likewise, approaches used to study the prevalence of undesirable

ACCEPTED MANUSCRIPT

interactions between components of nutritional beverage emulsions during various manufacturing processes and their shelf life are discussed to provide a detailed overview of the current methodology used in understanding, controlling and predicting stability of nutritional beverages. This review also provides a guide to common challenges experienced with formulation and stabilisation of emulsion-based beverages (in both liquid and powder finished product formats). It also presents and critically assesses the suitability of the principal analytical techniques available for identification of the underlying mechanisms responsible for stability challenges, and in the development of strategies to address these challenges, in nutritional beverage emulsions.

2. Interfacial structures of emulsions

2.1. Formation of emulsion interfaces

Surface active compounds (e.g., emulsifiers) are essential to provide a reasonable stability and homogeneity to a system composed of two (or more) immiscible phases (i.e., oil and water in food emulsions) due to their affinity for both hydrophobic and hydrophilic phases (McClements, 2015). The presence of emulsifiers, their rapid migration to, and adsorption at, the oil/water (O/W) interface reduces the interfacial tension and effectively impedes the drive towards coalescence of oil globules. In addition, a physical barrier is formed by the adsorbed compounds at these interfaces, providing further protection against coalescence. The term surface active ingredient covers a broad range of components, the effectiveness of which for formation and stabilisation of an emulsion system depends on their amphiphilic balance, molecular size and structure. Proteins are common, naturally occurring emulsifiers, widely used in a broad range of food applications; however, other compounds such as lipid-based low molecular weight surfactants (e.g., phospholipids, mono- and di-glycerides, esterified glycerides) are also commonly used (McSweeney, 2008). In general, smaller emulsifiers

ACCEPTED MANUSCRIPT

display higher mobility, compared to larger emulsifiers; smaller emulsifiers tend to dominate the interface in a shorter time. In addition, these small emulsifiers can display higher packing density at the interface (i.e., have a more densely populated interface), compared to large, more structured surfactants (e.g., protein), resulting in lower interfacial tension (Pugnaloni, Ettelaie, and Dickinson, 2005).

To assess the ability of an emulsifier to stabilise an interface, analysis of interfacial tension is typically performed; such analysis provides information on the emulsifier adsorption rates as well as on the extent of reduction in interfacial tension. A comprehensive review of different approaches used to study surface and interfacial tension (γ_S and γ_I, respectively) has been published by Drelich, Fang, and White (2002) and the main principles of these approaches, with some examples of their applications, are summarised briefly in the current review. The most commonly used methods for the analysis of interfacial tension can be divided into 2 categories, based on the measurement principle employed: (1) direct measurement of the repulsive force between two immiscible phases and (2) analysis of the shape of a droplet as affected by interfacial tension. Both of these approaches are based on quantification of the force resisting an increase in the surface area (i.e., surface tension) promoted by forces acting in the opposing direction (e.g., pull, gravitational or centrifugal forces; Fig. 2).

The repulsive forces acting at the interface between two immiscible phases can be quantified using a microbalance or capillary pressure approaches. For the former approach, a probe (typically Wilhelmy plate or Du Noüy ring), connected to the microbalance, is placed directly above the surface/interface and is brought into contact with it. Resultant wetting of the probe by the liquid, due to the capillary forces (Fig. 2 a), causes an increase in the surface area; at the same time the inherent drive of the system to reduce the surface area is acting in the opposite direction. The net effect is that a pull force acts on the probe, which is quantified by the

ACCEPTED MANUSCRIPT

microbalance and used to calculate the surface/interfacial tension. The presence of a surface active compound results in a decrease of that pull force, owing to the reduced surface free energy upon its adsorption at the interface. Using the Du Noüy ring attachment allows only a single point measurement, where the force required to detach the ring from the lamella is related to the γ_I. On the other hand, the Wilhelmy plate attachment facilitates the study of the dynamics of the interface by continuous measurement of γ_I without the detachment step, making it a recommended geometry for measuring surface properties of emulsifiers.

The Wilhelmy plate approach can be used to study the effect of structural modifications of emulsifiers on their interfacial properties (e.g., adsorption and flexibility of the interfacial layer), as documented for heat-denatured β-lactoglobulin (β-lg; Kim, Cornec, & Narsimhan, 2005) or for pH-denatured whey proteins (i.e., β-lg; α-lactalbumin, α-la; bovine serum albumin, BSA; Jara, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2014). In both cases structural changes to the proteins resulted in altered interfacial properties, where more opened denatured structure of individual building blocks, compared to native protein, promoted formation of cohesive and, at the same time flexible interfaces. In a similar way, Turgeon, Gauthier, Molle, and Leonii (1992) studied the modification of interfacial properties of β-lg by enzymatic hydrolysis; the molecular structure of the resultant peptides was assessed using reversed phase-high performance liquid chromatography and analysis of surface hydrophobicity. They concluded that the greatest surface activity for hydrolysed β-lg was observed at low M_w (i.e., ~2000 Da) combined with clustering in distinct polypeptide regions of hydrophilic and hydrophobic residues. Effectively, that work showed that analysis of surface tension, combined with the other methods, allowed a more complete evaluation of hydrolysed whey protein fractions with improved surface activity. Surface tension analysis is often used for studying the compatibility between different surface active components in a system, where it can help with

ACCEPTED MANUSCRIPT

assessing the shelf life stability of emulsions. Cai and Ikeda (2016) studied the competitive displacement of native or conjugated whey protein, by low molecular weight (LMW) surfactant, Tween 20, at an air-water (A/W) surface. Modification of the structure of emulsion interfacial layer by conjugation of proteins with the network-forming polysaccharide, gellan, provided a resistance to protein displacement from the interface by the LMW surfactant (Cai & Ikeda, 2016). Competitive displacement behaviour, and its effect on the shelf life of nutritional beverage emulsions, can also be characterised using surface rheology approaches (Dickinson, 2001); this approach typically uses modified surface tension methods and will be discussed later in this section.

The second category of methods employed to measure surface/interfacial tension is based on the shape of a liquid droplet. In this approach, the interfacial tension is calculated from the droplet dimensions and the volume of the liquid used to form the droplet. The shape of a liquid droplet is resultant from the two opposing forces acting on it; surface tension, which pulls the liquid upwards towards a spherical drop, and the force of gravity, which pulls the liquid downwards causing an elongation of a pendant drop or widening of a sessile drop (Fig. 2 b; Drelich, Fang, and White, 2002). It is worth noting that the main limitation for using pendant and sessile drop methods is their low sensitivity for low surface/interfacial tension systems (Sagis and Scholten, 2014). It is often possible to combine the surface tension measurement, using the pendant drop approach, with surface rheology analysis, whereby the sample dosing system allows deformations to be induced in the interfacial area by fluctuating the volume of the drop at controlled amplitude and frequency – the resultant response to the sinusoidal compression and expansion of the interface is related to the elasticity and flexibility of the interface. The pendant drop method has been used to study the dynamics of surface adsorption for lipid- and protein-based surfactants, and modifications of those (e.g., pre-aggregated β-lg)

ACCEPTED MANUSCRIPT

and the dilatational properties of the interfacial films formed by these surfactants (Dombrowski, Johler, Warncke, & Kulozik, 2016; Kaltsa, Paximada, Mandala, & Scholten, 2014). The information on surface behaviour of emulsifiers generated using the pendant drop approach can give valuable insights into the dynamics of assembly of the interfacial layer, its structural properties (i.e., rigidity, flexibility, interfacial tension) and changes the emulsion interfaces undergo post formation (e.g., due to competitive displacement and changes in the pH, ionic strength and temperature).

Drop detachment is a more traditional variant of the pendant drop method, where the force of gravity is utilised to measure the surface/interfacial tension; in this approach the weight or volume of the liquid required to detach the drop from a needle is measured. This information is then calculated to give surface tension; the force required to detach the droplet is directly proportional to its surface tension (i.e., higher γ_S will require the droplet to be bigger/heavier) (Dunkhin, Kretzschmar, and Miller, 1995). This method was traditionally used to study the surface and interfacial tension before more advanced, camera-based devices became available. The spinning drop method is a more advanced variant of the liquid drop shape analysis, used for a low γ_I range; in this method drop deformations are measured as a result of radial pressure gradients acting on the drop in a rapidly spinning tube (Fig. 2 c). The spinning drop method is used for studying the interfacial tension between two liquids, where the continuous high density phase is placed in the measurement cell (i.e., transparent horizontal tube) and a drop of the low density phase is injected into the tube as it is spinning (Thiessen and Man, 2000). The extent of drop deformation (i.e., elongation) is inversely proportional to the γ_I; the higher the γ_I the more spherical the shape of the spinning drop. The spinning drop method is especially useful for systems with very low γ_I (<0.01 mN m^{-1}), typical for microemulsions and emulsions predominately stabilised by LMW surfactants. An application of the method for screening of

ACCEPTED MANUSCRIPT

co-surfactants (Tween 20, Tween 80, glycerol, sorbitan monooleate, glyceryl monooleate and saponin) with ultra-low γ_I (i.e., as low as 0.0002 mN m^{-1}) for application in microemulsions for oil recovery was reported by Jeirani et al. (2013).

Analysis of interfacial rheology typically involves a modification of one of the approaches discussed above; it allows the generation of dynamic information on the structure of the adsorbed layer, its flexibility and strength (i.e., viscoelastic properties) by measuring the deformation of the interface as a function of force and time (Bos and van Vliet, 2001; Karbaschi et al., 2014). The approaches used to study interfacial rheology are, generally based on oscillating a probe (e.g., Du Noüy ring) placed at the interface (Kim et al., 2005) or the drop volume/shape approaches (Zinoviadou, Scholten, Moschakis, & Biliaderis, 2012); these techniques allow exertion of a deformation force on the interface, while measuring responses of the interface to such a deformation. Studying interfacial rheology can provide valuable information on different surfactants and mixed surfactant systems, especially for applications in processing stability of emulsions (i.e., rigidity of the interfacial layer and stability against coalescence on collisions during liquid pumping and transport). The influence of emulsifiers on the rheological properties of interfaces in food systems have been reviewed by Murray (2002) and Karbaschi et al. (2014). A good understanding of the interactions between emulsifiers adsorbed at the O/W interfacial layer allows a better control of the overall structure of the interfaces of emulsions, which is largely responsible for subsequent stability. Assembly and interactions between emulsifiers are largely dictated by the structure of these building blocks and can be investigated using most of the interfacial rheology measurement approaches; Tamm and Drusch (2017) studied differences in the dilatational elastic moduli of O/W interfaces resultant from structural modification of β-lg by enzymatic hydrolysis (3 and 6%) and by complexing hydrolysed β-lg with pectins at a range of methoxylation (32, 35 and 64%).

ACCEPTED MANUSCRIPT

Hydrolysis of protein diminishes its structure, often negatively impacting the viscoelastic properties of interfaces formed thereof, compared to the native protein. However, as shown by Tamm and Drusch (2017), the viscoelastic properties can be improved by promoting interactions between the emulsifier (i.e., hydrolysed β-lg) and a polysaccharide (i.e., pectin) in order to develop more cohesive interfacial networks. Facilitating strong hydrophobic interactions between the interfacial building blocks (i.e., emulsifiers) is another strategy for increasing the strength of the interfacial layer; Hong and Fischer (2016) showed that stabilisation of canola oil-based O/W emulsions by a mixture of both hydrophilic and hydrophobic colloids (clays) resulted in strong association between the two and, significantly increased interfacial modulus, improving stability to coalescence of the emulsions.

Analysis of the interfacial properties of emulsions can provide valuable information like emulsifier adsorption rates, compatibility between emulsifiers in a multi-emulsifier system or the strength of the interfacial layer, for producing a homogenous and stable emulsion system. However, it must be noted that these analyses have different limitations (e.g., unsuitability for low γ_I values, only model interfaces analysed) and they also measure changes at the interface (e.g., adsorption and displacement) over a relatively short period of time and do not necessarily relate to all changes that can take place in these systems over storage and shelf life. Hence, it is advised that this approach is combined with other analyses (e.g., monitoring of particle size distribution and/or separation rates) that measure changes in the system during processing or storage. These other analytical approaches will be discussed in this review to give a comprehensive overview of the relationship between changes in the structure of the interfacial layer of an emulsion and resultant stability of the system.

ACCEPTED MANUSCRIPT

2.2. Modes of oil globule stabilisation

Emulsifiers adsorbed at emulsion interfaces increase thermodynamic stability of these systems by reducing the surface free energy; the adsorbed layers also provide barrier properties that further stabilise emulsions. These barrier stabilisation mechanisms can be divided into (1) electrostatic (repulsion of oil globules possessing a similar charge) and (2) steric (physical restriction of contact between oil globules by extending into the continuous phase hydrophilic domains of the adsorbed emulsifier) interfacial barriers. Adsorption of solid particles on the interfacial layers can also provide a physical barrier against aggregation of oil globules (Pickering stabilisation; McClements 2015), however these particles are not typically classified as emulsifiers and are not discussed in this review.

Electrostatic repulsion is one of the primary mechanisms of stabilisation of milk protein-based emulsions owing to the charge on the protein adsorbed at the interfaces of oil globules. Such charged interfaces provide repulsion between oil globules and effectively prevent their coalescence and globule-globule interactions post-homogenisation (Damodaran, 2005). For a protein-based emulsifier, the electrostatic charge (zeta potential, ζ) is highest at a pH far from its isoelectric point (pI) and it is also influenced by the type and concentration of salts (i.e., ionic strength of the system); hence, it is important to characterise the potential of an emulsifier to exert an electrostatic barrier under desired environmental conditions. Zeta potential is typically determined by measuring the mobility of particles in an electric field, hence the devices available for ζ analysis normally combine light scattering and electrostatic field approaches. During a ζ measurement, a dilute liquid sample is placed in a sealed cell containing two electrodes, and as current is passed through the sample the movement (i.e., electrophoretic mobility) of particles is measured and expressed as ζ. A strong relationship between ζ of oil globules and system pH in nanoemulsions stabilised by LMW surfactants (lecithin and

ACCEPTED MANUSCRIPT

saponin) has been shown by Ozturk, Argin, Ozilgen, and McClements (2014), where decreasing the pH from 8 to 2 resulted in a reduction in the ζ from -60 to -5 mV and led to a decrease in emulsion stability. Aggregation of oil globules at high salt concentrations (i.e., >100 mM NaCl) in those nanoemulsions, as a result of electrostatic screening, was also reported (Ozturk et al., 2014). The electrostatic screening effect describes accumulation of monovalent ions of an opposite charge at the surface of the oil globules, neutralising the charge on the globules and effectively disabling the electrostatic barrier protecting the emulsion from globule-globule interactions. On the other hand, the presence of divalent ions in an emulsion can facilitate flocculation of oil globules by formation of crosslinks, mediated by such ions (Dickinson, 2001); this can often be a challenge in nutritional beverages fortified with soluble minerals (e.g., calcium fortified milk protein-based beverages). The electrostatic barrier is sensitive to the composition and properties of the emulsion interfacial layer, which is prone to changes during processing (e.g., heat-induced protein deposition) or storage (e.g., surfactant displacement). Modification of the individual emulsifiers or of the overall interfacial layer to control the ζ of oil globules is a commonly-used strategy to improve stability of emulsions against oil globule interactions.

Nowadays, consumers seek new sensory experiences, which often extend beyond the traditional, neutral pH dairy-based beverages; these applications pose challenges for emulsion stability due to low electrostatic repulsion between protein-stabilised oil globules. In an effort to meet that demand, food manufacturers need to look for science-based approaches in order to address the challenges encountered with stability of such products. Similarly, emulsion stability to acidic environment is often needed when using microemulsions as delivery vehicles for sensitive bio-components, where they have to be stable under the acidic environment of the stomach. Neirynck, Van der Meeren, Bayarri Gorbe, Dierckx, and Dewettinck (2004) studied

ACCEPTED MANUSCRIPT

the electrophoretic mobility (i.e., ζ) of conjugated whey protein isolate (WPI) and anionic pectin near the pI of β-lg (pH 5.5) to determine their ability to form and stabilise O/W emulsions under these adverse conditions. Improvement in stability of emulsions formed with the conjugated WPI at the acidic pH, was reported, owing to the greater negative charge of oil globules in that environment compared to emulsions stabilised by WPI alone. Manipulation of the electrostatic charge of oil globules in an emulsion can also be achieved using multilayer deposition of charged species at the emulsion interface, as shown by Zhao, Wei, Wei, Yuan, and Gao (2015), where they stabilised O/W emulsions with the cationic protein, lactoferrin, and formed a secondary interfacial layer by electrostatic adsorption of anionic polysaccharides (from pectin or soybean). The authors reported changes in the ζ of oil globules as a function of lactoferrin and polysaccharide concentrations, which ranged from +40 to -40 mV. A similar approach was investigated by Liu, Wang, Sun, McClements and Gao (2016), where layer-by-layer electrostatic deposition of lactoferrin (cationic) and polyphenolics (anionic) was performed for β-carotene emulsions. A number of variants of the interface with different deposition sequences were tested by the authors and these interfacial configurations were discussed in the context of electrostatic and steric repulsion and resultant stability to heating and storage of the emulsions. Shimoni, Shani Levi, Levi Tal, and Lesmes (2013) showed that increasing the ζ of oil globules by complexation of lactoferrin nanoparticles with carrageenan yielded good stability of those systems to simulated gastric conditions; the study specifically identified the benefits of synergies between electrostatic and steric stabilisation for emulsion stability in an acidic environment. Enzymatic hydrolysis of protein can affect the ζ of oil globules; Adjonu, Doran, Torley, and Agboola (2014) compared ζ of oil globules in emulsions stabilised by intact and hydrolysed (degree of hydrolysis 10-11%) WPI and reported higher extent of electrostatic stabilisation for the native WPI-based emulsions, which gave the emulsions enhanced stability during storage compared to emulsions formed using the whey

ACCEPTED MANUSCRIPT

protein hydrolysate (WPH). Thermal processing of emulsions often presents challenges to emulsion stability due to decreasing pH with increasing temperature, effectively reducing the electrostatic repulsion forces; increasing temperature also increases reaction rates (e.g., protein denaturation and aggregation) and increases mobility of the emulsion components, further promoting their interactions.

Electrostatic stabilisation is sensitive to changes in pH, temperature, and concentration of ions, therefore, approaches aimed at providing steric stabilisation to oil globules have been gaining significant interest in nutritional beverage emulsions. In recent work by Drapala et al. (2016a,b) steric stabilisation of oil globules in model infant formula emulsions, achieved by the incorporation of WPH-maltodextrin conjugates, resulted in significantly improved stability of these emulsions to thermal processing (75-100°C for 15 min) and to storage (4-40°C for 10-14 d), compared to emulsions stabilised using WPI, WPH, or WPH with the addition of LMW surfactants (i.e., lecithin and CITREM). Such improved stability was attributed to the protrusion of the maltodextrin moiety of the emulsifier into the continuous phase of the emulsion, thereby physically restricting contact between oil globules during heating and on storage.

3. Emulsion quality – homogeneity and size distribution

The size of oil globules in an emulsion is a major factor governing the quality of the system and its stability to gravity-induced separation as described by Stokes' law (see Introduction). Emulsion quality and its stability during shelf life are strongly related to its homogeneity, specifically the size and distribution of oil globules in the emulsion system. Thus, formation of an emulsion with sufficiently small oil droplets is a key initial requirement for stability of such systems; typically, emulsions with mean oil globule diameter <1 μm, that display a monomodal distribution, are considered to be stable to phase separation (McClements, 2015). The size of

ACCEPTED MANUSCRIPT

oil globules in food emulsions is also important for their sensory properties; parameters such as mouthfeel, flavour release and colour are related to size distribution of the oil globules in the system (Benjamins, Vingerhoeds, Zoet, de Hoog, and van Aken, 2009; van Aken, 2010).

While it is important to be able to monitor changes that can take place in an emulsion system during processing (e.g., heating, pumping and short-term storage) and subsequent storage, it is also desirable to be able to predict the long-term storage stability of freshly-produced emulsions. The main approach used to do this is to monitor changes in the PSD using static light scattering (i.e., laser diffraction), where particles dispersed in a liquid medium (typically water) scatter laser light at different angles, depending on their size. The presentation of PSD results obtained with laser diffraction instruments typically includes a size distribution profile (Fig. 1 I), values for particle size at the 10, 50 and 90% quantiles of the distribution ($D_{v,0.1}$, $D_{v,0.5}$ and $D_{v,0.9}$, respectively), particle mean diameters (volume-based, $D_{4,3}$ and surface area-based, $D_{3,2}$), specific surface area and distribution span (Span = $D_{v,0.9}$-$D_{v,0.1}$/$D_{v,0.5}$). The use of distribution profiles for presentation of the PSD results is useful for assessing the homogeneity of the system and tracking changes caused by interactions between the components (i.e., monomodal *vs* bi- or multi-modal distribution). As an example, analysis of PSD data from WPH-based O/W emulsions has been used to optimise the conditions for preparation of WPH ingredients with good emulsifying properties (Van der Ven, Gruppen, de Bont, & Voragen, 2001). The analysis of PSD was also used in evaluation of a new processing technology (i.e., inline high-shear rotor-stator mixer) used to formulate nutritional beverage emulsions (O'Sullivan, Drapala, Kelly, & O'Mahony, 2018). It may sometimes be useful to construct a cumulative distribution plot, to show the population size at a given percentile value; such representation of the PSD data allows comparison of the range (i.e., span) of size distribution between samples, which is especially useful in systems displaying a monomodal size

ACCEPTED MANUSCRIPT

distribution. The cumulative distribution profiles can also be used to track changes in the PSD in response to changes in environmental conditions, prolonged storage (Kasran, Cui, & Goff, 2013) or simulated gastric digestion (Shimoni et al., 2013).

The information on the particle size at the extremities of the distribution can be extracted by studying the $D_{v,0.1}$ and $D_{v,0.9}$ parameters; these parameters are important when monitoring changes in sample PSD (e.g., after certain treatments or during shelf life stability testing). Alba, Ritzoulis, Georgiadis, and Kontogiorgos (2013) showed that an increase in $D_{v,0.1}$ for model Okra-based acidic (pH 3) emulsions during storage can be used as an indicator of Ostwald ripening, where a certain population of oil globules grows in size at the expense of other, smaller globules in the system. Conversely, an increase in the $D_{v,0.9}$, with little changes to the other quantile parameters would typically indicate formation of a small number of large particles in the sample. In an emulsion system, this normally indicates flocculation or coalescence of oil globules (Drapala, Auty, Mulvihill, and O'Mahony, 2015; Łuczak and Fryźlewicz-Kozak, 2013; McClements, 2015). Detrimental changes to emulsions, evidenced by an increase in the $D_{v,0.9}$, can be further characterised using a dissociating agent (e.g., sodium dodecyl sulphate; SDS) as the dispersing media during the analysis of PSD (Bazmi, Duquenoy, and Relkin, 2007; Liang et al., 2014). Such an approach allows discrimination between reversible and irreversible interactions between oil globules, such as flocculation and coalescence, respectively. Identifying the type of interactions between oil globules provides information on mechanisms responsible for emulsion destabilisation; examples include increased hydrophobic interactions on denaturation of whey proteins leading to flocculation of oil globules (Drapala et al., 2016a) and competitive displacement arising from incompatibility between emulsifiers leading to coalescence of oil globules in model infant formula emulsions (Drapala et al., 2015).

ACCEPTED MANUSCRIPT

The two different approaches used for calculating the mean particle (i.e., oil globule) diameter are based on the particle volume ($D_{4,3}$, de Brouckere mean diameter) or on the particle surface area ($D_{3,2}$, Sauter mean diameter). The differences between these parameters lay in the method of their calculation, as detailed below:

$$\text{Volume-weighted mean diameter:} \quad D_{4,3} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$$

$$\text{Surface-weighted mean diameter:} \quad D_{3,2} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$$

Where the d_i represents particle diameter in each size class, and the n_i represents the number of particles in each size class per unit volume of emulsion (McClements, 2015). $D_{4,3}$, is the most commonly used parameter when discussing PSD results for polydisperse systems (i.e., majority of food emulsions) as it shows where the mass/volume fraction of the system lies; the $D_{4,3}$ is sensitive to changes at the extremes of the distribution (i.e., especially the large particles with large volume) as well as the span and the homogeneity of the distribution. The $D_{4,3}$ parameter is suitable for screening of emulsifiers for their ability to form small oil globules during homogenisation and to prevent interactions between oil globules on processing and storage; small changes in the size distribution can be easily detected with this volume-based mean parameter (Martinet et al., 2005; Mwangi, Ho, Tey, and Chan, 2016; Ye, Hemar, and Singh, 2004). Often subtle but significant changes (e.g., coalescence) in the size distribution of oil globules are not detected using the $D_{3,2}$ parameter but are detected using the $D_{4,3}$ parameter. Conversely, $D_{3,2}$ is more sensitive to the small particles in the distribution (i.e., those with greatest surface area to volume ratio), it can also be used for studying changes to the thickness and/or structure of the interfaces of oil globules in an emulsion. One example of such use of the $D_{3,2}$ parameter is in work from Van der Meeren, El-Bakry, Neirynck, and Noppe (2005), where the authors measured particle size distribution of soybean oil-based coffee cream emulsions containing protein and lecithin before and after heat treatment; two separate

ACCEPTED MANUSCRIPT

techniques were used by those authors, photon correlation spectroscopy and static laser light diffraction, to obtain harmonic intensity-weighted average hydrodynamic diameter and $D_{3,2}$, respectively. In that work, the authors reported increases in the hydrodynamic diameter and no change in the $D_{3,2}$, from which they concluded that heat treatment (i.e., in-container sterilisation; >12 min at 119°C, 45 min total heating time) resulted in deposition of protein on the surface of emulsion oil globules. Specific surface area, measured by laser diffraction, can provide indirect information on changes in the particle size, i.e., a large surface area would indicate a large number of small particles and the opposite for small surface area. However, measurement of surface area using laser diffraction does not take into account the porosity and topography of the surface and other methods (e.g., Brunauer–Emmett–Teller relationship or mercury porositometry methods used for solid particles) are more appropriate if the surface area is the principal focus (Alghunaim, Kirdponpattara, and Newby, 2016; Arvaniti et al., 2014; Williams, 2007).

A significant volume of information can be generated using the laser diffraction technique for a beverage emulsion system; it is often possible to discriminate between emulsion destabilisation pathways such as coalescence, flocculation and Ostwald ripening, as well as provide information on the performance of protein-based emulsifiers in these systems (e.g., heat-induced aggregation, protein-mediated bridging flocculation and changes in the interfacial protein load). Euston, Finnigan, and Hirst (2001) measured the rate of apparent aggregation of emulsion globules by monitoring the change in the ratio between initial number of emulsion droplets and the number of emulsion droplets after heating. Analysis of PSD allows tracking of the evolution of particle size distribution in emulsions as a function of changes in environmental conditions (e.g., temperature, pH, and ionic strength), therefore simulating the emulsion destabilisation processes relevant to their manufacture, storage and digestion. Liang

ACCEPTED MANUSCRIPT

et al. (2014) reported changes to the casein micelle and oil globule populations in O/W emulsions as a result of a range of heat treatments (0-20 min at 140°C), where a shift from bimodal to multimodal size distribution and a progressive shift of the peaks towards the large particle region of the size distribution profile were observed as the holding time increased. Particle size analysis is one of the most useful tools for assessment of strategies to improve thermal stability of emulsion beverages as exemplified by Chevallier et al. (2016), where the use of whey protein microgel-casein mixtures was successful in forming and stabilising whey protein rich (6%) O/W emulsions. Laser diffraction is often used in combination with other analytical techniques, (e.g., microscopy, protein load analysis or zeta potential analysis) to provide more holistic information about the system and changes that take place within. Some of these other approaches will also be discussed in the following sections of this review.

4. Phase separation and emulsion stability

Emulsions display an inherent tendency to destabilise by phase separation owing to the differences in the density between their main components (oil and water). The rate of separation is influenced by four parameters – density, viscosity, particle size and acceleration due to gravity (Walstra, 1994). Of these four parameters, three are directly related to the emulsion system itself and can be, to some extent, controlled by the formulation and the processes used (phase densities, oil globule size and viscosity of the continuous phase). Since most nutritional beverage emulsions are produced using either innate or added emulsifiers in the formulation and homogenisation (typically), the initial emulsion quality is generally good and it is difficult to determine its creaming rate, and effectively shelf life, under ambient storage conditions. Hence, approaches utilising exaggeration of external conditions (i.e., centrifugation, increased temperature) are used to facilitate generation of information on emulsion stability under accelerated conditions.

ACCEPTED MANUSCRIPT

In this review, approaches for measuring emulsion stability to creaming under accelerated conditions will be discussed. A range of commercially available analytical centrifuges allow for acceleration of the phase separation process by controlling the centrifugal speed and the temperature while monitoring movement of the particles (e.g., oil globules, protein particles and other colloids) through the measurement cell in real-time. The principle of this method and calculations used are comprehensively detailed by Lerche and Sobisch (2007; 2011). Briefly, a liquid sample is introduced to a transparent measurement cell and placed in the centrifuge, while transmission of light through the cell is measured as a function of time and movement of the particles towards the top (creaming) or bottom (sedimentation) of the cell can be observed. Collected data represents the changes in the light transmission through the cell during analysis, integral transmission (cumulative changes in the transmission through the cell) and front tracking (movement of the phase boundary) profiles, as shown in Fig. 1 III. Creaming velocity of the sample can be calculated from the front tracking profile and recalculated to a corresponding creaming velocity under ambient conditions (Drapala et al., 2016b); similarly the evolution of the thickness of the cream layer can be calculated from the front tracking profiles. It should be noted that the analytical centrifugation approach speeds up the separation of components in the system due only to size of particles in the dispersed phase, viscosity of the continuous phase and the difference in density between the dispersed and continuous phases. In addition, progressive creaming and sedimentation can result in changes in viscosity during the course of the analysis. Velocity of movement of particles in the sample is also influenced by the centrifugal force, as is their movement regime (i.e., particles moving directly upward or downward and not being influenced by Brownian motion). Other analytical approaches based on light scattering, where the emulsion separation rate is accelerated solely by increased temperature of the system, can also be employed to assess emulsion stability (Degrand, Michon, and Bosc, 2016; Lesaint, Glomm, Lundgaard, and Sjöblom, 2009).

ACCEPTED MANUSCRIPT

Application of the analytical centrifuge approach for emulsion systems is useful when screening of emulsifiers or when reformulating existing products, where it can provide information on the effects of the new ingredients on the creaming rates of the system, compared to the original formulation. Liu et al. (2016) studied the stability of walnut oil-based emulsions to various unit operations, changes in composition and storage conditions such as freeze-thaw cycles and pH fluctuation; these authors used the integral transmission data to investigate the effects these conditions had on the creaming behaviour of emulsions as a function of the emulsifier used. Shimoni et al. (2013) used the space- and time-resolved extinction profiles (STEP) and calculated creaming velocities to describe differences in the stability of coarse ($D_{4,3}$ ~ 65-85 μm) and fine ($D_{4,3}$ ~ 4-6 μm) olive oil-based emulsions stabilised by lactoferrin nanoparticles (i.e., Pickering emulsions) after homogenisation and after in vitro digestion. The authors used the separation velocity data to calculate the harmonic mean droplet size, as detailed previously by Detloff, Sobisch, and Lerche, (2006) and Lerche and Sobisch (2011), and correlated the size of oil globules with the creaming rates and zeta potential (ζ). Shimoni et al. (2013) reported that the nanoparticles were more successful in stabilising coarse compared to fine emulsions; it was also reported that addition of carrageenan significantly increased the ζ of oil globules and reduced the creaming rates in fine emulsions stabilised by the nanoparticles; however, no viscosity data was provided for these systems. To be able accurately compare changes in creaming rates for reformulated emulsions using the accelerated separation approach, it is essential to measure and account for changes in viscosity arising from reformulation. An example of this approach being adopted in the stability analysis of nutritional beverage emulsions is the work of Lei, Liu, Yuan, and Gao, (2014) who used the analytical centrifuge approach to study the effect of emulsifier type on the physicochemical properties of β-carotene emulsions; the authors correlated changes in the system viscosity and oil globule size with the differences in measured creaming rates. In another study, Meshulam, Slavuter,

ACCEPTED MANUSCRIPT

and Lesmes (2014) investigated destabilisation of inulin-stabilised emulsions by saliva addition and the authors showed that the creaming velocity of emulsions were not affected by differences in the ζ of oil globules achieved by changing the system pH (i.e., pH 2-10). This finding is not surprising as accelerated conditions used in the analytical centrifuge approach cause movement of the system components in accordance with Stokes' law. Hence, the changes typically observed under quiescent conditions, such as repulsive interactions of oil globules due to low contribution of electrostatic stabilisation, are excluded from the analysis due to its time-scale being too short for their role to be important in separation. On the other hand, modification of the steric stabilisation system for oil globules can potentially yield different stability to separation under accelerated conditions owing to increased thickness of the interfacial layer (i.e., increased surface load of high density components), effectively slowing down the separation due to lowered density difference between the dispersed and continuous phases (van Lent, Le, Vanlerberghe, and Van der Meeren, 2008; Klein et al., 2010). Extensive protrusion of the steric layer on the surface of oil globules into the aqueous phase can potentially affect the separation rate by physically restricting the upward motion of oil globules in the emulsion. Stability of an emulsion to creaming can be modified by controlling the density of its constituent oil droplets, which can be achieved by selection and/or modification of the oil component to allow effective interactions of its carbonyl groups with polar functional groups of emulsifiers (e.g., amine, amide, carboxyl and hydroxyl groups) (Klein, Aserin, Svitov, and Garti, 2010). Another strategy for controlling the oil globule density was shown by Ruiz-Rodriguez, Meshulam, and Lesmes (2014), who studied the effect of incorporation of silica nanoparticles into oil globules on emulsion creaming rates. These authors showed that by increasing the density of the dispersed phase they were able to decelerate creaming and even achieve sedimentation of oil globules in an aqueous phase at high levels of inclusion (≥ 1%, w/w) of silica nanoparticles. In an analogous manner, an increase in the protein load at the

ACCEPTED MANUSCRIPT

emulsion interface will give rise to increased density of emulsified oil globules and, effectively lower difference in the densities between the two phases, resulting in slower creaming rates (van Lent et al., 2008).

Based on the same principle, analytical centrifugation can provide information on the rate and extent of sedimentation in an emulsion system, where deposition of material at the bottom of the product container is, generally, undesirable. This can often be the case in nutritional beverage formulations (e.g., infant, sports nutrition or elderly nutritional products), often produced from dried protein base ingredients, where the protein ingredient can display poor solubility and undergo changes in hydration during beverage storage (Crowley et al., 2015; De Wit, 1990; Pelegrine and Gaspareto, 2005). Crowley, Kelly, and O'Mahony (2014) discussed sedimentation rates for casein micelles in reconstituted skim milk powder, which were found to increase with calcium fortification, as measured using an analytical centrifugation approach. Sedimentation is often a considerable challenge for plant protein-based beverages, and since such products have been gaining significant commercial interest, it is of relevance to measure and predict both creaming and sedimentation behaviour in such emulsion-based products. In a study by Makinen, Uniacke-Lowe, O'Mahony, and Arendt, (2015) separation rates for bovine milk and plant-based milk substitutes were compared using an analytical centrifugation approach. The authors reported that creaming was the main mechanism of separation for the bovine milk, while both creaming and sedimentation were observed for the plant-based systems. The analytical centrifugation approach has also been used to study the reconstitution properties of model infant formula powders (Murphy et al., 2015) and physical stability of milk protein-carbohydrate nutritional beverages (Chen and O'Mahony, 2016).

It should be pointed out that, despite the fact that the analytical centrifugation approach is often used to investigate shelf life stability of emulsions, it can only reflect separation based on the

ACCEPTED MANUSCRIPT

current-state properties of the system, without considering changes that take place in complex multicomponent systems (e.g., coalescence, flocculation, aggregation, competitive displacement and Maillard reaction) over time and which are influenced by storage conditions (e.g., temperature and time). Thus, when the creaming behaviour of an emulsion-based beverage product over its shelf life is the focus, single point testing is not enough to provide the necessary information and sample incubation needs to be incorporated into the experimental design. Storage of emulsion samples at one or more elevated temperatures (compared to normal storage conditions for the product) should be coupled with testing of samples at different storage times for creaming velocity (ideally combined with measurement of PSD and viscosity) in order to construct a stability map as a function of time (and storage temperature) (Lerche and Sobisch, 2011). This approach allows data collection for the evolution of creaming rates in the product stored and tested under accelerated conditions, to give a predictive shelf life stability model for a given system. Effectively, a correlation can be developed to estimate product shelf life under its typical storage conditions (Lerche and Sobisch, 2011).

5. Interactions between emulsion components – rheological characterisation

Rheological properties strongly influence the processing behaviour and shelf life stability of liquid foods, as well as their appearance, texture, mouthfeel and flavour release (Fischer and Windhab, 2011). Studying the rheological properties of an emulsion-based beverage can provide indirect information on the size and shape of its components as well as on the interactions between these components (e.g., oil globules, protein aggregates, hydrocolloids). A typical approach for measuring the rheological properties of a liquid is to measure the resistance of the system to applied stress (e.g., rotational or oscillatory stress), where the stress causes deformation of the system; extensive literature is available on the fundamental

ACCEPTED MANUSCRIPT

principles of the approach (Chung and McClements, 2014; Erni, Fischer, and Windhab, 2007; McClements, Monahan, and Kinsella, 1993; Norton, Spyropoulos, and Cox, 2010) and only selected concepts, of relevance to emulsion structure, will be considered in this review. In brief, the greater the force resisting deformation, the higher the viscosity of the system and the linearity of the system deformation depends on the interactions of its components. Lack of such interactions results in a linear response (i.e., typical for water; Newtonian flow, where the relationship between the apparent viscosity and shear rate is linear), while a nonlinear response indicates disruption (shear thinning) or formation (shear thickening) of inter-component interactions on application of the stress. Shear thinning behaviour is typical for food protein systems, owing to associative interactions between proteins, forming weak structures that are interrupted/broken when the sample is sheared (Williams, 2007). In general, the magnitude of shear thinning behaviour increases with increasing protein concentration. While dilute emulsions, typically display near-Newtonian flow behaviour (Sünder, Scherze, and Muschiolik, 2001), a deviation from Newtonian behaviour to shear-thinning is usual for concentrated emulsions (Liang, Patel, Matia-Merino, Ye, and Golding, 2013). Effectively, increasing the concentration of dispersed phase particles (e.g., oil globules, protein and other constituents), changes the rheological properties of the system due to increasing potential for interactions between the dispersed particles (Fischer, Pollard, Erni, Marti, and Padar, 2009). The viscosity of emulsions increases with increasing concentration of the dispersed phase (i.e., volume fraction, φ), where increasing φ can result in a change in the flow regime from turbulent to laminar flow (Tadros, 2013), until a critical φ is reached (~0.63). At φ greater than the critical φ the emulsion cannot flow easily due to the high packing density of globules (Piorkowski and McClements, 2014). The rotational rheological approach is generally used for studying the viscosity and flow behaviour, while the oscillatory approach can be used to provide information

ACCEPTED MANUSCRIPT

on the viscoelastic properties of the system without applying rotational shear; the latter is often used for concentrated, high volume fraction emulsion systems (Tadros, 2013).

The rheological properties of emulsions are dictated by the concentration, shape, and size of constituents of the dispersed phase as well as their ability to participate in various interactions (e.g., hydrophobic interactions, covalent bonds, electrostatic and steric interactions) with other components. Such interactions are inherent to food-based emulsions in liquid format, which are generally comprised of a heterogeneous mixture of biopolymers. In particular, nutritional beverage emulsions contain a variety of particles with a broad range of sizes and structures; oil globules, proteins (e.g., casein micelles, globular protein, denatured and aggregated protein), carbohydrates, LMW emulsifiers and other smaller components (ions, vitamins) that can influence the flow behaviour and viscosity of emulsions. In O/W emulsions, oil globules are, typically, the largest physical components; theoretically, a spherical shaped particle has minimal effect on flow behaviour as it does not cause high disturbance in flow, compared to irregularly shaped particles that tend to rotate before assuming an optimal orientation in regards to the direction of flow (Fig. 3; Mueller, Llewellin, and Mader, 2010; Sandler and Wilson, 2010). In addition, the spherical conformation would, in theory, indicate a minimal extent of steady state interactions, as opposed to components with a linear or branched conformation (i.e., polypeptide chain of protein or polysaccharide chain of carbohydrate) (Neelima, Sharma, Rajput, and Mann, 2013; Nobel, Weidendorfer, and Hinrichs, 2012). However, the interfaces of oil globules are seldom smooth and inert, since they are populated by surface-active species (e.g., proteins, peptides, LMW surfactants), often making their contribution to the rheological properties of the system significant. Oil globules can often participate in interactions with other globules (e.g., flocculation, aggregation, complexation) or with components of the continuous phase (e.g., aggregation, segregative separation and complexation), or a combination of both

ACCEPTED MANUSCRIPT

(Chevallier et al., 2016; Drapala et al., 2016a, 2016b; Liang et al., 2013). These interactions are particularly important during unit operations applied in the preparation of emulsion-containing nutritional beverage products (e.g., heating, pumping, short-time storage and spray drying). Thermal processing typical for these systems (thermisation, pasteurisation, UHT) causes an increase in protein-mediated interactions due to protein denaturation and aggregation (Chevallier et al., 2016; Liang et al., 2013); the increase in the temperature also provides higher mobility of solutes in the liquid, thus accelerating the rate of physicochemical changes in the system (e.g., surfactant exchange, interactions and structural rearrangements at the surface) (Ryu and Free, 2003). In recent work, Buggy, McManus, Brodkorb, McCarthy and Fenelon (2017) studied physical stability of model infant formula emulsions enriched with α-la by measuring viscosity of emulsions as affected by the process configuration, where heat treatment (65°C × 30 s) was carried out either before or after homogenisation (2-stage, 21 MPa total pressure). Significantly higher viscosity of emulsions heated post-homogenisation, compared to pre-homogenisation, was associated with the presence of significantly larger aggregates of protein and oil globules in the former system (as measured by electrophoresis and laser diffraction, respectively). Shear-thinning behaviour of dilute emulsions has been associated with disruption of weak interactions between the components of the continuous and dispersed phases in an emulsion system (Ruiz-Rodriguez et al., 2014); it can be used to discriminate between reversible and irreversible (i.e., flocculation *vs* coalescence) associations between oil globules in an emulsion (Dickinson, 2001; McClements, 2007). The use of rheological measurements is a powerful approach for evaluation of strategies aimed at limiting interactions between emulsion components on heating; such an approach was reported by Liang et al. (2013), who studied the effect of pre-aggregation of whey proteins on the heat stability of milk protein concentrate- (MPC) stabilised emulsions. Using steady-state flow measurements (shear rate ramp from 0.001-500 s^{-1}), the authors reported lower apparent

ACCEPTED MANUSCRIPT

viscosity in emulsions stabilised by pre-aggregated protein, compared to emulsions stabilised by unheated protein. The authors were also able to identify reversible changes in these emulsions on heating, as exhibited by a shear thinning behaviour, which they associated with disruption of heat-induced oil globule clusters on shearing. In another study, Liang et al. (2014) compared the effects of addition of different sugars (glucose, maltose, trehalose and sucrose) on the heat stability of MPC-stabilised emulsions. A range of rheological measurements, including shear rate ramping, were used by these authors to study the steady-state flow properties, the effect of viscosity ratio between the dispersed and continuous phases on droplet break-up in emulsions and the forces of attractive between the system components for unheated and heated samples. Integration of these rheological approaches with particle size distribution and microstructural analyses allowed the authors to identify aggregation of casein micelles, oil globules and a combination of both as factors affecting stability of these emulsions. Similarly, Lei et al. (2014) used the viscoelastic response of β-carotene-containing O/W emulsions stabilised by chitosan, physical complexes of chitosan and epigallocatechin-3-gallate (EGCG) and chitosan-EGCG conjugates to predict the extent of flocculation of oil globules in these systems. These authors were able to correlate the rheological data (i.e., shear and yield stress, consistency index and flow behaviour index) with the differences observed in stability of emulsions to creaming, reporting slower emulsion separation rates for systems stabilised by the chitosan-EGCG complexes, which displayed non-Newtonian flow behaviour and had the highest viscosity. A novel approach for studying rheological properties of emulsions was reported by Degrand et al. (2016), where multi-speckle diffusing-wave spectroscopy (MS-DWS) was used to characterise evolution of stability in rapeseed oil-based O/W emulsions stabilised by Tween 20 or whey protein concentrate (WPC). The authors used the elasticity index for describing the rheological behaviour of emulsions and extracting information about the local organisation of oil globules in the emulsion. Effectively, the MS-DWS approach

ACCEPTED MANUSCRIPT

allowed successful monitoring of flocculation of oil globules (bridging flocculation or depletion-flocculation) and rearrangements in the protein network.

6. Emulsion microstructure

Visualisation of microstructure of emulsions provides valuable information on the structural assembly of components of the system and allows a better understanding of the nature of changes taking place in the system. Several microscopic approaches, based on different technologies, are available (e.g., optical microscopy, OM; confocal laser scanning microscopy, CLSM; confocal raman microscopy, CRM; scanning and transmission electron microscopy, SEM and TEM, respectively; or atomic force microscopy, AFM) and choosing the right technique for a given sample depends on the physical format of the sample, the information and the desired level of detail required. CLSM is one of the most insightful microscopic techniques used for studying structure of food emulsion systems; it allows cross-sectional localisation of individually labelled components (e.g., protein, lipid) of a sample. In addition microscopic techniques such as OM, CRM, SEM, TEM and AFM, can also provide valuable information about emulsions, their morphology (classes, shapes and sizes of particles present), localisation of individual components (e.g., clustering of minerals) or overall 3-dimensional structure of oil globules in the emulsion. Therefore, a brief discussion of these techniques, in the context of the most recent developments relating to nutritional beverage emulsion applications, will be provided in this section.

6.1. Confocal laser scanning microscopy

The first publications using CLSM for studying a food system were focused on O/W emulsions, where the displacement of sodium caseinate by monoacylglycerols from an O/W interface was the focus (Heertje, Nedelof, Kendrickx, and Lucassen-Reynders, 1990; Heertje, Van Aalst, Blonk, Nederlof, and Lucassesn-Reynders, 1996). Several components of a sample can be

ACCEPTED MANUSCRIPT

visualised simultaneously, provided the emission wavelengths do not overlap. Comprehensive information on the principles of CLSM analysis as well as important considerations for its use in various food matrices are available elsewhere (Auty, Morris, and Groves, 2013; Auty, Twomey, Guinee, and Mulvihill, 2001; Everett and Auty, 2008). Labelling the protein and lipid components of an emulsion can provide important information on the microstructure of emulsion-based systems and more elaborate approaches can be used to label other components, such as polar lipids (Fig. 4) and glycoproteins (Bourlieu et al., 2015; Lopez, Madec, and Jimenez-Flores, 2010; Lopez and Ménard, 2011) or a specific protein class (Li, Auty, O'Mahony, Kelly and Brodkorb, 2016; Sørensen et al., 2007). Visualisation of the locations of components within the system matrix can aid understanding of the structure of native food matrices (e.g., human or bovine milk fat globule membranes) to allow effective engineering of food systems to replicate naturally-occurring structures. Lopez, Cauty, and Guyomarc'h (2015) used CLSM to investigate differences in the microstructure of oil globules in native human and bovine milks and in commercial infant formula (IF) products. They identified that, apart from differences in size of oil globules, the differences between human/bovine and infant formula milks were due to differences in the composition and structural organisation of the emulsion interfacial layers. Localisation of a specific component in a food matrix can also be helpful to study the pathways for rearrangement of the system during its processing.

CLSM is often used to study changes taking place in food systems as influenced by processing conditions, storage time (i.e., shelf life stability), or conditions during ingestion and digestion; application of CLSM can significantly aid interpretation of data obtained using other analytical techniques. Processing conditions can affect the structure and properties of a food system, for example, powder-based nutritional beverage emulsion-containing systems undergo several processing steps (e.g., homogenisation, heat treatments, concentration and spray drying) before

ACCEPTED MANUSCRIPT

they are packaged as a final product. The effect of homogenisation on the composition and organisation of the O/W interfacial layer in bovine milk was reported by Lopez et al. (2015); selective staining of polar lipids and protein allowed to observe a homogenisation-driven shift from polar lipid- to protein-dominant emulsion interfaces. Differences in the thickness of interfacial layers in emulsions stabilised by β-casein and WPI were reported by Li et al. (2016); with the aid of image analysis of CLSM micrographs, the authors observed thicker interfaces for β-casein-stabilised emulsions compared to those stabilised with whey protein. Sørensen et al. (2007) used CLSM to study surface behaviour and interfacial barrier properties in casein- and whey protein-based emulsions, made using different homogenisation conditions. The authors reported that modification of homogenisation temperature (50 and 72 °C) and pressure (5.0, 15.0 and 22.5 MPa) resulted in different interfacial composition of oil globules in the fish oil-enriched milk emulsions, as evidenced by regions of co-localisation of lipid and protein (casein or lactoferrin) and protein aggregation; importantly, differences in the structural assembly of the interfaces in emulsions observed by the authors were linked with different oxidative stability of these systems during storage.

The use of CLSM for studying changes in emulsion microstructure under simulated digestion conditions is very effective in helping in the design of value-added emulsion-based products containing sensitive nutritional components (e.g., long chain fatty acids, vitamins), where the conditions responsible for the release of the encapsulated component are of interest. The changes in microstructure of emulsions during *in vitro* digestion of infant formula were studied by Bourlieu et al. (2015) with the aid of CLSM, where fluorescent labelling of protein, neutral and polar lipids facilitated the monitoring of changes in the system during simulated digestion. Reorganisation of the system components and aggregation of protein and oil globules in model infant formula emulsions during their digestion, as demonstrated by microscopy, were used to

ACCEPTED MANUSCRIPT

study the kinetics of digestion of these systems as affected by their processing (i.e., homogenisation and pasteurisation conditions). Displacement of emulsifiers from the interface and changes in the microstructure of the system during processing, and under model digestion conditions, were also reported by Yang et al. (2013). Qiu, Zhao, Decker and McClements (2015) presented CLSM micrographs of fish oil-based O/W emulsions stabilised with different proteins at different stages of *in vitro* digestion (i.e., before digestion, in mouth, in stomach and in the intestine). Visualisation of disruption of the emulsions at these 4 stages of digestion allowed the authors to determine the role of interfacial composition on emulsion stability and fat release during digestion. In recent work, Gallier et al. (2015) used CLSM and TEM techniques to evaluate the interfacial composition of oil globules in a concept infant formula product and compared it with those of traditional infant formulae and human milk. The concept infant formula emulsion was designed to mimic the polar lipid-dominant interfacial layer known to exist in human milk; using these microscopic techniques provided guidance for the microstructural design of interfaces of oil globules in the concept product. Microstructural approaches can be powerful in engineering food structures for specific biological functions through the control of digestive and metabolic properties of nutritional beverage emulsions. Understanding the relationship between food structure and sensory properties can be aided by the application of CLSM approaches, which can effectively enable design of new formulation/structure strategies for reducing the fat content of products. Abhyankar, Mulvihill and Auty (2014) used this combined analytical approach to study the deformation and release of oil globules in emulsion-filled gels.

Microstructural analysis of emulsions using CLSM is a powerful approach for identification of the mechanisms responsible for their destabilisation such as coalescence, flocculation or protein aggregation (Fig. 1 II). The cross-sectional analysis of oil globules provides valuable

ACCEPTED MANUSCRIPT

information on the arrangement and properties of the emulsion interfacial layer, its thickness, density and continuity. Progression of thermally-induced changes to the structure of model infant formula emulsions was studied with the aid of CLSM by Drapala et al. (2016a), whereby the microscopic technique allowed visualising of heat-induced protein deposition on the oil globule surfaces and protein-mediated bridging flocculation of oil globules during heating. Formation of buoyant white flecks in whey protein-based infant formula was reported by Drapala et al. (2016a); the flecks were shown to consist of globules entrapped within a protein network using CLSM. Similarly, CLSM was used to visualise and study associative interactions between protein and oil globules in infant formula products induced by processing steps in their manufacture (i.e., heat treatments performed for the microbial safety of these products) (Lopez et al. 2015), where the mechanism responsible was shown to be aggregation of protein and interactions between protein and oil globules in the formation of lipoprotein complexes (i.e., white flecks).

The use of CLSM to investigate the distribution of protein and oil in emulsion-based powders can provide important information on heat-induced changes (e.g., aggregation and coalescence of protein molecules and oil globules, respectively) in formulation constituents during unit operations (e.g., heating) and storage of the resultant emulsion-containing products. In a recent study, Drapala, Auty, Mulvihill, and O'Mahony (2017) used CLSM to determine differences in the physical stability to spray drying of model infant formula emulsions stabilised by different emulsifier systems. The authors fluorescently-labelled the protein and neutral lipids in the dried powder particles to visualise the distribution of oil globules within the powder matrices; inhomogeneous and irregular distribution of oil in the powders was linked to poor thermal stability of emulsions and to competitive displacement between surfactants used in the formulations. The presence of white flecks (i.e., white buoyant particles, visible to the naked

ACCEPTED MANUSCRIPT

eye) is a common defect in reconstituted dairy-based emulsion powders and the underlying reasons for their formation are not yet clearly understood. Most compelling theories point towards protein-mediated association of oil globules induced during further processing (e.g., heat treatment, evaporation and spray drying) of the emulsions post homogenisation. Preliminary results from our research group confirmed heat-induced flocculation of oil globules as a factor responsible for formation of white flecks (Schmidmeier, O'Gorman, Drapala, & O'Mahony, 2017), where CLSM analysis of the white flecks showed them to have a porous protein network, densely packed with individual oil globules (Fig. 5). Using CLSM to visualise the structural assembly of such product defect materials provides important information in reverse-engineering of nutritional beverage formulations for the development of strategies aimed at elimination of such defects.

Time-related changes in an emulsion system govern its shelf life stability; generally, the displacement of emulsifiers at the interfaces of oil globules can lead to coalescence and, effectively, phase separation. Displacement of whey proteins from interfaces of infant formula emulsions by LMW surfactants (i.e., lecithin) has been observed with the aid of CLSM by Drapala et al. (2015). Changes in the continuity of the protein layer at the emulsion interface were observed during storage (14 d at 4°C) and the incompatibility of the emulsifiers in the system resulted in poor stability, as evidenced by coalescence of oil globules (Drapala et al., 2015). The shelf life of food emulsions is often increased by removal of water using spray drying, effectively immobilising the oil globules and other components of the continuous phase; stability of such 'immobilised' emulsions to storage can be assessed with the aid of microscopic techniques (Lim, Burdikova, Sheehan, and Roos, 2016; Lim, Griffin, and Roos, 2014; Lim and Roos, 2016). McCarthy et al. (2013) used CLSM to study changes in oil

ACCEPTED MANUSCRIPT

distribution in infant formula powders during storage, where changes in the size of oil globules and the amount of surface free oil were attributed to lactose crystallisation in the powders.

6.2. Optical microscopy

Despite CLSM being the most commonly utilised microscopic technique for studying the structure of emulsions, other microscopic techniques are also useful in studying these complex, multi-phase systems. One of the most commonly available and, yet the most underutilised technique is optical microscopy; OM generally requires very little sample preparation and basic familiarity with its principles are sufficient for performing analysis. Optical microscopes are suitable for observing the overall morphology of beverage emulsions (i.e., size of oil globules and protein aggregates; Lu, Xiao, & Huang, 2017), can provide information on the types of interactions between oil globules (i.e., coalescence *vs* flocculation; Goibier, Lecomte, Leal-Calderon, & Faure, 2017) and identify reasons for their destabilisation (e.g., presence on undissolved lactose crystals causing rupture of oil globules). Incorporation of OM into routine analyses of nutritional beverage emulsion formulations is recommended in commercial research and development departments.

6.3. Electron microscopy

Electron microscopy (EM) techniques are often used to study the microstructure of emulsions and a detailed review of their principles and applications in emulsion systems is available in Klang, Matsko, Valenta, and Hofer (2012); a brief discussion of these techniques will be provided in this review, including some recent examples of their application in emulsion systems. The main benefits of EM approaches are their ability to provide powerful magnifications (500,000-5,000,000× for SEM and TEM, respectively), thereby delivering highly detailed information on the structure of a surface (i.e., topography; SEM) or of the distribution of components within the interior of a sample, based on differences in electron

ACCEPTED MANUSCRIPT

density between the constituents components (TEM). On the other hand, challenges associated with the use of EM for studying emulsions are linked with the need to immobilise the sample, which can often result in changes to the colloidal system; rapid freezing of the sample is a common approach for SEM visualisation of emulsion systems (i.e., cryo-SEM). In emulsions, SEM can be used to visualise the topography of the interfacial layer of oil globules, which can be very useful when engineering these interfaces for improved emulsion stability. Lu et al. (2017) used cryo-SEM to visualise Pickering particles (i.e., milled starch granules) at the interfaces of oil globules in emulsions; they observed the presence of irregularly shaped starch nanoparticles (~100 nm) anchored onto these interfaces. In a similar way, Xiao, Lu, and Huang (2017) visualised, using SEM, the interfacial structure of kafirin- (i.e., plant protein emulsifier) stabilised water-in-oil-in-water emulsions (W/O/W) in an effort to relate interfacial structure with stability of the emulsion systems under simulated gastric conditions.

A different approach focused on structural characterisation of interfacial layers in O/W emulsions, using TEM, was taken by Xie et al. (2016), where differences in the structural density of the interface and the core of the oil globules was visualised. The authors used TEM to obtain information about interactions between two emulsifiers (i.e., lecithin and oleic acid) in the system at different ratios; irregularities in the interfacial layer were associated with the ratio-dependent incompatibilities between the two emulsifiers. EM (both SEM and TEM) can provide very detailed information about the internal and interfacial structures of milk fat globules in human milk, as shown recently by Gallier, Acton, Garg and Singh (2017). The authors visualised distinct interfacial regions dominated either by ordered lipid domains (i.e., sphingomyelin and cholesterol) or by glycoprotein and large cytoplasmic regions containing numerous vesicles and cytoplasmic material within the globule. Naturally-excreted milk fat globules (human and animal) are considered excellent vesicles for transport and delivery of

ACCEPTED MANUSCRIPT

nutrients (Singh & Gallier, 2017); expanding the knowledge base on their structures brings emulsion scientists closer to successfully engineering similar vesicles.

6.4. Confocal Raman microscopy

CRM is a very powerful microscopic technique as it allows visualisation of a broad range of components localised within the structure of the sample; the combination of microscopy and spectroscopy allows a signal spectrum to be obtained which is then transformed into detailed information about the chemical composition of the given region of the sample (Cailletaud et al., 2017). In recent work Munoz-Ibanez et al. (2016) used CRM to localise maltodextrin, acacia gum and sunflower oil in spray dried emulsion powder particles; the microstructural results provided important information on the assembly of the multicomponent structure during the short time needed for formation of these particles (i.e., during atomisation and spray drying). CRM was used by Goibier et al. (2017) to track changes taking place in an emulsion system (i.e., crystallisation of the surfactants at the emulsion interface). In their approach, specific spectral bands corresponding to soybean oil, monoglycerides and water were selected to visualise their location within an O/W emulsion; the presence of thick monoglyceride-rich interfaces was associated with crystallisation of the surfactant and improving stability of the emulsion to coalescence. CRM can also be used for quantifying the concentration of a specific component, as reported by Wan Mohamad, Buckow, Augustin, and McNaughton (2017), where the concentration of β-carotene in O/W emulsions was determined using this microscopic technique. CRM is a promising technique for detailed qualitative and quantitative study of the structure of multicomponent emulsion systems; this approach does not require elaborate sample preparation and the use of expensive sample dyes is not needed. On the other hand, the main limitations of CRM are associated with the heterogeneity of an emulsion system, often resulting in overlapping component spectra, and the need to immobilise the

ACCEPTED MANUSCRIPT

sample matrix prior to the analysis to ensure accurate localisation of components within the sample.

6.5. Atomic force microscopy

When it comes to the visualisation of emulsion interfaces, AFM can provide detailed information about the topography and structure of these interfacial layers. AFM has been used to study the interactions between protein- and lipid-based emulsifiers, where competitive interactions are common and these can lead to poor emulsion stability (Berton-Carabin, Genot, Gaillard, Guibert, & Ropers, 2013; Morris & Gunning, 2008; Yao et al., 2018). Interactions between gum Arabic and Tween 80 at model O/W emulsion interfaces were observed using AFM by Yao et al. (2018), where, depending on the inclusion ratios of these emulsifiers, differences in the structure of the resultant interfaces (i.e., smoothness/roughness) and characteristics of the network formed at these interfaces were observed. Similarly, Berton-Carabin et al. (2013) reported on differences in the roughness and homogeneity of interfacial layers in O/W emulsions stabilised by β-lg, β-casein and combinations of each of these proteins with the lipid-based LMW surfactant dilauroyl phosphatidylcholine (DLPC). The authors reported that the presence of DLPC resulted in significant changes to the structure of the interfacial layer, compared to the protein-only interfaces, with higher heterogeneity, and decreased continuity of the interfacial layer observed for the former. The use of AFM to visualise separate protein and LMW surfactant domains at emulsion interfaces has been well documented by Morris and Gunning (2008), who showed the competitive dispalcement of protein (β-lg) by LMW surfactant (Tween 20) from O/W interfaces. The authors used AFM to study the adsorption of LMW surfactant in gaps within the adsorbed protein network, subsequent growth of the surfactant-dominant areas and compression of the interconnected protein network up to the point of forcing the protein out to the continuous phase of the

ACCEPTED MANUSCRIPT

emulsion, leaving the interface dominated by the LMW surfactant. Therefore, the use of AFM is of interest in the study of mixed emulsifier (i.e., protein and LMW) stabilised emulsions which are prone to coalescence, where this analytical tool can provide some insights into the optimum emulsifier ratios and other strategies to help in limiting such detrimental changes.

7. Other considerations

A number of other analytical techniques may also be applied when studying nutritional beverage emulsion systems; these are aimed at assessing the performance of emulsifiers and the physicochemical properties and stability of the emulsions. These techniques can include, but are not restricted to, measurement of surface hydrophobicity and reactive groups of an emulsifier, measurement of the interfacial protein load and composition, heat coagulation time of an emulsion or the oxidative stability of an emulsion.

Measurement and modification of the hydrophilic-hydrophobic balance of an emulsifier can provide control over its surface activity and influence its emulsion capacity and activity properties (Hamada and Swanson 1994; Lei, Zhao, Selomulya, and Xiong, 2015; Morand, Dekkari, Guyomarc'h, and Famelart, 2012). This approach typically involves spectrofluorometric analysis of surface hydrophobicity, where the hydrophobic groups of an emulsifier are quantified based on their interactions with the fluorescent probe anilino naphthalene sulfonic acid (ANS) (Bonomi, Iametti, Pagliarini, and Peri, 1988).

Food emulsions stabilised by whey protein often display poor stability to thermal processing due to high reactivity of the free thiol groups of β-lg (Simmons, Jayaraman, and Fryer, 2007; Wijayanti, Bansal, and Deeth, 2014) as evidenced by formation of buoyant lipoprotein complexes (i.e., white flecks) (Drapala et al., 2016a). The level of free thiol groups in a protein sample can be measured by the spectrophotometric method of Hoffmann and van Mil (1997)

ACCEPTED MANUSCRIPT

and Alting, Hamer, De Kruif, Paques and Visschers (2003). This approach is useful when focusing on improvement of heat stability of emulsions by controlling the reactivity of its protein component. The protein load (i.e., mg protein per m^2 surface area of fat) and composition of the interfacial layer of the emulsion can also be determined; this approach typically involves separation of the oil globules from the continuous phase of the emulsion by means of centrifugation, followed by analysis of protein content by standard analytical methods (e.g., Lowry method, Kjeldahl method, protein assay kits) (Alba et al., 2013; Patton and Huston, 1986; Sünder et al., 2001; Surel et al., 2014). However, this approach for determination of the surface composition of oil globules in emulsions has its limitations due to its invasive nature, leading to changes to the emulsion interfacial layer (e.g., coalescence of oil globules during centrifugation, displacement and rearrangement of protein at the interface during the washing steps) and it may not provide representative information on the system studied (Holzmüller, Müller, Himbert, and Kulozik, 2016). It is also possible to measure the thickness of the interfacial layer and formation of multilayer structures at the O/W interface using a range of techniques, including spectroscopy, light scattering, microscopy, gravimetric and reflectivity techniques, as detailed by Guzey and McClements (2006).

Heat stability of emulsions is often assessed using various modifications of a method by Miller and Sommer (1940), where a liquid sample is placed in an oil bath at high temperatures (120-140°C) until onset of coagulation is observed (O'Connell and Fox, 2000). The incubation time of the sample until coagulation is noted and it represents the heat coagulation time (HCT) and HCT typically decreases as the pH of the sample approaches the pI of the protein stabilising the emulsion. This method is commonly used to assess the stability of emulsions to heat treatment during processing, especially for protein-stabilised nutritional beverage formulations destined for UHT treatment (Sievanen, Huppertz, Kelly, and Fox, 2008).

ACCEPTED MANUSCRIPT

Oxidative stability is an important consideration when designing emulsion-based food systems; its role is especially significant in high added-value nutritional formulations that typically contain unsaturated fatty acids, which are prone to oxidation. A range of assays and techniques can be used to monitor the progression of lipid oxidation in emulsions under accelerated storage conditions, these are typically targeted at monitoring the levels of oxidation products. Spectrophotometric assays (peroxide value; *para*-anisidine value; and thiobarbituric acid reactive substances, TBARS assays) are typically used to quantify the levels of primary or secondary oxidation products (i.e., hydroperoxides or aldehydes and ketones, respectively) (Djordjevic, McClements, and Decker, 2004; Mei, McClements, Wu, and Decker, 1998; Qiu et al., 2015). Gas chromatography (GC) and GC-mass spectrometry methods are also commonly used to monitor oxygen consumption (Lethuaut, Métro, and Genot, 2002) or production of volatile oxidation products in nutritional beverage emulsions (e.g., headspace propanal or hexanal) (Cho, McClements, and Decker, 2002; Lee and Decker, 2011).

Nutritional beverage emulsions are complex and dynamic systems and numerous techniques directed at the assessment of their physicochemical properties, the prediction of their stability and digestive fate are available in the scientific literature. Generally, studies focused on emulsion systems involve a combination of two or more different analytical approaches to provide relevant, meaningful information. These approaches cover a broad spectrum of emulsion evaluations, from characterisation of emulsion building blocks, the dynamics of emulsion formation and system microstructure, to the identification and control of the interactions between components of an emulsion. Designing a study focused on emulsion stability should incorporate techniques capable of measuring thermodynamic-driven changes affecting the system, as well as those that can take place under environmental conditions (e.g., physical stress, temperature extremes, high ion concentrations and low pH) representative of

ACCEPTED MANUSCRIPT

the typical composition, processing and storage of nutritional beverage emulsions. Good initial quality of an emulsion may not necessarily confer good emulsion stability due to interactions between its components promoted by the processing conditions or by changes that affect the emulsion during storage. The use of emulsion systems for nutrient delivery, where the oil globules play the role of delivery vessels, requires a detailed understanding of the formation and subsequent stability of the system to ensure effective protection and targeted, predictable release of the transported bioactive component. Microstructural approaches have been shown to be particularly useful for such studies as they enable detailed profiling of the interfacial layers and allow study of the evolution of microstructure at different stages during the digestion process. Analytical approaches used for assessment of physicochemical and stability properties of emulsions range from simple assays to sophisticated techniques that require access to expensive analytical equipment. Choosing the most suitable approach will ultimately depend on the specific focus of the study and the facilities available. A combination of several complementary techniques is typically required to provide comprehensive information for nutritional beverage systems and to facilitate engineering of a bio-functional and stable food product.

Acknowledgements

This review is a part of a PhD thesis funded by the Irish Department of Agriculture, Food and the Marine through the Food Institutional Research Measure (FIRM; 10/RD/Optihydro/UCC/702).

ACCEPTED MANUSCRIPT

References

Abhyankar, A.R., Mulvihill, D.M., and Auty, M.A.E. (2014). Combined confocal microscopy and large deformation analysis of emulsion filled gels and stirred acid milk gels. *Food Structure*, 1, 127-136.

Adjonu, R., Doran, G., Torley, P., and Agboola, S. (2014). Formation of whey protein isolate hydrolysate stabilised nanoemulsion. *Food Hydrocolloids*, 41, 169–177.

Alba, K., Ritzoulis, C., Georgiadis, N., and Kontogiorgos, V. (2013). Okra extracts as emulsifiers for acidic emulsions. *Food Research International*, 54, 1730-1737.

Alghunaim, A., Kirdponpattara, S., and Newby, B.Z. (2016). Techniques for determining contact angle and wettability of powders. *Powder Technology*, 287, 201–215.

Alting, A.C., Hamer, R.J., De Kruif, C.G., Paques, M., and Visschers, R.W. (2003). Number of thiol groups rather than the size of the aggregates determines the hardness of cold set whey protein gels. *Food Hydrocolloids*, 17, 469-479.

Antipova, A.S., Semenova, M.G., Belyakova, L.E., and Il'in, M.M. (2001). On relationships between molecular structure, interaction and surface behavior in mixture: Small-molecule surfactant + protein. *Colloids and Surfaces B: Biointerfaces*, 21, 217-230.

Arvaniti, E.C., Juenger, M.C.G., Bernal, S. A., Duchesne, J., Courard, L., Leroy, S., … De Belie, N. (2014). Determination of particle size, surface area, and shape of supplementary cementitious materials by different techniques. *Materials and Structures*, 48, 3687–3701. 3

Auty, M.A.E., Morris, V.J., and Groves, K. (2013). Confocal microscopy: principles and applications to food microstructures. In: *Food microstructures: microscopy, measurement and modelling*. Morris V. J., and Groves K. (eds). Woodhead Publishing, Cambridge, UK, pp. 96-131.

Auty, M.A.E., Twomey, M.Y.R.A., Guinee, T.P., and Mulvihill, D.M. (2001). Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. *Journal of Dairy Research*, 68, 417-427.

Bazmi, A., Duquenoy, A., and Relkin, P. (2007). Aeration of low fat dairy emulsions: Effects of saturated–unsaturated triglycerides. *International Dairy Journal*, 17, 1021-1027.

Benjamins, J., Vingerhoeds, M.H., Zoet, F.D., de Hoog, E.H.A., and van Aken, G.A. (2009). Partial coalescence as a tool to control sensory perception of emulsions. *Food Hydrocolloids*, 23, 102–115.

Bonomi, F., Iametti, S., Pagliarini, E., and Peri, C. (1988). A spectro-flourimetric approach to the estimation of the surface hydrophobicity modifications in milk proteins upon thermal treatment. *Milchwissenschaft*, 43, 281–285.

Bos, M.A, and van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a review. *Advances in Colloid and Interface Science*, 91, 437–71.

Bourlieu, C., Ménard, O., De La Chevasnerie, A., Sams, L., Rousseau, F., Madec, M.N., … Dupont, D. (2015). The structure of infant formulas impacts their lipolysis, proteolysis and disintegration during in vitro gastric digestion. *Food Chemistry*, 182, 224-235.

ACCEPTED MANUSCRIPT

Buggy, A.K., McManus, J.J., Brodkorb, A., McCarthy, N.M., and Fenelon, M.A. (2017). Stabilising effect of α-lactalbumin on concentrated infant milk formula emulsions heat treated pre- or post-homogenisation. Dairy Science and Technology, 96, 845–859.

Cai, B., and Ikeda, S. (2016). Effects of the conjugation of whey proteins with gellan polysaccharides on surfactant-induced competitive displacement from the air-water interface. *Journal of Dairy Science*, 99, 1–10.

Chen, B., and O'Mahony, J.A. (2016). Impact of glucose polymer chain length on heat and physical stability of milk protein-carbohydrate nutritional beverages. *Food Chemistry*, 211, 474-482.

Chevallier, M., Riaublanc, A., Lopez, C., Hamon, P., Rousseau, F., Cauty, C., Pasco, M., Croguennec, T. (2016). Improving the heat stability of whey protein microgel-emulsion with a small quantity of casein. . Presented at IDF World Dairy Summit 2016, Rotterdam, NLD (2016-10-16 - 2016-10-21).

Cho, Y.J., McClements, D.J., and Decker, E.A. (2002). Ability of surfactant micelles to alter the physical location and reactivity of iron in oil-in-water emulsion. *Journal of Agricultural and Food Chemistry*, 50, 5704–10.

Chung, C., and McClements, D.J. (2014). Structure-function relationships in food emulsions: Improving food quality and sensory perception. *Food Structure*, 1, 106–126.

Crowley, S.V., Desautel, B., Gazi, I., Kelly, A.L., Huppertz, T., O'Mahony, J.A. (2015). Rehydration characteristics of milk protein concentrate powders. *Journal of Food Engineering*, 149, 105–113.

Crowley, S.V., Kelly, A.L., and O'Mahony, J.A. (2014). Fortification of reconstituted skim milk powder with different calcium salts: Impact of physicochemical changes on stability to processing. *International Journal of Dairy Technology*, 67, 474–482.

Damodaran, S. (2005). Protein Stabilization of Emulsions and Foams. *Journal of Food Science*, 70, R54–R66.

Degrand, L., Michon, C., and Bosc, V. (2016). New insights into the study of the destabilization of oil-in-water emulsions with dextran sulfate provided by the use of light scattering methods. *Food Hydrocolloids*, 52, 848–856.

Detloff, T., Sobisch, T., and Lerche, D. (2006). Particle size distribution by space or time dependent extinction profiles obtained by analytical centrifugation. *Particle and Particle Systems Characterization*, 23, 184-187.

De Wit, J.N. (1990). Thermal stability and functionality of whey proteins. *Journal of Dairy Science*, 73, 3602-3612.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces. B, Biointerfaces*, 20, 197–210.

Djordjevic, D., McClements, D.J., and Decker, E.A. (2004). Oxidative Stability of Whey Protein-stabilized Oil-in-water Emulsions at pH 3 : Potential ω-3 Fatty Acid Delivery Systems (Part B). *Journal of Food Science*, 69, 356-362.

ACCEPTED MANUSCRIPT

Dombrowski, J., Johler, F., Warncke, M., and Kulozik, U. (2016). Correlation between bulk characteristics of aggregated β-lactoglobulin and its surface and foaming properties. *Food Hydrocolloids*, 61, 318–328.

Drapala, K.P., Auty, M.A.E., Mulvihill, D.M., and O'Mahony, J.A. (2015). Influence of lecithin on the processing stability of model whey protein hydrolysate-based infant formula emulsions. *International Journal of Dairy Technology*, 68, 322–333.

Drapala, K.P., Auty, M.A.E., Mulvihill, D.M., and O'Mahony, J.A. (2016a). Improving thermal stability of hydrolysed whey protein-based infant formula emulsions by protein–carbohydrate conjugation. *Food Research International*, 88, 42-51.

Drapala, K.P., Auty, M.A.E., Mulvihill, D.M., and O'Mahony, J.A. (2016b). Performance of whey protein hydrolysate-maltodextrin conjugates as emulsifiers in model infant formula emulsions. *International Dairy Journal*, 62, 76–83.

Drapala, K.P., Auty, M.A.E., Mulvihill, D.M., and O'Mahony, J.A. (2017). Influence of emulsifier type on the spray-drying properties of model infant formula emulsions. *Food Hydrocolloids*, 69, 56–66.

Drelich, J., Fang, C., and White, C.L. (2002). Measurement of interfacial tension in fluid-fluid systems. *Encyclopedia of Surface and Colloid Science*, 3, 3158-3163.

Dunkhin, S.S., Kretzschmar, G., and Miller, R. (1995). Experimental technique to study adsorption kinetics (Chapter 5). In: *Dynamics of Adsorption at Liquid Interfaces - Theory, Experiment, Application* (First, Vol. 1). Mobius, D. and Miller, R. (eds.), Amsterdam: Elsevier. pp. 140-201.

Erni, P., Fischer, P., and Windhab, E.J. (2007). Role of viscoelastic interfaces in emulsion rheology and drop deformation. *Journal of Central South University of Technology*, 14, 246-249.

Euston, S.R., Finnigan, S.R., and Hirst, R.L. (2001). Aggregation kinetics of heated whey protein-stabilised emulsions: effect of low-molecular weight emulsifiers. *Food Hydrocolloids*, 15, 253–262.

Everett, D.W., and Auty, M.A.E. (2008). Cheese structure and current methods of analysis. *International Dairy Journal*, 18, 759–773.

Fischer, P., Pollard, M., Erni, P., Marti, I., and Padar, S. (2009). Rheological approaches to food systems. *Comptes Rendus Physique*, 10, 740–750.

Fischer, P., and Windhab, E.J. (2011). Rheology of food materials. *Current Opinion in Colloid and Interface Science*, 16, 36–40.

Gallier, S., Acton, D., Garg, M., & Singh, H. (2017). Natural and processed milk and oil body emulsions: Bioavailability, bioaccessibility and functionality. *Food Structure*, 13, 13–23.

Gallier, S., Vocking, K., Post, J.A., Van De Heijning, B., Acton, D., Van Der Beek, E.M., and Van Baalen, T. (2015). A novel infant milk formula concept: Mimicking the human milk fat globule structure. *Colloids and Surfaces B: Biointerfaces*, 136, 329–339.

ACCEPTED MANUSCRIPT

Guzey, D., and McClements, D.J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science*, 128–130, 227–48.

Hamada, J.S., and Swanson, B. (1994). Deamidation of food proteins to improve functionality. *Critical Reviews in Food Science and Nutrition*, 34, 283-292.

Heertje, I., Nedelof, J., Kendrickx, H.A.C.M., and Lucassen-Reynders, E. (1990). The observation of the displacement of emulsifiers by confocal scanning laser microscopy. *Food Structure*, 9, 305-316.

Heertje, I., Van Aalst, H., Blonk, J.C.G., Nederlof, D.J. and Lucassesn-Reynders, E.H. (1996). Observations on emulsifiers at the interface between oil and water by confocal scanning light microscopy. *Lebensmittel Wissenschaft und Technologie*, 29, 217-226.

Hoffmann, M.A.M., and van Mil, P.J.J.M. (1997). Heat-induced aggregation of β-lactoglobulin: role of the free thiol group and disulfide bonds. *Journal of Agricultural and Food Chemistry*, 45, 2942-2948.

Holzmüller, W., Müller, M., Himbert, D., and Kulozik, U. (2016). Impact of cream washing on fat globules and milk fat globule membrane proteins. *International Dairy Journal*, 59, 52–61.

Hong, J.S., and Fischer, P. (2016). Bulk and interfacial rheology of emulsions stabilized with clay particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 508, 316–326.

Iritani, E., Katagiri, N., Aoki, K., Shimamoto, M., and Yoo, K.M. (2007). Determination of permeability characteristics from centrifugal flotation velocity of deformable oil droplets in O/W emulsions. *Separation and Purification Technology*, 58, 247-255.

Jara, F.L., Carrera Sánchez, C., Rodríguez Patino, J.M., and Pilosof, A.M.R. (2014). Competitive adsorption behavior of β-lactoglobulin, α-lactalbumin, bovine serum albumin in presence of hydroxypropylmethylcellulose. Influence of pH. *Food Hydrocolloids*, 35, 189–197.

Jeirani, Z., Mohamed Jan, B., Si Ali, B., Noor, I. M., See, C. H., and Saphanuchart, W. (2013). Formulation, optimization and application of triglyceride microemulsion in enhanced oil recovery. *Industrial Crops and Products*, 43, 6–14.

Kaltsa, O., Paximada, P., Mandala, I., and Scholten, E. (2014). Physical characteristics of submicron emulsions upon partial displacement of whey protein by a small molecular weight surfactant and pectin addition. *Food Research International*, 66, 401–408.

Karbaschi, M., Lotfi, M., Kragel, J., Javadi, A., Bastani, D., and Miller, R. (2014). Rheology of interfacial layers. *Current Opinion in Colloid and Interface Science*, 19, 514–519.

Kasran, M., Cui, S.W., and Goff, H.D. (2013). Covalent attachment of fenugreek gum to soy whey protein isolate through natural Maillard reaction for improved emulsion stability. *Food Hydrocolloids*, 30, 552–558.

Kim, D.A., Cornec, M., and Narsimhan, G. (2005). Effect of thermal treatment on interfacial properties of β-lactoglobulin. *Journal of Colloid and Interface Science*, 285, 100–109.

ACCEPTED MANUSCRIPT

Klein, M., Aserin, A., Svitov, I., and Garti, N. (2010). Enhanced stabilization of cloudy emulsions with gum Arabic and whey protein isolate. *Colloids and Surfaces B: Biointerfaces*, 77, 75-81.

Lee, J., and Decker, E.A. (2011). Effects of metal chelator, sodium azide, and superoxide dismutase on the oxidative stability in riboflavin-photosensitized oil-in-water emulsion systems. *Journal of Agricultural and Food Chemistry*, 59, 6271–6276.

Lei, F., Liu, F., Yuan, F., and Gao, Y. (2014). Impact of chitosan-EGCG conjugates on physicochemical stability of β-carotene emulsion. *Food Hydrocolloids*, 39, 163–170.

Lei, L., Zhao, Q., Selomulya, C., and Xiong, H. (2015). The effect of deamidation on the structural, functional, and rheological properties of glutelin prepared from Akebia trifoliata var. australis seed. *Food Chemistry*, 178, 96–105.

Leick, S., Henning, S., Degen, P., Suter, D., and Rehage, H. (2010). Deformation of liquid-filled calcium alginate capsules in a spinning drop apparatus. *Physical Chemistry Chemical Physics*, 12, 2950-2958.

Lerche, D., and Sobisch, T. (2007). Consolidation of concentrated dispersions of nano- and microparticles determined by analytical centrifugation. *Powder Technology*, 174, 46-49.

Lerche, D., and Sobisch, T. (2011). Direct and accelerated characterization of formulation stability. *Journal of Dispersion Science and Technology*, 32, 1799–1811.

Lesaint, C., Glomm, W.R., Lundgaard, L.E., & Sjöblom, J. (2009). Dehydration efficiency of AC electrical fields on water-in-model-oil emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 352, 63-69.

Lethuaut, L., Métro, F., and Genot, C. (2002). Effect of droplet size on lipid oxidation rates of oil-in-water emulsions stabilized by protein. *Journal of the American Oil Chemists' Society*, 79, 425–430.

Li, M., Auty, M.A.E., O'Mahony, J.A., Kelly, A.L., and Brodkorb, A. (2016). Covalent labelling of β-casein and its effect on the microstructure and physico-chemical properties of emulsions stabilised by β-casein and whey protein isolate. *Food Hydrocolloids*, 61, 504-513.

Liang, Y., Matia-Merino, L., Patel, H., Ye, A., Gillies, G., and Golding, M. (2014). Effect of sugar type and concentration on the heat coagulation of oil-in-water emulsions stabilized by milk-protein-concentrate. *Food Hydrocolloids*, 41, 332–342.

Liang, Y., Patel, H., Matia-Merino, L., Ye, A., and Golding, M. (2013). Structure and stability of heat-treated concentrated dairy-protein-stabilised oil-in-water emulsions: A stability map characterisation approach. *Food Hydrocolloids*, 33, 297–308.

Lim, A.S.L., Burdikova, Z., Sheehan, D.J., and Roos, Y.H. (2016). Carotenoid stability in high total solid spray dried emulsions with gum Arabic layered interface and trehalose-WPI composites as wall materials. *Innovative Food Science and Emerging Technologies*, 34, 310–319.

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

ACCEPTED MANUSCRIPT

Lim, A.S.L., and Roos, Y.H. (2016). Spray drying of high hydrophilic solids emulsions with layered interface and trehalose-maltodextrin as glass formers for carotenoids stabilization. *Journal of Food Engineering*, 171, 174–184.

Liu, F., Wang, D., Sun, C., McClements, D.J., and Gao, Y. (2016). Utilization of interfacial engineering to improve physicochemical stability of β-carotene emulsions: Multilayer coatings formed using protein and protein-polyphenol conjugates. *Food Chemistry*, 205, 129–139.

Liu, S., Sun, C., Xue, Y., and Gao, Y. (2016). Impact of pH, freeze-thaw and thermal sterilization on physicochemical stability of walnut beverage emulsion. *Food Chemistry*, 196, 475–85.

Lopez, C., Cauty, C., and Guyomarc'h, F. (2015). Organization of lipids in milks, infant milk formulas and various dairy products: role of technological processes and potential impacts. *Dairy Science and Technology*, 95, 863–893.

Lopez, C., Madec, M.N., and Jimenez-Flores, R. (2010). Lipid rafts in the bovine milk fat globule membrane revealed by the lateral segregation of phospholipids and heterogeneous distribution of glycoproteins. *Food Chemistry*, 120, 22–33.

Lopez, C., and Ménard, O. (2011). Human milk fat globules: polar lipid composition and in situ structural investigations revealing the heterogeneous distribution of proteins and the lateral segregation of sphingomyelin in the biological membrane. *Colloids and Surfaces. B, Biointerfaces*, 83, 29–41.

Łuczak, A., and Fryźlewicz-Kozak, B. (2013). Methods of research into hair conditioners stability. *Technical Transactions: Chemistry*, 1, 29–38.

Makinen, O.E., Uniacke-Lowe, T., O'Mahony, J.A., and Arendt, E.K. (2015). Physicochemical and acid gelation properties of commercial UHT-treated plant-based milk substitutes and lactose free bovine milk. *Food Chemistry*, 168, 630–638.

Martinet, V., Valentini, C., Casalinho, J., Schorsch, C., Vaslin, S., and Courthaudon, J.L. (2005). Composition of interfacial layers in complex food emulsions before and after aeration: effect of egg to milk protein ratio. *Journal of Dairy Science*, 88, 30–9.

McCarthy, N.A., Gee, V.L., Hickey, D.K., Kelly, A.L., O'Mahony, J.A., and Fenelon, M.A. (2013). Effect of protein content on the physical stability and microstructure of a model infant formula. *International Dairy Journal*, 29, 53–59.

McCarthy, N.A., Kelly, A.L., Mahony, J.A. O., Hickey, D.K., Chaurin, V., and Fenelon, M.A. (2012). Effect of protein content on emulsion stability of a model infant formula. *International Dairy Journal*, 25, 80–86.

McClements, D.J. (2015). Emulsion rheology. In: *Food emulsions: principles, practices, and techniques*. Boca Raton: CRC press, pp. 383-436.

McClements, D.J. (2015). Emulsion stability. In: *Food emulsions: principles, practices, and techniques*. Boca Raton: CRC press, pp. 289-382.

McClements, D.J., Monahan, F.J., and Kinsella, J.E. (1993). Effect of emulsion droplets on the rheology of whey protein isolate gels. *Journal of Texture Studies*, 24, 411-422.

ACCEPTED MANUSCRIPT

McSweeney, S.L. (2008). Emulsifiers in infant nutritional products. In: *Food Emulsifiers and Their Applications* (2nd ed.), Hasenhuettl, G. L., and Hartel, R. W. (eds.). New York: Springer Science+Business Media, pp. 233–261.

McSweeney, S.L., Healy, R., and Mulvihill, D.M. (2008). Effect of lecithin and monoglycerides on the heat stability of a model infant formula emulsion. *Food Hydrocolloids*, 22, 888–898.

Mei, L., McClements, D.J., Wu, J. and Decker, E.A. (1998). Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food Chemistry*, 61, 307-312.

Meshulam, D., Slavuter, J. and Lesmes, U. (2014). Behavior of emulsions stabilized by a hydrophobically modified inulin under bio-relevant conditions of the human gastro-intestine. *Food Biophysics*, 9, 416-423.

Miller, P.G. and Sommer, H.H. (1940). The coagulation temperature of milk as affected by pH, salts, evaporation and previous heat treatment. *Journal of Dairy Science*, 23, 405-421.

Morand, M., Dekkari, A., Guyomarc'h, F., and Famelart, M.H. (2012). Increasing the hydrophobicity of the heat-induced whey protein complexes improves the acid gelation of skim milk. *International Dairy Journal*, 25, 103–111.

Mueller, S., Llewellin, E.W., and Mader, H.M. (2010). The rheology of suspensions of solid particles. *Proceedings of The Royal Society A*, 466, 1201–1228.

Munoz-Ibanez, M., Nuzzo, M., Turchiuli, C., Bergenståhl, B., Dumoulin, E., and Millqvist-Fureby, A. (2016). The microstructure and component distribution in spray-dried emulsion particles. *Food Structure*, 8, 16–24.

Murphy, E.G., Roos, Y.H., Hogan, S.A., Maher, P.G., Flynn, C.G., and Fenelon, M.A. (2015). Physical stability of infant milk formula made with selectively hydrolysed whey proteins. *International Dairy Journal*, 40, 39-46.

Murray, B.S. (2002). Interfacial rheology of food emulsifiers and proteins. *Current Opinion in Colloid and Interface Science*, 7, 426–431.

Mustapha, N.A., Ruttarattanamongkol, K., and Rizvi, S.S.H. (2012). The effects of supercritical fluid extrusion process on surface hydrophobicity of whey protein concentrate and its relation to storage and heat stability of concentrated emulsions. *Food Research International*, 48, 470–477.

Mwangi, W.W., Ho, K.W., Tey, B.T., and Chan, E.S. (2016). Effects of environmental factors on the physical stability of pickering-emulsions stabilized by chitosan particles. *Food Hydrocolloids*, 60, 543–550.

Neelima, Sharma, R., Rajput, Y.S., and Mann, B. (2013). Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk: A review. *Dairy Science and Technology*, 93, 21–43.

Neirynck, N., Van der Meeren, P., Bayarri Gorbe, S., Dierckx, S., and Dewettinck, K. (2004). Improved emulsion stabilizing properties of whey protein isolate by conjugation with pectins. *Food Hydrocolloids*, 18, 949–957.

Nobel, S., Weidendorfer, K., and Hinrichs, J. (2012). Apparent voluminosity of casein micelles determined by rheometry. *Journal of Colloid and Interface Science*, 386, 174–180.

ACCEPTED MANUSCRIPT

Norton, I.T., Spyropoulos, F., and Cox, P. (eds.). (2010). Practical food rheology: an interpretive approach. John Wiley and Sons.

O'Connell, J.E., and Fox, P.F. (2000). The two-stage coagulation of milk proteins in the minimum of the heat coagulation time-pH profile of milk: effect of casein micelle size. *Journal of Dairy Science*, 83, 378–86.

O'Sullivan, J.J., Drapala, K.P., Kelly, A.L., and O'Mahony, J.A. (2017). The use of inline high-shear rotor-stator mixing for preparation of high-solids milk-protein-stabilised oil-in-water emulsions with different protein:fat ratios. *Journal of Food Engineering*, 222, 218-225.

Ozturk, B., Argin, S., Ozilgen, M., and McClements, D.J. (2014). Formation and stabilization of nanoemulsion-based vitamin e delivery systems using natural surfactants: Quillaja saponin and lecithin. *Journal of Food Engineering*, 142, 57–63.

Pan, Y., Tikekar, R.V, and Nitin, N. (2013). Effect of antioxidant properties of lecithin emulsifier on oxidative stability of encapsulated bioactive compounds. *International Journal of Pharmaceutics*, 450, 129–137.

Patton, S., and Huston, G.E. (1986). A method for isolation of milk fat globules. *Lipids*, 21, 170-174.

Pelegrine, D.H.G., and Gasparetto, C.A. (2005). Whey proteins solubility as function of temperature and pH. *LWT-Food Science and Technology*, 38, 77-80.

Phoon, P.Y., San Martin-Gonzalez, M.F., and Narsimhan, G. (2014). Effect of hydrolysis of soy β-conglycinin on the oxidative stability of O/W emulsions. *Food Hydrocolloids*, 35, 429–443.

Piorkowski, D.T., and McClements, D.J. (2014). Beverage emulsions: Recent developments in formulation, production, and applications. *Food Hydrocolloids*, 42, 5–41.

Pugnaloni, B.L.A., Ettelaie, R., and Dickinson, E. (2005). Computer Simulation of Interfacial Structure and Large-Deformation Rheology during Competitive Adsorption of Proteins and Surfactants. In: *Food Colloids: Interactions, Microstructure and Processing*. Dickinson, E. (ed.). Cambridge: The Royal Society of Chemistry, pp. 131–142.

Qiu, C., Zhao, M., Decker, E.A., and McClements, D.J. (2015). Influence of protein type on oxidation and digestibility of fish oil-in-water emulsions: Gliadin, caseinate, and whey protein. *Food Chemistry*, 175, 249–257.

Regost N. (2016). Characterization of "white flecks materials" in reconstituted infant formula. Presented at IDF Parallel Symposia 2016, Dublin, Ireland (2016-04-11 - 2016-04-13).

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

Ryu, D.Y., and Free, M.L. (2003). The importance of temperature and viscosity effects for surfactant adsorption measurements made using the electrochemical quartz crystal microbalance. *Journal of Colloid and Interface Science*, 264, 402–406.

Sagis, L.M.C., and Scholten, E. (2014). Complex interfaces in food: Structure and mechanical properties. *Trends in Food Science and Technology*, 37, 59–71.

ACCEPTED MANUSCRIPT

Sandler, N., and Wilson, D. (2010). Prediction of granule packing and flow behavior based on particle size and shape analysis. *Journal of Pharmaceutical Sciences*, 99, 958-968.

Sarkar, A., Arfsten, J., Golay, P.A., Acquistapace, S., and Heinrich, E. (2016). Microstructure and long-term stability of spray dried emulsions with ultra-high oil content. *Food Hydrocolloids*, 52, 857–867.

Shimoni, G., Shani Levi, C., Levi Tal, S., and Lesmes, U. (2013). Emulsions stabilization by lactoferrin nano-particles under in vitro digestion conditions. *Food Hydrocolloids*, 33, 264–272.

Schmidmeier, C., O'Gorman, C., Drapala, K.P., and O'Mahony, J.A. (2017). Root cause analysis of white flecks defect in reconstituted fat filled milk powders. Presented at 10th NIZO Dairy Conference, Papendal, The Netherlands (2017-10-01 - 2017-10-03).

Sievanen, K., Huppertz, T., Kelly, A.L., and Fox, P.F. (2008). Influence of added calcium chloride on the heat stability of unconcentrated and concentrated bovine milk. *International Journal of Dairy Technology*, 61, 151–155.

Simmons, M.J.H., Jayaraman, P., and Fryer, P.J. (2007). The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. *Journal of Food Engineering*, 79, 517–528.

Singh, H., & Gallier, S. (2017). Nature's complex emulsion: The fat globules of milk. *Food Hydrocolloids*, *68*, 81–89.

Sørensen, A.D.M., Baron, C.P., Let, M.B., Brüggemann, D.A., Pedersen, L.R.L., and Jacobsen, C. (2007). Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: oxidation linked to changes in protein composition at the oil-water interface. *Journal of Agricultural and Food Chemistry*, 55, 1781–1789.

Sünder, A, Scherze, I., and Muschiolik, G. (2001). Physico-chemical characteristics of oil-in-water emulsions based on whey protein-phospholipid mixtures. *Colloids and Surfaces. B, Biointerfaces*, 21, 75–85.

Surel, C., Foucquier, J., Perrot, N., Mackie, A., Garnier, C., Riaublanc, A., and Anton, M. (2014). Composition and structure of interface impacts texture of O/W emulsions. *Food Hydrocolloids*, 34, 3-9.

Tadros, T.F. (2013). Emulsion Formation, Stability, and Rheology. In: *Emulsion Formation and Stability*, John Wiley and Sons, pp.1–76.

Tamm, F., and Drusch, S. (2017). Impact of enzymatic hydrolysis on the interfacial rheology of whey protein/pectin interfacial layers at the oil/water-interface. *Food Hydrocolloids*, 63, 8–18.

Tcholakova, S., Denkov, N.D., Ivanov, I.B., and Campbell, B. (2006). Coalescence stability of emulsions containing globular milk proteins. *Advances in Colloid and Interface Science*, 123–126, 259–93.

Thiessen, D.B., and Man, K.F. (2000). Surface tension measurement. In: *Measurement, instrumentation and sensors handbook*. CRC Press LLC, Boca Raton, pp. 31-1.

ACCEPTED MANUSCRIPT

Turgeon, S.L., Gauthier, S.F., Molle, D., and Leonii, J. (1992). Interfacial Properties of Tryptic Peptides of β-Lactoglobulin. *Journal of Agricultural and Food Chemistry*, 40, 669–675.

van Aken, G.A. (2010). Relating food emulsion structure and composition to the way it is processed in the gastrointestinal tract and physiological responses: what are the opportunities? *Food Biophysics*, 5, 258–283.

Van der Meeren, P., El-Bakry, M., Neirynck, N., and Noppe, P. (2005). Influence of hydrolysed lecithin addition on protein adsorption and heat stability of a sterilised coffee cream simulant. *International Dairy Journal*, 15, 1235–1243.

van der Ven, C., Gruppen, H., de Bont, D.B., and Voragen, A.G. (2001). Emulsion properties of casein and whey protein hydrolysates and the relation with other hydrolysate characteristics. *Journal of Agricultural and Food Chemistry*, 49, 5005–5012.

van Lent, K., Le, C.T., Vanlerberghe, B., and Van der Meeren, P. (2008). Effect of formulation on the emulsion and whipping properties of recombined dairy cream. *International Dairy Journal*, 18, 1003-1010.

Vega, C., and Roos, Y.H. (2006). Invited review: Spray-dried dairy and dairy-like emulsions--compositional considerations. *Journal of Dairy Science*, 89, 383–401.

Walstra, P. (1994). Physical chemistry of milk fat globules. In: *Advanced Dairy Chemistry Volume 2: Lipids*. Fox, P.F. (ed.). London: Chapman & Hall. pp. 131–178.

Wijayanti, H.B., Bansal, N., and Deeth, H.C. (2014). Stability of Whey Proteins during Thermal Processing: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 13, 1235–1251.

Williams, A.M. (2007). Instant Milk Powder Production: Determining the Extent of Agglomeration. Ph.D. thesis, Massey University, Palmerston North, New Zealand.

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

Yang, R., Zhang, X., Li, F., Ding, L., Li, B., Sun, H., and Gan, Y. (2013). Role of phospholipids and copolymers in enhancing stability and controlling degradation of intravenous lipid emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 436, 434–442.

Ye, A, Hemar, Y., and Singh, H. (2004). Enhancement of coalescence by xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins. *Food Hydrocolloids*, 18, 737–746.

Ye, A., and Singh, H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: influence of polysaccharides. *Food Hydrocolloids*, 20, 269–276.

Zhao, J., Wei, T., Wei, Z., Yuan, F., and Gao, Y. (2015). Influence of soybean soluble polysaccharides and beet pectin on the physicochemical properties of lactoferrin-coated orange oil emulsion. *Food Hydrocolloids*, 44, 443–452.

Zinoviadou, K.G., Scholten, E., Moschakis, T., and Biliaderis, C.G. (2012). Properties of emulsions stabilised by sodium caseinate–chitosan complexes. *International Dairy Journal*, 26, 94-101.

ACCEPTED MANUSCRIPT

Figure captions

Figure 1. Schematic representation of changes that can affect (A) a typical infant formula emulsion as a result of (B) density difference (creaming of oil globules), (C) thermal processing (protein mediated aggregation of oil globules) and (D) long term storage (coalescence of oil globules) as evidenced by various analytical techniques typically used to characterise emulsions: (I) particle size analysis measured using laser diffraction; (II) confocal laser scanning microscopy (CLSM) images showing the distribution of protein (red) and neutral lipids (green) in emulsions; (III) accelerated creaming stability analysis with an analytical centrifuge; and (IV) simulated heat treatment with dynamic sample viscosity information obtained using a rheometer, equipped with a starch pasting cell. *CLSM Image source*: authors' image database.

Figure 2. Schematic representations of principles of typical analytical techniques used for determination of interfacial tension (γ_I): (a) microbalance technique with the Wilhelmy plate geometry; (b) pendant (left) and sessile (right) drop techniques using image analysis software; (c) spinning drop method using image analysis software.

Adapted from: Drelich, Fang and White (2002); Leick, Henning, Degen, Suter and Rehage (2010); Thiessen and Man (2000).

ACCEPTED MANUSCRIPT

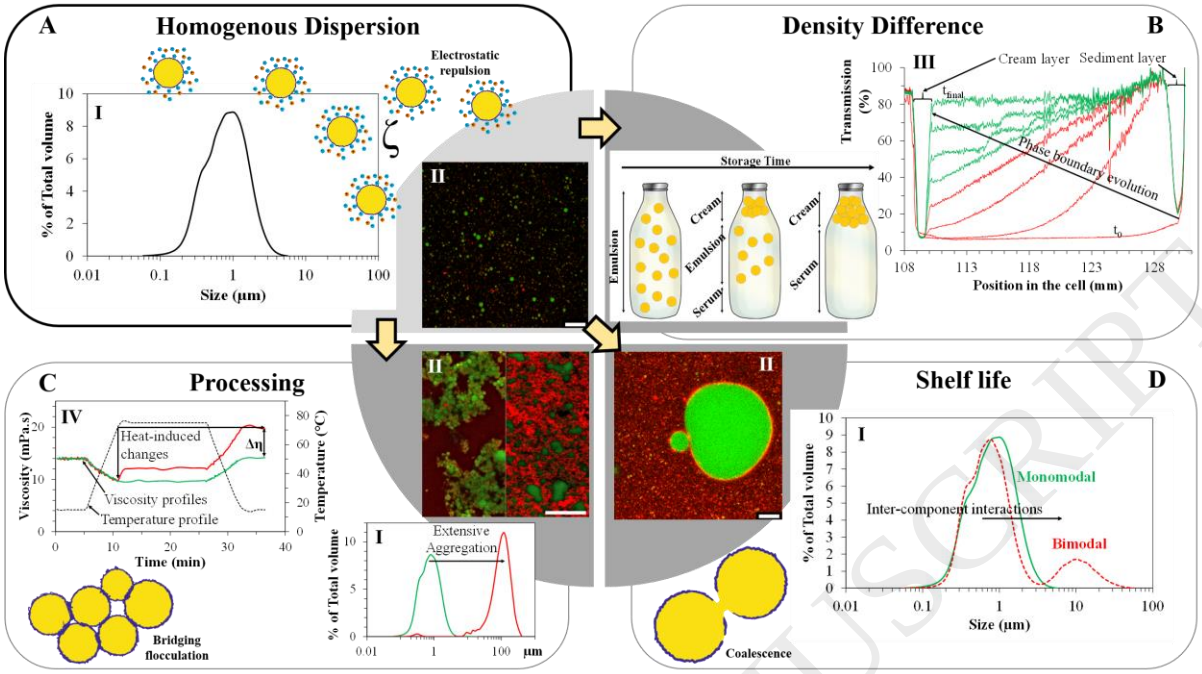
Figure 3. Schematic representation of re-arrangement of macrocomponents in food emulsions (e.g., oil globules, proteins/peptides, carbohydrates) as affected by application of shear (i.e., horizontal flow). Flow behaviour of food emulsions is affected by the changes in the orientation/structures of its macrocomponents: deformation (i.e., elongation) of oil globules, disruption of aggregates, extension of structured colloids (e.g., proteins/peptides) and orientation of linear/branched carbohydrates.

Figure 4. Interfacial location of phospholipids (bright yellow regions) on the surface of an oil globule in a model infant formula emulsion (15.5, 35.0 and 70.0 g L-1 of protein, lipid and carbohydrate, respectively; prepared as detailed by Drapala et al., 2015). The emulsion was stabilised by moderately hydrolysed whey protein (degree of hydrolysis = 10.7%) and soybean lecithin (3% of the total lipid weight). The lecithin (i.e., phospholipids) were stained with fluorescent dye Rhod-DOPE (Avanti Polar Lipids) according to the protocol of Lopez et al. (2010); scale bar = 5 μm; distance between the cross-sections of the globule (i.e., z-distance) = 0.5 μm. *Image source*: authors' image database.

Figure 5. Microstructure of white flecks – a common product defect in spray-dried emulsions as visualised with confocal laser scanning microscopy (CLSM). Protein and fat component have been labelled individually with fluorescent probes, Fast Green and Nile Red, respectively. The use of selective channels allowed observation of [A] dense porous protein network and [B] oil globules distributed within the structure of the flecks. [C] Overlay of both channels allowed visualisation of the distribution of the two components in flecks and provided information on the mechanisms of destabilisation in these emulsions. *Image source*: authors' image database.

ACCEPTED MANUSCRIPT

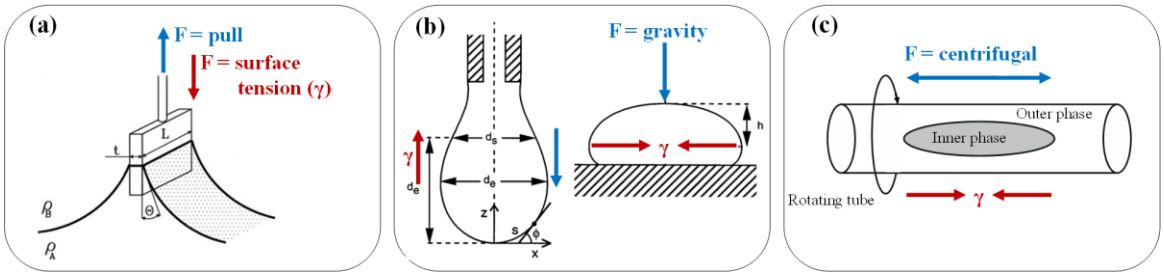
Fig 1



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

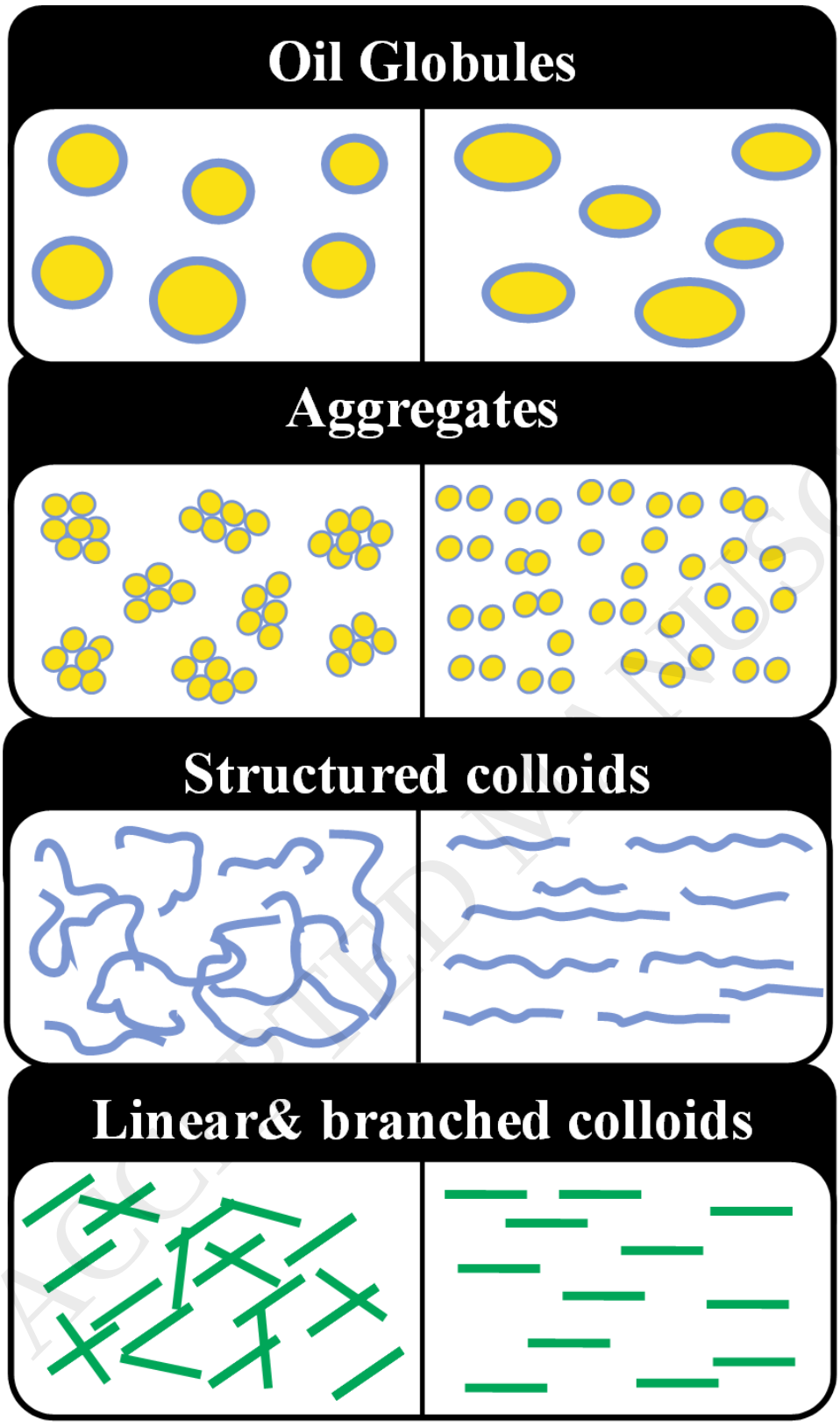
Fig 2



ACCEPTED MANUSCRIPT

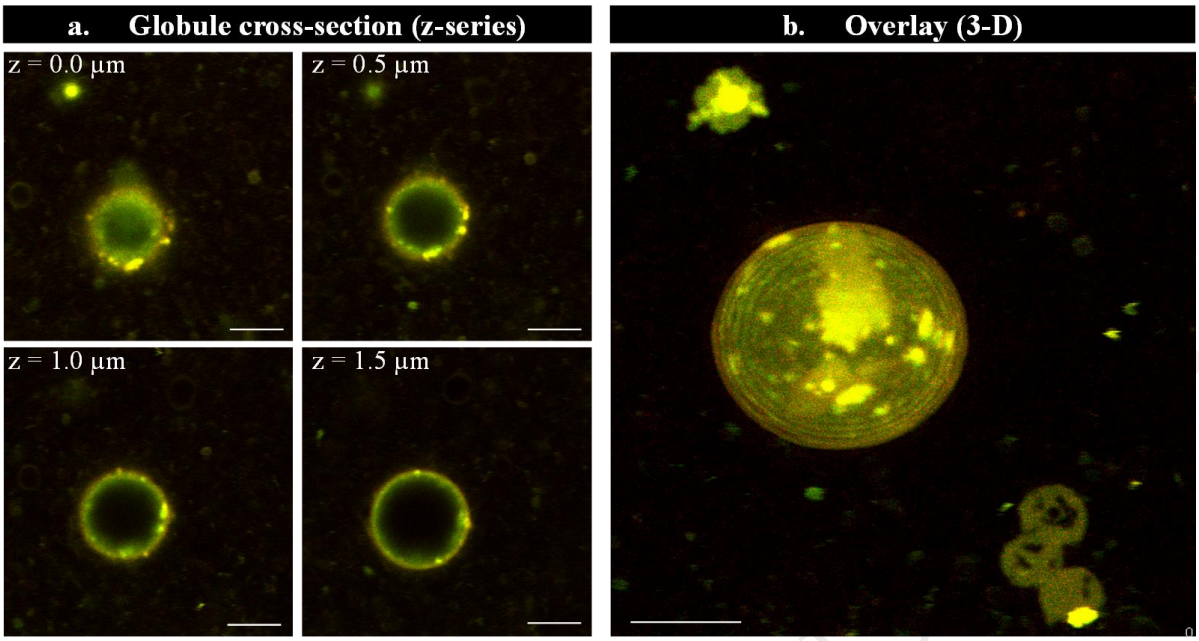
ACCEPTED MANUSCRIPT

Fig 3



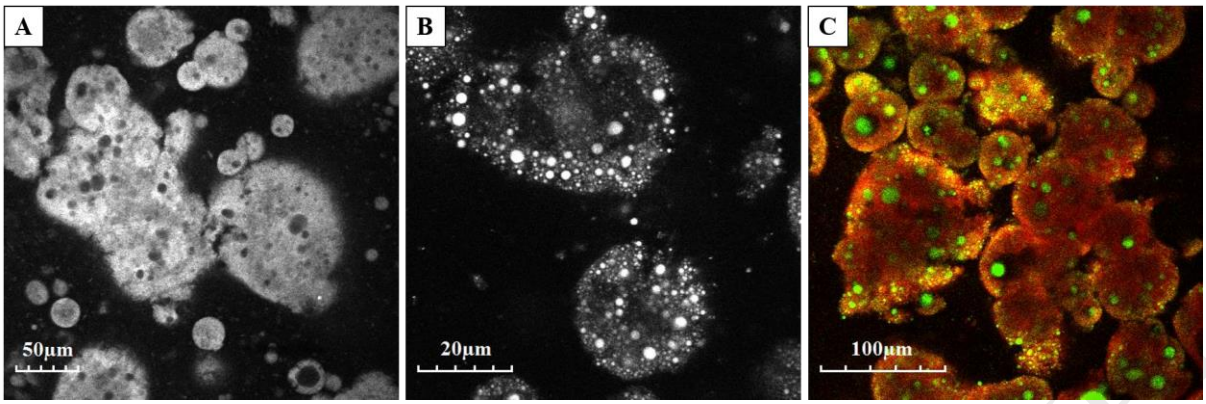
ACCEPTED MANUSCRIPT

Fig 4



ACCEPTED MANUSCRIPT

Fig 5



ACCEPTED MANUSCRIPT