**Natural Emulsifiers - Biosurfactants, Phospholipids, Biopolymers,
and Colloidal Particles: Molecular and Physicochemical Basis of
Functional Performance**

David Julian McClements^{a,b*}, Cansu Ekin Gumus^a

^a *Department of Food Science, University of Massachusetts, Chenoweth Laboratory, Amherst,
MA, USA*

^b *Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203
Jeddah 21589 Saudi Arabia*

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***Corresponding Author**

Address: Department of Food Science, University of Massachusetts, Chenoweth Laboratory,
Amherst, MA, 01003 USA

Tel: 413 545 1019; Fax: 413 545 1262; E-mail: mcclements@foodsci.umass.edu

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19 **Abstract**

20 There is increasing consumer pressure for commercial products that are more natural,
21 sustainable, and environmentally friendly, including foods, cosmetics, detergents, and personal
22 care products. Industry has responded by trying to identify natural alternatives to synthetic
23 functional ingredients within these products. The focus of this review article is on the
24 replacement of synthetic surfactants with natural emulsifiers, such as amphiphilic proteins,
25 polysaccharides, biosurfactants, phospholipids, and bioparticles. In particular, the
26 physicochemical basis of emulsion formation and stabilization by natural emulsifiers is
27 discussed, and the benefits and limitations of different natural emulsifiers are compared.
28 Surface-active polysaccharides typically have to be used at relatively high levels to produce small
29 droplets, but the droplets formed are highly resistant to environmental changes. Conversely,
30 surface-active proteins are typically utilized at low levels, but the droplets formed are highly
31 sensitive to changes in pH, ionic strength, and temperature. Certain phospholipids are capable of
32 producing small oil droplets during homogenization, but again the droplets formed are highly
33 sensitive to changes in environmental conditions. Biosurfactants (saponins) can be utilized at
34 low levels to form fine oil droplets that remain stable over a range of environmental conditions.
35 Some nature-derived nanoparticles (*e.g.*, cellulose, chitosan, or starch) are effective at stabilizing
36 emulsions containing relatively large oil droplets. Future research is encouraged to identify,
37 isolate, purify, and characterize new types of natural emulsifier, and to test their efficacy in food,
38 cosmetic, detergent, personal care, and other products.

39 *Keywords:* Emulsifiers; Natural: Proteins; Polysaccharides; Phospholipids; Biosurfactants;
40 Pickering Stabilization

41

1. INTRODUCTION

Oil-in-water emulsions are an integral part of many commercial products used in the food, supplements, personal care, cosmetic, detergent, and pharmaceutical industries [1-3]. The lipid droplets in these products strongly contribute to their desirable physicochemical and sensory attributes, such as appearance, texture, stability, aroma, taste, and mouthfeel [4]. For example, the addition of lipid droplets to an aqueous solution increases its turbidity and viscosity. The lipid droplets in emulsions may also be utilized as delivery systems to encapsulate, protect, and release non-polar active ingredients, such as hydrophobic colors, flavors, vitamins, nutrients, nutraceuticals, pharmaceuticals, antimicrobials, and antioxidants [3, 5-7]. Oil-in-water emulsions are thermodynamically unstable systems that consist of small lipid droplets dispersed within an aqueous medium. To produce commercial products with sufficiently long shelf lives and with resistances to the environmental stresses they may encounter during their utilization it is necessary to incorporate stabilizers, such as emulsifiers, thickening agents, gelling agents, weighting agents, or ripening inhibitors [4]. Emulsifiers are particularly important ingredients for forming stable emulsions with appropriate shelf lives and functional attributes. Many of the emulsifiers currently used industrially to stabilize oil-in-water emulsions are synthetic surfactants, such as Tweens or Spans [8-10] or animal-based emulsifiers, such as gelatin, egg protein, whey protein, or caseinate [11-14]. However, there has been increasing consumer demand for more natural, environmentally friendly, and sustainable commercial products [15-17], and so many manufacturers have been reformulating their products to replace synthetic surfactants with more label-friendly natural alternatives [18] or to replace animal proteins with

plant proteins [19, 20]. In particular, manufacturers would often like to create new products entirely from natural ingredients so that they can make “all-natural” claims on their labels.

This article reviews the physicochemical basis for the ability of emulsifiers to form and stabilize oil-in-water emulsions, because this information is critical for understanding the requirements of any natural emulsifier that will be used as an alternative to a synthetic one. It then outlines a series of standardized tests that can be used to test and compare emulsifiers, which is useful for establishing the suitability of a particular emulsifier for different applications, and for comparing the relative performance of natural and synthetic emulsifiers. Finally, a review of the different kinds of natural emulsifiers available for use in foods is given (*i.e.*, proteins, polysaccharides, phospholipids, biosurfactants, and bioparticles), and their advantages and disadvantages are highlighted. This article mainly focuses on the development of natural emulsifiers that can be used in food emulsions (Table 1), but a great deal of the material discussed is also pertinent to other types of commercial emulsion-based products. It should also be stressed that the utilization of emulsifiers in the food industry is of great economic importance, with the market for these ingredients being estimated to be around \$2.1 billion in 2012 and predicted to rise to around \$2.9 billion by 2018 [21]. Consequently, the identification of natural alternatives to synthetic emulsifiers has considerable economic implications.

2. PHYSICOCHEMICAL PRINCIPLES OF EMULSIFIER PERFORMANCE

Emulsifiers play two key roles in the creation of successful emulsion-based products (**Figure 1**): (i) they facilitate the initial *formation* of fine lipid droplets during homogenization; (ii) they enhance the *stability* of the lipid droplets once they have been formed [4]. An

understanding of the factors that impact these two distinct roles is essential for assessing the potential performance of natural emulsifiers.

2.1. Emulsion Formation

2.1.1. Principles of Homogenization

Oil-in-water emulsions may be formed using either high- or low-energy approaches [22, 23]. High-energy approaches utilize mechanical devices (“homogenizers”) that disrupt and intermingle the oil and water phases leading to the production of fine lipid droplets [24, 25]. The most commonly utilized mechanical devices in the food industry for forming emulsions are high shear mixers, colloid mills, high-pressure valve homogenizers, microfluidizers, and sonicators [24-27]. Most natural emulsifiers are suitable for use with most types of mechanical homogenizers; however, there are some examples where one must be careful. Polysaccharides or proteins may be depolymerized or denatured within sonicators due to the high local temperature and pressure gradients generated, which can adversely affect their functional performance [28]. Globular proteins may also be denatured and aggregate within high-pressure homogenizers or microfluidizers, which again alters their functional performance [29]. Low-energy homogenization relies on the spontaneous formation of emulsions when the composition or environment of certain emulsifier-oil-water mixtures is changed in a particular way [30, 31]. The most commonly used low-energy approaches for producing emulsions are the phase inversion temperature (PIT), spontaneous emulsification (SE), and emulsion inversion point (EIP) methods [32, 33]. Most natural emulsifiers cannot be used to form emulsions using low-energy approaches, and so this section focuses on the role of emulsifiers in emulsion formation using high-energy approaches.

2.1.2. Role of Emulsifier

The role of the emulsifier in emulsion formation can be understood by examining the major physicochemical events that occur within a homogenizer (**Figure 2**). For the sake of clarity, only a high-pressure valve homogenizer will be considered here since it is the most commonly used mechanical device to form small lipid droplets industrially (**Figure 3**). Nevertheless, fairly similar physicochemical processes occur within other types of homogenizers [23, 26]. Initially, the emulsifier is usually dissolved within the aqueous phase (although this is not always the case), and then the oil and aqueous phases are combined and intermingled using a high-shear mixer, which leads to the formation of a coarse emulsion. This coarse emulsion contains relatively large droplets (typically $d > 1 \mu\text{m}$) that are coated by emulsifier, with the remaining emulsifier molecules being dispersed within the aqueous phase. The coarse emulsion is then pumped through a small valve in the homogenizer at high pressure, which produces powerful disruptive forces (cavitation, turbulence, and shear) that break up the larger droplets into smaller ones [34]. The dimensions of the droplets initially produced inside the homogenizer depend on the relative magnitude of the disruptive forces and the interfacial restoring forces [35, 36].

Facilitation of Droplet Fragmentation: A homogenizer must generate disruptive forces large enough to overcome the Laplace pressure (ΔP_L) of the droplets:

$$\Delta P_L = \frac{4\gamma}{d} \quad (1)$$

Here, γ is the oil-water interfacial tension and d is the droplet diameter [4]. Large droplets are fragmented into smaller droplets when the disruptive forces are appreciably higher than the Laplace pressure [35, 36]. The intensity of the disruptive forces required to break down droplets

therefore tends to increase as γ increases or d decreases. As a consequence, smaller droplets will be produced during homogenization at fixed energy intensity (e.g., operating pressure) as the interfacial tension decreases.

An emulsifier can therefore expedite the production of fine droplets inside a homogenizer by rapidly adsorbing to the droplet surfaces and depressing the interfacial tension. The greater the ability of an emulsifier to reduce γ , the smaller will be the droplets that can be generated using fixed homogenization conditions, such as pressure and number of passes [35, 37]. However, the emulsifier adsorption rate must be faster than the droplet fragmentation rate, otherwise the droplets will not be fully coated with emulsifier before a droplet break up event occurs [24, 25, 38]. There are major differences between the ability of natural emulsifiers to rapidly adsorb to lipid droplet surfaces during homogenization and therefore in their ability to rapidly decrease the interfacial tension during homogenization, which leads to considerable differences in the size of the droplets that can be generated within a homogenizer (see later). In addition, some biopolymers are not as efficient at screening the thermodynamically unfavorable contact between the oil and water phases as small molecule surfactants, and therefore lead to higher interfacial tensions and larger droplets during homogenization [39, 40]. For example the interfacial tension is typically $< 5 \text{ mJ m}^{-2}$ for synthetic surfactants but around $15\text{-}25 \text{ mJ m}^{-2}$ for amphiphilic biopolymers (Table 2).

Inhibition of Droplet Coalescence: Once the large droplets have been broken down into smaller ones it is important to prevent their coalescence within the homogenizer (**Figure 2**). Immediately after a large droplet has been broken down into two or more smaller ones the new droplet surfaces formed are not completely covered with emulsifier due to the increase in oil-

water interfacial area [35, 41]. The stability of lipid droplets to coalescence inside a homogenization chamber depends on the degree of surface coverage [42]. If the surfaces can be completely covered by the amount of available emulsifier, and the emulsifier is effective at generating sufficiently strong repulsive forces (*e.g.*, steric or electrostatic), then relatively stable droplets can be produced. However, if the droplets can only be partially covered by the available emulsifier, then they are liable to coalesce when they collide, which leads to larger droplets exiting the homogenizer [42]. Consequently, it is important that the lipid droplet surfaces are saturated with emulsifier molecules before they collide with their neighbors [41, 43, 44]. Another important feature of an emulsifier is therefore its adsorption rate relative to the droplet collision rate. Emulsifiers that rapidly adsorb to the surfaces of the lipid droplets tend to be more effective at inhibiting droplet coalescence inside a homogenizer [45]. This is one of the reasons that synthetic or natural [24] small molecule surfactants are so effective at forming emulsions containing small droplets since they are able to rapidly adsorb to the droplet surfaces during homogenization, thereby rapidly lowering the interfacial tension and rapidly forming a protective coating [24, 46, 47]. On the other hand, some natural emulsifiers (such as polysaccharides) are relatively large molecules that adsorb to lipid droplet surfaces relatively slowly and are therefore less efficient at creating fine droplets [48, 49].

To form small droplets and to optimize energy efficiency, it is important that there is adequate emulsifier present to completely cover the surfaces of the lipid droplets formed inside the homogenizer [42]. A certain amount of emulsifier can only cover a certain amount of oil-water surface area, which depends on oil content, droplet size, and the packing of emulsifier

molecules at the droplet surfaces [42]. The smallest mean droplet diameter ($d_{\min}$) that can theoretically be achieved during homogenization is given by the following equation [26]:

$$d_{\min} = \frac{6 \cdot \Gamma_{\text{sat}} \cdot \phi}{c_s} \quad (2)$$

Here, $d_{\min}$ is the surface-weighted mean diameter (d_{32}), Γ_{sat} is the emulsifier surface load at saturation (in kg m^{-2}), ϕ is the disperse phase volume fraction (unitless), and c_s is total emulsifier concentration in the emulsion (in kg m^{-3}). This equation assumes that stable droplets can only be formed when they are fully coated with emulsifier, that droplet diameter is not limited by the strength of the disruptive forces produced by the homogenizer, and that all the emulsifier adsorbs to the lipid droplet surfaces. A prediction of the dependence of the mean droplet diameter on emulsifier concentration for emulsifiers with different surface loads is shown in **Figure 4**. This prediction shows that the droplet diameter decreases with increasing emulsifier concentration, and that the minimum droplet size that can be produced at a given emulsifier concentration increases with increasing surface load. Typically, the surface load of natural emulsifiers follows the order (Table 2): small molecule surfactants (such as saponins) < globular proteins (such as whey protein) < flexible proteins (such as caseinate) < polysaccharides (such as gum arabic) [50, 51]. Consequently, one would expect saponins to form much smaller droplets than gum arabic when used at the same concentration. Experimental measurements of the mean droplet diameter *versus* emulsifier concentration support these theoretical predictions (**Figure 5**). In practice, it is often not possible to reach the theoretically predicted minimum droplet size because the emulsifiers do not adsorb rapidly enough, some of the emulsifier remains in the water phase,

some droplet coalescence occurs, or the homogenizer is unable to generate sufficiently strong disruptive forces.

Another important factor to consider during emulsion formation is the dependence of the droplet size on homogenization pressure [24, 52]. Typically, the mean droplet diameter decreases with increasing pressure, but the dependence of this relationship depends on emulsifier type and concentration [26]. A number of possible situations are highlighted in **Figure 6**:

- (i) **Excess Emulsifier**: If there is an excess of emulsifier present, then the droplet diameter will continue to decrease with increasing homogenization pressure. Eventually, the upper limit for droplet disruption by the homogenizer is reached, and the droplet size will not decrease any further. In this case, droplet size is determined by homogenization pressure and there is typically a log-log relationship between them. Droplet size also depends on the ease of droplet disruption. In oil-in-water emulsions the ease of droplet disruption tends to increase with diminishing interfacial tension and dispersed-to-continuous phase viscosity ratio [52, 53]. Thus, natural emulsifiers that are better at decreasing the interfacial tension tend to lead to smaller droplets [50, 51].
- (ii) **Limited Emulsifier**: If there is only a limited amount of emulsifier present, then the droplet size decreases with increasing homogenization pressure until a certain droplet size is reached [42]. At this point, all of the emulsifier initially added to the system is adsorbed to the droplet surfaces, and so the droplet size cannot be reduced any further since there is not enough emulsifier to cover any more droplets. As a result, any smaller droplets formed within the homogenizer will not be fully covered with

emulsifier, and so they will tend to coalesce with each other. In this case, the minimum droplet size that can be produced is mainly determined by the initial emulsifier concentration added.

- (iii) **Over-processing:** In some situations, the droplet size may initially decrease with increasing homogenization pressure, but then increase, which is often referred to as “over-processing” [45]. There is often a considerable increase in the temperature of a sample during homogenization at high pressures due to frictional losses. High pressures and temperatures sometimes cause an increase in droplet diameter due to a reduction in emulsifier functionality, *e.g.*, due to depolymerization or unfolding of biopolymer chains or due to dehydration of surfactant head-groups. These effects are likely to be highly system specific. As mentioned earlier, some proteins and polysaccharides are susceptible to depolymerization or unfolding in certain types of homogenizers, and therefore this effect has to be taken into account when deciding the most appropriate homogenization method for a specific natural emulsifier.

2.2. Emulsion Stability

Once the droplets in an oil-in-water emulsion have been formed during homogenization it is important to keep them stable throughout the expected lifetime of the product [4, 54, 55]. Emulsions may become unstable through numerous physicochemical processes, which are often highly dependent on the nature of the emulsifier used to stabilize the system (**Figure 7**). Some of the most important ways that emulsifiers can influence emulsion stability are outlined below, again with special emphasis on the behavior of natural emulsifiers.

2.2.1. Gravitational separation

To a first approximation, the velocity (v) at which the droplets move due to gravitational forces in a dilute emulsion is given by Stokes' Law [4]:

$$v_{Stokes} = -\frac{g(\rho_2 - \rho_1)d^2}{18\eta_1} \quad (3)$$

Here, g is the gravitational field, d is the droplet diameter, ρ is density, η is shear viscosity, and the subscripts 1 and 2 refer to the continuous and dispersed phases, respectively. The sign of the Stokes velocity is an indication of whether the droplets cream (+) or sediment (-).

Emulsifiers may influence gravitational separation through both direct and indirect means. First, the mean diameter of the droplets in an emulsion is influenced by the effectiveness of an emulsifier at rapidly adsorbing to the droplet surfaces during homogenization thereby facilitating droplet fragmentation and inhibiting droplet coalescence (**Section 2.1**). Emulsifiers vary considerably in their ability to produce fine droplets inside an homogenizer [47, 56], which will therefore influence their subsequent creaming stability. Second, emulsifiers may alter the effective density of the droplets by forming a dense interfacial coating around them [22, 32]. Typically, emulsifiers have a higher density than water, whereas oil has a lower density. Consequently, the presence of an emulsifier layer tends to reduce the difference in density between the droplets and surrounding medium, thereby reducing the creaming velocity (Equation 3). However, this effect is only really significant in emulsions that contain relatively small droplets and thick interfacial layers [57].

2.2.2. Droplet Aggregation

The droplets in an oil-in-water emulsion may aggregate through numerous mechanisms (**Figure 7**), with the most common being flocculation, coalescence, and partial coalescence [26, 58]. Flocculation involves the association of two or more droplets into a clump, with each individual droplet retaining its original dimensions [54]. Coalescence is the process whereby two or more droplets merge together to form a single larger droplet [42]. Partial coalescence is the process whereby two or more partially crystalline lipid droplets form a clump, which is often initiated by protrusion of fat crystals inside one droplet into the fluid region of neighboring droplets [55]. In this case, the droplets do not fully merge together because of the mechanical strength of the three-dimensional fat crystal network inside them [55, 59].

The nature of the emulsifiers present in an emulsion may influence droplet aggregation in numerous ways. First, emulsifier type plays a major role in determining the attractive and repulsive colloidal interactions between the droplets [60]. The droplets in an emulsion tend to aggregate when the attractive interactions dominate, but be stable when the repulsive interactions dominate [4]. Typically, emulsifiers inhibit droplet aggregation by generating strong electrostatic and/or steric repulsive interactions (**Figure 8**). However, in some cases they may promote droplet aggregation by generating attractive interactions between the droplets, such as hydrophobic attraction when they have exposed non-polar regions [61] or depletion attraction when there are high levels of non-adsorbed emulsifier [62]. A brief summary of some of the most important properties that may influence the colloidal interactions between oil droplets coated by natural emulsifiers is given below:

Electrostatic interactions: The electrostatic repulsive interactions acting between lipid droplets suspended in water depends on the surface charge density, as well as on solution conditions, such as ionic strength and solvent type [4, 60]. Typically, the higher the surface charge density and the lower the ionic strength the stronger and longer range is the electrostatic interaction. The nature of the emulsifier molecules surrounding the lipid droplets in an emulsion strongly influences the surface charge density, as well as its pH-dependence. For example, the magnitude of the electrical charge (ζ -potential) on protein-coated droplets goes from highly positive at low pH, to zero at intermediate pH, to highly negative at high pH (**Figure 9**). For instance, legume proteins are constituted of around 70% globulin and 30% albumin [63-65]. The isoelectric point for globulins is around pH 4.5, whereas it is around pH 6 for albumins, and so the net isoelectric point for the overall system is around pH 4.9 [66]. Consequently, protein-based emulsifiers are usually only suitable for preventing droplet aggregation due to electrostatic repulsion at pH values sufficiently above or below their isoelectric point [67].

Steric repulsion: The magnitude and range of the steric repulsion between droplets is largely determined by the thickness and packing of the emulsifier molecules at their surfaces [4, 60]. Typically, the denser the packing and the thicker the interface, the stronger and longer range is the steric repulsion. Emulsifiers differ considerably in their molecular organization at oil-water interfaces, which influences their ability to generate steric repulsion between droplets. For example, polysaccharides that form thick interfacial layers (such as gum arabic) are highly effective at inhibiting droplet aggregation through steric interactions [48, 49]. Conversely, globular proteins (such as whey proteins) that form thin interfacial layers are not effective at preventing droplet aggregation through steric repulsion alone because the range of the van der

Waals attraction exceeds the range of the steric repulsion. In this case, droplet aggregation may be inhibited by ensuring the globular proteins have a strong electrical charge (previous section) or by covalently attaching hydrophilic chains that increase the effective thickness of the interface [54, 68]. Interfacial thickness, and therefore steric interactions, can be increased by choosing natural emulsifiers with large extended structures or by using electrostatic deposition to form multilayered interfaces [58, 69, 70]. The presence of a thick interfacial layer may also inhibit partial coalescence by preventing fat crystals penetrating from one droplet to another droplet [71].

Hydrophobic interactions: After adsorption to the surfaces of oil droplets, certain types of emulsifiers have non-polar regions that remain exposed to the surrounding water, which generates a hydrophobic attraction between the droplets that can promote aggregation [4, 60]. Amphiphilic proteins have both polar and non-polar groups along the polypeptide backbone, and after they adsorb to lipid droplet surfaces the non-polar groups tend to protrude into the oil phase, whereas the polar groups tend to protrude into the water phase. Nevertheless, some of the non-polar groups on the surfaces of the adsorbed proteins may still be directed towards the water phase, and therefore cause the droplet surfaces to have some hydrophobic character. In addition, globular proteins (such as whey, soy, and pea proteins) may undergo conformational changes after adsorption to droplet surfaces (“surface denaturation”) or after an emulsion is heated (“thermal denaturation”), which leads to an increase in the number of non-polar groups exposed to the surrounding aqueous phase [61, 72-74]. As a result of this increase in surface hydrophobicity, a strong hydrophobic attraction is often generated between protein-coated droplets that promotes droplet flocculation (**Figure 10**). Hydrophobic interactions are typically

less important for lipid droplets coated by biosurfactants or phospholipids because there are few non-polar groups exposed at the droplet surfaces. There may be some contribution to the overall colloidal interactions from hydrophobic interactions for polysaccharides that have exposed non-polar groups, but this is likely to be highly dependent on the nature of the polysaccharide used, and there have been few studies in this area.

Covalent interactions: Some natural emulsifiers have chemically reactive functional groups capable of forming covalent bonds with other emulsifiers on the same or on different lipid droplets depending on solution and environmental conditions. One of the commonest examples of this phenomenon are globular proteins (such as whey, soy, and egg proteins) that have free sulfhydryl groups (-SH) or disulfide bonds (-S-S-) that can react with each other [75, 76]. If covalent bonds are formed among proteins adsorbed to the same droplet surfaces, then they can improve the aggregation stability of emulsions [61, 72]. Conversely, if the covalent bonds are formed between proteins adsorbed onto different droplets, then they can lead to flocculation with the droplets being held together by strong covalent bonds [76]. In general, covalent interactions are relatively strong short-range interactions, and therefore they can only form when the reactive groups are in close proximity. Consequently, they may work in concert with other physical interactions, such as van der Waals, electrostatic, hydrophobic, or hydrogen bonding interactions. For example, protein-coated droplets may come into close contact due to a reduction in electrostatic repulsion or an increase in hydrophobic attraction, and then the covalent bonds form between the adsorbed layers on the different droplets [61, 72]. The formation of covalent bonds depends on the presence of chemically reactive functional groups, as well as the precise solution and environmental conditions of the system. This type of interaction therefore tends to be less

important for many natural surfactants, phospholipids, and polysaccharides because they have less chemically reactive functional groups.

Overall interactions: The overall colloidal interaction depends on the contribution of the various types of attractive and repulsive interactions between the droplets (Figure 11). Typically, there is always a van der Waals attraction between lipid droplets that will favor their aggregation, which may be supplemented by other types of attractive interaction such as hydrophobic or depletion attraction. Consequently, the emulsifier layer must generate some kind of repulsive force that is strong enough to overcome these attractive interactions. Emulsifiers that can generate repulsive interactions that are stronger and longer range than the attractive interactions can completely inhibit droplet aggregation by preventing them from coming close together [4, 54]. On the other hand, droplet aggregation may occur in emulsions containing emulsifiers that are unable to generate sufficiently strong or long-range repulsive interactions. In this case, weak flocculation, strong flocculation, or coalescence may occur depending on the nature of the emulsifier layer and its resistance to disruption.

Understanding the major types of colloidal interactions that operate in a particular natural emulsifier-stabilized system is particularly important for understanding the major factors that will influence its aggregation stability. Emulsifier-coated lipid droplets that are primarily stabilized by electrostatic repulsion tend to be highly sensitive to pH and ionic strength, *e.g.*, proteins, phospholipids, and ionic surfactants [54]. Conversely, those primarily stabilized by steric repulsion are much less sensitive to changes in environmental conditions, such as amphiphilic polysaccharides [56]. Emulsifiers that undergo conformational changes upon

heating (such as globular proteins) may be susceptible to droplet aggregation due to an increase in hydrophobic attraction [72].

Impact on partial coalescence: Some natural emulsifiers can impact the tendency for partial coalescence to occur in emulsions containing partly crystalline droplets [55]. Firstly, some emulsifiers alter the nucleation and crystallization of emulsified lipids by acting as templates, thereby altering the number, size, and location of the fat crystals present at the oil-water interface [77]. Secondly, some emulsifiers form thick interfacial coatings around lipid droplets that can prevent a crystal from one droplet penetrating into the liquid portion of another droplet, *e.g.*, caseinate can form thick interfacial layers that inhibit partial coalescence [71, 78]. As a result, the type of natural emulsifier used may have a strong influence on the stability of emulsions to partial coalescence. In some cases, partial coalescence leads to emulsion instability and should therefore be inhibited by using natural emulsifiers that form thick interfacial layers that prevent fat crystal penetration. In other cases, partial coalescence is an important stage in the production of food products, such as margarine, butter, ice cream, and whipped cream. In this case it may be important to use a natural emulsifier that forms a thin interfacial layer that is easy to penetrate, such as a biosurfactant or phospholipid.

2.2.3. Ostwald ripening

Ostwald ripening (OR) causes instability in those oil-in-water emulsions where the oil phase has some solubility in the water phase, which is the case for flavor oils, essential oils, and short chain triglycerides [53, 79, 80]. OR leads to a progressive increase in the mean droplet size over time as a result of diffusion of oil molecules from small to large droplets through the intervening

381 water phase [81]. The rate of droplet growth due to OR can be described by the following
 382 equation [82]:

$$383 \quad d(t)^3 = d_0^3 + \left(\frac{64\gamma V_m^2}{9RT} \right) S(\infty)Dt \quad (4) \quad \text{Here}$$

384 $S(\infty)$ is the equilibrium water-solubility of the oil phase in the aqueous phase, $d(t)$ is the droplet
 385 diameter at time t , d_0 is the initial droplet diameter, V_m is the molar volume of the oil molecules,
 386 and γ is the oil-water interfacial tension. This equations indicates the OR rate is strongly
 387 influenced by the water-solubility of the oil phase, but it also depends on some emulsifier
 388 properties.

389 Emulsifiers may influence the OR rate in oil-in-water emulsions through various
 390 mechanisms. First, the rate of OR is proportional to the oil-water interfacial tension (Equation 4),
 391 and so the more effective an emulsifier is at decreasing the interfacial the more effective it
 392 should be at inhibiting droplet growth through this mechanism [81]. Small molecule surfactants
 393 tend to be better at reducing the interfacial tension than proteins or polysaccharides, and may
 394 therefore be more effective at inhibiting OR through this mechanism. Second, some emulsifiers
 395 can form rigid shells around oil droplets that can inhibit Ostwald ripening by mechanically
 396 retarding droplet shrinkage or growth [80, 83]. Third, some emulsifiers are capable for forming
 397 colloidal structures (such as micelles) that can increase the solubility of the oil phase in the
 398 aqueous phase, thereby increasing the OR rate [84]. The type of natural emulsifier used may
 399 therefore have an influence on the tendency for OR to occur in emulsions.

2.2.4. Lipid Oxidation

Lipid oxidation is an important factor causing loss of product quality and nutrients in many foods [85, 86]. Moreover, potentially toxic reaction products, such as carcinogenic or inflammation-inducing substances, may be formed in foods as a result of lipid oxidation [86, 87]. Lipid oxidation in oil-in-water emulsions is a particular problem when the oil phase contains appreciable levels of polyunsaturated lipids, such as ω -3 oils or carotenoids [88-90]. Lipid oxidation typically involves an interaction between an unsaturated lipid and oxygen leading to the formation of hydroperoxides and their breakdown products [91]. The lipid oxidation reaction can be divided into four major steps: initiation, propagation, decomposition, and termination [91]. This reaction may be initiated by autooxidation, photosensitizer-induced oxidation, or enzyme-induced oxidation depending on system composition and environmental conditions. Controlling the rate of lipid oxidation in emulsions has proved to be a major challenge, and many different strategies have been developed, including controlling environmental conditions (such as oxygen, light, and temperature), controlling ingredient quality, adding antioxidants, adding chelating agents, and engineering the droplet interface [85, 86, 88, 92]. The interfacial layer formed by emulsifiers around lipid droplets has a major impact on the stability of emulsions to lipid oxidation [93, 94]. Some emulsifiers have been shown to inhibit lipid oxidation, whereas others have been shown to accelerate it. For example, proteins can inhibit lipid oxidation by scavenging free radicals, chelating pro-oxidative transition metals, or physically forming a barrier to separate lipids from other reactive species [95]. The metal-catalyzed decomposition of lipid hydroperoxides is a major oxidation pathway in emulsions [86]. Lipid hydroperoxides are surface-active molecules that migrate to droplet surfaces after formation, where they decompose

422 by a metal-catalyzed pathway. Proteins can inhibit lipid oxidation in emulsions by hindering the
423 access of metals to the interface by electrostatic repulsion or by creating a steric barrier due to
424 their thickness and denseness [95, 96]. Some proteins are able to bind transition metals and
425 thereby alter their ability to promote lipid oxidation [97, 98]. If the proteins are present within
426 the aqueous phase, then they will keep the transition metals away from the lipid substrate and
427 inhibit oxidation. However, if the proteins are adsorbed to the droplet surfaces, they may bring
428 the transition metals into close proximity to the droplet surfaces and thus promote oxidation.
429 Proteins that can inhibit lipid oxidation include casein, whey protein, egg protein, gelatin, soy
430 protein, bovine serum albumin, zein, and potato protein [95]. Saponins and certain types of
431 phospholipids may also be effective at inhibiting lipid oxidation in emulsions because of their
432 free radical scavenging capacity [99-101]. In addition, colloidal particles used to stabilize
433 Pickering emulsions have been reported to inhibit lipid oxidation by forming thick interfacial
434 layers and physically separating the pro-oxidant compounds in the continuous phase from the
435 lipid hydroperoxides located at the droplet interface [102].

436 Environmental and solution conditions are known to affect the anti- or pro-oxidative
437 properties of emulsifiers. For instance, lipid oxidation is inhibited by adsorbed proteins at pH
438 values below their isoelectric due to their ability to electrostatically repel transition metals, but
439 may be promoted above their isoelectric point due to their ability to electrostatically attract
440 transition metals [103, 104]. Conversely, the opposite may be true for non-adsorbed proteins
441 since they can pull transition metals away from the droplet surfaces when they bind them. Thus,
442 the ratio of free-to-adsorbed emulsifier may have to be controlled, as well as solution and
443 environmental conditions, for emulsions prone to lipid oxidation.

In summary, some natural emulsifiers may promote lipid oxidation whereas others may inhibit it depending on their molecular properties, location, and environmental conditions. Consequently, the selection and application of an appropriate natural emulsifier is particularly important in commercial products that are prone to lipid oxidation.

2.3. Gastrointestinal fate

Emulsions are often used as delivery systems to encapsulate lipophilic bioactive components within commercial products [5, 105, 106]. However, it is important that any delivery system is able to release the bioactive component at the appropriate site of action after the product has been ingested. In some cases, a lipid may be encapsulated so well, that it is not released within the gastrointestinal tract (GIT) and therefore does not have its potential beneficial effects. The nature of the emulsifier used can have a pronounced influence on the GIT fate of emulsions, and selection of an appropriate natural emulsifier may therefore be important for commercial products that are intended for oral delivery of bioactive components.

In order to select an appropriate emulsifier it is useful to have an understanding of the behavior of emulsions within the GIT after ingestion. Initially, an emulsion-based product will enter the oral cavity where it will spend a few seconds or so depending on the nature of the product [22, 107, 108]. On entering the mouth, an emulsion is mixed with saliva and may experience changes in pH, ionic strength, shearing, and temperature, as well as being exposed to mucin and the surfaces of the tongue, palate, and cheeks. After swallowing, the bolus travels through the esophagus and into the gastric cavity, where it encounters highly acidic gastric fluids that contain minerals and digestive enzymes (such as pepsin and lipase) [109]. In addition, the

465 lipid droplets may be exposed to complex fluid flows and forces due to the motility of the
466 stomach [110]. Typically, an emulsion may spend from a few minutes to a few hours in the
467 stomach depending on its composition and physicochemical properties, as well as those of the
468 surrounding matrix.

469 After a food has been sufficiently disrupted within the gastric cavity, the resulting chyme
470 passes through the pylorus sphincter (a biological valve) and into the small intestine, where the
471 pH increases due to the secretion of pancreatic fluids containing alkaline bicarbonate salts [111,
472 112]. The pancreatic fluids also contain digestive enzymes (such as lipase, amylase, and
473 protease) that hydrolyze the lipids, starches, and proteins in the chyme. In addition,
474 phospholipids and bile salts are mixed with the chyme, which serve to displace some of the
475 existing emulsifiers from the droplet surfaces, and to solubilize the free fatty acids formed during
476 lipolysis [113]. The changes in the environment of the lipid droplets as they pass through the
477 GIT cause alterations in their composition, size, and aggregation state [112]. Droplet
478 composition may be changed due to displacement of some of the original emulsifiers from the
479 droplet surfaces, or due to hydrolysis of the lipids or emulsifiers. Droplet size may be changed
480 due to lipid hydrolysis, coalescence, or fragmentation processes. The droplet aggregation state
481 may be altered due to flocculation induced by bridging, depletion, or electrostatic screening
482 mechanisms. Many of these processes depend on the nature of the emulsifier used to stabilize
483 the original lipid droplets, and can therefore be modulated by selection of an appropriate natural
484 emulsifier. Consequently, it may be possible to design food emulsions with improved nutritional
485 aspects, such as increased bioavailability, targeted release, or enhanced satiety response.

The rate and extent of lipid digestion within the small intestine is one of the most important factors affecting the release, solubilization, and transport of encapsulated bioactive components [5]. Oil type has a major impact on the potential gastrointestinal fate of emulsions [114-116], but will not be considered further because it is not directly related to natural emulsifier properties. Droplet size also influences the rate of lipid digestion, with smaller droplets (bigger surface area) being digested more rapidly than larger ones [117, 118]. Consequently, natural emulsifiers that produce emulsions containing smaller lipid droplets are more effective at ensuring rapid lipid digestion and bioactive release within the GIT [119, 120]. Studies have also shown that lipid digestion may be influenced by the nature of the emulsifier used to stabilize the droplets. Lipid digestion may be inhibited when an emulsifier coating restricts the adsorption of lipase to the oil droplet surfaces, thereby preventing it from coming into close contact with the lipids [121-124]. For example, the initial rate of lipid digestion has been reported to be much slower for caseinate-coated oil droplets than for lactoferrin- or Tween-coated ones (**Figure 12**) because the caseinate-coated droplets were highly flocculated when they entered the small intestine, which restricted the ability of the lipase to reach the lipid phase [125]. Other studies have also shown that emulsions that are highly aggregated when they enter the small intestine have slower lipid digestion rates [126, 127]. As mentioned earlier, caseinate-stabilized emulsions are highly susceptible to flocculation within the stomach, which can influence their aggregation state and digestion in the small intestine [128]. On the other hand, saponin-stabilized emulsions are more stable to droplet aggregation in the stomach, and therefore have a higher surface area and faster digestion rate in the small intestine [129].

As well as acting on the lipid phase within oil droplets, digestive enzymes may also act upon the emulsifier molecules that coat the droplets. For example, proteases within the stomach (pepsin) or small intestine (trypsin and chymotrypsin) may hydrolyze the layer of protein molecules adsorbed to lipid droplet surfaces, thereby affecting their susceptibility to lipid digestion [130-132]. Studies have also shown that the type of natural emulsifier coating the lipid droplets in an emulsion may influence the extent of lipid digestion and the type of lipid digestion products produced, *i.e.*, the ratio of monoacylglycerols, diacylglycerols and triacylglycerols [133]. In this study, the extent of lipid digestion was greater for gum arabic stabilized emulsions than for whey protein stabilized ones, which was attributed to the ability of the whey protein molecules to partly inhibit the adsorption of the lipase molecules.

A number of other studies have compared the ability of different natural emulsifiers to influence the lipid digestion process under simulated GIT conditions. The free fatty acid release was reported to be faster when oil-in-water emulsions were stabilized by proteins than by lecithin [119], and when emulsions were stabilized by saponins than by Tween 20 [134]. There have been a number of recent studies on the potential GIT fate of oil-in-water emulsions stabilized by natural colloidal particles ("Pickering emulsions"). For instance, the rate of lipid digestion was found to be slower for oil droplets coated by chitin nanoparticles than for droplets coated by whey protein or caseinate [135]. On the other hand, coating oil droplets with lactoferrin nanoparticles appeared to have little influence on their rate of lipid digestion [136]. These differences may be because chitin nanoparticles are indigestible, whereas lactoferrin nanoparticles are digested by proteases. Overall, these studies suggest that it may be possible to

528 alter the GIT fate of emulsions by choosing appropriate natural emulsifiers to coat the lipid
529 droplets.

530 **2.4. Summary of Role of Natural Emulsifiers**

531 In summary, a natural emulsifier must be a surface-active molecule or colloidal particle that
532 can rapidly adsorb to the surfaces of the oil droplets produced during homogenization. After
533 adsorption, the emulsifier should rapidly depress the interfacial tension so as to facilitate droplet
534 disruption and the generation of fine droplets, and it should form a coating that protects the
535 droplets from aggregation. In addition, the emulsifier may have to be selected to provide
536 protection against the chemical degradation of encapsulated lipids (such as the oxidation of
537 polyunsaturated lipids), as well as guaranteeing that the lipids are completely digested and
538 absorbed within the GIT. The level of emulsifier needed to form an emulsion containing droplets
539 with a particular size is largely determined by its surface load (Γ), which may vary appreciably
540 for natural emulsifiers.

541 **3. EXPERIMENTAL METHODS FOR COMPARING PERFORMANCE OF NATURAL** 542 **EMULSIFIERS**

543 If a manufacturer would like to select the most appropriate natural emulsifier to use in a
544 particular commercial product, they need to have some standardized analytical tests that can be
545 used to compare different emulsifier types. In addition, if a researcher identifies a new type of
546 natural emulsifier, then they need some standardized means of comparing its performance with
547 existing emulsifiers. In this section, some practically viable analytical tests for characterizing

and comparing the performance of natural emulsifiers according to their capability to form and stabilize emulsions is given.

3.1. Emulsion Formation

Practically, two of the most important attributes of an emulsifier related to emulsion formation are: (i) the minimum amount of emulsifier needed to form an emulsion with a given droplet size; and, (ii) the smallest droplet size achievable under a specified set of homogenization conditions. Information related to these attributes can be obtained using fundamental and/or empirical methods depending on the needs of the investigator [26].

3.1.1. Fundamental methods

Fundamental information about emulsifier properties can be obtained by measuring their effectiveness at reducing the interfacial tension of an oil-water interface [26]. Typically, the interfacial tension is measured as a function of increasing emulsifier level, and then the surface pressure *versus* emulsifier concentration profile is calculated (**Figure 13**). The surface pressure (Π) is defined as the difference in interfacial tension between a clean interface and an interface in the presence of emulsifier: $\Pi = \gamma_0 - \gamma$. In general, the surface pressure rises from zero in the absence of emulsifier to Π_{sat} when the interface is saturated with emulsifier. A number of valuable pieces of information can be obtained from a plot of Π versus emulsifier concentration:

- *Saturation Surface Pressure*: The value of Π_{sat} gives an indication of how effectively an emulsifier can reduce the interfacial tension after it adsorbs to the droplet surfaces, which is related to how easily droplets are fragmented within a homogenizer. The greater Π_{sat} , the smaller the size of the droplets generated under fixed homogenization

conditions (assuming there is enough emulsifier available and that it adsorbs rapidly enough).

- *Surface Activity*: Practically, the surface activity (SA) of an emulsifier can be taken to be the reciprocal of the emulsifier concentration at which the surface pressure reaches 50% of the saturation value: $SA = 1/C_{50\%}$. The thermodynamic affinity of an emulsifier for an oil-water interface increases as its surface activity increases. At a molecular level, the surface activity depends on how effectively the emulsifier shields the thermodynamically unfavorable oil-water interactions that occur at the interface, which depends on interfacial packing efficiency.
- *Surface Load*: The surface load of an emulsifier can be calculated from the gradient of an interfacial tension *versus* logarithm of emulsifier concentration plot (**Figure 13**). As mentioned earlier, the surface load is related to the level of emulsifier needed to stabilize a given amount of interfacial area.

As discussed in Section 2, the dimensions of the droplets leaving a homogenizer depend on the speed at which emulsifier molecules are able to adsorb to the droplet surfaces during homogenization. Information about the kinetics of emulsifier adsorption (under quiescent conditions) can be obtained by acquiring interfacial tension *versus* time profiles [26, 137]. Nevertheless, the time scales that can be accessed in conventional interfacial tension meters is not usually fast enough to accurately mimic the highly dynamic events occurring within a homogenizer. The stability of emulsifier-coated droplets within a homogenizer depends on interfacial properties such as thickness and charge, which can be measured using a variety of analytical tools, such as dynamic light scattering and particle electrophoresis [26].

3.1.2. Empirical methods

Fundamental methods are useful for providing quantitative information about the interfacial properties of natural emulsifiers that can be related to their molecular characteristics and that can be compared between different laboratories. However, they usually provide little insight into how a particular emulsifier functions in practice under commercial homogenization conditions. Consequently, empirical methods based on test conditions that more closely mimic the way an emulsifier is actually used in practice are useful [26]. For example, if a manufacturer were preparing a commercial emulsion-based product using a particular homogenizer, then standardized laboratory conditions could be established to mimic this process. In this case, a coarse oil-in-water emulsion could be prepared with a composition similar to the commercial product (*e.g.*, oil content, oil type, aqueous phase composition, pH, and ionic strength). This coarse emulsion would then be passed through a homogenizer operated under standardized conditions that mimic the industrial process (*e.g.*, homogenizer type, operating pressure, and number of passes), and the mean droplet diameter (d_{32}) would be measured. This procedure is repeated for emulsions containing a range of emulsifier levels, and then the data is plotted as mean droplet diameter *versus* emulsifier concentration (**Figure 4**). This kind of plot is particularly useful for characterizing and comparing the properties of different natural emulsifiers (**Figure 5**). For example, it can be used to identify the amount of emulsifier required to produce droplets of a particular size. Since the droplet size (d_{32}) and disperse phase volume fraction (ϕ) of emulsions are typically known, then the effective surface load (Γ) of an emulsifier can be estimated by fitting the above equation to the experimental data. Indeed, plotting d_{32} versus $1/C$ should result in a linear line that goes through the origin. The slope of this line should

be $6\Gamma\phi$, and therefore the surface load is given by: $\Gamma = \text{slope}/6\phi$. This approach assumes that the droplet size is limited by the amount of emulsifier present, rather than by the disruptive forces that can be generated by the homogenizer, and therefore only the data at relatively low emulsifier concentrations should be used in the analysis. In addition, it assumes that the interfacial composition and structure does not change with increasing emulsifier concentration, *e.g.*, due to multilayer formation. Despite these limitations this approach is a useful means of comparing emulsifiers under similar conditions that mimic commercial processes. For example, based on the data shown in **Figure 5** the surface load of quillaja saponins, whey protein, and gum arabic are 0.001, 1, and 25 mg m⁻², respectively (Table 2). Hence, a much lower concentration of the quillaja saponins is required to form an emulsion than for the other natural emulsifiers.

3.2. Emulsion Stability

Emulsions are thermodynamically unstable colloidal dispersions that may breakdown through numerous instability pathways, including creaming, sedimentation, flocculation, coalescence, and Ostwald ripening [26, 42, 55, 58, 81]. The type of natural emulsifier used to stabilize an oil-in-water emulsion has a major influence of type of instability mechanisms that the droplets are most susceptible to [16]. Analytical tools and experimental protocols are therefore needed to characterize and compare the stability of emulsions stabilized by different kinds of natural emulsifiers [138].

3.2.1. Analytical methods for measuring emulsion stability

Numerous analytical tools exist for measuring the stability of emulsions, which have been reviewed in detail elsewhere [4, 138]. For this reason, only a concise overview of the major

methods is given here. A particularly important factor that influences the stability of many emulsions is the size and aggregation state of the droplets they contain. Particle size is usually measured using specialized analytical instruments, such as those based on light scattering, particle counting, or microscopy. Typically, an emulsion sample is diluted (if required) and then placed within the measurement chamber of the instrument. The instrument then analyzes the sample and provides information about the particle size distribution and mean particle diameter (often within a few minutes).

The electrical properties of the interfaces formed by natural emulsifiers have a major impact on emulsion stability and performance. There are several methods available to measure the electrical characteristics of emulsion droplets, but the simplest and most widely used method is based on micro-electrophoresis [26]. Instruments based on this principle measure the direction and velocity of colloidal particles in a well-defined electrical field, and then use this information to calculate the sign and magnitude of the ζ -potential. The thickness of the interfacial layer formed by a natural emulsifier plays an important role in determining the steric repulsion between droplets, as well as their protective and release characteristics. X-ray and neutron scattering or reflection techniques can be utilized to determine the thickness of the interfacial layer, but they require specialized instrumentation that is often not widely available. Interfacial thickness can sometimes be determined using dynamic light scattering instruments by determining the difference in particle diameter between naked and emulsifier-coated latex beads [139].

Information about the aggregation state of the droplets in emulsions is usually obtained using microscopy methods, such as optical or electron microscopy [26]. This kind of structural

information is particularly useful for distinguishing between droplet flocculation, coalescence, and Ostwald ripening. The susceptibility of an emulsion to gravitational separation can be established by simple visual observation, or using specialized instruments that scan the droplet concentration as a function of sample height (*e.g.*, using a laser).

3.2.2. Emulsion testing protocols

An important criteria to consider when choosing a natural emulsifier for a particular application is to determine whether it will form emulsions that remain stable under the solution conditions found in commercial products (*e.g.*, pH, ionic strength, and ingredient profile), as well as under the various environmental changes that a product experiences throughout its lifetime (*e.g.*, temperature variations, water activity, and mechanical forces) [138]. It is therefore useful to develop standardized testing protocols to identify the solution and environmental conditions that an emulsion containing droplets coated by a particular natural emulsifier will remain stable. Initially, a stock emulsion is produced using the emulsifier to be tested using conditions where the system is known to be stable (*e.g.*, pH, ionic strength, temperature, *etc.*). This stock emulsion is then used to prepare samples that are exposed to a range of solution conditions and environmental stresses:

pH: The stock emulsion is used to prepare samples with pH values spanning the range that might be encountered within commercial products or within the gastrointestinal tract (*e.g.*, 2 to 8).

Ionic strength: The stock emulsion is used to prepare samples with a range of ionic strengths by adding different quantities of salts (*e.g.*, 0 to 500 mM NaCl; 0 to 50 mM CaCl₂). The type and

677 levels of salts chosen should represent those that an emulsion may experience within a
678 commercial product or during passage through the gastrointestinal tract.

679 *Thermal processing:* The stock emulsion is adjusted to a certain pH and ionic strength
680 (chosen to mimic the values of the commercial product it may be used in), and then a series of
681 samples are prepared that are exposed to different temperatures (*e.g.*, 0 to 90 °C) for a specific
682 time (*e.g.*, 20 minutes), or that or exposed to a certain temperature (*e.g.*, 90 °C) for varying times
683 (*e.g.*, 0 to 30 minutes). Alternatively, thermal processing conditions that mimic an industrial
684 process such as pasteurization, sterilization, or cooking can be used.

685 *Freeze-thaw stability:* The stock emulsion is adjusted to a certain pH and ionic strength, and
686 then samples are exposed to freezing (*e.g.* -20 °C for 24 hours) and thawing (*e.g.* +20 °C for 24
687 hours). This procedure may be repeated numerous times to simulate thermal fluctuations that
688 might be experienced by a commercial product. The holding temperatures chosen are important
689 because the water and fat phases may crystallize at different temperatures.

690 *Mechanical stress:* The stock emulsion is adjusted to a certain pH, ionic strength and
691 temperature, and then samples are exposed to standardized mechanical stress conditions *e.g.*,
692 shearing at a constant rate (*e.g.*, 500 s⁻¹) for a fixed time (*e.g.*, 20 minutes); exposing samples to a
693 series of fixed shear rates (*e.g.*, 0 to 500 s⁻¹) for a fixed time at each shear rate (*e.g.*, 5 minutes);
694 or shearing at a constant rate (*e.g.*, 500 s⁻¹) for increasing times (*e.g.*, 0 to 60 minutes).

695 *Light stability:* The stock emulsion is adjusted to a certain pH, ionic strength and
696 temperature, and then samples are exposed to standardized ultraviolet or visible radiation of a
697 known intensity *versus* wavelength profile.

After exposure to these environmental stresses, changes in the particle size, aggregation state, and creaming stability can be measured, as well as other relevant characteristics, such as rheology, optical properties, flavor profile, or chemical degradation.

4. NATURAL EMULSIFIERS

In the context of oil-in-water emulsions, the term “emulsifier” refers to amphiphilic substances that have the ability to adsorb to oil droplet surfaces, reduce the interfacial tension, and protect them from aggregation [26]. The most frequently utilized food-grade emulsifiers are proteins, polysaccharides, phospholipids, and small molecule surfactants [8-10]. Nevertheless, there has recently been great interest in identifying food-grade colloidal particles to stabilize food emulsions through a Pickering mechanism [140-143]. Food emulsifiers vary considerably in their abilities to form and stabilize oil-in-water emulsions depending on their unique chemical and structural properties [4]. An ideal natural emulsifier needs to rapidly adsorb to the oil droplet surfaces generated during homogenization, appreciably decrease the oil-water interfacial tension (to facilitate droplet fragmentation), and generate a protective coating (to inhibit droplet coalescence within the homogenizer) (Section 2.1). Moreover, the emulsifier coating should keep the lipid droplets stable under the conditions that a commercial product might confront during its production, transport, storage, and utilization (Sections 2.2 and 3.2). In this section, natural emulsifiers that are already used in commercial food products are reviewed, as well as some that are currently being investigated for their potential application. In addition, the major factors that affect the functionality of different food emulsifiers are discussed so that their potential range of application can be established.

4.1. Phospholipids

4.1.1. Molecular and physicochemical characteristics

Phospholipids are polar lipids naturally found in animal, plant, and microorganism cell walls [144]. In nature, phospholipids form semi-permeable membranes that play important roles in the separation, protection, and transportation of cellular constituents, as well in cellular integrity and signaling [145]. Phospholipids consist of a glycerol backbone with two fatty acids and a phosphoric acid moiety attached [144]. The fatty acid chains make up the non-polar lipophilic tail of the emulsifier, whereas the phosphoric acid moiety and any attached groups form the polar hydrophilic head. Because phospholipids have appreciable non-polar and polar regions within the same molecule they are amphiphilic molecules that can adsorb to oil-water interfaces and stabilize lipid droplets [146, 147]. When a phospholipid adsorbs to an oil-water interface the non-polar fatty acid tails protrude into the oil phase, whereas the polar hydrophilic head-groups protrude into the surrounding aqueous phase (**Figure 14**). In some circumstances, phospholipids form monolayers around oil droplets, but in other circumstances they may form multiple bilayers (with the phospholipids lined up head to head), which may impact the stability and properties of emulsions [146, 148].

The phospholipid-based functional ingredients used as emulsifiers in commercial products are usually called *lecithins* [9, 147]. Lecithins can be isolated from numerous biological sources, with the most common being soybeans, eggs, milk, rapeseed, canola seed, cottonseed, and sunflower [149]. Commercial lecithins typically contain a combination of various phospholipids and other lipophilic materials (such as triglycerides, glycolipids, and sterols), but they can be fractionated to create more refined ingredients [150]. The most common phospholipids found in

commercial lecithin ingredients are: phosphatidylcholine (PC), phosphatidyletanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA) [144]. The hydrophilic head-groups of phospholipids are typically either anionic (PI and PA) or zwitterionic (PC and PE), with the charge depending strongly on pH. The non-polar tail groups of phospholipids usually have two fatty acids, which can vary in the number of carbon atoms and double bonds they contain. In some commercial lecithin ingredients (“lysolecithins”), one of the fatty acid tails is removed to alter their functional characteristics [151]. There is therefore a question about whether lysolecithins can truly be considered to be natural emulsifiers.

4.1.2. Factors affecting emulsion formation and stability

Unlike most other natural emulsifiers, phospholipids may be dispersed in the oil or the aqueous phase prior to homogenization. The most appropriate phase to disperse the phospholipids is governed by the food application, and depends on the nature of the phospholipids, oil, and aqueous phase and would have to be determined empirically.

Oil-in-water emulsions have been formed using sunflower lecithins, but the dimensions of the oil droplets created was reported to be relatively large (30 to 160 μm), which may be because only a high-shear mixer was used to homogenize them [150]. Another study using sunflower lecithins to form oil-in-water emulsions showed that most of the phospholipids were adsorbed to the droplet surfaces when used at low concentrations, but that phospholipid vesicles were formed in the aqueous phase at higher concentrations [152]. These vesicles may cause an undesirable increase in emulsion viscosity, and may also lead to instability through depletion effects. The oil droplets created in this study were again relatively large (40 to 100 μm) due to the fact that only a high shear mixer was used to prepare the emulsions. Nevertheless, recent studies in our

laboratory have shown that sunflower lecithins can be used to form emulsions containing small droplets ($d_{32} < 200$ nm) when a high-pressure homogenizer (microfluidizer) is used to fabricate them (**Figure 15**).

Another recent study showed that oil-in-water emulsions containing relatively small droplets ($d_{32} < 400$ nm) could be fabricated by microfluidization using soy lecithin as an emulsifier [153]. Under neutral pH conditions, the lecithin-coated droplets were highly negatively charged, which led to good aggregation stability because of the strong electrostatic repulsion between them. However, under highly acidic conditions (pH 1.6), the droplets were not stable to aggregation because the phospholipid head-groups lost their negative charge ($pK_a = 1.5$). Soy lecithin has also been used to create vitamin E-enriched emulsions containing small droplets ($d < 200$ nm) using microfluidization [50]. Without salt addition, the lecithin-coated lipid droplets were stable to aggregation from pH 8 to 3, but became highly flocculated at pH 2. Again, this phenomenon can be attributed to the fact that the phospholipid head-groups lost much of their negative charge at these low pH values thereby reducing the electrostatic repulsion between them. At neutral pH, the emulsions underwent appreciable droplet aggregation when the salt concentration exceeded about 100 mM NaCl, presumably due to electrostatic screening of the anionic phospholipid head-groups by cationic sodium ions. Without salt addition, these emulsions were stable to heat treatment (30 to 90 °C, 30 min), which can again be attributed to the strong electrostatic repulsion between the strongly anionic droplets at pH 7.

A number of other studies have also examined the emulsifying properties of lecithins. The mean droplet diameter has been reported to decrease with increasing lecithin concentration during homogenization; with the droplet size produced depending on homogenization method

and operating conditions [150, 152, 154, 155]. Emulsion stability has also been related to the molecular composition of the phospholipids used, *e.g.*, the ratio of PC to PE [150]. Phospholipid ingredients with high levels of PC were reported to produce smaller oil droplets [154]. Formulation-composition maps have been developed to predict the optimum lecithin-oil-water ratios needed to produce stable emulsions [156]. Certain types of phospholipids may also be effective at retarding the oxidation of emulsified lipids because of their natural free radical scavenging capacity [99, 100].

Some commercial lecithin ingredients are not particularly good at stabilizing oil-in-water emulsions when used in isolation because they have low or intermediate hydrophilic-lipophilic balance numbers (HLB= 2 to 8). Nevertheless, these ingredients can be combined with other natural emulsifiers to form stable emulsions. For example, lecithin has been combined with caseins to form antimicrobial emulsions [157], with caseins to form fish oil emulsions [158], with whey proteins to form lutein-loaded emulsions [159], and with monoacylglycerols to form infant formula emulsions [160]. Indeed, many commercial lecithin-based ingredients intended for utilization in the food industry come as blends of different surface active agents. The functionality of lecithin may also be improved by utilizing cosolvents, such as ethanol, which alter the properties of the surfactant monolayer (such as optimum curvature) thereby facilitating emulsion formation and stability [161]. Alternatively, natural lecithins can be modified by chemically or enzymatically cleaving one of the fatty acid tails from the glycerol backbone to create more polar surfactants (“lysolecithins”) that are suitable for forming and stabilizing emulsions, especially when used in combination with other emulsifiers [151, 162]. Indeed, lysolecithins have been used in the food, pharmaceutical and supplements industries to create

nutritional emulsions designed to provide a mixture of minerals, vitamins, proteins, and lipids. These emulsions may be designed to be taken orally or intravenously. A number of studies have shown that stable oil-in-water emulsions can be formed using lysolecithins when utilized in isolation or in combination with other emulsifiers [162-164]. Nevertheless, these emulsions may become unstable under certain pH ranges or at high ionic strengths due to a reduction in the electrostatic repulsion between the droplets. [162]

The physical and chemical stability of lecithin-coated lipid droplets can also be improved by coating them with oppositely charged biopolymers to form multilayer emulsions, *e.g.*, cationic chitosan has been used to coat anionic lecithin-coated droplets [165-167]. The same approach can be used to alter the potential gastrointestinal fate of lecithin-coated lipid droplets [168].

4.2. Biosurfactants

4.2.1. Molecular and physicochemical characteristics

Saponins are natural small molecule surfactants that are isolated from the bark of a tree (*Quillaja saponaria*). These biosurfactants typically contain a complex mixture of different amphiphilic constituents that have been shown to form micelles when dispersed in water, and that can facilitate the formation and stability oil-in-water emulsions [169-173]. The dominant amphiphilic components identified within the natural extracts from the *Quillaja saponaria* tree are saponins [173-175]. The saponins are amphiphilic because they have regions that are hydrophilic (*e.g.*, sugar groups) and regions that are hydrophobic (*e.g.*, phenolic groups) distributed within a single molecule [171, 176]. An emulsifier derived from the quillaja saponin extract (Q-Naturale[®], Ingredion, Bridgewater, NJ) is available commercially for application

within the food industry. This ingredient is typically provided in either a powdered form or dissolved within an aqueous solution. It has been reported that the critical micelle concentration (CMC) of quillaja saponins is around 0.025 wt%, and that each molecule occupies about 1 nm^2 at the interface [173], which corresponds to a surface load of about 2.8 mg m^{-2} . The same study reported that the surface tension at saturation was around 40 mN m^{-1} , and that adsorption of the surfactant molecules to interfaces was much slower than predicted by simple diffusion, which suggested that there was a large energy barrier to adsorption. This study also reported that adsorbed saponins form relatively strong elastic interfaces with a surface dilatational elasticity around 280 mN/m and a surface shear elasticity around 26 mN/m . Finally, it has been shown that the interfacial rheology of saponin layers depends on the nature of the oil phase, with the interfacial elasticity increasing with increasing hydrophobicity [177].

4.2.2. Factors affecting emulsion formation and stability

Numerous studies have reported that quillaja saponin is a particularly efficacious emulsifier for forming and stabilizing oil-in-water emulsions. This biosurfactant can form emulsions containing small oil droplets ($d < 200 \text{ nm}$) that are stable to aggregation over a range of conditions (pH, ionic strength, and temperature) that make it suitable for application in a wide variety of foods [50, 172, 178]. For instance, it has been shown that quillaja saponin can form vitamin E-enriched nanoemulsions ($d < 200 \text{ nm}$) (**Figure 5**), that may be used as delivery systems to fortify foods and other products with oil-soluble vitamins [50]. In the absence of salt, saponin-coated oil droplets had high aggregation stability from pH 8 to 3, but flocculated at pH 2. At the higher pH values, the droplets were prevented from aggregating because of the high negative charge on them, but once the pH fell below a certain value the oil droplets became less

negatively charged and so became flocculated (**Figure 9**). At neutral pH, the droplets were highly unstable to flocculation at elevated salt levels (≥ 400 mM NaCl, pH 7) due to the reduction in electrostatic repulsion caused by electrostatic screening. The saponin-coated oil droplets also had good heat stability (30 to 90 °C, 30 min, no salt, pH 7) due to the strong steric and electrostatic repulsion between them. Quillaja saponins have also been shown to protect oil droplets from aggregation when the lipid phase crystallizes, which is important for preventing partial coalescence and for the production of solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLC) [179]. Part of the ability of saponins to form stable emulsions may be due to the fact that they form interfacial layers with a high dilatational elasticity [173], which may inhibit droplet deformation and coalescence. A study of the ability of different kinds of emulsifiers to produce nanoemulsions and emulsions by low energy methods (emulsion phase inversion) reported that quillaja saponins were ineffective because they could not be dissolved in the oil phase [180], which is important for this type of emulsion formation method. Moreover, simulated GIT studies have shown that lipid droplets stabilized by saponins are still rapidly digested [134]. Finally, saponin-stabilized oil-in-water emulsions showed better lipid oxidation stability than those stabilized by synthetic emulsifiers, which was attributed to their free radical scavenging capacity [101].

4.3 Proteins

4.3.1. Molecular and physicochemical characteristics

Proteins are biopolymers consisting of strings of amino acid units covalent linked by peptide bonds [91, 181, 182]. The type, number, and position of amino acids in the polypeptide chain determine the molecular, physicochemical, and functional properties of food proteins. Most

proteins contain a mixture of polar and non-polar amino acids and are therefore amphiphilic molecules that can attach to oil-water interfaces and stabilize lipid droplets in emulsions [11, 14, 16, 183]. The relative balance of polar and non-polar groups exposed on their surfaces governs the surface activity of proteins [184]. If the surface hydrophobicity is too low, then the driving force for protein adsorption is not strong enough to overcome the loss of entropy associated with adsorption. Conversely, if the surface hydrophobicity is too high, then the proteins tend to aggregate, become water-insoluble, and lose their surface activity. Consequently, an optimum level of surface hydrophobicity is typically required for a protein to be a good emulsifier.

Most proteins also have a mixture of anionic, neutral, and cationic amino acids along their polypeptide chains, which determines their electrical characteristics under different pH conditions [11, 182]. The electrical characteristics of a protein have a major influence on its functional properties in emulsions. In particular, *electrostatic repulsion* plays a critical role in preventing protein-coated oil droplets from aggregating [16, 26, 54]. In addition, electrostatic interactions have an impact on the stability of emulsions to lipid oxidation, since anionic droplet surfaces may attract cationic transition metals that catalyze the oxidation of lipids within the droplets [185, 186]. The distribution of the charges on the surfaces of proteins is also important since this influences the adsorption of other charged species, *e.g.*, charged biopolymers can adsorb to the surfaces of similarly charged droplets if they have sufficiently large patches of opposite charge [187, 188].

Proteins may adopt various conformations in aqueous solutions and at oil-water interfaces depending on the balance of van der Waals forces, hydrophobic interactions, electrostatic interactions, hydrogen bonding, covalent bonds, steric effects, and entropy effects [11, 16, 74,

189]. This balance is determined by solution and environmental conditions, such as pH, ionic strength, dielectric constant, and temperature. Consequently, the conformation of a protein in solution or at an interface may change when these conditions are altered. The two most common conformations of surface-active proteins used as emulsifiers in the food industry are globular and random coil [58]. Globular proteins have fairly compact spheroid structures where the majority of non-polar groups are located within the interior, and the majority of polar groups are present at the exterior [190]. Nevertheless, many globular proteins still have surface activity because some of the non-polar groups remain exposed at their surfaces, which gives a driving force for adsorption to oil-water interfaces [191]. There are a wide variety of surface-active globular proteins that can be used as emulsifiers, including whey, soy, egg, and plant proteins (**Table 1**). Random coil proteins have a more open flexible structure, although there may still be some regions that have local order such as helical or sheet structures. The most common random coil proteins used as emulsifiers in foods are casein and gelatin (**Table 1**). The structure of proteins often changes after they adsorb to oil-water interfaces because the resulting change in their environment alters the delicate balance between the different molecular interactions and entropy effects [191]. For example, globular proteins may unfold after they adsorb to droplet surfaces and expose groups normally located in their interiors, such as non-polar and sulfhydryl groups [74, 75, 192, 193]. As a result, the proteins may react with other proteins adsorbed to the same or different lipid droplets through hydrophobic or disulfide bonds, which may influence the stability of the droplets to coalescence and flocculation. After adsorption to oil droplet surfaces protein molecules tend to adopt a configuration where many of the hydrophilic groups protrude into the water phase, whereas many of the hydrophobic groups protrude into the oil phase (**Figure 14**).

The most common proteins used as food emulsifiers are whey proteins and caseins from bovine milk [194, 195]. In addition, other proteins derived from animal sources are also widely used in some food products, such as gelatin and egg proteins [16]. Nevertheless, there is a major push towards identifying, isolating and characterizing alternative types of proteins that can be used as emulsifiers in foods, particularly those from plant sources, such as soy, pea, lentil, chickpea, bean and canola proteins [15, 16]. The various kinds of proteins that may be utilized as emulsifiers are summarized in **Table 1**.

4.3.2. Factors affecting emulsion formation and stability

Proteins differ considerably in their abilities to form and stabilize oil-in-water emulsions, with some proteins being highly effective at producing stable emulsions containing small droplets, and others being highly ineffective [15, 16, 26]. These differences in performance are due to differences in the molecular and physicochemical characteristics of proteins from diverse sources. These characteristics depend on their biological origin, as well as the isolation, processing, and storage conditions used. If a protein is too hydrophilic, then it will not have an appreciable surface activity, *e.g.*, certain types of gelatin [196]. Conversely, if a protein is too hydrophobic, then it may be insoluble in water and form aggregates that have poor surface activity, *e.g.*, zein [197]. Proteins that are water-soluble and that do have sufficient surface activity still differ in their effectiveness at forming and stabilizing emulsions due to differences in their adsorption rates, surface activities, surface loads, saturation surface pressures, interfacial thickness, surface hydrophobicity, and electrical characteristics [193, 198]. For example, β -lactoglobulin can form smaller droplets than lactoferrin under similar conditions (emulsifier

concentration, homogenization pressure, and number of passes) [199], which may be attributed to its smaller surface load, faster adsorption kinetics, and/or higher surface pressure.

For food proteins, the surface tension values are typically between about 22 to 42 mN m⁻¹ and the interfacial tension values are typically between about 8 and 22 mN m⁻¹ depending on oil type [200]. Surface loads for food proteins are usually around 2 to 4 mg m⁻² depending on protein type and concentration and system conditions, such as pH, ionic strength, and temperature [200]. Many globular proteins form viscoelastic gel-like interfaces after they adsorb to surfaces due to intermolecular cross-linking with their neighbors, *e.g.*, it has been reported that β -lactoglobulin forms an interface with a surface dilatational modulus around 150 mN m⁻¹ [201]. After adsorption to droplet surfaces globular proteins may undertake conformational changes in response to their new molecular environment, which leads to exposure of hydrophobic groups and sulfhydryl groups. As a result, neighboring protein molecules may form hydrophobic or disulfide bonds with each other [202]. On the other hand, more flexible proteins tend to form layers that are more viscous than elastic, such as casein [202].

Adsorbed proteins usually form interfacial layers that are rather thin (< 10 nm) compared to those formed by adsorbed polysaccharides (> 10 nm), which means that steric repulsion alone is often not sufficiently long-range to inhibit droplet aggregation [16, 54, 58, 67]. Instead, protein-coated droplets are often stabilized against aggregation by having a high electrical charge, which may generate a strong and long-range electrostatic repulsion under appropriate solution conditions, *i.e.*, sufficiently low ionic strength. Hence, protein-coated droplets are highly susceptible to flocculation under conditions where their surface charge is reduced, such as high salt levels or pH values close to the isoelectric point [14, 67]. On the other hand, they may still

be stable to coalescence due to the strong short-range steric repulsion generated by the adsorbed protein layer. The thickness of the interfacial layer will tend to increase with increasing molecular weight of the protein, but will also depend on its conformation (such as globular versus flexible). Thus proteins with high molecular weights and extended structures are often better at preventing aggregation by generating stronger steric repulsion. Globular proteins tend to expose non-polar groups when they are held at temperatures above their thermal denaturation temperature, which can increase the surface hydrophobicity of the droplets [61, 72]. As a result, the hydrophobic attraction between the droplets becomes stronger, and can lead to aggregation if any repulsive forces (such as electrostatic repulsion) operating in the system are not strong (**Figure 10**). In addition, sulfhydryl groups may be exposed when a globular protein unfolds, which results in the formation of covalent linkages between other proteins adsorbed on the same or different droplets [61, 72]. Proteins differ considerably in the number of non-polar and sulfhydryl groups they have exposed at their surfaces, which therefore influences their ability to form hydrophobic and disulfide bonds.

Proteins adsorbed to oil droplets surfaces have been shown to protect the underlying oil phase from lipid oxidation [203-205]. This may occur due to a number of physicochemical mechanisms associated with the adsorbed protein layer, including free radical scavenging, chelation, steric hindrance, and electrostatic repulsion [92]. Whey proteins, soy proteins and caseinate have been shown to inhibit lipid oxidation in oil-in-water emulsions [95]. Chickpea and lentil proteins have also been shown to inhibit lipid oxidation in emulsions [206, 207]. The metal-catalyzed decomposition of lipid hydroperoxides is the dominant oxidation pathway in emulsions [86]. Copper and iron are pro-oxidative transition metals that are widely found in

foods. Some proteins can form complexes with transition metals and thus influence the fate of the lipid oxidation in foods [95].

In the food industry, the most widely utilized protein emulsifiers are whey proteins and caseins isolated from bovine milk [16, 194]. Whey proteins consist of a mixture of globular proteins, whereas caseins consist of a mixture of flexible proteins. Both types of protein have good water-solubility, high surface-activity, and the ability to stabilize oil-in-water emulsions over a range of conditions. Caseins are highly effective at stabilizing oil-in-water emulsions at neutral pH, provided there are not high levels of multivalent cations (such as calcium) in the system, because they can generate strong electrostatic and steric repulsion. However, they are often unsuitable for utilization at intermediate pH values because of their tendency to become highly flocculated around their isoelectric point. One advantage of using caseins over whey proteins is that they typically have much better heat-stability because they do not unfold upon heating [208, 209]. Both the yolk and white of eggs contain a mixture of surface-active globular proteins that are able to form and stabilize emulsions [16, 210]. Other animal-based proteins, such as gelatin, have also been shown to be effective emulsifiers under certain circumstances [196, 211, 212].

Recently, there has been interest in finding plant-based alternatives to these animal-based proteins for labeling, economic, allergenicity, and functionality reasons [16]. Consequently, researchers have examined various types of plant-based proteins, including those isolated from soy, peas, lentils, beans, chickpeas, and corn [16]. Some of these proteins have been shown to have potential as emulsifiers, although in many cases the proteins have to be physically, chemically, or enzymatically modified before they are effective. In certain cases, the

1003 modification method used means that the resulting emulsifiers can no longer be considered to be
1004 natural. In addition, the performance and economic viability of any new protein-based
1005 emulsifiers needs to be established under the demanding conditions experienced within many
1006 food products. Protein-based emulsifiers are available as fairly crude extracts (such as whey
1007 protein concentrates) or as more purified extracts (such as β -lactoglobulin or α -lactalbumin).
1008 Typically, the more pure the extract, the more expensive is the ingredient. The properties of
1009 different protein-based emulsifiers are summarized in Table 1.

1010 Consumers are changing their dietary preferences and are leaning more towards clean labels
1011 [213]. In particular, there is a shift towards plant-based proteins rather than animal-based ones
1012 [214] because of their wide availability, low-cost, consumer desirability, and nutritional benefits
1013 [215, 216]. In addition, whey proteins and caseins have been reported to be food allergens [132],
1014 while some plant proteins are not. Therefore, there is an increase in studies on sources of novel
1015 protein sources, such as faba bean, lentil, pea, and chickpeas [15, 206, 207, 214, 217]. Legume
1016 proteins are globular proteins that can stabilize emulsions by forming relatively thick and
1017 charged layers around oils droplets that generate strong steric and electrostatic repulsion [19].
1018 Soybean proteins have been widely used as food emulsifiers because of their high solubility and
1019 good surface activity [218]; however, there is a high risk of allergic reactions combined to soy.
1020 Chickpea, pea, lentil and faba bean proteins have particularly strong potential as food emulsifiers
1021 because of their non-genetically modified production style, high nutritional value, and low risk
1022 of allergic reactions [19, 65, 66, 214, 217, 219-222].

1023 As mentioned earlier, some proteins have been shown to be particularly effective at
1024 improving the stability of emulsions to lipid oxidation [223]. Lipid oxidation is typically

1025 inhibited by the proteins at pH values below the pI of the protein due to electrostatic repulsion of
1026 the cationic transition metals by the cationic droplet surfaces [103]. The pI of legume proteins
1027 usually ranges from around pH 4.3 to 5.0, so at neutral pH the net charge on the legume proteins
1028 is negative. As a consequence, they may be less effective as antioxidants because there is an
1029 electrostatic attraction between the cationic transition metals and anionic droplets, which brings
1030 these pro-oxidants into close proximity to the lipids.

1031 Legume proteins typically have lower digestibility than proteins from other sources, which
1032 could affect the bioavailability of any encapsulated lipids [224]. The hydrolysis of vegetable
1033 proteins has been reported to lead to the formation of larger peptides than those formed by
1034 animal proteins [225]. Conversely, pea proteins were reported to be completely digested in *in*
1035 *vivo* studies [226]. The digestibility of lentil and faba bean proteins was reported to be more
1036 extensive to that of chickpeas [227].

1037 It should be noted that the functional properties of proteins may vary considerably
1038 depending on their native structures, but also on the way they are isolated, purified, stored, and
1039 processed, since these steps may alter their molecular conformation, aggregation state, purity,
1040 and functional properties. Indeed, this is often an important consideration when developing new
1041 protein-based ingredients: producing a final ingredient with well-defined and consistent
1042 properties from batch-to-batch.

4.4 Polysaccharides

4.4.1. Molecular and physicochemical characteristics

Polysaccharides are natural polymers consisting of one or more types of monosaccharide linked together by glycosidic bonds [91, 181, 182]. Polysaccharide molecules vary considerably in their molar masses, degree of branching, electrical charge, hydrophobicity, and polarity, which alter their physicochemical attributes and functional performance. Some polysaccharides have polypeptides (glyco-proteins) or lipids (glyco-lipids) covalently attached to them, which often influences their ability to act as emulsifiers. Many polysaccharides are not good emulsifiers because they are mainly comprised of hydrophilic monosaccharides and are therefore not particularly surface active [58]. Nevertheless, some polysaccharides do contain an appropriate mixture of non-polar and polar groups and are therefore amphiphilic molecules that can adsorb to oil droplet surfaces and thereby stabilize emulsions. The non-polar groups may be part of the carbohydrate molecule (*e.g.*, methylated groups) or they may be non-carbohydrate moieties (*e.g.*, lipids or proteins) that are covalently or physically attached to the carbohydrate molecules.

By far the most widely used natural polysaccharide emulsifier in the food industry is gum arabic [228-230]. Gum arabic is amphiphilic because it has a non-polar polypeptide backbone with a number of polar polysaccharide chains attached. After adsorption to oil droplet surfaces, the polypeptide chain protrudes into the oil phase, whereas the polysaccharide chains dangle into the water (**Figure 14**). This leads to the formation of a relatively thick hydrophilic coating around oil droplets, which gives them good stability against aggregation due to strong steric repulsion (**Figure 8**). A new form of gum arabic, based on a controlled heating and humidity process, has been shown to have improved emulsification properties [231]. Two other

1065 polysaccharide-based emulsifiers used in the food industry are modified starch and modified
1066 cellulose, which have non-polar hydrocarbon chains covalently attached to polysaccharide chains
1067 [232]. However, these emulsifiers are not natural since their synthesis involves the chemical
1068 modification of starch or cellulose molecules, and so they will not be considered further here.

1069 A number of researchers have focused on the identification of new sources of amphiphilic
1070 polysaccharides suitable for use as emulsifiers. Pectin fractions isolated from various sources
1071 (beet, citrus, apple, and okra) have been shown to have surface activity and the ability to stabilize
1072 oil-in-water emulsions [233-235]. Pectin fractions with higher levels of protein were reported to
1073 be more effective at forming small droplets during homogenization, which can be attributed to
1074 the fact that the proteins have non-polar groups that help anchor the molecules to the oil phase.
1075 Pectins isolated from sugar beet have been shown to be particularly effective at forming and
1076 stabilizing oil-in-water emulsions, which has mainly been attributed to the presence of non-polar
1077 groups (ferulic acid groups) and proteins associated with the polysaccharide chain [236-238].
1078 Indeed, a comparison of the emulsifying properties of beet pectin and gum arabic showed that
1079 beet pectin could be used at appreciably lower levels and that it produced emulsions that were
1080 more stable to environmental conditions [239]. Corn fiber gum can be used to fabricate oil-in-
1081 water emulsions containing relatively small stable droplets [240, 241]. This polysaccharide
1082 contains some non-polar hydrophobic groups (possibly polypeptide and/or phenolic groups)
1083 attached to a polar polysaccharide backbone. Another polysaccharide that appears to be a highly
1084 effective emulsifier is water-soluble yellow mustard mucilage, which has been shown to form
1085 stable emulsions at much lower levels than gum arabic [242]. Chitosan, a cationic polysaccharide
1086 typically derived from crustacean shells, has also been shown to be capable for facilitating

emulsion formation and stability [243]. Other sources of polysaccharide that have been shown to be effective as emulsifiers include those isolated from soybeans [239, 244], basil seeds [245], gum tragacanth [246], and olives [247]. Further work is needed to thoroughly test these emulsifiers under standardized conditions, and to establish their potential commercial applications, economic feasibility, batch-to-batch consistency, and reliability of source.

4.4.2. Factors affecting emulsion formation and stability

Many amphiphilic polysaccharides have relatively large molecular weights and dimensions, and therefore have high surface loads (Γ). As a result, relatively high amounts are required to produce small droplets during homogenization (**Figure 5**). For example, typically a 1:1 mass ratio of emulsifier-to-oil is required to form small droplets using gum arabic ($\Gamma = 26 \text{ mg m}^{-2}$) [231] compared to less than 1:10 for whey proteins ($\Gamma = 2 \text{ mg m}^{-2}$). A similar challenge is likely to exist for other types of amphiphilic polysaccharides that have high molecular weights, although it has been reported that some of them can be used at appreciably lower amounts than gum arabic [239, 242].

The relatively thick and hydrophilic biopolymer layers formed by polysaccharide-based emulsifiers often means that they are mainly stabilized by steric repulsion [26, 58]. Nevertheless, many polysaccharides do have an appreciable electrical charge, which can impact their ability to act as emulsifiers, *e.g.*, by influencing their interactions with charged mineral ions, surfactants, proteins, or other polysaccharides. Indeed, the electrical charge on polysaccharides is critical for the assembly of many types of structured emulsions, such as filled hydrogels, coacervates, or multilayer emulsions (**Figure 17**) [1].

The fact that polysaccharide-coated lipid droplets are primarily stabilized by steric repulsion means that the emulsions tend to be much less affected by changes in pH and ionic strength than protein-coated droplets [26, 58]. For example, gum arabic-coated droplets are stable to droplet flocculation over a range of pH values (3 to 9), salt conditions (0 to 500 mM NaCl and 0 to 25 mM CaCl₂), and temperatures (30 to 90 °C) [48, 49, 51, 56]. The high stability of these systems to environmental stresses can again be attributed to the strong steric repulsion between them, and is one of their major advantages over other types of natural emulsifiers.

4.5 Natural Colloidal Particles

A considerable research effort has recently been directed to the identification of food-grade colloidal particles that can be used to stabilize oil-in-water emulsions through a Pickering stabilization mechanism [140, 248]. This type of colloidal particle tends to become strongly attached to oil-water interfaces because their surfaces are partially wetted by both oil and water phases (**Figure 17**). When the colloidal particles are wetted better by the aqueous phase than the oil phase they tend to protrude into the water and can therefore stabilize oil-in-water emulsions (**Figure 17**).

Some examples of nanoparticles and microparticles derived from natural sources that have potential to stabilize oil-in-water emulsions through a Pickering mechanism include chitin [249, 250], cellulose [251], starch [252], zein [253], pea protein [254], soy protein [255], kafirin [256] and cocoa [257] particles. Comprehensive overviews of different kinds of food-grade colloidal particles that have been investigated are given elsewhere {Berton-Carabin, 2015 #315; Lam, 2014 #195; Rayner, 2014 #194}. A major advantage of using colloidal particles to stabilize emulsions

1129 is that they can lead to systems that are very stable to droplet coalescence. On the other hand, a
1130 major drawback is that they can typically only be used to form emulsions containing relatively
1131 large oil droplets ($d > 2 \mu\text{m}$). This means that the droplets do not have very good stability against
1132 gravitational separation. In addition, colloidal particles used to stabilize Pickering emulsions may
1133 inhibit lipid oxidation by forming thick interfacial layers and physically separating the
1134 prooxidant compounds in the continuous phase from the lipid hydroperoxides located at the
1135 droplet interface [102]. Consequently, there is currently great interest in identifying alternative
1136 sources of natural food-grade colloidal particles that can be used to stabilize emulsions with
1137 small droplets {Berton-Carabin, 2015 #315; Lam, 2014 #195; Rayner, 2014 #194}. Ideally, these
1138 should be ultrafine particles that rapidly adsorb to the droplet surfaces during homogenization,
1139 and form small oil droplets coated by a layer of colloidal particles that protrude into the aqueous
1140 phase.

1141 The GIT fate of Pickering emulsions stabilized by natural colloidal particles has not been
1142 widely studied. One *in vitro* study showed that lipid digestion was retarded in emulsions
1143 containing oil droplets coated by chitin nanocrystals [259]. Another study showed that the rate
1144 of lipid digestion in emulsions containing oil droplets coated by kafirin nanoparticles was
1145 between that of bulk oil emulsions containing oil droplets coated by a synthetic surfactant [256].
1146 This effect was attributed to the fact that the protein nanoparticles were digested by proteases in
1147 the simulated GIT, which led to droplet coalescence and therefore a decrease in droplet surface
1148 area. These studies show that the potential gastrointestinal fate of Pickering emulsions depends
1149 on the nature of the colloidal particles used, which highlights the need for further studies in this
1150 area.

1151 **4.6. Emulsifier Complexes**

1152 The ability of some natural emulsifiers to form and stabilize emulsions can be improved by
1153 using them in combination with other emulsifiers, *e.g.*, proteins-polysaccharides, surfactants-
1154 proteins, or surfactants-polysaccharides. Emulsifiers can be used in combination using a number
1155 of different approaches (**Figure 16**):

- 1156 • *Co-adsorption*: In this case, the two emulsifiers are both adsorbed to the lipid droplet
1157 surfaces as individual molecules [70, 260]. The resulting interface may consist of a
1158 homogeneous mixture of the two different emulsifiers, or it may have regions rich in one
1159 emulsifier and depleted in another. The emulsifiers may be both incorporated into the
1160 system prior to homogenization by dispersing them in the oil and/or water phases.
1161 Alternatively, one emulsifier may be added before homogenization, and the other
1162 emulsifier added after homogenization. The overall composition of the interface will
1163 depend on the relative affinity of the two emulsifiers for the oil-water interface (their
1164 surface activities), as well as their relative concentrations.
- 1165 • *Complexation*: In this case, the two components (which may be two emulsifiers or an
1166 emulsifier and another molecule) form a complex through physical or non-physical
1167 interactions, such as electrostatic, hydrogen bonding, hydrophobic forces, or covalent
1168 bonding [70]. The complexes may be formed before or after homogenization. In the first
1169 case, the two components are mixed together in the aqueous phase to form a complex,
1170 and then the aqueous phase is homogenized with an oil phase. In the second case, one of
1171 the components (an emulsifier) is used to form an emulsion containing emulsifier-coated
1172 lipid droplets, and then the other component is added to form a complex.

- *Layer-by-layer deposition*: Initially, an emulsion is fabricated by homogenizing oil, water, and emulsifier together [261]. The emulsifier used should have some ionizable groups, so that the emulsifier-coated droplets have an electrical charge. This emulsion is then mixed with a solution containing polymers or particles that have an opposite charge to the emulsifier-coated droplets, which causes them to be adsorbed onto the droplet surfaces through electrostatic attraction. The resulting “multilayer” emulsion typically has an opposite charge to the original emulsion. The electrostatic deposition process can be repeated a number of times to form a series of layers around the droplets, which may improve their stability and functional performance. Nevertheless, the system composition and structure must be carefully controlled during the electrostatic deposition process to avoid droplet aggregation [261].

There are appreciable differences between the emulsifying abilities of individual natural emulsifiers. For instance, when used at low levels, protein emulsifiers are often more effective at generating fine oil droplets during homogenization than polysaccharide emulsifiers. Conversely, polysaccharide emulsifiers are usually more effective at generating oil droplets that are stable to a broader range of environmental conditions, such pH, ionic strength, temperature, and freezing. Some of the approaches mentioned above may therefore be used to form emulsifier combinations that can overcome the challenges using individual emulsifiers. Indeed, it has been reported that protein-polysaccharide complexes are better emulsifiers than either of the biopolymers used on its own [58, 70, 261]. It has been shown that considerable improvements in the stability of oil-in-water emulsions to pH changes, salt addition, heating, freezing, and drying [261]. As an example, depositing an anionic polysaccharide (pectin) onto the surfaces of protein-coated lipid

1195 droplets improves the pH stability of the emulsions (**Figure 18**). In this example, the pectin
1196 molecules form a coating around the droplets that increases the steric and electrostatic repulsion
1197 between the droplets, and therefore helps prevent the droplets from aggregation. The complexes
1198 formed by proteins and polysaccharides may be held together by physical or covalent bonds, and
1199 they may be created prior to, during, or after the homogenization process. Commercial
1200 emulsifiers based on protein-polysaccharide complexes will have to meet regulatory
1201 requirements, be economically feasible, and provide enhanced functionality before they are used
1202 in the food industry.

1203 **5. CHALLENGES WITH NATURAL EMULSIFIERS**

1204 The previous sections have mainly focused on the physicochemical properties of natural
1205 emulsifiers, but there are also a number of other factors that may limit the widespread application
1206 of natural emulsifiers in foods.

1207 First, it is important to identify an economically viable source of the natural emulsifier. This
1208 may be a sustainable underutilized natural material, or it may be a byproduct of some other food
1209 processing operation. Second, it is important to identify appropriate isolation methods that can
1210 be used to extract and purify the emulsifier so that it can be used as a food ingredient. These
1211 methods will be highly dependent on the nature of the natural emulsifier and the material from
1212 which it is isolated, and may include processes such as disruption (physical, chemical, or
1213 enzymatic), solvent extraction, selective precipitation, filtration, and centrifugation. Ideally, the
1214 processes developed should be economically viable, sustainable, capable of scale up, robust, and
1215 reproducible. Third, it is important that the emulsifier has consistent functional performance

1216 from batch-to-batch, which means that the molecular characteristics and composition of the final
1217 ingredient are kept constant. Many natural ingredients vary considerably in their molecular and
1218 functional properties depending on factors such as the species they were isolated from, weather
1219 and soil conditions, time of year they were isolated, extraction methods etc. Fourth, it is
1220 important that the emulsifier can be reliably obtained in sufficiently high quantities and at a
1221 reliable cost. Some natural emulsifiers are sourced from regions of the world where there are
1222 political instabilities that may threaten ingredient availability and lead to large fluctuations in
1223 ingredient costs. Fifth, the ingredient should be generally recognized as safe (GRAS) so that it
1224 can be used in foods.

1225 **6. CONCLUSIONS AND FUTURE DIRECTIONS**

1226 There is a strong demand from consumers for “all-natural” foods and beverages, which has
1227 driven researchers in the food industry to identify natural alternatives to many synthetic
1228 ingredients currently utilized in foods. In addition, there is a movement from animal-based
1229 ingredients to more label friendly plant based ones. This article has focused on recent progress
1230 in the identification and characterization of natural emulsifiers, such as biosurfactants,
1231 phospholipids, proteins, polysaccharides, and colloidal particles. Many of these natural
1232 emulsifiers are capable of forming oil-in-water emulsions containing relatively small droplets
1233 that are stable over a range of environmental conditions, and may therefore be suitable for
1234 utilization within commercial food products. Nevertheless, there are still challenges to overcome
1235 for many types of natural emulsifiers. Proteins are capable of forming small droplets at low
1236 usage levels, but the droplets formed are often highly susceptible to aggregation at certain pH
1237 values, high ionic strengths, or after thermal processing. Conversely, high levels of

polysaccharides are typically needed to form emulsions containing small droplets, but the droplets formed have excellent stability to environmental stresses, such as pH, ionic strength, and temperature changes. Biosurfactants, such as saponins, are capable of forming small droplets at low levels that are stable to a wide range of environmental conditions, and may therefore be particularly suitable for food applications.

For certain applications in the food industry it would also be useful to identify natural emulsifiers that have enhanced functional performance, such as stability to freezing/thawing, protection of encapsulated components against chemical degradation, or controlled release properties. Consequently, there is still a need for researchers to search the natural world for new sources of emulsifiers. Based on our current understanding of the structure-function relationships of emulsifiers, these molecules should have a number of characteristics: they should be water-dispersible and amphiphilic; they should be relatively small so that they can rapidly adsorb to droplet surfaces during homogenization; and, they should form thick hydrophilic layers to give good steric stabilization. Each newly identified natural emulsifier should be carefully characterized in terms of its ability to form and stabilize emulsions. Ideally, standardized methods, such as the ones proposed in Section 2, should be used to compare the effectiveness of different natural emulsifiers.

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1258

1259 **TABLES**

1260 **Table 1:** Comparison of properties of emulsifiers isolated from various natural sources that may
 1261 be utilized within the food industry. The information in the table was taken from a variety of
 1262 sources (McClements 2015, Brady 2013, Damodaran 2007).

1263

Emulsifier	Molecular Properties	Emulsion properties
Small molecule surfactants		
<i>Quillija saponins</i>	Surface active because they contain both hydrophilic (<i>e.g.</i> , sugars) and hydrophobic (<i>e.g.</i> , phenolics) regions	Can form small droplets at low levels using high-pressure homogenization. Emulsions unstable at highly acidic conditions (pH < 3), and at high ionic strengths. Stable to heating.
Phospholipids		
<i>Lecithin</i>	Surface active because of polar head-group (phosphate moiety) and non-polar (two fatty acids) tail group	Can forms fairly small droplets at low levels using high pressure homogenization. Unstable under acidic conditions (pH < 3), and at

		high ionic strength. May breakdown at high temperatures.
<i>Lysolecithin</i>	Surface active because of polar head-group (phosphate moiety) and non-polar (one fatty acid) tail group	Can forms fairly small droplets at low levels using high-pressure homogenization. Unstable under acidic conditions ($\text{pH} < 3$), and at high ionic strength. May breakdown at high temperatures.
Proteins		
- <i>Whey protein</i>	Mixture of globular proteins from milk $\text{MW} \approx 18 \text{ kDa}$; $\text{pI} \approx 5$, $T_m \approx 80^\circ\text{C}$	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>β-lactoglobulin</i>	Globular protein from whey protein $\text{MW} \approx 18.4 \text{ kDa}$; $\text{pI} \approx 5.4$; $T_m \approx 83^\circ\text{C}$	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably

		below T_m .
- <i>α-lactalbumin</i>	Globular protein from whey protein MW $\approx$ 14.2 kDa; pI $\approx$ 4.4; $T_m \approx$ 83 °C	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>Bovine serum albumin</i>	Globular protein from whey protein MW $\approx$ 66.3 kDa; pI $\approx$ 5.1; $T_m \approx$ 75 °C	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>Lactoferrin</i>	Globular glyco-protein from whey protein MW $\approx$ 80 kDa; pI $\approx$ 8; $T_m \approx$ 60 and 85 °C	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>Caseinates</i>	Mixtures of flexible proteins	Unstable at pH near pI, and at high ionic strength. Stable at pH well

	from milk MW $\approx$ 24 kDa; pI $\approx$ 5	below or above pI, at low ionic strength, and to heating.
- α_s -casein	Flexible protein from milk. MW $\approx$ 23.6 kDa; pI $\approx$ 5.1	Unstable at pH near pI, and at high ionic strength. Stable at pH well below or above pI, at low ionic strength, and to heating.
- β -casein	Flexible protein from milk. MW $\approx$ 24.0 kDa; pI $\approx$ 5.5	Unstable at pH near pI, and at high ionic strength. Stable at pH well below or above pI, at low ionic strength, and to heating.
- Egg protein	Mixture of globular proteins from egg white or yolk	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- Ovalbumin	Globular protein from egg white MW $\approx$ 45 kDa; pI $\approx$ 4.5; $T_m \approx$	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low

	80°C	ionic strength, and at temperatures appreciably below T_m .
- <i>Lysozyme</i>	Globular protein from egg white MW $\approx$ 14.3 kDa; pI $\approx$ 11.3; $T_m \approx$ 72 °C	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>Legume proteins</i> (Soy, pea, lentil, chickpea, faba bean etc.)	Mixture of globular proteins from legumes with variable molecular weights. pI $\approx$ 4.3-5.0; $T_m \approx$ 82-90 °C	Unstable at pH near pI, at high ionic strength, and at temperatures $> T_m$. Stable at pH well below or above pI, at low ionic strength, and at temperatures appreciably below T_m .
- <i>Gelatin</i>	Fairly hydrophilic flexible proteins from animal sources (collagen). Variable molecular weight depending on processing conditions. pI $\approx$ 5 (Type B) or 8 (Type A);	Often not very surface active due to high hydrophilic character. Some types of gelatin can be used successfully as emulsifiers.

	$T_m \approx 10-30\text{ }^{\circ}\text{C}$	
Polysaccharides		
<i>Gum arabic</i>	Branched glycoprotein MW $\approx 1,000\text{ kDa}$; $pK_a \approx 3.5$	Requires a high surfactant-to-oil ratio, but forms droplets stable to a wide range of pH, ionic strength, and temperature
<i>Beet Pectin</i>	Branched anionic hydrophilic polysaccharide with hydrophobic ferulic acid groups.	Requires a high surfactant-to-oil ratio, but forms droplets stable to a wide range of pH, ionic strength, and temperature
<i>Citrus Pectin</i>	Branched anionic hydrophilic polysaccharide with hydrophobic protein groups attached.	Requires a high surfactant-to-oil ratio, but forms droplets stable to a wide range of pH, ionic strength, and temperature

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1266 **Table 2.** Interfacial properties of selected synthetic and natural emulsifiers: surface tension at
1267 saturation, and surface load. It should be noted that the interfacial tension will depend on the
1268 nature of the oil phase.

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Emulsifier	Surface Tension (mN/m)	Interfacial Tension (mN/m)	Surface Load (mg/m ²)	Surface Rheology (mN/m)		References
				Dilatational Elasticity	Shear Elasticity	
Tween 20	34-45	2	1.5 - 2.5	52-74	0	[1-4]
SDS	31-65	3		2-3		[3, 5-7]
Saponins	35-41	23	2.9	260-280	10-40	[3, 8, 9]
β-lactoglobulin	22	14.5 - 21.6	1.9 - 2.3	19-26	20	[4, 10-13]
β -casein	50	19	1.2- 3	17-20	0.2	[4, 14-18]
Gum arabic	47-55	7 - 47	6 - 26	15.5-54		[19-23]

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