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Review

Food proteins: A review on their emulsifying properties using a structure–function approach

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ABSTRACT

Proteins are of great interest due to their amphiphilic nature, which allows them to reduce the interfacial tension at the oil–water interface. The incorporation of proteins at the oil–water interface has allowed scientists to utilise them to form emulsions (O/W or W/O), which may be used in food formulations, drug and nutrient delivery. The systematic study of the proteins at the interface and the factors that affect their stability (*i.e.*, conformation, pH, solvent conditions, and thermal treatment) has allowed for a broader use of these emulsions tailored for various applications. In this review, the factors affecting the stability of emulsions using food proteins will be discussed. The use of polysaccharides to complex with proteins will also be explored in relation to enhancing emulsion stability.

Contents

1. Introduction

Emulsions are defined as a dispersion of two or more immiscible liquids in which one of the liquids is dispersed in the other as small droplets (0.1–100 μm) (Dickinson, 1992; Dickinson & Stainsby, 1982; Friberg & Larsson, 1997). Typically in the food industry, emulsions are either oil-in-water (O/W) mixtures, in the case of milk, creams, salad dressings, mayonnaise and soups, or water-in-oil (W/O) mixtures, in the case of margarine and butter. In more advanced systems, multiple emulsions (W/O/W or O/W/O) (Garti, 1997; Muschiolik, 2007; Pal, 2011; Sajjadi, Zerfa, & Brooks, 2002) or nanoemulsions (Date, Desai, Dixit, & Nagarsenker, 2010; Divsalar et al., 2012; Shakeel, Shafiq, Haq, Alanazi, & Alsarra, 2012) can be formed, which are particularly advantageous for targeted drug/nutraceutical delivery applications (Divsalar et al., 2012; Shakeel et al., 2012). Though emulsions typically form droplet sizes in the 0.1–100 μm range, nanoemulsions contain dispersed droplet sizes in the ⩽100 nm range (Shakeel et al., 2012). Emulsions are formed by inducing mechanical shear to the mixture either using a homogeniser, a valve (high pressure) homogeniser or through sparging, in order to create small droplets of one liquid dispersed in the other (Schultz, Wagner, Urban, & Ulrich, 2004).

Emulsions are also formed in the presence of an emulsifier, which comprises of both hydrophobic and hydrophilic components that become integrated at the oil–water or water–oil interface to lower the interfacial tension (Bos & van Vliet, 2001). Emulsifiers may be in the form of low molecular weight synthetic (*e.g.*, monoglycerides (Goldstein & Seetharaman, 2011)), sucrose esters (Tual et al., 2006), polyglycerol esters (Su, Flanagan, Hemar, & Singh, 2006)) or natural (*e.g.*, soy or egg lecithin (Palacios & Wang, 2005)) molecules, or be comprised of larger macromolecules, such

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Table 1
Mechanistic studies utilising plant and animal proteins as emulsifiers.

Protein	Factors investigated	Measuring parameters	References
Soy and wheat gluten	Protein-type, pH, temperature, alteration to pH post-emulsification	Rheology, droplet size	Bengoechea, Romero, Aguilar, Cordobés, and Guerrero (2010)
Wheat (gliadins)	Hydrolysis, pH, ionic strength	Surface hydrophobicity, emulsion activity index, emulsion stability index, foam capacity, foam drainage	Agyare, Addo, and Xiong (2009)
Wheat (gluten), bovine serum albumin, casein	Hydrolysis, ionic strength, pH	Foam stability, foam capacity, creaming kinetics, droplet size, resistance to coalescence	Popineau, Huchet, Larré, and Bérot (2002)
Soy and corn germ protein fluor	Protein type (soy isolate vs. fluor vs. concentrate), pH, temperature	Emulsifying capacity, emulsion stability	Wang and Zayas (1992)
Soy	Temperature	Protein aggregate size, droplet size, colorimetry, interfacial composition, emulsion viscosity, TEM	Keerati-u-rai and Corredig (2009)
Egg	Protein-type (egg yolk vs. whole egg), temperature, addition of sugar, protein concentration, addition of salt (ionic strength)	Turbidity, droplet size, aggregate size (Native-PAGE electrophoresis)	Campbell, Raikos, and Euston (2005)
Egg	Protein-type (egg yolk vs. egg white), addition of xanthan gum, storage time	Droplet size, creaming test, optical microscopy, emulsion viscosity, SDS–PAGE	Drakos and Kiosseoglou (2006)
Egg	Addition of polysaccharide, pH	Surface charge, protein adsorption, droplet size	Padala, Williams, and Philips (2009)
Milk (whey)	Oil concentration, protein concentration, emulsion viscosity, addition of salt, heat treatment	Accelerated creaming test, emulsion viscosity, droplet size	Djordjevic, Kim, McClements, and Decker (2004)
Milk	Protein-type (casein vs. whey), pH modification post-emulsification, addition of salt, heat treatment	Droplet size	Hunt and Dalgleish (1995)
Milk (caseinate)	Preparation method (single step vs. two step), addition of surfactant	Creaming stability, emulsion activity index, droplet size	Einhorn-Stoll, Weiss, and Kunzek (2002)
Lentil	Protein concentration, ionic strength	Interfacial tension, surface hydrophobicity, emulsion stability index, emulsion activity index	Joshi et al. (2012)
Pea	Protein type (commercial preparations vs. isolate) pH, protein concentration	Emulsion stability, foam stability, droplet size	Aluko, Mofolasayo, and Watts (2009)
Pea, fava bean, cowpea, French bean, and soybean	Protein type, protein concentration, ionic strength, comparison of subunits	Thermal stability, SDS–Page, hydrophobicity, protein solubility, emulsion stability	Kimura et al. (2008)
Soy, pea, lentil, chickpea, and faba bean	Protein-type, extraction (isoelectric vs. salt extraction)	Surface charge, surface hydrophobicity, protein solubility, interfacial tension, emulsion capacity, emulsion activity index, emulsion stability index, droplet size, creaming stability	Karaca, Low, and Nickerson (2011a)
Chickpea	Protein extraction (ultrafiltration vs. isoelectric), concentration, protein fractionation)	SDS–Page, interfacial tension, interfacial rheology, aggregate size, droplet size, concentration of protein at interface, emulsion stability, zeta-potential	Papalamprou, Doxastakis, and Kiosseoglou (2010)
Milk (whey), canola and flaxseed	Protein-type, extraction (isoelectric vs. salt extraction)	Emulsion capacity, emulsion stability, creaming stability, emulsion activity index, surface charge, surface hydrophobicity, interfacial tension, protein solubility, droplet size	Karaca, Low, and Nickerson (2011b)
Canola	Protein concentration, pH, ionic strength, addition of hydrocolloid	Emulsion activity index, emulsion stability index	Uruakpa and Arntfield (2005)
Gelatin	Hydrolysis, oil polarity, addition of surfactant, pH	Surface tension, interfacial tension, surface charge, emulsion stability	Olijve, Mori, and Toda (2001)
Gelatin and milk (whey)	Protein content (alone and in mixtures), pH, protein concentration	Surface charge, droplet size, surface protein concentration, surface protein content, emulsion stability, emulsion rheology, optical microscopy, oil oxidative stability	Taherian, Britten, Sabik, and Fustier (2011)

as proteins (Damodaran, 2006). Emulsifiers act to form viscoelastic films around dispersed droplets to keep the emulsions stable over time.

Commonly used proteins used by the food industry for their emulsifying abilities include: whey protein isolate (Hu, McClements, & Decker, 2003; Li et al., 2012; Ye & Singh, 2006), casein (Dalgleish, 1993; Hu et al., 2003; Mulvihill & Murphy, 1991), ovalbumin (Galazka, Dickinson, & Ledward, 2000; Mine, Noutomi, & Haga, 1991; Nakamura, Kato, & Kobayashi, 1992), soy (Hu et al., 2003; Palazolo, Sorgentini, & Wagner, 2005; Puppo et al., 2011) and bovine serum albumin (Castelain & Genot, 1996; Derkach

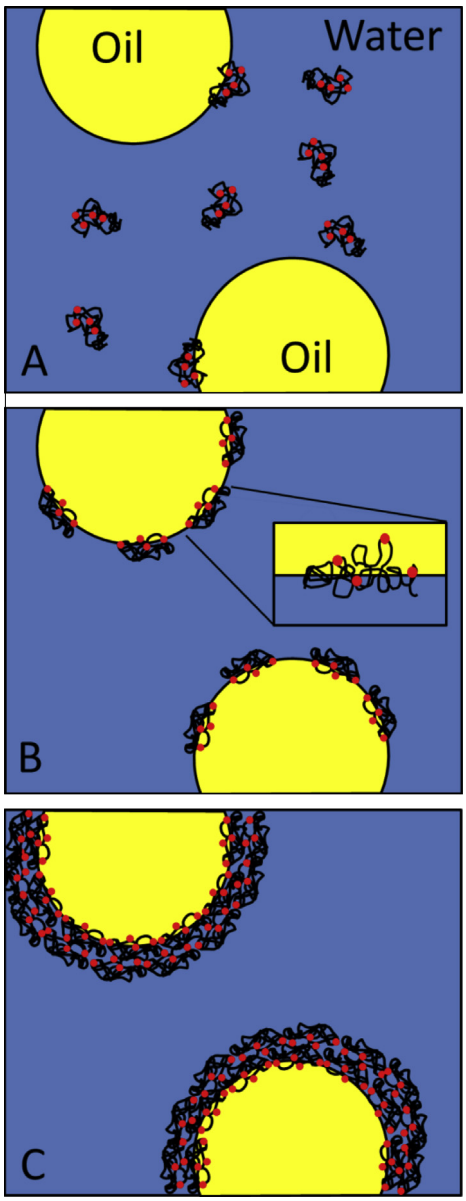

Fig. 1. A depiction of globular proteins migrating to the water–oil interface (A) followed by reorientation (B) and viscoelastic film formation (C). The red dots represent hydrophobic moieties found in proteins.

et al., 2007; Kim, Choi, Shin, & Moon, 2003). In addition, the emulsifying properties of emerging protein ingredients, such as from legume (faba bean, lentil, pea and chickpea) (Karaca, Low, & Nickerson, 2011a) and oilseed (canola (Aluko & McIntosh, 2001; Karaca, Low, & Nickerson, 2011b; Uruakpa & Arntfield, 2005) and flaxseed (Karaca et al., 2011b; Khalloufi, Corredig, & Alexander, 2009; Wang, Li, Wang, Adhikari, & Shi, 2010)) crops have also been studied. Table 1 gives a summary of various mechanistic studies involving both plant and animal proteins as emulsifiers.

2. Protein-stabilised emulsions

Proteins are of particular interest in terms of their emulsifying properties, due to their amphiphilic nature (*i.e.,* having both hydrophobic and hydrophilic groups) and film-forming abilities (Foegeding & Davis, 2011). Unlike small molecular weight emulsifiers that diffuse rapidly to the interface to give excellent emulsion

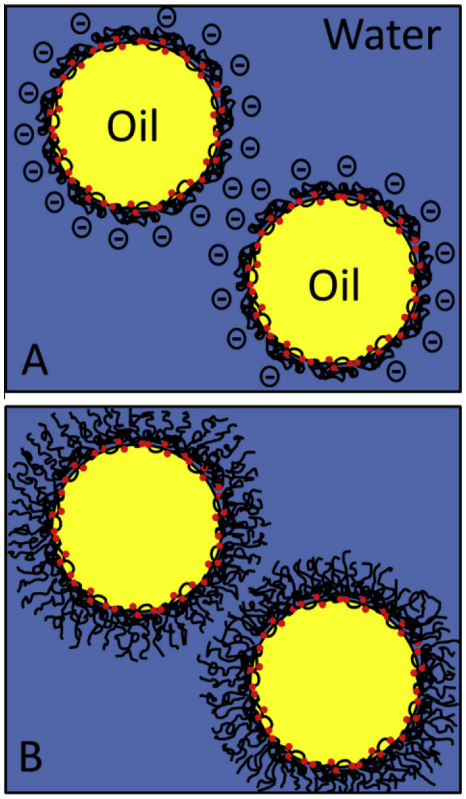

Fig. 2. Mechanisms for emulsion stability by (A) electrostatic repulsion and (B) steric stabilisation. The red dots represent hydrophobic moieties found in proteins.

forming abilities, proteins tend to be bulkier and diffuse at a much slower rate (Fig. 1A) (McClements, 2005). Once at the interface, some level of partial denaturation (or unravelling) is often needed in order to expose buried hydrophobic amino acids to the surface (Fig. 1B). Proteins then re-align themselves to position their surface hydrophobic amino acids within the oil phase and hydrophilic amino acids within the aqueous phase (Walstra, 2003). Consequently, emulsion capacity (amount of grams oil per grams protein that can be held, prior to a phase inversion taking place) tends to be lower than with small molecular weight molecules (Surh, Decker, & McClements, 2006). However, once at the interface, strong viscoelastic films can be developed (Fig. 1C) that resist mechanical stresses, and provides electrostatic (Fig. 2A) (depending on the solvent conditions) and steric (depending on the protein) stabilisation (Fig. 2B) (Tcholakova, Denkov, Ivanov, & Campbell, 2006a).

The physiochemical properties of proteins play an important role in determining their emulsifying abilities (Bueno, Pereira, Menegassi, Arêas, & Castro, 2009; Moure, Sineiro, Domínguez, & Parajó, 2006; Papalamprou, Doxastakis, & Kiosseoglou, 2010). For instance, surface hydrophobicity influences the ability for the protein to adsorb to the oil side of the interface, where greater integration typically leads to higher emulsion capacities (Kim, Decker, & McClements, 2005). In contrast, surface charge of the protein influences the solubility of the proteins within the aqueous phase, where high solubility is desired for having greater diffusion rates to the interface (Karaca et al., 2011a). Once the viscoelastic film is formed, droplets can assume a negative or positive charge depending on whether the emulsion pH is above or below the protein's isoelectric point (and low ionic strength), respectively. High electrostatic repulsion between oil droplets tends to lead to greater emulsion stability, whereas under pH conditions close to the protein's isoelectric point (or high ionic strength) droplet flocculation/aggregation dominates leading to coalescence and instability

(McClements, 2005). Depending on the protein's size, structure and conformational freedom, 'loops' or 'tails' of protein segments may radiate from the interface comprised of mainly hydrophilic amino acids, to create steric stabilisation, physically restricting droplets from coming together (Damodaran, 1996; Tcholakova et al., 2006a). The presence of protein within the continuous phase also acts to increase emulsion viscosity, reducing the mobility and diffusing of oil droplets within the emulsion (Jafari, Beheshti, & Assadpoor, 2012).

Researchers have investigated the use of partial protein hydrolysis to enhance their emulsifying properties. A variety of protein sources (milk (Agboola & Dalgleish, 1996; Chobert, Bertrand-Harb, & Nicolas, 1988; Ramkumar, Singh, Munro, & Singh, 2000), soy (Chen, Chen, Ren, & Zhao, 2011; Jung, Murphy, & Johnson, 2005; Lamsal, Jung, & Johnson, 2007), and wheat (Bombara, Anon, & Pilosof, 1997; Popineau, Pineau, Evon, & Bérot, 1999; Wang, Zhao, Yang, & Jiang, 2006)) have been studied for the effects of hydrolysis on emulsion characteristics. Partial hydrolysis has been used to improve the emulsifying properties of the protein by increasing solubility, revealing hidden hydrophobic groups, increasing its surface hydrophobicity and reduce its molecular weight, which allows for better adherence to the oil–water interface (Govindaraju & Srinivas, 2006; Radha & Prakash, 2009; Tsumura, 2009). Protein hydrolysis and the associated improvement in functionality are dependent on the degree of hydrolysis where factors such as the time, temperature and enzyme selected control the resulting product characteristics and functionality (Chobert et al., 1988; Jung et al., 2005; Lamsal et al., 2007). When proteins are highly hydrolysed, the high concentration of hydrolysed protein tends to saturate the continuous phase rather than adhere to the water–oil interface (Conde & Patino, 2007). This indicates that the use of protein hydrolysis for increased functionality is limited to low degrees of hydrolysis (Conde & Patino, 2007). In a comparative study of an air-in-water emulsion, partially hydrolysed proteins were found to decrease both the interfacial viscosity and elasticity while increasing the adherence of the proteins to the interface and improving foam stability (Ipsen, Otte, Sharma, Nielsen, & Hansen, 2001).

3. Destabilising mechanisms in protein-stabilised emulsions

Over the past decade, mechanisms driving instability within protein-stabilised emulsions have been well documented (Ercelebi & Ibanoğlu, 2007; Reiffers-Magnani, Cuq, & Watzke, 2000; Van Aken, Bomhof, Zoet, Verbeek, & Oosterveld, 2011), along with a number of comprehensive reviews (Capek, 2004; McClements, 2012; Tcholakova et al., 2006a; Van Aken, Blijdenstein, & Hotrum, 2003). Emulsions are inherently thermodynamically unstable systems, where over time the two phases move towards a more stable state that minimises free energy (*i.e.*, complete phase separation into a denser aqueous layer on the bottom and a less dense upper layer) (Capek, 2004). Under conditions where electrostatic repulsion is reduced (*i.e.*, near the isoelectric point of the protein film or in the presence of high ionic strengths), flocculation and aggregation of oil droplets can lead to partial or complete coalescence (Tcholakova et al., 2006a). The latter refers to the process where films of neighbouring droplets fuse and exchange their material, ultimately leading to the formation of a larger droplet. Due to density differences, the larger droplets gravitationally separate and migrate upwards, a process known as creaming (Robins, 2000). Although not dominant, Ostwald ripening also can occur in protein-stabilised emulsions, whereby small oil droplets diffuse through the continuous phase to merge with larger ones (Taylor, 1998).

In addition to thermodynamic instability, emulsions destabilise in the presence of competing biopolymers or changes in protein conformation during ageing. Commercial protein preparations (isolates, concentrates) are often mixtures of proteins. The presence of other proteins and/or surfactants influences the interfacial properties by competitive adsorption which is often a factor of the environmental conditions (*i.e.*, pH, ionic strength, *etc.*) in the continuous phase of an emulsion (Tcholakova, Denkov, Sidzhakova, & Campbell, 2006b). In a study on milk proteins, the dominant protein at an interface is dependent on the concentration of proteins (Sharma & Singh, 1998). Furthermore, the authors demonstrated that there were preferences to adherence on the interface even between proteins among different casein or whey types. Changes in environmental conditions affect the surface hydrophobicity of the protein and its affinity towards the interface (Rampon et al., 2004). Therefore the protein with the highest affinity towards an interface may displace another protein at the interface. Surfactant molecules (*i.e.*, tweens, spans, *etc.*) often displace proteins at the interface as their small molecular weight, manouevrability and strong affinity towards the interface allow them to displace proteins (Diftis & Kiosseoglou, 2004; Van Aken, 2003). It was found that though a surfactant may lead to the displacement of proteins from the interface, only certain surfactants, such as Span 80, would lead to coalescence by spontaneous rupture of the thin film formed between droplets (Van Aken, 2003). It was noted by Van Aken (2003) that though Span 80 is not capable of forming an emulsion itself, its displacement of the proteins at the interface led to a rapid destabilisation of the emulsion. Finally, the stability of an emulsion decreases over time, due to changes in the protein conformation in addition to the thermodynamic drive for phase separation (Tcholakova et al., 2006a). During ageing, proteins form non-covalent bonds, such as hydrogen and hydrophobic bonds, with neighbouring proteins at the interface, due to conformational changes of the protein over time (Fang & Dalgleish, 1997; Kim et al., 2005; Tcholakova et al., 2006a). This conformational change and the new non-covalent bonds formed lead to a decrease in emulsion stability (Fang & Dalgleish, 1997; Kim et al., 2005; Tcholakova et al., 2006a).

4. Protein dynamics and affinity to the oil–water interface

The oil–water interface can be considered as a planar surface with infinitesimal thickness, however the interfacial region/film is quite dynamic in nature (McClements, 2005). For instance, within this region there are water–water (*via* hydrogen bonding), oil–oil (*via* van der Waals forces) and water–oil (*via* a hydrophobic effect, whereby water molecules orient away from the non-polar triglycerides to minimise contact) interactions taking place (Mishchuk, Sanfeld, & Steinchen, 2004). With the addition of a protein to the oil–water interface, and depending on its structure/conformation, polar and non-polar amino groups may become dissolved in each phase, despite in some cases being unfavourable. Proteins will then re-orient and re-align to minimise the number of thermodynamically unfavourable interactions, resulting in a decrease in interfacial tension (Bos & van Vliet, 2001). The latter is defined as the free energy required to increase the area of an interface by a unit amount (J/m^2 or N/m) (Bos & van Vliet, 2001). By adhering to the interface, proteins form a viscoelastic film that helps stabilise the dispersed droplets (Tcholakova et al., 2006a). β-Lactoglobulin has been reported to be capable of forming a viscoelastic film at the oil–water interface where the interfacial protein film was found to be strengthened following high pressure treatment (Dickinson & James, 1999). However, the types of bonds formed (*i.e.*, hydrophobic, van der Waals, *etc.*) within the protein film will also influence whether or not it will remain at the interface (Tcholakova et al., 2006a). It has been reported that when soy proteins were heat-treated at 90 °C, proteins exhibited great surface

hydrophobicity and formed disulphide bonds with neighbouring proteins, which enhanced their emulsion stability (Wang et al., 2012). Conversely, excessive hydrophobic bonding among soy proteins treated at 120 °C caused aggregates to form, which reduced their emulsifying capabilities (Wang et al., 2012). The concentration of proteins occupying the interface tends to be at equilibrium with those in the continuous phase; therefore altering levels present may destabilise/stabilise the equilibrium and the concentration of proteins at the interface (McClements, 2005). For example, increases in protein concentration in the continuous phase often correlate with an increase in protein concentration at the interface (Conde & Patino, 2007). Furthermore, changes in concentration have been found to cause changes in the interfacial tension at the oil–water interface (Romero et al., 2011a).

Proteins contain many ionisable groups which alter the electrical characteristics of an interface of oil droplets once adsorbed (Charalambous & Doxastakis, 1989; Damodaran, 1996; Jönsson, Lindman, Holmberg, & Kronberg, 1998; Linfield, 1976; Magdassi, 1996; Myers, 1988; Richmond, 1990). These ionisable groups may contain acidic (COOH, $COO-$, or H^+) or basic (NH_2 or NH_3^+) moieties which are positively or negatively charged depending on the pH and ionic strength of the aqueous solvent (Charalambous & Doxastakis, 1989; Damodaran, 1996; Jönsson et al., 1998; Linfield, 1976; Magdassi, 1996; Myers, 1988; Richmond, 1990). In a recent study of emulsions stabilised with potato proteins, a change in pH from 2 to 8 improved emulsion stability by changes in the protein surface charge and improved the viscoelastic properties of the interface (Romero et al., 2011a). The improved emulsion stability was attributed to enhanced electrostatic repulsion of potato proteins at pH 8 and the increased elasticity of the proteins at the oil–water interface which resisted mechanical deformation of the droplets (Romero et al., 2011a). At the isoelectric pH, the net charge is neutral, which causes a tendency for the protein to aggregate (Foegeding & Davis, 2011). It is the charge and magnitude of these ionisable groups which affect the stability and physicochemical properties of protein-coated oil droplets (Guzey & McClements, 2007). When the interface is highly charged, the stability is enhanced due to electrostatic repulsion between droplets (Guzey & McClements, 2007). Charged surfaces may also attract various molecules, such as ions, antioxidants, and flavours, which may screen the charge on the interface (McClements, 2005). If the charge at the interface is neutral due to charge screening by ionisable molecules, the stability of the emulsion will be reduced due to the tendency for flocculation (Foegeding & Davis, 2011). Charged interfaces may also bind to divalent ions which may destabilise the emulsion by crosslinking proteins on different droplets, causing aggregation to occur (McClements, 2005).

The composition of the continuous phase also dictates how well the protein adsorbs to the interface with respect to quantity and time (Tcholakova et al., 2006a). When proteins adsorb, they often undergo conformational changes to reduce the interfacial tension between the two immiscible liquids (Bos & van Vliet, 2001). Conformational changes are dependent on the structure of the protein and solvent conditions, such that open random-coil structures (*e.g.,* β-casein and gelatins A, B, and F (Bohin, Vincken, Van der Hijden, & Gruppen, 2012)) may rapidly undergo changes while globular structures (*e.g.,* β-lactoglobulin, bovine serum albumin (Baier & McClements, 2005), and ovalbumin (Najbar, Considine, & Drummond, 2003)) undergo slower changes due to physical restraints (Dickinson, 1992; Malmsten, 2003; Norde, 2003). Proteins that adsorb at high rates may undergo little to no changes in conformation prior to the saturation of the interface to produce a thick interfacial layer under high concentrations (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). Yet the thickness of the interfacial layer is not arbitrary, such that a study on caseins revealed that although the minimum surface coverage was 1 mg/m^2 with a thickness of 5 nm, the maximum surface coverage was reported as 3 mg/m^2 with an interfacial layer of 10 nm (Fang & Dalgleish, 1993). It has been reported that the four types of caseins have been found to exhibit varying thicknesses at the water–oil interface (Dalgleish, 1993). Caseins occupy a thickness from 5.4 nm for α_{s1}-casein to 11.1 nm for β-casein (Dalgleish, 1993). Due to changes in conformation, proteins at the interface may display differing proteolytic rates and profiles at the water–oil interface compared to in solution (Agboola & Dalgleish, 1996; Dufour, Dalgalarrondo, & Adam, 1998). The degree of proteolysis was reported to be affected by the polarity of the oil phase due to the conformation of the protein at the water–oil interface (Maldonado-Valderrama, Wilde, Mulholland, & Morris, 2012). It was previously reported that, for β-lactoglobulins, hydrolysis at the interface was much great than in the bulk solution, which was attributed to change in conformation at the interface which enabled better access to proteolytic sites (Agboola & Dalgleish, 1996).

Solvent composition *(i.e.*, pH and ionic strength) also affects the ability for proteins to have an affinity for and adhere to the interface (Chen & Dickinson, 1993). Proteins which interact strongly with the interface will adsorb and undergo little to no change in conformation, whereas those with weak interactions will adsorb and undergo conformational changes to minimise unfavourable interactions (McClements, 2005). At extreme pHs, proteins tend to unravel or disassociate, which exposes more of its hydrophobic core, allowing it to better adhere to the interface (Tcholakova et al., 2006b). When a protein unravels, previously hidden sulphydryl groups may form disulphide bonds with itself or other proteins (Wu, Xiong, & Chen, 2011). This is beneficial if disulphide bonds formed between proteins on the same interface (Dickinson, 1992; Dickinson & Matsumura, 1991) and detrimental for proteins on different droplets (Kim, Decker, & McClements, 2002a, 2002b; McClements, Monahan, & Kinsella, 1993). When disulphide bonds are formed at the oil–water interface, enhanced emulsion stability is achieved (Wu et al., 2011). In a study where the sulphydryl groups on proteins were successively chemically blocked, emulsions formed yielded less stable emulsions. The interfacial protein layer was found to be ruptured and the oil content 'leaked' into the continuous phase (Wu et al., 2011). In contrast, a study using lentil protein isolates indicated that emulsion stability improved when the disulphide bonds were reduced (Joshi et al., 2012). The reduction in disulphide bonds reduced the amount of inter-interface bonding which leads to coalescence (Joshi et al., 2012). This indicates that though bonding at the interface is important to improve the viscoelastic properties of the interface, which increases emulsion stability, the bonds formed may also occur between two droplet interfaces which are detrimental to emulsion stability.

Changes in the interfacial layer with regards to composition, thickness and the bonds formed between them can also influence the interfacial rheology and tension at the oil–water interface, which dictates how well the interface deforms under stress (McClements, 2005). The interfacial tension measures how effective the protein is at reducing unfavourable interactions, whereas interfacial rheology determines how well proteins are arranged and interacting at the interface; this takes into account any bonds (*i.e.*, hydrophobic, disulphide, *etc.*) formed between proteins at the interface (McClements, 2005). Interfacial rheological properties consist of both elastic and fluid-like properties determined by applying a shear stress to the interface to give an interfacial elastic constant and an interfacial viscosity (McClements, 2005). It has been reported from research done on crayfish protein isolates that though changes in pH may have a minor effect on the interfacial tension and the thickness of the interfacial layer, the changes to protein conformation may drastically affect the viscoelasticity of the interfacial layer (Romero et al., 2011b). It was found by Romero and co-workers (2011b) that crayfish protein isolates exhibited

much more elasticity at pH 8 than at pH 2, allowing for greater emulsion stability; this demonstrates how the bonds formed and conformational changes of the protein affect the viscoelasticity of the interfacial layer and indicates that interfacial dynamics is an important factor contributing to emulsion stability. The rheological properties of the interface have been found to affect the bulk viscoelasticity of the creams formed (Mackie, Ridout, Moates, Husband, & Wilde, 2007). Furthermore, protein-based emulsions have been found to have improved emulsion stability in comparison to surfactant-based emulsion, which was attributed to differences in the interfacial organisation of the protein or emulsifier (Mackie et al., 2007). Proteins formed an elastic interfacial layer, due to the bonds formed between adjacent proteins, which increased the elasticity of the emulsion (Mackie et al., 2007). The superior emulsifying properties of proteins are attributed to their slower changes in conformation at the interface, which lead to a lower density network, in comparison to surfactant-based emulsions (Mackie et al., 2007). In contrast, surfactant-based emulsions undergo faster conformational changes at the interface, due to their small molecular weight which gives a well-packed, concentrated cream layer (Mackie et al., 2007).

5. Effect of polysaccharides on protein-stabilised emulsions

The addition of anionic polysaccharides to protein-stabilised emulsions may result in either a positive or negative effect on emulsion stability. The level of protein–polysaccharide interactions can depend on a large number of factors, including biopolymer characteristics (*i.e.*, size, conformation, mixing ratio, biopolymer-type, and type and distribution of reactive sites), solvent conditions (*i.e.,* pH, salts and temperature), total biopolymer concentration and emulsion preparation method (Liu, Elmer, Low, & Nickerson, 2010; Neirynck, van der Meeren, Gorbe, Dierckx, & Dewettinck, 2004; Padala, Williams, & Philips, 2009; Stone & Nickerson, 2012; Tippetts & Martini, 2012; Yin, Deng, Xu, Huang, & Yao, 2012). A comprehensive review on complex coacervation involving biopolymer interactions has been given by Schmitt and co-workers (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998), and as such is not within the scope of this review. Emulsion preparation in mixed systems can greatly influence the composition, structure and dynamic behaviour of biopolymers at the interface. For instance, homogenisation involving a mixed protein–polysaccharide aqueous solution (under specific solvent conditions) and an oil phase leads to the formation of a homogenous biopolymer film at the interface (Fig. 3A) (Weinbreck, Minor, & de Kruif, 2004). In contrast, using a multi-step process, in which a primary emulsion involving an aqueous protein and oil mixture is first formed, followed by the addition of a polysaccharide solution (under specific solvent conditions) to a pre-formed emulsion (Fig. 3B), can result in a multi-layer biopolymer interface based on the principle of layer-by-layer deposition (Guzey & McClements, 2006; Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2005).

The addition of a polysaccharide to protein-based emulsions has been reported to enhance emulsion stability. Polysaccharides have been found to electrostatically associate with proteins forming a coacervate (Liu, Low, & Nickerson, 2009). Researchers have reported that emulsions made with mixtures of protein and polysaccharide were less susceptible to destabilisation by flocculation, compared to those formed by multi-layer deposition (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). The choice of polysaccharide (*i.e.*, type and size) has also been found to greatly

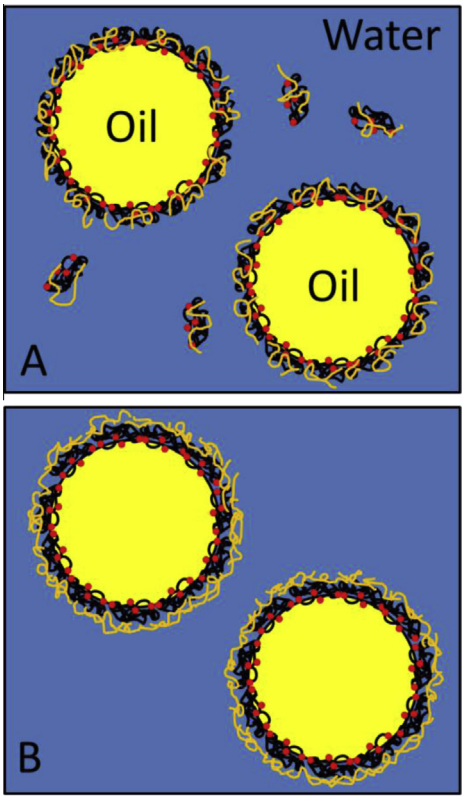

Fig. 3. Representations of a (A) homogenous mixed polysaccharide–protein emulsion and (B) and emulsion formed using a layer-by-layer deposition technique. The red dots represent hydrophobic moieties found in proteins whereas orange strands represent polysaccharides.

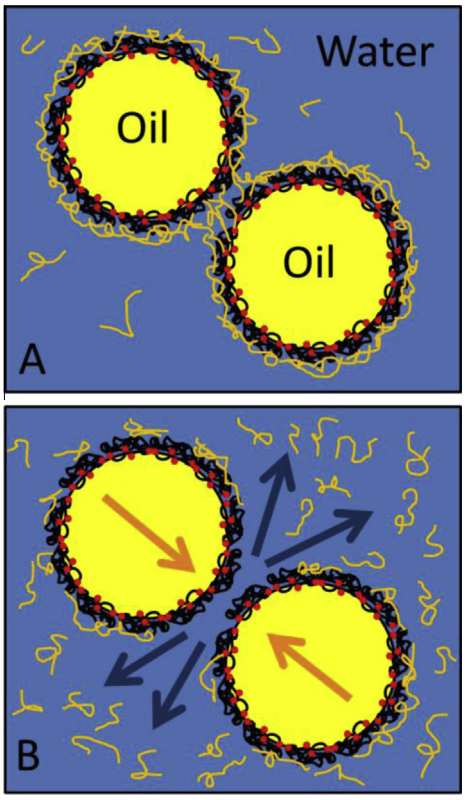

Fig. 4. Mechanisms for protein–polysaccharide emulsion destabilisation as represented by (A) bridging flocculation and (B) depletion flocculation. The red dots represent hydrophobic moieties found in proteins whereas orange strands represent polysaccharides.

affect the stability of the emulsion dependent on solvent conditions (*i.e.*, pH and ionic strength) (Hayati, Man, Tan, & Aini, 2009). Other factors such as the biopolymer characteristics (*e.g.*, protein surface charged groups), concentration, ratio, and solvent conditions (*e.g.*, pH, temperature and ionic strength) all play a significant role as to the formation of a coacervate (Chourpa, Ducel, Richard, Dubois, & Boury, 2006; Turgeon, Beaulier, Schmitt, & Sanchez, 2003; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). In a study where soy soluble polysaccharides were mixed with soy protein isolate, greater emulsion stability using this protein–polysaccharide mixture was attributed to the capacity for polysaccharides to coat the proteins to prevent protein–protein interactions between two droplets (Tran & Rousseau, 2013). When polysaccharides bind to proteins at the interface, they change the rheology of the emulsion depending on the polysaccharide used (Hayati et al., 2009). For instance, the addition of gum Arabic to an egg-protein-stabilised emulsion turned the rheology of the emulsion from a Newtonian fluid to a Bingham plastic while the use of the other polysaccharides (*i.e.,* locust bean gum, guar gum, carboxymethylcellulose and xanthan gum) resulted in shear thinning behaviour (Hayati et al., 2009). Stone and Nickerson (2012) investigated the nature of the protein–polysaccharide interaction and have reported that for three forms of carrageenan (kappa-, iota-, and lambda-) that the adsorption of carrageenan onto the whey protein isolate depended on their linear charge densities and conformation. Harnsilawat, Pongsawatmanit, and McClements (2006) found that protein–polysaccharide complexes increase emulsion stability when the polysaccharide was able to coat the protein's surface. Mixed protein–polysaccharide emulsions have also been reported to have better free-thaw stability in freeze-dried emulsions (Mun, Cho, Decker, & McClements, 2008) and have been utilised to improve the stability of double emulsions used for drug delivery (O'Regan & Mulvihill, 2010; Perrechil & Cunha, 2012).

In contrast, mixed biopolymer systems also lead to emulsion instability. Due to the electrostatic nature of this complexation, emulsions stabilised by protein–polysaccharide coacervates are sensitive to changes in pH and ionic strength (Jourdain et al., 2008; Tran & Rousseau, 2013). For instance, bridging flocculation whereby a polysaccharide molecule electrostatically complexed with a protein film surrounds more than one oil droplet, leading to aggregation (Fig. 4A) (Blijdenstein, Van Winden, Van Vliet, Van der Linden, & Van Aken, 2004). This process has been reported for bovine serum albumin–dextran sulphate (Dickinson & Pawlowsky, 1996), pea protein-high methoxyl pectin (Gharsallaoui, Yamauchi, Chambin, Cases, & Saurel, 2010), faba bean protein–dextran (Dickinson & Semenova, 1992) and β-lactoglobulin–sodium alginate (Pongsawatmanit, Harnsilawat, & McClements, 2006) stabilised emulsions. Depletion flocculation may also occur where a non-adsorbing polysaccharide in solution causes flocculation when the non-adsorbing polysaccharide is above a specific concentration (Fig. 4B) (Chanamai & McClements, 2006; Jenkins & Snowden, 1996). At the depletion flocculation concentration, when two adjacent droplets approach each other, the space between the two droplets is devoid of polysaccharide, which drives an osmotic gradient to remove the solvent between the two droplets, causing flocculation (Chanamai & McClements, 2006; Jenkins & Snowden, 1996). Depletion flocculation occurs when both the protein and polysaccharide are both similarly charged (*i.e.*, cationic or anionic) and is characterised by an increased stability below and decreased stability above the respective critical concentration (Chanamai & McClements, 2006; Jenkins & Snowden, 1996). This process has been reported for faba bean protein–dextran (Dickinson & Semenova, 1992), ovalbumin–carrageenan (Galazka et al., 2000), soy protein isolate-high methoxyl pectin (Roudsari, Nakamura, Smith, & Corredig, 2006) and sodium caseinate–kappa-carrageenan (Vega, Dalgleish, & Goff, 2005) stabilised emulsions. Both mechanisms of instability have also been reviewed by Dickinson (2003), and Syrbe and co-workers (Syrbe, Bauer, & Klostermeyer, 1998).

6. Summary

An understanding of structure–function mechanisms underpinning protein-stabilised emulsions is essential for improving their use in food products, and for broadening the use of underutilised protein ingredients (*e.g.*, pea, lentil, chickpea, canola, *etc.*) by the food industry. By controlling processing, solvent and biopolymer conditions, the interface could be controlled and possibly tailored for more high-value controlled delivery applications by the food and/or pharmaceutical industries. Control over the electrical properties, tension and the interfacial rheological properties could lead to greater advances in protein-based emulsifiers.

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