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Biopolymeric-based emulsions and their effects during processing, digestibility and bioaccessibility of bioactive compounds in food systems

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ABSTRACT

This article reviews the major type of interactions between proteins and polysaccharides which occur either by covalent bonding or non-covalent interactions. Covalent interactions are specific and occur due to glycosylation of amino group of proteins and carboxylic group of polysaccharides, while non-covalent interactions are non-specific and generally occur via electrostatic interaction, hydrogen bonding, hydrophobic and van der Waals interactions. Furthermore, this review presents the recent research conducted on the development of various proteins (animal, milk, egg, and plant based proteins)-polysaccharide conjugates, their functional properties as emulsifiers and industrial applications. Recent applications of the protein-polysaccharide conjugates to encapsulate, protect, improve bioavailability and control delivery of bioactive components are also discussed.

1. Introduction

The potential of the protein-polysaccharide complex as encapsulation and delivery vehicles for bioactive compounds, nutrients, and drugs, has gained great attention in the field of food, cosmetics and pharmaceutical industries (Jones & McClements, 2011). Proteins are known to be surface active in nature and due to amphiphilic nature act as emulsifier whereas, polysaccharides with hydrophilic nature can act as thickening agent and stabilizer (Koksel, Masatcioglu, Kahraman, Ozturk, & Basman, 2008). In food emulsion systems, polysaccharides are commonly used as stabilizers, texturizers, and health-promoting ingredients. Presence of polysaccharides in the emulsion impacts the potential gastrointestinal (GI) fate of the lipid digestion through numerous physiochemical mechanisms: (i) polysaccharides may form protective coating and inhibit the accessibility of lipase to the lipid droplets; (ii) polysaccharides may alter the colloidal interactions between the lipid droplets; (iii) polysaccharides may sequester GI components such as bile salts, fatty acids, phospholipids, or other digestive enzymes; and (iv) polysaccharides may change the mass transport of digestive components due to their ability to increase the viscosity or form hydrogel networks (Chang & McClements, 2016).

The interaction between polysaccharides and proteins is a natural phenomenon in food systems for improving the texture, stability, and quality of wide range of colloidal systems including, emulsions, gels, dispersions, foams and their mixed variants (Semenova, 2017). The combination of protein and polysaccharide in food system results in synergistic effects with various applications for the development of new nano, micro or macrostructures, improvement of the food system and cost reduction. Protein and polysaccharide molecules are linked together by either covalent interaction (formation of Maillard reaction products) or number of non-covalent interactions (H-bonding, steric exclusion, electrostatic and hydrophobic). When present together in a system, the physical interactions between the polysaccharides and proteins are either attractive or segregative, depending on the environmental conditions such as pH, temperature and concentration (Patino & Pilosof, 2011). The physicochemical properties of these biopolymers and their interactions are also influenced by numerous other factors such as molar mass, molecular conformation, polydispersity, charged density, concentration, pH, ionic strength, temperature, solvent quality, and nature of interactions (Goh, Sarkar, & Singh, 2014) (see Table 1).

In many food systems, lipids play a vital role to regulate the physicochemical properties, provide texture and flavor. In the human body, dietary lipids act as a source of energy, essential fatty acids, and fat-soluble vitamins. However, various health problems including obesity, cardiovascular disease, diabetes and others, are related to the high lipid digestion and absorption within the GI tract (Bellesi, Ruiz-Henestrosa, Maldonado-Valderrama, Santaella, & Pilosof, 2018). Therefore, understanding the mechanism and controlling the lipids digestibility within the human GI tract is gaining interest in food and pharmaceutical industries to design a lipid-based delivery system to encapsulate, protect, improve bioavailability and control delivery of bioactive components

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Table 1
Summary of interaction mechanisms between different types of protein and polysaccharides, their analyses and applications.

Protein-polysaccharide	Conditions	Interactions	Analysis techniques	Functionality or application	References
Milk protein + Polysaccharide					
Whey protein concentrate + polysaccharides (sodium alginates and λ-carrageenan)	Protein (1.0%) and polysaccharide (0.0–1.0%) in aqueous solution at pH 7.0, mixed for 30 min at room temperature (20–23 °C)	Attractive interaction	Fluorescence spectroscopy, absorption spectroscopy, confocal laser scanning microscopy	Protein-polysaccharide interaction resulted in hybrid macromolecular entities (biopolymer network) which is the basis of the excellent interfacial viscoelastic properties and foaming characteristics.	Perez et al., 2009
Milk protein + polysaccharide (carboxymethylcellulose and guar gum)	Skimmed milk powder (11% w/w) and polysaccharide (0.0–0.4% w/w) were stirred at room temperature for 1 h.	Attractive interaction	Microstructure, surface adsorption, interfacial tension, creaming, rheological properties, fluorescence spectroscopy and zeta-potential	The associative interaction between milk protein and carboxymethyl cellulose resulted in three-dimensional network that strengthened the stability against extensive flocculation of the ice cream mix models.	Cheng et al., 2015
Sodium caseinate + arabic gum	Protein (0.1–0.5%) and polysaccharide (0.01–5%), Temperature 20 to 80 °C.	Hydrophobic interaction	Dynamic light scattering, Fluorescence, NMR spectroscopy	Sodium caseinate-arabic gum complex can potentially be used to form complex surface layers of emulsion droplets.	Ye, Edwards, Gilliland, Jameson, & Singh, 2012
Milk protein (sodium caseinate and whey protein isolate) + pectin (high and low degree of methylation)	pH 5.8 to 7.0, temperature 50 to 60 °C, humidity 65 and 80%, mixing ratio 1:1 to 1:5 for up to 15 days	Maillard reaction	Color, emulsifying activity, emulsion stability	The emulsifying properties of whey protein isolate were highly improved by conjugation with pectin.	Einhorn-Stoll et al., 2005
Sodium Caseinate + resistant starch	Mixed at 1:1, pH 7.5 heating at 100^0 C for 12 min followed by freeze drying	Maillard reaction	SDS-PAGE, encapsulation efficiency	Presence of resistant starch along with protein showed improved encapsulation efficiency of the fish oil microcapsules.	Chung et al., 2010
Gelatin + Polysaccharide					
Gelatin + cashew gum	pH 4 to 4.5	Electrostatic interaction (Coacervation)	Zeta-potential, encapsulation efficiency, accelerated stability study	Gelatin-cashew gum coacervates were able to encapsulate and enhance the stability of astaxanthin, and improve solubility, oxidative stability and dispersibility in selected food matrix model.	Gomez-Estaca et al., 2016
Fish Gelatin + arabic gum	pH 2.5 to 8.0 Gelatin to arabic gum ratio 10:90 to 90:10	Electrostatic interaction (Coacervation)	turbidimetry, methylene blue spectrophotometry, zeta potentiometry, dynamic light scattering, protein assay, state diagram and total biopolymer concentration	The interaction mechanism between fish gelatin and arabic gum showed applications in different areas such as microcapsule formation, textural modification, emulsion stabilization and fat replacer development.	Yang et al., 2012
Gelatin (hide) + iota-carrageenan	Stirring at 65 °C for 30 min.	Associative interaction	Methylene blue spectrophotometric method	The associative interaction lead to the formation of insoluble complexes and soluble aggregates depending on the biopolymer ratio.	Michon et al., 2002
Fish gelatin + arabic gum	Fish gelatin to arabic gum 1:1 pH 3.6, 5.0 and 9.0	Electrostatic attractive interaction	Confocal laser scanning microscopy, rheological characterization	The complexes formed exhibited greater creaming stability and higher viscoelastic moduli. Such complexes showed applications in the development of emulsion-based food products with improved stability and desirable textural attributes.	Anvari & Joyner, 2017
Plant protein + polysaccharide					
Potato protein + carboxymethylcellulose	pH 2.5	Electrostatic interaction (precipitation)	Protein solubility, emulsifying properties	The electrostatic interaction improved protein solubility and its ability to stabilize the emulsion against creaming and foam system against liquid drainage.	Vikelouda & Kiosseoglou, 2004
Pea proteins (vicilin and legumin) + arabic gum	pH adjustment	Electrostatic complexes	Turbidimetric, surface charge, fluorometric measurement	Complex formation between pea protein and arabic gum occurred over pH range where biopolymers exhibited opposing charges. The electrostatically bound protein-protein-arabic gum complex provided additional stability.	Klassen & Nickerson, 2012
Pea protein isolate + alginate	pH (1.5–7.0) Mixing ratio of protein: polysaccharide (1:1 to 20:1)	Associative phase separation (complex coacervation)	Turbidimetric analysis, electrophoretic mobility	Pea protein isolate-alginate, pH dependent complexes are useful in designing pH-sensitive	Klemmer et al., 2012

(*continued on next page*)

Table 1 (*continued*)

Protein-polysaccharide	Conditions	Interactions	Analysis techniques	Functionality or application	References
				biopolymer carriers in functional foods and bio-material application.	
Pea protein isolate + arabic gum	pH (4.3–2.4)	Complex coacervation	Turbidimetric acid titration	Pea protein isolate-Arabic gum complexes showed improvement in functional attributes such as pH solubility range, emulsifying and foaming stability compared to protein alone.	Liu et al., 2010
Soybean protein isolate + arabic gum	pH (2.5–4.5), Ionic ratio (0–2), soybean protein isolate/arabic gum ratio (2:5 to 9:5)	Complex coacervation	Absorbance measurement, coacervate yield	Microencapsulation of sweet orange oil by soy protein isolate-arabic gum complex showed well retention of flavor components and good protection of core material.	Jun-xia et al., 2011
Faba bean legumini + chitosan	Titrated to pH 7.3 for legumini or 6.1 for chitosan, mixed in various proportion, final pH adjusted to 6.3	Electrostatic interaction	Ultraviolet spectroscopy, viscometry calorimetry, turbidimetric titration	Soluble complexes formed by interaction between legumin and chitosan showed increase in thermal stability, and emulsifying properties.	Plashchina et al., 2001.
Soy whey protein isolate + fenugreek gum	Dry heating (60 °C, 75% relative humidity for 3 days)	Covalent (Maillard reaction)	Protein solubility, SDS-PAGE, emulsion stability	Soy whey protein isolate-fenugreek gum conjugate improved the protein solubility and emulsifying properties at isoelectric pH of protein and high salt concentration compared to soy whey protein isolate alone.	Kasran et al., 2013
Soluble isolated wheat protein fraction + dextran	Dry heating (60 °C, 75% relative humidity for 5 days)	Covalent (Maillard reaction)	$-NH_2$ groups, SDS-PAGE, tryptophan fluorescence emission spectroscopy, interfacial layer thickness, and emulsion stability	Soluble isolated wheat protein- dextran conjugated complex contributed to a thicker interfacial layer and enhanced steric stabilization of emulsion droplets at acidic pH compared to protein alone.	Wong et al., 2011
Soy-protein isolate + culled banana resistant starch	Hydrated in warm water (60 °C, 1:1 ratio, w/w) followed by heating (100 °C for 90 min)	Maillard reaction	FTIR, emulsion stability	Soy protein isolate-culled banana resistant starch conjugate enhanced the microencapsulation efficiency, oxidative stability and masked the fishy flavor compared to soy protein isolate alone.	Nasrin & Anal, 2015
Rice protein hydrolysate + glucose, lactose, maltodextrin and dextran	Mixed at 1:1, pH 11, heating at 100 °C for different time periods	Maillard reaction	Emulsifying properties, solubility, fluorescence analysis, hydrophobicity, amino acid analysis, molecular weight distribution	The Maillard reaction product resulting from conjugation of rice protein hydrolysates and polysaccharides showed improvement in solubility, emulsification activity and stability.	Li et al., 2013
Peanut protein isolate + dextran	Mixed at 1:1 wt ratio, dry-heated at 60 °C and 79% relative humidity for 7 days	Maillard reaction	SDS-PAGE	Peanut protein isolate-dextran conjugate showed improvement in thermal stability of protein, compacted tertiary conformation and emulsifying and foaming properties compared to peanut protein isolate alone.	Liu et al., 2012

(McClements & Li, 2010). Various functional bioactive compounds need to be delivered in the designed edible forms, such as food, pharmaceutical and medical products. These bioactive components such as essential fatty acids, carotenoids, antioxidants, phytosterols and others differ in their molecular, physiochemical and physiological properties which address the need of designing the delivery system unique to each of these bioactive components (McClements, Decker, & Weiss, 2007).

In order to improve the dispersibility, stability, and bioavailability of lipophilic drugs and nutrients, oil-in-water emulsion system is one of the most effective delivery systems (Kotta et al., 2012). For this purpose, protein stabilized oil-in-water (o/w) emulsions have been used extensively. The digestion and adsorption of bioactive compounds are dependent on the physical and chemical stability of the emulsion, which in turn is influenced by a broad range of conditions, such as pH, temperature, ionic strength, and presence of other surface-active components and digestive enzymes. The emulsions stabilized by protein alone lose the stability under aforementioned conditions while the addition of polysaccharides into protein-stabilized emulsion leads to stability of the emulsion droplets under simulated gastric conditions (Lesmes & McClements, 2012; Xu et al., 2014; Yang et al., 2015). The composition and structure of the emulsions undergo numerous changes during passage through the GI tract as well. The bioavailability of the encapsulated core material and emulsified oil depends on the digestion of the emulsion in human GI system. The GI digestion of lipid droplets depends on the influence of emulsifiers on the stability of lipid droplets within the GI tract (Chang & McClements, 2016). The protein begins to hydrolyze in the gastric phase due to action of pepsin. The partially digested emulsion is further hydrolyzed by different enzymes (trypsin, chymotrypsin, and lipase), coenzyme (colipase) and surface-active components (bile salts and phospholipids) in the small intestine (Bellesi et al., 2018). This fact provides a great opportunity to design the target-oriented emulsion system with improved bio-accessibility and controlled release of encapsulated materials.

This review aims further to reveal the nature of interactions between proteins and polysaccharides and the fate of protein-polysaccharide stabilized emulsions during processing and in GI tract.

2. Mechanism of proteins and polysaccharides interactions

The major type of interactions between protein and polysaccharide involve bonding between naturally occurring complexes such as electrostatic interaction between oppositely charged polysaccharide and protein and conjugation by Maillard reaction (Evans, Ratcliffe, & Williams, 2013). Protein and polysaccharide interact together by covalent bonding or non-covalent bonding. Covalent bonding between carboxylic group of polysaccharides and the amino group of proteins is induced by the dehydration of the complex (e.g. Maillard reaction, Fig. 1) under controlled humidity or addition of a crosslinker. This interaction is also known as glycosylation of protein and polysaccharide and results in specific, strong and importantly permanent conjugates (Wijaya, Patel, Setiowati, & Van der Meeren, 2017). Maillard-type conjugates produced by the dry-heating of a mixture of these two kinds of biopolymers improve the protein solubility, colloidal stability and interfacial functionality (Patino & Pilosof, 2011).

When oppositely charged biopolymers are mixed together, attractive electrostatic interaction occurs leading to the formation of soluble or insoluble complexes depending on the strength of the interactions. At pH near to isoelectric point (pI) of the protein, the interaction between cationic proteins and anionic polysaccharides forms a relatively stable soluble colloidal complex (Wijaya et al., 2017). Since the opposite charges carried by the complex formed, are not equal, the resulting net charge allows the complex solubilization by interaction with solvent molecules (Fig. 2a). On the other hand, the binding of proteins and polysaccharides with an equal number of opposite charges results in electrically neutralized insoluble complex leading to phase separation known as associative phase separation between the complexes and solvent (Fig. 2b).

The associative phase separation results in a two-phase system consisting of a phase rich in both biopolymers and another phase depleted in both biopolymers. The biopolymer-rich phase may be either a coacervate or a precipitate, depending on the strength of the attraction and the nature of the polymers involved (McClements, 2006). Stronger electrostatic complexes are formed in the mixtures of negatively charged polysaccharides and positively charge proteins (pH < pI), while weaker reversible electrostatic complexes are formed in the mixture of negatively charged polysaccharides and proteins carrying nearly net zero charge (pH ≈ pI) or net negative charge (pH > pI) (Patino & Pilosof, 2011).

When two polymers with the charge of same sign are mixed together at very low ionic strength, repulsive electrostatic interaction occurs. At low concentration, two biopolymers are co-soluble in a single phase (Fig. 2c). However, when the biopolymer concentration exceeds a certain level, thermodynamic incompatibility or segregative phase separation occurs resulting in two-phase solution with one of the phases being rich in one type of biopolymer and depleted in the other type, and vice versa. The behavior of biopolymer blends under different solution and environmental conditions can be conveniently characterized in terms of phase diagrams which can be utilized to optimize the biopolymer composition required to produce a solution with a particular microstructure and physicochemical properties (McClements, 2006) (Fig. 2d). The thermodynamic incompatibility generally results in the condition when the protein is in the presence of a neutral polysaccharide or of an anionic polysaccharide bearing a charge of the same sign as the protein (close to neutrality); obviously, the main parameters involved in the mechanisms are pH and ionic strength (Doublier, Garnier, Renard, & Sanchez, 2000).

The prime attribute of an emulsion for industrial application includes ability of emulsion to prevent both physical and chemical deformities during storage at normal or extreme conditions. Nevertheless, the emulsions are thermodynamically unstable i.e. tendency to separate into constituent oil and aqueous phase over prolong time. Fig. 3 illustrates the major kinds of instability processes exhibited by o/w emulsions: creaming, sedimentation, flocculation, coalescence and Ostwald ripening. The tendency of the droplets to flocculate, sediment or colloid causes serious physical instability in emulsion. Therefore, to exhibit stability, emulsion should resist the breakdown into constituent oil and aqueous phases, as indicated growth in average droplet size or change in their spatial distribution (Dickinson, 2009). The theoretical analysis of emulsion instability mechanism has been discussed in detail in some of the recent reports (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; Dickinson, 2009; McClements, 2007, 2015).

3. Protein-polysaccharide biopolymer for emulsion

An emulsion system comprises of two immiscible liquids mainly oil and water where one liquid is dispersed as discrete spherical droplets into the other (Kakran & Antipina, 2014). Based on phase distribution, emulsion can be either oil-in-water (o/w, oil as dispersed phase) or water-in-oil (w/o, water as dispersed phase). Besides these, multilayer emulsions such as oil-in-water-in-oil (o/w/o) or water-in-oil-in-water (w/o/w) are generally prepared with the aim to control release, reduce total fat content, or to isolate one ingredient from another. Recently, fabrication of emulsion with smaller droplet diameter (d < 100 nm) is gaining interest compared to conventional emulsion with droplet diameter ranging between 100 nm and 100 μm in most of the food products (McClements, 2015).

Colloidal delivery systems, such as emulsions, nanoemulsions, and microemulsion, are gaining interest in food and beverage industries for encapsulation of bioactive compounds. However, each of these systems has specific physicochemical properties that distinct them from each other. Emulsions droplets size is large, i.e., mean radii > 100 nm, hence emulsions are thermodynamically unstable and tend to appear either

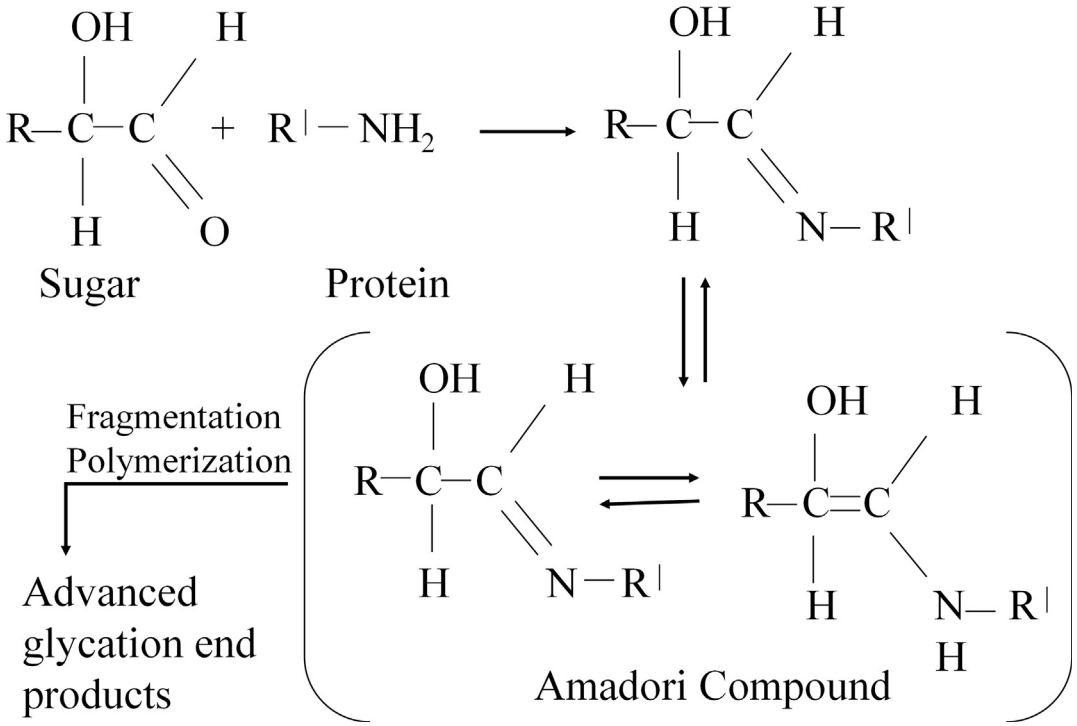

Fig. 1. Schematic representation of reaction between reducing group of polysaccharides with a free amine group of proteins (Adopted from Evans et al., 2013).

turbid or opaque. Nanoemulsions have relatively small droplet size i.e., mean radii < 100 nm, but they are also thermodynamically unstable. Microemulsions exhibit smallest droplet size (mean radii < 50 nm) therefore, they scatter light weakly and appear either transparent or slightly turbid (Rao & McClements, 2011a). Microemulsions are thermodynamically stable systems that typically consist of oil, surfactant (co-surfactant/co-solvent) and water (Flanagan & Singh, 2006). Microemulsions, therefore, have the advantages of thermodynamic and long-term stability, optical transparency, and ease of preparation. Application of microemulsions as potential delivery systems for lipophilic or poorly water-soluble drugs and other bioactive compounds is gaining interest in functional foods and pharmaceuticals (Lin, Lin, Chen, Yu, & Lee, 2009). The development of food grade nanoemulsions and microemulsions is gaining interest as numerous foods and beverage products are desired to be either transparent or slightly cloudy (Rao & McClements, 2011b).

The kinetically stable emulsions are formed for a reasonable period of time by using intense mechanical forces and/or using surface active agents, also known as emulsifiers that adsorb in the interfacial layer (Kuhn & Cunha, 2012). Biopolymers such as proteins and polysaccharides are generally used for the stabilization of the dispersed phase that protects them against phase separation (Adjonu, Doran, Torley, & Agboola, 2014; Geetha & Tyagi, 2012). Due to the amphiphilic property i.e., presence of both hydrophilic and hydrophobic moieties, proteins have the tendency to lower the surface tension and adsorb strongly at the oil-water interface. The adsorbed protein molecules stabilize the emulsion system through electrostatic and or steric repulsion which in turn prevents droplet agregation and coalescence

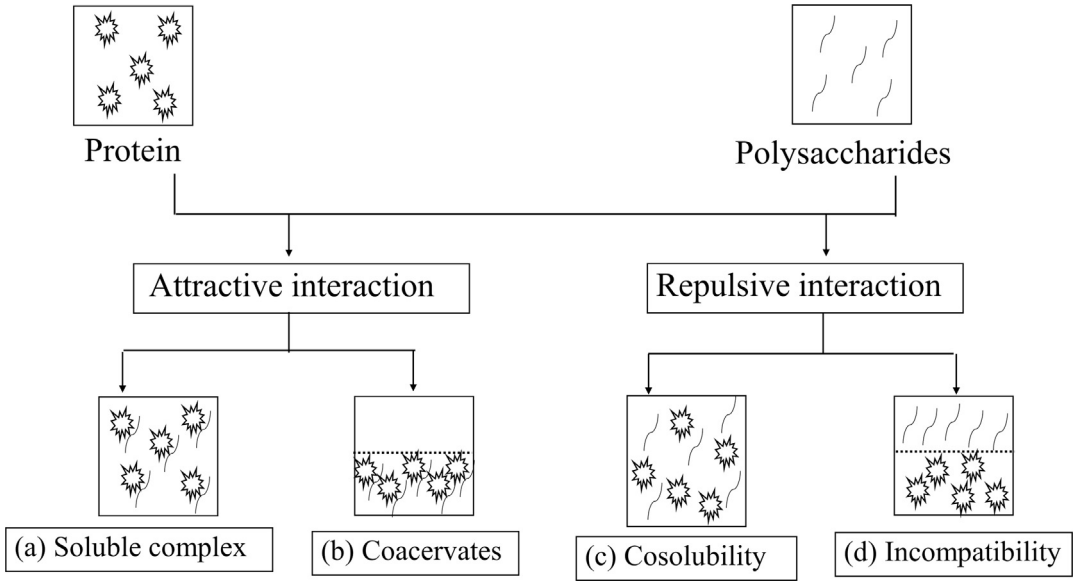

Fig. 2. Schematic representation of protein-polysaccharide interactions (Adopted from McClements, 2006).

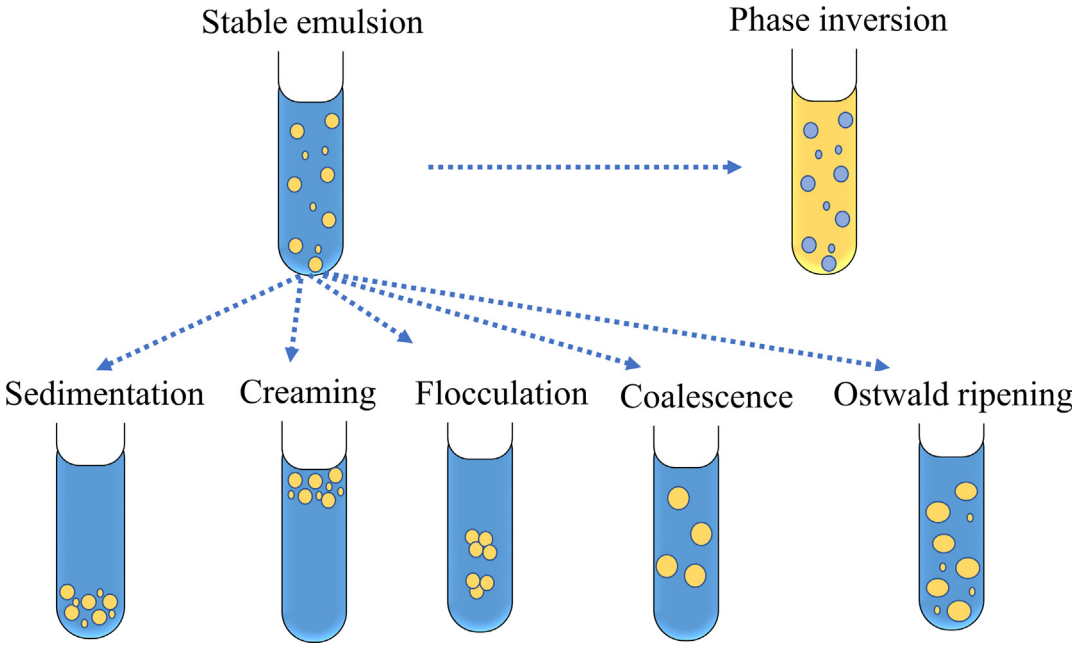

Fig. 3. Schematic presentation of emulsion instability mechanisms.

(Evans et al., 2013). However, industrial application of proteins as emulsifiers are limited as proteins may lose emulsifying properties at different conditions, including high ionic strength, presence of organic solvent, high temperature, or acidic conditions (Akhtar & Ding, 2017). On the other hand, polysaccharides act as emulsion stabilizer over the wide range of environmental conditions including ionic strength, temperature, pH etc. The addition of polysaccharides in emulsion, even regulate the rheology of the dispersed phase, influencing the emulsion stability and the creaming behavior (Tippetts, Shen, & Martini, 2013).

As shown in Fig. 4, the emulsions stabilized by protein-polysaccharide conjugate have thicker stabilizing layers compared to emulsion stabilized by protein alone. The molecular weight of the polysaccharide determined the thickness of the steric stabilizing layer (Akhtar & Ding, 2017). The protein-polysaccharide conjugations stabilize the emulsions and emulsion-based food products (Dickinson, 2008; McClements, 2010; Nasrin & Anal, 2014; Shrestha, Sadiq, & Anal, 2018).

3.1. Animal protein-polysaccharides stabilized emulsion

3.1.1. Gelatin-polysaccharides stabilized emulsion

Collagen is a fibrous animal protein responsible for the structural sustainability of animal tissues. Gelatin is derived from animal collagen (pig, cow, fish) by hydrolyzing either in acidic or alkaline condition, resulting in Type A gelatin (pI ~ 7 to 9) and Type B gelatin (pI ~ 5), respectively. Gelatin is slightly surface active and exhibits high molecular weight (Bouyer et al., 2012). Gelatin is widely used in the food industries for gelation and viscosity enhancement, and in the pharmaceutical industries for the manufacture of soft and hard capsules. It is composite mixture of three protein fractions: free α-chains, β-chains; the covalent linkage between two α-chains and γ-chains, and the covalent linkage between three α-chains. It lacks appreciable internal structures due to which in aqueous solution at sufficiently high temperatures the peptide chains have random configurations similar to the behavior of synthetic liner-chain polymers (O'Sullivan, Murray, Flynn, & Norton, 2016).

Collagen and gelatin have widely been used as emulsifiers due to

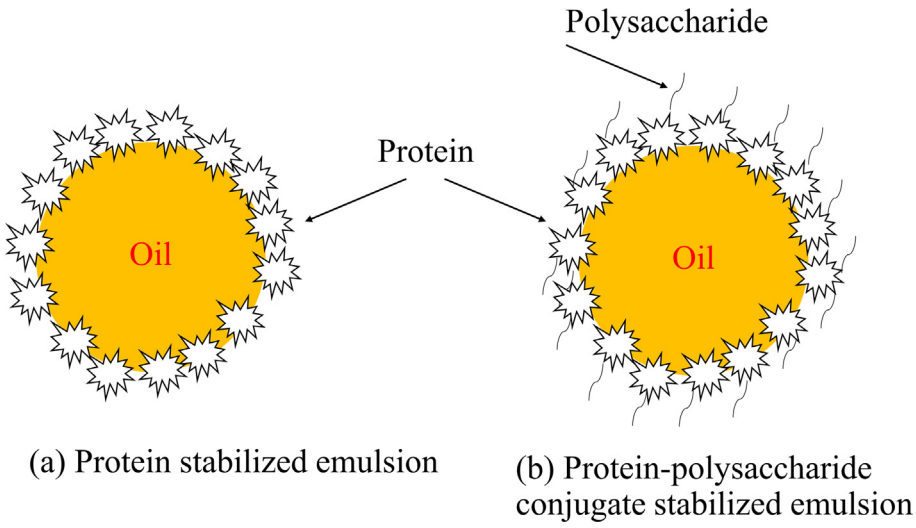

Fig. 4. Schematic presentation of emulsions stabilized by protein alone (a) and protein-polysaccharide conjugate (b) (adopted from Akhtar & Ding, 2017).

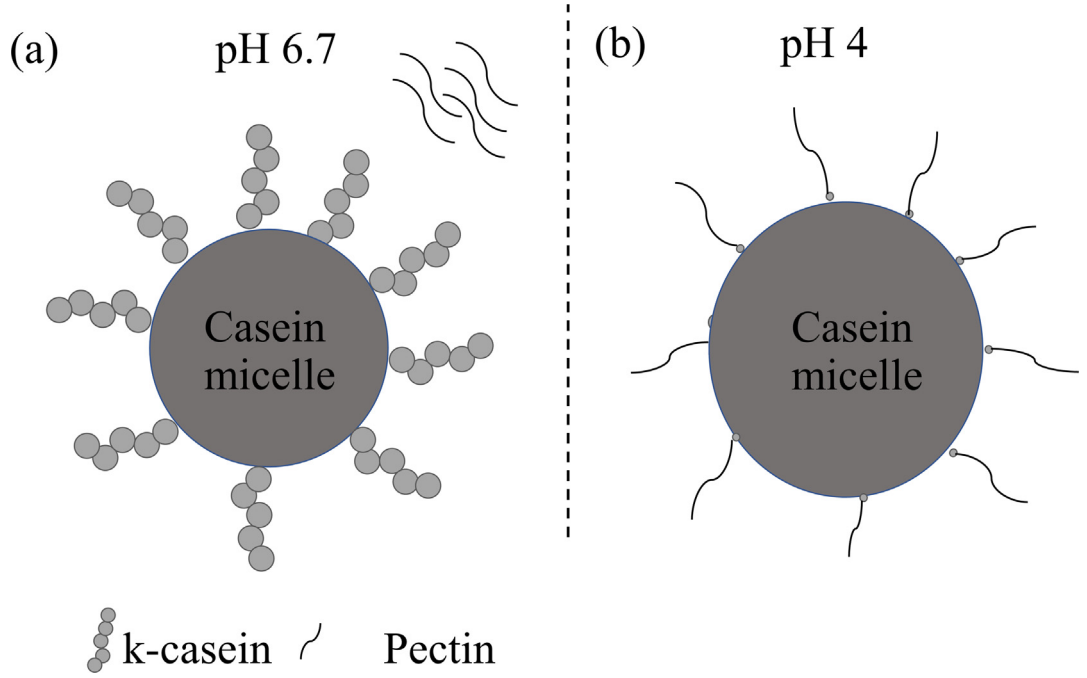

Fig. 5. Schematic representation of (a) stability of casein micelle and (b) replacement of κ-casein on lowering pH (adopted from Tromp et al., 2004).

their properties to form, stabilize and produce desirable physicochemical properties in oil-in-water emulsions and found to improve the elasticity, consistency, and stability of the foods (Dhakal, Koomsap, Lamichhane, Sadiq, & Anal, 2018; Graça, Raymundo, & de Sousa, 2016). The relatively high isoelectric point of Type A gelatin signifies its ability to form oil-in-water emulsions with positive charge over a wider range of pH values as compared to conventional protein emulsifiers (soy or whey proteins). Further, Type A gelatin helps to stabilize the oil-in-water emulsion with high oxidative stability due to its ability to repel iron ions from oil droplet surfaces over the wide pH range of food. However, gelatin alone as sole emulsifier produces relatively large droplet size. This imparts the need of gelatin to either be used together with anionic surfactants or modified hydrophobically by attachment of non-polar side-groups (Surh, Decker, & McClements, 2006).

Over the mammalian gelatin, fish gelatin derived from warm- and cold-water fish skin, bones and fins, is gaining interest as it facilitates fish processing industry byproduct utilization and has no concern regarding ethnic- or safety associated with the transmission of pathogenic vectors such as bovine spongiform encephalopathy (Feng, Lai, & Yang, 2014). Nevertheless, fish gelatin is not suitable for complete replacement of animal gelatin as it has weaker gelling strength and lower gelling and melting temperatures. Therefore, in order to improve its thermal and rheological properties, fish gelatin is conjugated with different polysaccharides, such as κ-carrageenan, hydroxypropyl methylcellulose, pectin, and chitosan (Yang, Anvari, Pan, & Chung, 2012). Coacervation, colloidal phenomenon due to interaction between positively charged proteins and negatively charged polysaccharides, is one of the techniques utilized for encapsulation of thermolabile compounds. Coacervates formed by interaction between gelatin and cashew gum (pH 4 to 4.5) were used to encapsulate astaxanthin extracted from shrimp forming multinucleated polymorphic microcapsules. The microcapsules revealed an improvement in astaxanthin stability under accelerated stability study and its suitability for product development (Gomez-Estaca, Comunian, Montero, Ferro-Furtado, & Favaro-Trindade, 2016). Among different polymer combination, gelatin and arabic gum have been extensively studied. In a research work by Yang et al. (2012) fish gelatin underwent complex coacervation with arabic gum depending on pH and gelatin to arabic gum ratio, possibly via conformational change. Michon, Konaté, Cuvelier, and Launay (2002) revealed the existences of associative interaction between gelatin and iota-carrageenan either when the carrageenan is in coil or helix conformation, further forming soluble aggregates or insoluble complexes based on gelatin to iota-carrageenan ratio.

Many food products are based on concentrated emulsions containing both proteins and polysaccharides with the dispersed phase size within the colloidal range. Rheological and microstructural properties of emulsions are regulated by presence of biopolymers and dispersed oil phase such that Newtonian fluid at oil phase volume fractions < 0.4 changes to highly flocculated and concentrated emulsion (thick cream-like with a yield stress and gel-like properties) at oil phase volume fraction > 0.4. Concentrated emulsion prepared from fish gelatin-arabic gum complexation at different pH range (3.6, 5.0, and 9.0) showed dependency of emulsion rheological properties on biopolymer association (Anvari & Joyner, 2017).

3.1.2. *Milk protein-polysaccharides stabilized emulsion*

Bovine milk protein is mainly composed of casein (80%) and whey proteins. In casein micelle (aggregated structure of, α_{s1}-, α_{s2}-, β- and κ-caseins), a large proportion of κ-casein located on the surface that imparts steric stability by protruding into the serum phase with its hydrophilic glycosylated portion. The interior of the casein micelles is not accessible to larger molecules; therefore, the polyelectrolyte layer of κ-casein is responsible for the attraction or repulsion interaction with polysaccharides molecules (Corredig, Sharafbafi, & Kristo, 2011).

In milk (pH 6.7), κ-casein chains protrude from the surface of the casein micelle and casein micelles remain in the suspended state due to steric repulsive interaction. The κ-casein chains protrude in order to maximize their entropy which cause a repulsive mutual interaction between the micelles. This phenomenon, also known as steric stabilization is illustrated in Fig. 5. On the other hand, during milk acidification, at pH close to the isoelectric point (pH 4.6), the extended conformation of κ-casein chain collapses, resulting in aggregation of the casein micelles due to loss of the native stabilization mechanisms (Tromp, de Kruif, van Eijk, & Rolin, 2004).

This signifies the importance of the addition of stabilizer, such as high methoxyl pectin, soybean soluble polysaccharides (SSPS), propylene glycol alginate (PGA), carboxymethylcellulose (CMC) etc., in acidified milk drinks (pH 3.6 to 4.6) in order to prevent the flocculation

of milk proteins and subsequent macroscopic whey separation (Du et al., 2009). This adsorption of pectin with casein at low pH is used in steric stabilization of acidified milk drinks. When pectin is added in acidified milk systems, pectin adsorbs onto micelle surface at the charged blocks, while the uncharged block stretches and form entropy-rich loops extending to the aqueous solution. The resulting repulsive interaction between the micelle at low pH is similar to steric repulsion caused by κ-casein at native condition (Tromp et al., 2004). At lower pH, pectin molecules adsorb onto the casein micelle as the result of an electrostatic interaction. In contrast, pectin does not adsorb onto casein micelles at neutral pH leading to segregated phase separation (Maroziene & De Kruif, 2000).

Carrageenan is used to maintain the colloidal stabilization at pH of milk. The κ-carrageenan interacts synergistically with milk proteins i.e. casein micelles to enhance viscosity and gelation. The interaction of κ-carrageenan with casein micelles may be due to (i) the interaction between negatively charged κ-carrageenan with a positively charged region of κ-casein between the residues 97 and 112, and (ii) the formation of weak κ-carrageenan gel that holds casein micelles suspended, even though concentrations required for stability are below the critical gelling concentration (Spagnuolo, Dalgleish, Goff, & Morris, 2005). The associative interaction between κ-casein and κ-carrageenan results in the prevention of macroscopic phase separation and destabilization. κ-carrageenan adsorbs on the surface of the casein micelles. The helix-helix association between the chains cause the molecules to bridge with each other, resulting in weak three-dimensional network and structure stabilization (Corredig et al., 2011).

Perez, Carrara, Sánchez, Patino, and Santiago (2009), studied the molecular dynamics of the mixed system of whey protein concentrate (1.0%, w/v) with anionic polysaccharides (sodium alginates and λ-carrageenan, in range 0.0–1.0%, w/v) separately, in the aqueous phase at pH 7.0. The nature of the interaction was observed to be dependent on the type and relative concentration of polysaccharide. The interaction of whey protein isolate and polysaccharides showed effect on the adsorption of mixed biopolymers at the air-water interface and on foaming characteristics. Cheng, Ma, Li, Yan, and Cui (2015) studied the effect of milk protein-polysaccharide (carboxymethylcellulose and guar gum) interaction on the stability of ice cream model emulsions systems based on microstructure, surface adsorption, interfacial tension, creaming, rheological properties, fluorescence spectroscopy and zeta-potential. The attractive interaction between milk protein and carboxymethylcellulose exhibited lower creaming rate and enhanced apparent viscosity compared to milk protein and guar gum mixture system.

The protein-polysaccharide interaction by controlled dry heating method is suitable for the production of emulsifiers. Einhorn-Stoll, Ulbrich, Sever, and Kunzek (2005) formed complexes by controlled dry heating from milk proteins; whey protein isolate and sodium caseinate with pectin to achieve high and low degree of methylation, with the aim of producing conjugate with high emulsifying properties. Whey protein isolate and high methoxyl pectin, mixed at the ratio 1:3 when incubated at 60 °C and 80% humidity for 10 days showed the best emulsifying properties compared to sodium caseinate conjugated with pectin. The formation of the conjugate is therefore, influenced by the thermodynamic compatibility between the protein and the polysaccharide. Sodium caseinate and resistant starch were conjugated by two methods; heating and physical blending (Chung, Sanguansri, & Augustin, 2010).

3.1.3. Egg protein-polysaccharides stabilized emulsion

Eggs are the important part of the human diet and are rich in nutritional components such as proteins, lipids, vitamins-A, B and D (thiamin, riboflavin and niacin) and minerals. Eggs have a complex biological structure and divided into three main parts that includes: the egg yolk (27.5%), egg white or albumen (63%) and shell membranes between the albumen and the inner shell surface (9.5%) (Wu, 2014). Egg white protein is widely used in the food industries as functional ingredient due to its emulsifying, foaming and gelation properties. The main protein fractions of egg white protein include ovalbumin (around 55%), ovotransferrin (around 12%) and ovomucin (around 11%) along with other protein factions (O'Sullivan et al., 2016). Eggshells and eggshell membranes (ESM) are the main by-products generated from egg processing industries. However, these ESM are known to be good sources of bioactive protein hydrolysates and peptides with excellent solubility, good foaming capacity and emulsification activity (Jain & Anal, 2017).

Limonene oil-in-water emulsions prepared from the arabic gum-egg white protein mixtures (1:0.05 w/w) at pH 3.5 produced emulsion with smaller droplet size compared to the emulsion at pH 7.5. At pH 3.5, egg white protein has an overall net positive charge and arabic gum has a net negative charge leading to electrostatic attraction while at pH 7.5, both biopolymers have net negative charge; hence, they are not expected to interact (Padala, Williams, & Phillips, 2009). Drakos and Kiosseoglou (2006) studied the influence of xanthan gum concentration on the physicochemical stability of oil-in-water emulsion, emulsified by egg white protein at pH 3.8 and 150 mM NaCl. Droplet aggregate formation, rheological changes, and serum separation of the emulsion with storage time were examined to determine the complex stability. Although egg white protein exhibited higher creaming stability compared to yolk, addition of xanthan gum at a concentration higher than 0.1% resulted in fairly stable emulsion.

The functional properties of proteins are enhanced by conjugation rather than mere coexistence with polysaccharide. For protein-polysaccharide conjugation Maillard-type reaction, that involves condensation of a carbonyl group of reducing sugar and amino group of protein, is considered to be the most suitable method for food applications (Choi, Kim, Park, & Moon, 2005). Maillard reaction products (MRP) resulting from egg protein (glycosylated albumin, ovomucoid and lysozyme) and sugar (fructose, inulin) under heat treatment (60 °C, 79% relative humidity for 3 days), exhibited changes in chemical characteristics and improvement in functional properties compared to the unheated protein-sugar mixtures (Jing, Yap, Wong, & Kitts, 2011). Egg white protein-pectin conjugate via the Maillard reaction (60 °C and 79% relative humidity) when compared with the physical mixture of two biopolymers, showed higher emulsion viscosity and stability and showed significant effect on foam volume and stability (Al-Hakkak & Al-Hakkak, 2010). In a research work by Jain and Anal (2018) eggshell membrane (ESM) protein hydrolysates were conjugated with culled banana resistant starch (RS) by heating the mixture at 100 °C for 90 min. The emulsion formed using the ESM protein hydrolysates and RS conjugate showed better emulsifying capacity and retardation in lipid oxidation in comparison with emulsion prepared using ESM protein hydrolysates alone.

3.2. Plant protein-polysaccharides stabilized emulsion

Plant proteins are preferred alternatives to animal proteins as functional ingredients in food formulation. Some of the common sources of plant proteins include; legumes (peas, soybeans, lupins, chickpeas, peanuts), cereals (wheat, maize, rice, rye, oat, sorghum), oilseeds (sunflower, rapeseed, sesame, cottonseed, safflower), root vegetables (potato, cassava, sweet potato, leaves (alfalfa, cassava, amaranth, aquatic plants), fruits (grape seed, tomato seed, papaya kernel) (Gonzalez-Perez & Arellano, 2009). The trend in application of plant protein isolates in food industries as functional ingredients to improve the texture, stability and the nutritional quality is increasing gradually. However, the applications of vegetable-based proteins are mostly limited to soybean protein (Makri, Papalamprou, & Doxastakis, 2005).

In mixed biopolymer systems, associative phase separation (complex coacervation) resulting from protein-polysaccharide interaction, affects the protein solubility profile. Further, in protein-stabilized oil-in-water (o/w) emulsion system, presence of polysaccharide may lead to either steric stabilization or bridging flocculation destabilization

depending on the nature of the biopolymers, solvent nature and degree of complexation with the protein adsorbed at interface (Liu, Elmer, Low, & Nickerson, 2010). Electrostatic interactions between potato protein and carboxymethylcellulose resulted in precipitation of potato protein at pH 2.5 from waste effluent of potato processing industry. The protein precipitate along with polysaccharide showed improved protein solubility, stability of emulsion system against creaming and foam system stability against liquid drainage (Vikelouda & Kiosseoglou, 2004).

Pea proteins are comprised of two major protein components: 7S (vicilin) and 11S (legumin), and usually combined with other stabilizers/emulsifiers to improve the sensitivity to factors such as pH and ionic strength (Bouyer et al., 2012). Liu, Low, and Nickerson (2009) studied the effect of pH, salt, and biopolymer ratio on the formation of pea protein isolate-arabic gum complexes based on turbidity measurement. Complex formation between the biopolymers was due to electrostatic attractive forces with secondary stabilization by hydrogen bonding (Liu et al., 2010). Klemmer, Waldner, Stone, Low, and Nickerson (2012) investigated the formation of complex coacervation (both soluble and insoluble complexes) between pea protein isolate and alginate as a function of pH (1.5–7) and protein to polysaccharide mixing ratio (1:1 to 20:1) based on turbidimetric analysis, electrophoretic mobility, and Raman spectroscopy. Optimum associative phase separation was observed at pea protein isolate and alginate mixing ratio between 4:1 and 8:1 with maximum interaction at pH 2.75. These findings signify application of protein-polysaccharide complex coacervates in the design of pH-sensitive biopolymer carriers for use in functional food and bio-material applications.

Soybean proteins have two most important protein components: glycinin (11S globulin) and β-conglycinin (7S globulin) (Gonzalez-Perez & Arellano, 2009). Jun-xia, Hai-yan, and Jian (2011) studied the effect of pH, ionic strength and polymer ratio in the coacervation interaction between soybean protein isolate and arabic gum based on absorbance values and coacervate yield (%). The complex coacervation of soybean protein isolate/arabic gum was used as carrier system for microencapsulation of sweet orange oil.

Amide-covalent bonding occurring between amino groups of protein and carbonyl group of polysaccharides during Maillard reaction results in the formation of the strongest type of protein-polysaccharide interaction. The resulting conjugate is found to be stable against a wide range of pH, ionic strength and temperature. Emulsifying properties, functional properties, and solubility of the conjugate have been proved to be improved compared to protein alone. Kasran, Cui, and Goff (2013) prepared soy whey protein isolate-fenugreek gum conjugates by Maillard reaction under controlled dry state condition (60 °C, 75% relative humidity for 3 days). The conjugates exhibited improved solubility and better emulsifying properties. Nasrin and Anal (2014), conjugated soy protein isolate (SPI) and culled banana resistant starch (CBRS) (1:1, w/w), at 100 °C for 90 min to produce stable fish oil emulsion system. The fish oil emulsion prepared from the SPI - CBRS conjugate was able to mask the fishy odor and exhibited the lowest amount of peroxide and anisidine value. In a further research by Nasrin and Anal (2015), the fish oil emulsion was freeze-dried to get the microcapsules that were utilized to develop functional muffins. The muffins made with emulsion containing SPI - CBRS conjugate exhibited less fishy flavor in comparison to muffin made up of SPI and Hylon VII conjugate.

From deamidated wheat protein (30–35% deamidation) soluble wheat protein fraction were isolated and conjugated with dextran (D10 or D65) at 60 °C, 75% relative humidity for 5 days. The conjugates were further characterized for available $-NH_2$ groups, SDS-PAGE, tryptophan fluorescence emission spectroscopy, interfacial layer thickness, and emulsion stability (Wong, Day, & Augustin, 2011). Li et al. (2013) studied the interaction of rice protein hydrolysates with various carbohydrates (mono-, oligo- and polysaccharides) through Maillard reaction under wet reaction conditions. Functional properties, solubility and emulsifying properties, were improved for the Maillard reaction products resulting from rice protein hydrolysates (5% degree hydrolysis) and dextran T20. Liu, Zhao, Zhao, Ren, and Yang (2012) conjugated peanut protein isolate and dextran, mixed at 1:1 wt ratio dry-heated at 60 °C and 79% relative humidity for 7 days. The conjugate showed improved thermal stability, protein solubility (pH 4.5–6.0), emulsifying and foaming properties.

4. Digestibility and bioaccessibility of protein-polysaccharide stabilized emulsion

When the emulsion-based food and beverage products are ingested and pass through the GI tract, the composition and structure of emulsions in the product undergo numerous changes. These changes regulate the bioavailability of the encapsulated core material and emulsified oil. Understanding the behavior of emulsion under GI tract condition provide a great opportunity to design the emulsion system to improve the bio-accessibility and controlled release of encapsulated material (Chang & McClements, 2016). As the emulsion reaches intestine by passing through mouth and stomach i.e. via GI tract, it is exposed to wide range of physical and biochemical conditions for e.g. shear, temperature, dilution effect, pH, enzymes, bile salt etc. To understand physico-chemical changes in emulsion within the GI tract, various research studies are being focused on simulation of the physiological conditions (Sarkar, Goh, Singh, & Singh, 2009). Fig. 6 illustrates the possible changes in emulsion as it passes through the GI tract.

4.1. Mouth phase

Emulsion digestion starts from the mouth, where it comes in contact with the saliva in oral cavity and acted upon by various digestive enzymes (lingual lipase, amylase, protease), experiences series of physicochemical and physiological changes in pH, ionic strength, temperature and gets convert to suitable form for swallowing. The human saliva pH is around 5.5 to 6.1 during fasting and around 7 to 8 during feeding stage and it is secreted at the rate of 0.2–4 mL per min. It is mainly composed of water (99%), minerals (less than 1%) and proteins (0.1–0.2%) The protein fraction constitutes of different kinds of molecules, including enzymes, immunoglobulins, antibacterial protein, and highly glycosylated negatively charged proteins (mucin). Mucin are capable of inducing coalescence and flocculation of lipid droplets which further attributes to depletions and bridging mechanism (McClements & Li, 2010). The interaction mechanism of food with saliva is important for understanding oral processing of food emulsions. After mixing with saliva, role of droplet charge on the emulsion stability and viscosity has been established by using differently charged surfactants and proteins emulsifiers. In the presence of saliva, strongly negative charged emulsion did not flocculate, weakly negative charge emulsion (such as emulsion stabilized by β-lactoglobulin) reversibly flocculated and positively charged emulsion (such as emulsion stabilized by lysozyme) irreversibly flocculated (Silletti, Vingerhoeds, Van Aken, & Norde, 2008).

Chang and McClements (2016), reported that the incubation of emulsion stabilized by protein alone with artificial saliva, showed an increase in the mean particle size and decrease in zeta-potential of the emulsion. The increase in droplet size was mainly attributed due to depletion flocculation induced by mucin or inorganic salts of saliva, while decrease in zeta-potential was attributed due to electrostatic screening effects associated with the presence of salt ions in saliva. In contrast, addition of polysaccharide (fucoidan) to the emulsion showed no significant change in the mean particle size and zeta-potential. This effect can be attributed to the fact that in mouth phase both polysaccharides and lipid droplets were negatively charged, therefore, it was expected that the polysaccharides would not be electrostatically adsorbed onto the droplet surfaces. Mao, Boiteux, Roos, and Miao (2014) revealed that the emulsion stabilized by mixture of whey protein isolate and pectin showed lower volatiles release rate compared to

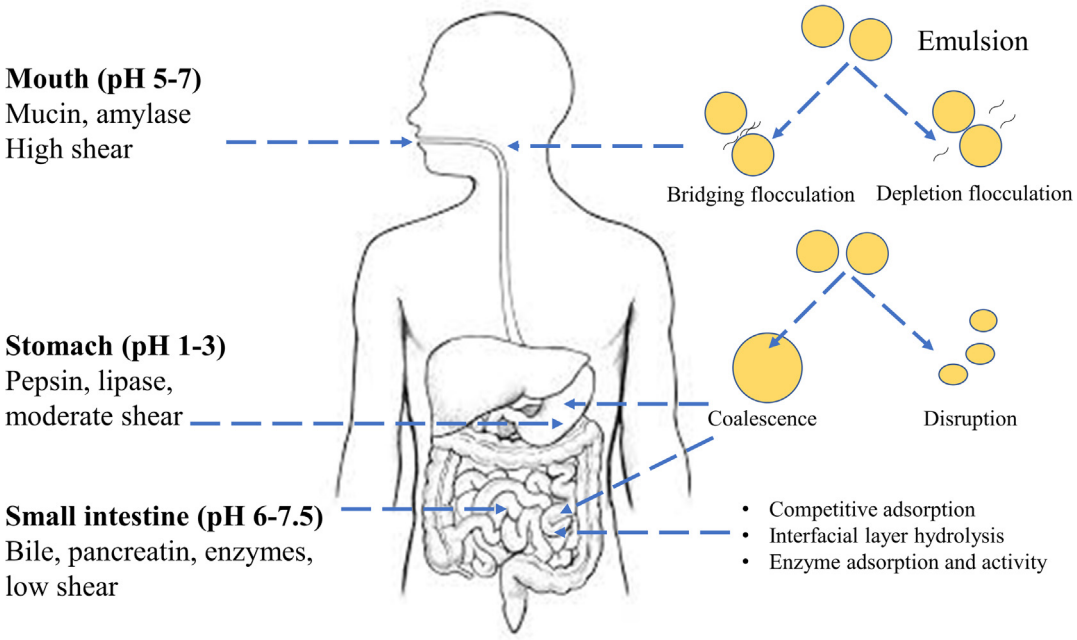

Fig. 6. Schematic representation of the possible changes in the emulsion within the gastrointestinal tract (adapted from Singh, Ye, & Horne, 2009).

emulsion stabilized by whey protein alone in saliva.

4.2. Gastric phase

After spending few seconds in mouth, the emulsion passes to stomach where it is subjected to highly acidic condition (pH 1 to 3) and mechanical agitation due to peristaltic movement. This movement causes mixing of emulsion with gastric juices containing different compounds such as proteolytic enzyme, lipolytic enzyme, gastric mucin, minerals, and other components. At low pH, protein-stabilized emulsion transforms into a cationic form and due to action of pepsin on the adsorbed protein, droplet charge would reduce and remove steric repulsion barriers leading to aggregation and coalescence of emulsion droplets (Singh et al., 2009). In the gastric phase, emulsions undergo series of physicochemical and structural changes due to interactions of pepsin with interfacial proteins and effect of low pH and ionic strength on the droplet charge (Singh & Sarkar, 2011). Digestion of emulsified lipid commences at the gastric phase by the action of gastric lipase and it accounts for 10–30% of total lipid hydrolysis. This initial lipid digestion on stomach is expected to subsequently help lipid digestion in small intestine (Hur, Decker, & McClements, 2009). β-lactoglobulin stabilized oil-in-water emulsion (1.0 wt% β-lactoglobulin and 20.0% soy oil) under simulated gastric conditions exhibited extensive droplet flocculation with some coalescence and change in zeta-potential values due to pH change and peptic hydrolysis of the interfacial layer (Sarkar et al., 2009). After incubation in the simulated stomach, significant change in droplet size was observed for the whey protein isolate (WPI) stabilized emulsion, while the whey protein isolate-beet pectin stabilized emulsion showed no significant change in the mean droplet diameters, indicated that the presence of beet pectin increases the stability of the emulsion droplet (Xu et al., 2014). Yang et al. (2015) reported an improvement in physical stability of citral containing emulsion stabilized by soy protein isolate-soy soluble polysaccharide Maillard reaction product (SPPMP). After incubation with simulated gastric fluid, SPI alone and mixture of SPI and soy soluble polysaccharide (SPP) stabilized emulsions showed multimodal droplet distribution and the mean droplet size was increased substantially within the early stage of incubation (30–60 min). In contrast, droplet size of SPPMP stabilized emulsion remained mono-modal and emulsion droplets exhibited very low magnitude of surface charges. The results indicated that the steric barrier provided by the protein-polysaccharide complex played an important role in stabilizing the emulsions in simulated gastric conditions.

4.3. Intestinal phase

The complete digestion of partially digested emulsion from stomach known as chyme, occurs in small intestine where it gets mixed with digestive fluid containing enzymes such as bile, pancreatic lipase, protease, colipase, salts and bicarbonate. The pH of the chyme increases from acidic to nearly neutral condition upon mixing with digestive juice. Bile salts and phospholipids act as surface-active and adsorb to the surface of lipids forming micelles. Bile salt thus assist in destruction of lipid droplet with agitation, and construction of micelles for transportation of hydrophobic molecules. Pancreatic lipase hydrolyzes the partially digested lipids releasing free fatty acids, monoacylglycerol, phospholipids, fat-soluble vitamins that are mixed micelles and transported to epithelium cells for absorption (Hur et al., 2009).

Under incubation with simulated intestinal fluid, β-carotene enriched emulsion, stabilized by whey protein isolate (WPI)-beet pectin (BP) conjugate showed stability to droplet flocculation and coalescence, and inhibition in the release of carrier oil and β-carotene compared to emulsion stabilized by WPI alone (Xu et al., 2014).

Zhang et al. (2015), studied the influence of the polysaccharides (alginate and chitosan) coating on the physicochemical properties, lipids digestibility and bioaccessibility of carotenoids-loaded emulsions under in vitro digestion model. After incubation with simulated intestinal fluid, emulsions stabilized by chitosan-soy protein isolate showed lower fatty acids release rate compared to emulsion stabilized by protein alone. At intestinal pH (pH 7), chitosan lost most of its positive charge and the pancreatic lipase could not get access to the lipids within the large chitosan aggregates. The result indicated that chitosan reduced the digestibility of emulsified lipids. Furthermore, emulsion stabilized by SPI alone showed lower bioaccessibility of carotenoids which was inferred due to the digestion and displacement of the adsorbed SPI coating due to action of digestive enzymes. Fan, Yi, Zhang, Wen, and Zhao (2017), studied nanoemulsions loaded with β-carotene by utilizing whey protein isolate (WPI) alone and WPI-dextran conjugates for lipid digestion and bioaccessibility. WPI-dextran conjugates decreased the extent of lipolysis and release of encapsulated β-carotene as compared to emulsions stabilized by WPI alone.

Micelles formed during the lipolysis are mainly composed of diglycerides, monoglycerides, free fatty acids, bile acids, and phospholipids. The micelles are essential for the transfer of the bioactive component into the intestinal cells for absorption by human cells. Smaller particle size, long chain and unsaturated fatty acid oils facilitates the transfer of bioactive compound into micelles and increase bioaccessibility (Fan et al., 2017). Zhang, Zhang, Zhang, Decker, and McClements (2015) examined the influence of addition of pectin on the rate and extent of lipid digestion in the emulsion stabilized by different emulsifiers. The initial rate of lipid digestion and the final amount of free fatty acids (FFA) release was reduced in presence of pectin (0.5%). Therefore the presence of polysaccharide may retard the rate of lipid digestion in the emulsion which might be attributed to different mechanisms such as binding of bile salts and phospholipids with polysaccharides that may change the lipid digestion process or retarding the transport of digested lipids from droplets to the intestinal wall, or formation of multi-layer that increase the thickness of the interfacial layer via the electrostatic interaction and prevent the lipase/co-lipase from adsorbing to the droplet surface and accessing the lipids inside the droplet (Singh et al., 2009).

5. Conclusion

The availability of wide range of food grade proteins and polysaccharides along with the versatility of protein-polysaccharide interaction mechanism, offers the scope in designing of tailor made colloidal particles. These particles have great applications in enhancing physical stability of the emulsion system, encapsulation of bioactive compound, controlled and targeted delivery of the bioactive compounds. Further, extensive *in vivo* research needs to be conducted to understand the behavior of protein-polysaccharide stabilized emulsions in human GI tract and the fate of the encapsulated bioactive compounds in the human digestive tract.

Conflicts of interest

The authors declare no conflict of interest.

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