Graphical abstract (for review)

Self-assembled protein-based systems for food applications

Coatings

Emulsions

Food additive delivery

Development of functional foods

Active substance

Hydrogels (Emulgels)

Controlled and sustained release of food additives

Nanoparticles

Self-assembled protein-based materials

Controlled and sustained release of food additives

Micelles-vesicles

Electrostatic and hydrogen bonding interactions

Self-assembled protein
Self-assembled proteins for food applications: A review

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Abstract

Background

The development of advanced food materials necessarily involves the building of well-known and oriented micro- and nanoarchitectures, which are obtained through the self-assembly of food grade (edible) polymers.

Scope and approach

Keeping this in view, proteins have proven to be more versatile building blocks than carbohydrate polymers for the manufacture of multifaceted and advanced systems for food applications.

Key findings and conclusions

Proteins from different sources (animal, vegetal and microbiological) can be self-assembled in several forms (films, hydrogels, micelles/vesicles and particles) to be targeted and tuned for various food applications such as biosensors, coatings, emulsions, controlled and sustained release of active food additives, development of functional foods, etc. Proteins can be self-assembled with each other, with carbohydrates or other proteins, and includes the use of enzymes and essential oils have achieved this physicochemical phenomenon that occurs between macromolecules via chemical interactions, mainly by hydrogen, hydrophilic and ionic bonding, which are determined by the conditions of ionic strength, mechanical force, pH, salt concentration and type, temperature, among others. This review aims to provide a comprehensive and concise analysis of the state of the art of self-assembled proteins for food applications, which have had a significant boom over the past five years in terms of the development of nanotechnology within the food industry.

Keywords: Active substance carriers; Advanced food materials; Coating; Controlled and sustained release systems; Emulgels; Encapsulation; Films; Functional foods; Layer-by-layer films; Protein architecture.
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<th>Abbreviations</th>
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<tr>
<td>AG: Arabic gum</td>
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<tr>
<td>Ca$^{2+}$: Calcium ions</td>
</tr>
<tr>
<td>CMP: Caseinomacropeptide</td>
</tr>
<tr>
<td>Cs: Chitosan</td>
</tr>
<tr>
<td>EWDP: Egg white derived peptides</td>
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<tr>
<td>EWP: Egg white proteins</td>
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<tr>
<td>GMP: Glycomacropeptide</td>
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<tr>
<td>H: Hydrogen</td>
</tr>
<tr>
<td>HEWL: Hen egg white lysozyme</td>
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<tr>
<td>IPP: Isoleucine-proline-proline</td>
</tr>
<tr>
<td>MEL-A: Mannosylerithritol lipid-A</td>
</tr>
<tr>
<td>Mw: Molecular weight</td>
</tr>
<tr>
<td>Na$^+$: Sodium ions</td>
</tr>
<tr>
<td>NaAlg: Sodium alginate</td>
</tr>
<tr>
<td>NaCas: Sodium caseinate</td>
</tr>
<tr>
<td>NPs: Nanoparticles</td>
</tr>
<tr>
<td>O/W: Oil-in-water emulsion</td>
</tr>
<tr>
<td>OVA: Ovalbumin</td>
</tr>
<tr>
<td>OVT: Ovotransferrin</td>
</tr>
<tr>
<td>PCD: Polycyclodextrin</td>
</tr>
<tr>
<td>pI: Isoelectric point</td>
</tr>
<tr>
<td>Pro: Proline</td>
</tr>
<tr>
<td>QPI: Quinoa protein isolates</td>
</tr>
<tr>
<td>SC: Soy $\beta$-conglycinin</td>
</tr>
<tr>
<td>SG: Soy glycinin</td>
</tr>
<tr>
<td>SL: Soybean lecithin</td>
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<tr>
<td>SLG: Short linear glucan</td>
</tr>
<tr>
<td>SPC: Soy phosphatidylcholine</td>
</tr>
<tr>
<td>SPI: Soy protein isolate</td>
</tr>
<tr>
<td>TA: Tannic acid</td>
</tr>
<tr>
<td>TE: Tulsi extract</td>
</tr>
<tr>
<td>TiO$_2$: Titanium dioxide</td>
</tr>
<tr>
<td>TPP: Tripolyphosphate</td>
</tr>
<tr>
<td>W/O: Water-in-oil emulsion</td>
</tr>
<tr>
<td>W/W: Water-in-water emulsion</td>
</tr>
<tr>
<td>WPI: Whey protein isolate</td>
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<td>WPNFs: Whey protein nanofibrils</td>
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1. Introduction

In recent years, there is a growing demand from consumers for healthier and more convenient food products. With this in mind, edible polymers such as carbohydrates, lipids and proteins have been used in the food industry as emulsifiers, thickeners, food packaging and coatings, among others (Gutiérrez, 2018a; Sedaghat Doost et al., 2019).

In particular, proteins are of great interest due to their nutritional value and versatility to modify their macromolecular structure (Ellis, & Lazidis, 2018; Garrido, Uranga, Guerrero, & de la Caba, 2018). This has allowed the development of stabilized emulsions, foams, gels and thickener solutions, as well as food packaging (Gómez-Estaca, Gavara, Catalá, & Hernández-Muñoz, 2016). The physicochemical properties of protein-based materials can be altered by different conditions, such as ionic strength, pH and temperature. Another option to modify the physicochemical properties of proteins is by self-assembly of protein structures with themselves or with others proteins, polysaccharides and active compounds (e.g. organic acids, flavonoids, phenolic compounds, among others), thus improving and creating novel structures with new functionalities which are not available by other means (Sedaghat Doost et al., 2019).

Self-assembly of proteins can be induced by means of reversible or non-reversible aggregation of protein segments driven by chemical reactions or non-covalent interactions, such as hydrogen (H) bonding, van der Waals forces, \( \pi-\pi \) stacking, as well as host-guest and hydrophobic interactions (Valencia, Zare, Makvandi, & Gutiérrez, 2019). The architectures formed could have several forms which can vary from nanometric to micrometric size (Anema, 2018; McManus, Charbonneau, Zaccarelli, & Asherie, 2016).
In the last years, several research studies have been focused on the development of additives, coatings, emulsions, films, functional foods, hydrogels, micelles/vesicles and particles based on the self-assembly of proteins (Belbekhouche, Bousserrhine, Alphonse, & Carbonnier, 2019; Diarrassouba et al., 2015; Li, He, et al., 2019; Loria, Pilosof, & Farías, 2018; Mantovani, Fattori, Michelon, & Cunha, 2016; Murmu & Mishra, 2017; Sedaghat Doost et al., 2019; Tsai & Weng, 2019; Visentini, Perez, & Santiago, 2019). These architectures have been formed by changing environmental conditions, such as ionic strength, mechanical force, pH, temperature and ion types. In addition, each protein has specific conditions for self-assembly. Keeping this in view, McManus et al. (2016) focused their review on specific aspects such as the physical mechanism of self-assembly of proteins, while Anema (2018) reviewed the self-assembly between lactoferrin with casein. It should, however, be noted that for our current knowledge, no review paper has comprehensively analyzed and reviewed the different mechanisms to induce self-assembly between protein-protein, protein-polysaccharide and protein-active compounds, nor their promising applications have been discussed in another review paper for the food sector. Therefore, the novelty and objective of this review article was to present the state of the art with respect to the main mechanisms for self-assembly of proteins used mainly for food applications.

2. Self-assembled proteins in food

Proteins constitute an essential nutrient for the good development and maintenance of human beings, and are an excellent resource for developing food grade materials (Cho & Jones, 2019). An interesting method to produce protein-based structures is through self-assembly, which comprises the spontaneous organization of macromolecules from
a disordered state to a highly well-organized state. These ordered structures are in a state of thermodynamic equilibrium which depends on environmental conditions, such as pH, pressure and temperature (Anema, 2018). Different materials can be prepared for various food applications through self-assembled proteins, from films and hydrogels to nanostructures (Bourbon, Pereira, Pastrana, Vicente, & Cerqueira, 2019). Surprisingly, some proteins can be self-organized into different supramolecular structures depending on the environmental conditions to which they are exposed (Anema, 2018). The self-assembly process of proteins is naturally ubiquitous, thus producing complex structures which are vital for many biological functions. In particular, self-assembled proteins in food systems have the ability to improve existing structures or create new ones. Self-assembly is accurate and reproducible, and requires a minimum energy use. Another advantage of the self-assembly method is that changing environmental conditions, such as ionic strength or pH, can trigger or reverse the formation of the supramolecular structures. Thus, this allows more targeted functionalities during processing and/or consumption (Anema, 2018).

Self-assembled proteins in food can be obtained from different sources, namely animals (Majorošová et al., 2019), microorganisms (Pham et al., 2018) and vegetables (Zhang et al., 2018). However, self-assembled or co-assembled multicomponent structures could also be produced from interactions between proteins of different origin (Abaee, Mohammadian, & Jafari, 2017), or even from protein-polysaccharide interactions (Gómez-Mascaraque, Llavata-Cabrero, Martínez-Sanz, Fabra, & López-Rubio, 2018). 

**Fig. 1** summarizes the different self-assembled proteins.
2.1. Self-assembled animal proteins

2.1.1. Milk proteins: Casein and whey protein

Among animal proteins, milk proteins are one of the most studied for the development of self-assembled structures (Allahdad, Varidi, Zadmard, & Saboury, 2018; Anema, 2018; Bao et al., 2019; Feng, Li et al., 2019; Yucel Falco, Geng, Cárdenas, & Risbo, 2017). Bovine milk contains about 3.5% protein, which can be classified into two main groups: 1) caseins, which constitute approx. 80% of the total milk protein, and 2) whey proteins, which are mainly β-lactoglobulin and α-lactalbumin, with lower amounts of bovine serum albumin: immunoglobulin and lactoferrin.

Caseins are a family of related phosphoproteins, which consist of four main proteins: αS1-, αS2-, β- and κ-casein. Caseins contain a high number of proline (Pro) moieties distributed in their primary structures and do not have disulfide bridges. Caseins can thus be considered unstructured or naturally denatured proteins (Anema, 2018). The isoelectric point (pI) of caseins is 4.6, which means caseins are negatively charged in milk (pH 6.6). Caseins show low water solubility and are naturally present in the form of self-assembled micelles (with diameters ranging from 50 to 600 nm) (Allahdad et al., 2018). The caseins in the micelles are held together through non-covalent bonds, such as hydrophobic interactions. Although the surface of the micelles are hydrophilic, its interior is hydrophobic, which favors its application as a natural carrier for hydrophobic molecules (Fig. 2) (Gupta, Arora, Sharma, & Sharma, 2019; Kimpel & Schmitt, 2015).

The degree of self-organization of caseins also depends on environmental conditions (Allahdad et al., 2018). For example, Loria, Pilosof, et al. (2018) studied different environmental factors (i.e. pH, temperature, type of salt and concentration) on self-
assembly of caseinomacropeptides (CMPs), which are end-amino acid moieties of κ-casein. CMP lacks cysteine and aromatic moieties compared to κ-casein, therefore, disulfide bonds cannot be formed. CMP assembly depends significantly on pH. CMP is present as individual molecules at pH 7, where electrostatic repulsive forces dominate over hydrophobic interactions. However, CMP self-assembly can be induced at pH below 4.5 by hydrophobic dimer formation, followed by electrostatic interactions, which ultimately lead to the development of a gel matrix. This process can occur spontaneously at room temperature, although by heating can be accelerated. The presence of calcium (Ca\(^{2+}\)) and sodium (Na\(^{+}\)) ions from calcium (CaCl\(_2\)) or sodium (NaCl) chloride salts, respectively, can also significantly affect the assembly properties of protein suspensions, since the electrostatic charges are screened and the hydrophobic parts of the CMP molecules can be associated (Loria, Pilosof, et al., 2018). The pH also has a significant effect on the spontaneous organization of casein, which has been thoroughly explained by Martinez, Farías, and Pilosof (2011) and Loria, Aragón, Torregiani, Pilosof, and Farías (2018).

On the other hand, whey protein isolate (WPI) is made up of approx. 80% of β-lactoglobulin and 15% of α-lactalbumin. WPI is widely used in the food sector due to its high nutritional value and functionality, and low cost (Mohammadian & Madadlou, 2016). The pH and temperature are important factors in the self-assembly of WPI (Nicolai, 2016). According to Nicolai (2016) when whey proteins in aqueous solutions are heated to more than 60 °C, the peptide chain gains mobility. This allows interaction of WPI chains with other whey proteins, which leads to the formation of bonds between proteins, thus being aggregated. Although there is no lower critical temperature for aggregation to occur, in practice aggregation is given too slowly below 60 °C to be observed (Nicolai, 2016).
Self-assembled micro and nanofibrils can be developed from WPI by prolonged heating at low pH (2.0) and ionic strength (Farjami, Madadlou, & Labbafi, 2016). However, proteins are hydrolyzed at this low pH, and the fibrils are formed by a fraction of the resulting peptides. In addition, at higher WPI concentrations (> 50 g/L), microgels are randomly associated into larger self-aggregates, and above a critical concentration (between 70 g/L and 80 g/L, depending on the pH) gels are formed (Murphy, Cho, Farkas, & Jones, 2015). WPI microgels are one of the protein micro- and nanoparticles that have recently attracted a growing interest for their potential applications in foods and pharmaceutics. In this sense, Nicolai (2016) reviewed self-assembled microgels from WPI or pure β-lactoglobulin. α-lactalbumin micelles can also be used in order to encapsulate or carry hydrophobic active compounds. For example, Du, Bao et al. (2019) and Jiang et al. (2018) formed amphiphilic peptides from partial enzymatic hydrolysis of α-lactalbumin, and then self-assembled into micelles under aqueous conditions.

2.1.2. Egg white proteins

Egg whites are widely used in the food industry because of their functional properties, such as foaming and gelling. The egg white proteins (EWP) comprise more than 80% of the total dry matter in egg white (mainly globulins, lysozyme, ovalbumin - OVA, ovomucin, ovomucoid and ovotransferrin - OVT). Therefore, research on the physicochemical properties EWP, such as its pI, has encouraged the investigation of its structure and how its functionality is affects for use in food processing (Strixner & Kulozik, 2011). So far, many studies have focused on the use of EWP to develop self-assembled materials. EWP is a potential biomaterial for the micro- and nano-carrier industry due to its excellent nutritional value, digestibility, self-assembly and
amphiphilic properties (Chang et al., 2019). With this in mind, Chang et al. (2019) prepared EWP particles by gelling at 90 °C, and observed that the final morphology of the particles, i.e. granular or fibrous particles depends mainly on the pH values. The dense, homogeneous and well crosslinked gel structure appears when the pH values are far from the pI of the EWP (4.8). In contrast, the structure of gels generated at pH values close to pI are generally stiffer and includes aggregate granular subunits.

The main protein component in egg white is OVA, a monomeric protein with amphiphilic characteristics, which makes it a highly efficient carrier for hydrophobic compounds. In this context, OVA nanoparticles (NPs) were prepared by Visentini et al. (2019) by means of a heat treatment at different pH conditions in order to study these systems as nanocarriers of polyunsaturated fatty acids.

Another important EWP is the OVT, which contains 686 amino acids, and can be reversibly bound to Fe$^{3+}$ cations in the presence of bicarbonate anions. The OVT-iron bond has been studied in detail by Wei et al. (2019). These authors evaluated different factors, such as ionic strength, pH, stirring speed and temperature on the assembly of OVT into amyloid fibrils. Amyloid fibrils have an important role in nanotechnology and biomaterials applications due to their unique physical and mechanical properties. Following Wei and Huang (2019) OVT amyloid fibrils do not show in vitro cytotoxicity, which implies their potential application in the food and pharmaceutical sectors.

Another relevant and well characterized model protein for the in vitro study of amyloid fibrillation is hen egg white lysozyme (HEWL), which represents a structural homologue of human lysozyme (Majorošová et al., 2019). These authors studied the self-assembly of HEWL into amyloid fibrils with magnetic NPs, which had radially-branched-dendritic structures under different conditions. The authors explained this
phenomenon through the diffusion-limited aggregation (DLA) theory, which is a theoretical model that explains the random aggregation of solid particles into branched structures. The DLA theory can be considered as a random irreversible growth model, from seed particles, which act as nucleation points for the organization of clusters. Therefore, the addition of magnetic NPs favors the self-assembly of proteins at an early stage, which eventually leads to the formation of regular protein patterns (Majorošová et al., 2019).

It is worth noting that despite significant advances in EWP self-assembly, this field is still booming and it is necessary to understand well the parameters that govern building these structures, since this may lead to interesting methods for obtaining advanced food systems from the controlled manufacturing of highly ordered complex assemblies.

2.1.3. Collagen and gelatin

Collagen is the most abundant protein in mammals, being the main component of connective tissue, such as bone, cartilage, cornea, ligaments, skin and tendons (Shen, Bu, Yang, Liu, & Li, 2018). The basic unit of collagen is the tropocollagen formed by a triple helical structure. Collagen can be self-assembled into well-organized fibrils via electrostatic, hydrophobic and H-bonding interactions (Leo, Bridelli, & Polverini, 2019). Self-assembly into fibrils is carried out under suitable conditions, i.e. high ion concentrations (especially phosphate) and moderately basic pH (9-11) (Maas et al., 2011). Leo et al. (2019) studied the self-assembly of rat tail collagen by using two different techniques: coupling molecular dynamics and ultraviolet-visible (UV-Vis) absorption. In this study, collagen self-assembly was evaluated at different pH values and the aggregation rate was estimated. These authors found that assembly mechanisms
depend significantly on pH. However, more research on the molecular lever is needed to
fully understand the effect of pH on the collagen chain interactions, which influence the
fibrillogenesis.

Gelatin is a protein obtained from the partial acid or alkaline hydrolysis of collagen. Thus, its structure is quite complex, being a mixture of fractions composed only of
amino acids linked by peptide bonds to form polymers with a molecular weight (Mw) in
the range of 15-400 kDa (Ali et al., 2019). The strength and viscosity of gelatin gel are
its most vital physical properties. The gelatin processing must be closely monitored in
order to obtain high gelling strength and avoid excessive degradation of the peptide

Gelatin has generally been used in the food industry as an additive to improve the
consistency, elasticity and stability of food products (Gómez-Mascaraque et al., 2018). The pharmaceutical industry has also long used gelatin for the encapsulation of drugs.
For this reason, gelatin has attracted interest as a wall biopolymeric material for the
micro- or nanoencapsulation of food additives (Ali et al., 2019). An interesting method
to develop carriers is through the simple mixing of the gelatin with the active food
additives in order to obtain their self-assembly. In general, different polyphenols are
encapsulated using gelatin, where the main force that explains the self-assembly is the
H-bonding. Other hydrophobic interactions play an important role in the self-
organization of the gelatin NPs, such as π-π stacking interactions between the benzene
rings in phenolic compounds and the aromatic amino acids in gelatin (Ali et al., 2019).
2.1.4. Other animal proteins

Some studies have also focused on the self-assembly of fish proteins. In particular, self-assembly of myosin (main muscle protein) from the silver carp was studied by Wang et al. (2018) and Wei et al. (2019), at different salt (NaCl) concentrations and pHs, respectively, and maintaining low temperature. These authors observed that ionic strength and salt concentration significantly affect the protein properties due to self-assembly of proteins. Specifically, Wang et al. (2018) confirmed that at a low NaCl concentration (below 2%), myosin is spontaneously assembled into dense filaments mainly through ionic rod-rod bonding. These assemblies were almost insoluble, which led to high turbidity of myosin solutions. However, ionic interactions broke down as the NaCl concentration increased, were bound to amino acids with opposite charges. The rupture of the intermolecular ionic bonds caused swelling and greater dissociation of myosin filaments, resulting in an increase in the interactions between myosin and water, and therefore, myosin slowly dissolved. An additional increase in salt concentration (above 6%) also led to many hydrophobic groups (e.g. sulfhydryl groups) of myosin being oriented towards the surface due to unfolding of the protein. As a consequence, this led to the formation of hydrophobic interactions, and turbidity and particle size increased significantly. Meanwhile, Wei et al. (2019) reported that the pH changes significantly altered the morphology of myosin assemblies, as a result of the degree of protonation and surface charge of myosin. At low pH, the low electrostatic repulsion promoted the assembly of myosin, which led to relatively high turbidity and UV absorption. In addition, the results of confocal laser scanning microscopy showed that the stiff structure assemblies were formed at low pHs. In contrast, under alkaline conditions (pH 7.0-9.0), negative charges increased the electrostatic repulsion and led to
a higher unfolding rate, thus exposing more hydrophobic moieties. This led to the formation of assemblies with fine and ordered structure. Therefore, the average particle size of myosin assemblies at high pH values was smaller than that found at low pH values. Finally, these authors concluded that the relative speed of unfolding and assembly of silver carp myosin under conditions of neutral pH (7.0) and low temperature was appropriate for the formation of fine and uniform structures, beneficial for gelation, which could be useful when silver carp myosin is used to produce surimi.

2.2. Self-assembled vegetal proteins

2.2.1. Zein

Zein is defined as a prolamine, which is the main storage protein in the corn endosperm, and is a readily available by-product from the corn sugar industry. Zein is soluble in aqueous solutions of ethanol, glycerol, ketones and extreme alkali conditions, but insoluble in water. The molecular structure of Zein has been studied thoroughly through different techniques (Zou et al., 2019), showing the great potential of this protein for the development of varied applications, since its self-assembly performance shows different structures. For example, its amino acid sequence contains more than 50% hydrophobic moieties that can be self-assembled into spherical particles, which makes it an ideal delivery matrix for bioactive compounds, drugs, oils and other nutraceutical and food ingredients (Chen et al., 2018; Wang & Zhang, 2019; Zhang, Khan, Cheng, & Liang, 2019). Zein can also be self-assembled into emulsion gels (De Vries, Nikiforidis, & Scholten, 2014; Zou, Thijssen, Yang, & Scholten, 2019).
There are three main zein fractions (α-, β-, and γ-), and a minor δ-zein fraction, being α-zein the most commercially available zein in the market. In particular, the α-helix conformation changes to a β-sheet by decreasing zein solubility. The β-sheet is then folded into an antiparallel structure due to hydrophobic interactions between neighboring β-sheets, thus showing the formation of stripes or ribbons. At low concentrations of zein, these ribbons are rolled up in rings, which grow and are rounded to form micro- and nano-spheres (De Vries et al., 2014).

The specific mechanism of self-assembly of zein begins in a rather hydrophilic environment, and this assembly behavior depends on the zein concentration and the specific balance between the polar and non-polar groups of the protein molecules and the environment. Hydrophobic interactions govern the zein aggregation, which can be altered with the polarity of the solvent (also referred as solvent quality). Zein self-assembly can also be controlled in a certain direction by including hydrophobic surfaces as nucleation sites (Zou et al., 2019). For example, the zein assembly results in a preferential direction, while a flat surface is used (Wang et al., 2004). In contrast, the zein assembly is produced in multiple directions when a curved surface is used. In line with this, Zou et al. (2019) studied different oil-solvent, oil-zein, solvent-zein and zein-zein interactions in order to analyze the core properties and the solvent quality. For this, four different types of oils with varied composition, hydrophobicity and viscosity were used as assembly cores for the preparation of emulsion gels in glycerol stabilized with zein. According to Zou et al. (2019) the zein protein network was the most dominant in the case of the more polar oils: an increase in the oil content made the gel network less resistant to fracture, and the decrease in solvent quality decreased gel strength and resistance. In contrast, the assembly of zein emulsion gels seemed to be more dominated by oil droplets in the net when more apolar oils were used. These drops
of oil provide resistance against breakage of the structure, and in this case, a decrease in solvent quality improved the gel resistance and strength. In addition, all zein emulsion gels were shown to be thermo-sensitive, and the gel strength increased due to network reorganization. This work showed that the properties of self-assembled zein emulsion gels can be easily targeted and tuned by modifying the hydrophobic interactions obtained by means of the solvent quality and the type of oil. These zein emulsion gels could provide fascinating characteristics for different food applications, such as controlled and sustained release of active food additives (Zou et al., 2019).

2.2.2. Wheat gluten

Gluten is mainly extracted from wheat (Díaz-Amigo & Popping, 2013), and obtained in smaller quantities from other cereals such as barley, oats or rye (Gutiérrez, 2018b). Gluten is basically used to improve the properties of flour for bread, and also, as an additive in baking products. However, with the growing production of wheat starch, wheat gluten has been studied for more diversified applications, both for the food industries and other sectors (Kong, Wu, Hua, Zhang, & Chen, 2019). With regard to the structure of wheat gluten, it comprises two different proteins: gliadins and glutenins. Gliadins are soluble in alcohol, while glutenins are insoluble, but both have high Mws. More than a half of peptide-linked amino acids in gluten proteins are glutamine and Pro. Therefore, they are probably important in the structure of gluten (Kong et al., 2019). Gliadins can be classified into four main types according to their amino acid sequences and their mobility at low pH in gel electrophoresis: α-, β-, γ-, and ω-gliadin (Herrera, Veuthey, & Dodero, 2016). It should be noted that gliadins are soluble in ethanol, but are water insoluble. This characteristic has been used for the formation of self-
assembled gliadin NPs, such as nanocapsules and nanofibrils, mainly obtained from extracts of gliadin in ethanol solution by means of the desolvatation technique. Following Herrera et al. (2016) pH plays an important role in the assembly of gliadins. These authors found that gliadins were spontaneously self-organized into micelle-like aggregates at pH 3.0. However, amorphous nanoparticle-like aggregates were observed at pH 7.0, which were probably stabilized by H-bonding between gliadin’s exposed amino acids and water. This pH-modulated transition from micelles to NPs was also reported for casein protein, although in the case of casein, the transition occurred when the pH decreased (Moitzi, Menzel, Schurtenberger, & Stradner, 2011).

Several studies have reported the gliadin assembly from different structures (Herrera et al., 2016; Niakousari et al., 2018; Sharif, Golmakani, Niakousari, Ghorani, & Lopez-Rubio, 2019). However, the formation of self-assembled glutenin structures has rarely been described. Glutelin consists of a concatenation of polypeptides stabilized through disulfide bonds. In general, glutenins are classified according to their Mw: low (10-70 kDa) and high (80-130 kDa) Mw glutenins (Anjum et al., 2007). Reddy et al. (2015) reported the development of glutenin NPs by phase separation, by adding water to the ethylene glycol solution of the hydrolyzed wheat glutenin. According to Kong et al. (2019) the assembly of glutenins is partly due to the formation of disulfide bonds between their chains. With this in mind, Li et al. (2019) developed a new type of redox-sensitive glutenin NPs. These authors studied the formation of the NPs by an antisolvent titration technique using hydrogen peroxide as an oxidative crosslinking agent, thus testing different concentrations of glutenin, as well as different periods of oxidation. The conclusion of this work suggested that the H-bonding and oxidative crosslinking interactions could have occurred, and caused the self-assembly or agglomeration of glutenin NP, and as a result the formation of particles with different morphologies was
observed. In addition, the formation of disulfide bonds was confirmed by means of Raman spectroscopy, i.e. the works from Kong et al. (2019) and Li et al. (2019) is on the same line. A hydrophilic compound model was also used to encapsulate Blue Nile A into glutenin particles, thus showing its high loading efficiency. Therefore, these glutenin NPs have great potential as redox-responsive carriers for controlled and sustained release of hydrophilic active compounds.

2.2.3. Soy protein

The importance of soy protein in the human diet has been growing over the years, as it has been recognized for its numerous beneficial nutritional functions (Tang, 2019). The main soy proteins comprise albumins and globulins, this latter being the most predominant, representing between 50 and 90% of the total soy proteins. Soy proteins can be classified by their sedimentation coefficient into four main fractions: 2S, 7S, 11S and 15S. Soy globulins are generally present in the 7S, 11S and 15S forms, while soy albumin appears in the 2S fraction. β-conglycinin (SC) and glycinin (SG) are the main soy globulins, which are known as 7S and 11S, respectively. Some reviews have addressed the SC and SG structure and physicochemical properties, as well as soy protein isolate (SPI), which is an important soy protein product (Nishinari, Fang, Guo, & Phillips, 2014; Tang, 2017).

In addition to their health benefits, which include lowering cholesterol, protective effects against diabetes, obesity, and kidney diseases, and anticarcinogenic activity, soy proteins have demonstrated other functionalities, such as their ability to aggregate, and their gelling and emulsifying properties. Currently, many studies have focused on the development of novel nanostructures based on soy proteins for the delivery of bioactive
compounds, especially those with reduced bioavailability or low water solubility (Abaee et al., 2017; Chen, Ou, & Tang, 2016; Pereira Souza, Deyse Gurak, & Damasceno Ferreira Marczak, 2017; Tang & Liang, 2017). Tang (2019) has extensively reviewed different methods to develop varied nanostructures from soy proteins, including the self-assembly mechanism.

In particular, some studies have focused on the effects of concentration, pH and temperature on the aggregation of soy proteins (Chen, Zhao, Chassenieux, & Nicolai, 2016; Chen, Zhao, Niepceron, Nicolai, & Chassenieux, 2017). These studies concluded that native soy globulin is self-assembled into aggregates whose size increases with increasing protein concentration and decreasing pH, and this process being reversible. However, protein bonds are relatively strong and cause very slow breakdown of the aggregates after dilution. The gelling rate of heat-denatured soy globulin also increases by increasing the temperature (Chen et al., 2017).

2.2.4. Other vegetal proteins

Recent studies have focused on new proteins from plant origin to prepare different self-assembly structures. For example, some authors have studied the self-assembly of quinoa seed proteins (Martínez et al., 2019; Ruiz, Xiao, Van Boekel, Minor, & Stieger, 2016). The value of quinoa (Chenopodium quinoa Willd.) seeds has recently increased due to its important health benefits, i.e. high content of antioxidant compounds and nutritional value. Quinoa seeds possess high amounts of lysine, an essential amino acid for humans (Nowak, Du, & Charrondière, 2016). Therefore, quinoa seeds show great technological potential, particularly due to their antioxidant, pigment and protein content. One of the main storage proteins in seeds is quinoa 11S, a globulin (also known
as chenopodin), which has a similar structure to SG. Quinoa 11S consists of six pairs of acid and/or basic polypeptides, with Mws in the range of 20-25 kDa and 30-40 kDa, respectively. These polypeptides are linked through disulphide bonds (Ruiz et al., 2016). Self-assembled structures of quinoa 11S can, for example, be used as a nanocarrier for betalatin (pigment) (Martínez et al., 2019). According to Martínez et al. (2019) the developed nanostructures showed a good potential for pigment delivery. However, hydrophobic protein-betalaine interactions interfered with the self-assembly mechanism between proteins (Martínez et al., 2019). Therefore, the interactions between the food additive and the protein matrix should be well studied, as they could interfere with the self-assembly between the proteins. It is worth clarifying that self-assembly of proteins can occur between the same or different proteins and proteins and additives, i.e. the interruption of a self-assembly mechanism of proteins could favor another self-assembly mechanism of the proteins. However, the least favorable condition for protein self-assembly is close to its pI.

2.3. Self-assembled microbial proteins

In general, microorganisms (bacteria and fungi) can produce biofilms based on extracellular DNA, polysaccharides and proteins (Bai & Rai, 2011; Gopu, Chandran, & Shetty, 2018). However, these biofilms are undesirable from a food quality and safety point of view, since they favor quorum sensing, thus allowing the resistance and growth of pathogenic and spoilage bacteria (Gutiérrez, 2019). However, some recent studies have shown that novel biomaterials can be designed for different applications from microorganisms.
2.3.1. Bacterial proteins

Certain bacteria species, such as *Escherichia coli*, *Mycobacterium tuberculosis*, *Salmonella typhimurium* and *Streptomyces coelicolor* are able to produce functional amyloids (TerAvest, Li, & Angenent, 2011; Payne et al., 2013). In the literature, some studies have focused on the self-assembly of amyloid proteins obtained from *E. coli* (Seker, Chen, Citorik, & Lu, 2017; Onur, Yuca, Olmez, & Seker, 2018). For example, Seker et al. (2017) developed amyloid curli nanofibers in living communities of *E. coli* as templates for nanomaterial assembly. Curli fibers showed great potential for the assembly of nanomaterials. Bacterial amyloid fibers could allow their application as nanomaterials, since at their ease to be genetically modified, their high aspect ratio and unique properties are attractive in this field. *E. coli* amyloid proteins were also studied by Onur et al. (2018), who developed self-organized nanofibers on solid surfaces. These authors concluded that recombinant production of protein/peptide ingredients can produce self-organized hierarchical structures, which could be designed with different functionalities according to the desired application, e.g. by fusion of bioactive peptides or enzymes, or other functional proteins using recombinant DNA techniques. Nonetheless, there is still a need to fully understand how to design a well-regulated system to control nanofiber systems with specific characteristics and functionalities. This challenge could be achieved with the support of fundamental research combined with nanotechnology and genetics.
2.3.2. Fungal proteins

Filamentous fungi can also secrete amphipathic proteins, called hydrophobins, which have the ability to be self-organized at hydrophobic/hydrophilic interfaces (HHIs), thus forming amphipathic structures. There are two main types of hydrophobins: class I and II. Class I hydrophobins consist of rodlets, which are robust fibrillar structures with an underlying cross-β amyloid organization, while class II hydrophobins are self-organized into amphipathic layers without fibrillar amyloid structure (Bayry, Aimanianda, Guijarro, Sunde, & Latgé, 2012). Pham et al. (2018) studied the self-assembly of six different class I hydrophobins from four different fungal species (Aspergillus fumigatus, A. nidulans, Magnaporthe oryzae and Neurospora crassa), which form functional amyloid fibrils with a rodlet morphology. The results of this study confirmed that hydrophobins have a significant conformational plasticity and that the HHIs where the self-assembly occurs, significantly affect the nature of the structures formed. Although high-resolution studies are required to understand the role of these self-assembled rodlets in fungal biology, which could result in the potential use of hydrophobins for biotechnological applications.

3. Different forms of self-assembled proteins in food

As already discussed, many proteins from different food sources can be used to develop highly organized structures through a self-assembly mechanism, and several factors, such as protein concentration and Mw, temperature and pH conditions, and even the solvent hydrophobicity can derive in different self-assembled structures of proteins with
various morphologies, such as films, hydrogels, micelles and particles (Fig. 3). In this section, different forms of self-assembled proteins in food will be reviewed.

3.1. Films

Films and coatings are thin layers based on continuous polymeric materials with a thickness of less than 0.3 mm. These materials are used as a barrier against chemical microbiological and physical contaminants, as well as to reduce carbon dioxide (CO$_2$), oxygen (O$_2$) and water vapor, and moisture transfer in fruits and vegetables (Valencia, Zare, et al., 2019). In recent years, due to the negative impact of non-biodegradable materials, most studies have focused on the development of biopolymer-based films, and more specifically, on proteins (Gómez-Estaca et al., 2016; Valencia, Lourenço, Bittate, & Sobral, 2016; Valencia & Sobral, 2018; Valencia, Luciano, Lourenço, Bittante, & Sobral, 2019). In general, protein-based films are widely used in the food industry because these materials have the best properties to produce packaging materials compared to other biopolymers (Álvarez et al., 2017). The wide diversity in physicochemical properties of protein-based films can be explained by the different combinations of the amino acids that make up the proteins. Protein-based films have acceptable mechanical properties, excellent fat barrier properties, good optical properties (transparency and gloss), low water vapor permeability at low and intermediate relative humidity and selective permeability to CO$_2$/O$_2$. However, protein-based films are water sensitive, which reduces their physicochemical properties and integrity (Gómez-Estaca et al., 2016). Keeping this in view, self-assembled protein-based films can be used to reduce the water sensitivity, as well as to improve the mechanical properties of these materials. Some approaches to manufacture self-
assembled protein-protein and protein-polysaccharide films have been studied. In this way, WPI was self-assembled by Tsai and Weng (2019) by using another protein (zein) in order to fabricate edible films. These composite films were made using a two-stage approach: first, WPI and zein were dissolved in ethanol and then spray dried to obtain self-assembled protein powders, and second, the self-assembled WPI-zein powder was then dissolved in deionized water to manufacture edible films by casting method. Following Tsai and Weng (2019), these multi-component self-assembled film systems had combined physicochemical properties compared to films made of each individual protein (WPI or zein). The same authors also concluded that self-assembly can contribute to the formulation of composite films exerting different characteristics, and the resulting co-assembled films can express the characteristics of the contributing materials (Tsai & Weng, 2019).

Composite films made from SPI were also obtained by Jensen, Lim, Barbut, and Marcone (2015) by self-assembly with cellulose at a 95:5 (SPI:cellulose) ratio using the casting methodology. These composite films derived from self-assembled SPI exhibited a more rigid mechanical behavior in terms of significant increases in tensile strength ($\sigma$) and Young’s modulus values, and a decreasing value of elongation at break compared to SPI films. The authors affirmed in this study that SPI-cellulose self-assembly could reduce the movement of the protein chains, thus explaining the mechanical behavior obtained (Jensen et al., 2015). A similar mechanical behavior was observed by Vejdán, Mahdí, Adeli, and Abdollahi (2016) for composite films made from self-assembling gelatin-agar, resulting in improvement of the $\sigma$ values by approx. 30% compared to gelatin film. The research work carried out by Arancibia, Alemán, López-Caballero, Gómez-Guillén, and Montero (2015) also fits well with the work done by Jensen et al. (2015) and Vejdán et al. (2016), i.e. self-assembly of protein-polysaccharide increases
the $\sigma$ values. In particular, Arancibia et al. (2015) observed that films manufactured by
the self-assembly of a protein concentrate obtained from shrimp waste and chitosan (Cs)
in the presence of Ca$^{2+}$ ions allows to obtain films with good antimicrobial and
antioxidant properties.

So far, preliminary studies on self-assembly of proteins have only been carried out on a
laboratory scale using the casting methodology. However, more studies should be
conducted to understand the mechanism of self-assembly in protein films, as well as the
use of other methods to promote their spontaneous organization within the films, but
being obtained by methodologies on an industrial scale. In this context, blown
extrusion, compression, electrospinning and injection molding could be explored
(Gutiérrez, & Alvarez, 2017a,b; Gantenbein, Masania, Woigk, & Tervoort, 2018; Yao et
al., 2019).

3.2. Hydrogels

Hydrogels can be defined as three-dimensional structures formed by the crosslinking of
natural or synthetic polymers through covalent, ionic or physical interactions
(Tomadoni, Casalongué, & Alvarez, 2019). These structures are hydrophilic and can
swell and absorb at least 90% in water or other fluids, without considerable changes in
their structure (Almeida, Carla, & Sato, 2019).

Proteins are raw materials widely used in the food industry as hydrogel agents due to
their amphiphilic nature which can be self-assembled in stable colloidal structures in
aqueous solutions (Du, Liu, Zhai, et al., 2019). Particularly, caseins and WPI have been
the most studied biopolymers for manufacturing self-assembled food grade hydrogels.
Li, Auty, et al. (2019) studied the effects of temperature (4-55 °C), the type of buffer
(sodium phosphate and imidazole-HCl buffers, both at pH 6.8) and the presence of CaCl$_2$ on the self-assembly of pure β-casein and β-casein concentrate to develop edible hydrogels. These authors observed larger particle size of pure β-casein and β-casein concentrate by increasing the temperature, thus suggesting that the self-assembling caseins via hydrophobic interactions. It should be noted that spherical and heterogeneous aggregates of β-casein were observed above 37 °C, which are reversed upon cooling. In addition, the turbidity and particle size of the self-assembled hydrogels had a similar aggregation behavior both in water and in imidazole buffer, although using the sodium phosphate buffer was greater, especially at higher Ca$^{2+}$ concentrations (Fig. 4a). According to Li, Auty, et al. (2019) self-assembly of β-casein can be carried out using β-casein concentrate in sodium phosphate buffer at high temperature and in the presence of CaCl$_2$. A similar temperature effect was identified by Nicolai and Chassenieux (2019) for the self-assembly of globulin hydrogels.

Following Morales, Martinez, and Pilosof (2015), the best condition to obtain self-assembled glycomacropeptide (GMP) and sodium caseinate (NaCas) hydrogels is by mixing these proteins (ratio 1:1) in an acid solution (pH 5) at 43 ºC. However, the self-assembled hydrogel was destabilized as the ratio of GMP increased in the formulation. This is possibly because GMP sequesters the Ca$^{2+}$ ions present in caseinate or because GMP interacts directly with the caseinate via hydrophobic interactions. Self-assembled hydrogels based on casein-peat protein using the same 1:1 ratio were also developed by Mession, Roustel, and Saurel (2017) mixing the protein solutions at pH 7 and 85 ºC for 60 min. followed by acidification at pH < 5.

Hydrogels from self-assembled WPI can also be formed as aggregates of spherical particles when heated in aqueous solutions at pH 5.8 (Fig. 4b) (Nicolai, 2016). These particles have a diameter between 100 nm and 1 μm and form highly stable microgels
In addition, the NaCl and CaCl$_2$ concentration increases the self-assembly of WPI and improves the hardness in these hydrogels (Nicolai, 2016). This behavior is associated with the reduction of the net negative charge \textit{per se} of proteins due to the ionic-type bonds with Na$^+$ and Ca$^{2+}$ (Guo, Ye, Lad, Dalgleish, & Singh, 2016; Nicolai, 2016). These self-assembled WPI hydrogels can resist gastric digestion, could thus be applied as carriers of free fatty acids with the aim of improving food digestion (Guo et al., 2016).

Another alternative to produce hydrogels is by associating different proteins or proteins with polysaccharides through electrostatic interactions, which can consequently lead to the formation of ionic hydrogels with better mechanical properties. In this way, proteins and polysaccharides must have opposite charge. This condition can be achieved at a pH value different from the pI of proteins, since that is where the proteins are partially ionized (Almeida et al., 2019; Du, Liu, Zhai, et al., 2019). For example, Ge et al. (2018) self-assembled gelatin with short linear glucan (SLG). Specifically, self-assembled hydrogels containing 5% (w/w) of SLG had two- and three-times higher hardness and maximum compressive stress values, respectively, compared to the corresponding values of the pure gelatin hydrogels (Ge et al., 2018). Probably, the formation of new H-bond interactions between the hydroxyl groups in the SLG and the amino groups in the gelatin could be the main reason for the properties of the self-assembled gelatin-SLG gels (Ge et al., 2018). Similar results were reported by Pérez, Wargon, and Pilosof (2006) for self-assembled gelatin with hydroxypropylmethylcellulose. Beyond the improvement of the mechanical properties of self-assembled hydrogels from proteins/polysaccharides, these systems can be used to load bioactive compounds. Recently, Almeida et al. (2019) and Du et al. (2019) self-assembled collagen-gellan gum-starch and casein-Cs hydrogels in order to improve the load of anthocyanins and
N-acetyl-L-cysteine/L-cysteine, respectively. These research papers concluded that self-assembled hydrogels have potential industrial applications as controlled release systems of encapsulated bioactive compounds, which can lead to food products with improved functional attributes. Taking this into account, Hu et al. (2017) self-assembled SPI with xanthan gum or carrageenan, and this delayed the digestibility of SPI. The SPI/xanthan and SPI/carrageenan mixtures could thus be applied to prepare anti-obesity drinks, where the digestion of SPI is delayed, thus decreasing the appetite (Hu et al., 2017).

Other self-assembled hydrogels containing active compounds, enzymes and surfactants have been developed. Some recent research studies in this field can be highlighted. For example, Xu, Teng, and Wang (2016) demonstrated that the enzyme tyrosine can be used to self-assemble caseinate hydrogels. Tyrosine-induced caseinate crosslinking was similar to glutaraldehyde caseinate self-assembly, however, this last conventional crosslinking agent is highly toxic, i.e. some enzymes can lead to non-toxic self-assembled protein hydrogels. Self-assembled hydrogels of β-lactoglobulins-mannosylitritol lipid-A (MEL-A) (surfactant) have also been developed by Fan et al. (2019). According to the authors, the interaction forces in the self-assembled structure were driven by hydrophobic interactions between the fatty acid chain or the acetyl groups and the hydrophobic groups of MEL-A and β-lactoglobulin, respectively, as well as by the H-bonding between the mannosyl-D-erythritol group of MEL-A and amino acids of β-lactoglobulin.

3.3. Micelles/vesicles

Micelles and vesicles are supramolecular aggregates containing an aqueous interior which is separated from the bulk solution. In the first system, the aqueous solution is
separated by an amphiphilic monolayer, while in the second system, two or more layers of amphiphilic compounds separate the solutions (Chen & Walde, 2010). In recent years, proteins have been used to produce new self-assembled materials in several well-defined functional micro- and nanostructures due to their amphiphilic properties (Anema, 2018; Chang et al., 2017). These reports provided a way to prepare protein-based micelles and vesicles for potential applications in the food industry. Some of them are discussed here.

Casein has been the most studied protein to produce self-assembled micelles. The presence of Ca$^{2+}$ or Na$^{+}$ from CaCl$_2$ and NaCl, respectively, can modify the electric charges of casein solutions and induce self-assembly of this protein. In general, Ca$^{2+}$ has a greater impact than Na$^{+}$, since casein micelles can be formed using concentration as low as 1.2 mmol of CaCl$_2$/g of casein (Loria, Pilosof, et al., 2018). Casein micelles have also been self-assembled with β-carotene (active compounds) by means of van der Waals interactions. Allahdad et al. (2018) found that these interactions are favored by a casein:β-carotene (1:1 w:w) ratio at alkaline pH, and lower temperatures and ionic strengths. The hydrophobicity of casein fractions based on their primary structures (β-, κ-, αs1- and αs2-) can also significantly affect the self-assembly of casein micelles with β-carotene. A lower hydrophobic order of casein (αs2- and αs1-) could even be self-assembled with β-carotene (Allahdad et al., 2018). Other self-assembled casein-based micelles have been developed to load vitamin D2 (Moeller, Martin, Schrader, Ho, & Lorenzen, 2018).

Lactalbumin is an amphiphilic protein which can be self-assembled in 20 nm monodispersed nanomicelles in aqueous solution. This system has also been used to load active compounds such as anthocyanins, curcumin and β-carotene via electrostatic and hydrophobic interactions. The active compounds did not alter the self-assembly of
lactalbumin and these micelles have high stability and controlled release in simulated gastrointestinal fluids (Du, Bao et al., 2019; Jiang et al., 2018).

There are few studies on self-assembled vesicles based on proteins and derivatives. Isoleucine-proline-proline (IPP) are peptides derived from milk protein and have antihypertensive properties. These peptides were self-assembled by Rezvani et al. (2019) using tween 80 and soy phosphatidylcholine (SPC) by using the thin film hydration followed by probe sonication and the modified ethanol injection microchannel techniques, respectively. Vesicles produced with SPC were smaller with a lower polydispersity index (78.6 ± 0.9 nm) than those prepared with tween 80 (90.5 ± 2.3 nm). However, vesicles with tween 80 exhibited a more sustained release behavior of IPP in simulated blood fluid than those prepared with SPC. Vesicles with tween 80 could be used for the development of functional beverages containing IPP (Rezvani et al., 2019).

3.4. Particles

Proteins are widely used in the food industry to stabilize foams and emulsions due to their bulking, gelling and thickening properties. However, these properties depend largely on the aspect ratio of the protein complexes (Mantovani et al., 2016). As explained above, depending on the pH and ionic strength, proteins can form complexes with smaller parts or larger aggregates. In general, smaller parts have a low volume fraction and can form a space-filling network in food products (Chen, Zhao et al., 2016; Mantovani et al., 2016). In this way, several research studies have focused on the self-assembly of proteins to manufacture raw materials with new architectures and with potential applications in the food sector. Some studies have addressed the self-assembly
of zein by means of electrostatic interactions, in the presence of Ca$^{2+}$ and Na$^+$ from CaCl$_2$ and NaCl, respectively (Sun, Chen, Dai, & Gao, 2017; Sun, Gao, & Zhong, 2018). Interestingly, self-assembled particle structures between zein and polysaccharides can be obtained. Dai, Sun, Wei, Mao, and Gao (2018) produced core-shell particles through H-bonds and electrostatic interactions between zein and arabic gum (AG) with a 1:1 ratio. These biopolymers were self-assembled using an anti-solvent precipitation method at pH 4, where zein and AG had a surface charge of +43 mV and -38 mV, respectively. The core-shell structure had a spherical shape with a particle size of 120 nm. These nanoparticles were applied to manufacture highly stable structures against coalescence for 30 days.

The nanofibrils have been produced by Mantovani et al. (2016) via the self-assembly between WPI and soybean lecithin (SL). Initially, SL and WPI were dissolved in acidified ultrapure water (pH 2), at room temperature, followed by heating at 80 °C for 20 h. Finally, the self-assembly of SL-WPI was stabilized at pH 2, 3, 5, 7 and 9. The self-assembled SL-WPI nanofibrils at pH 2, where the SL and WPI had a negative (-3 mV) and a positive (+35 mV) surface charge, respectively. The formation of electrostatic complexes between SL and WPI was probably not favored under pH > 2, due to the very low surface charge value of SL. Mantovani et al. (2016) concluded that the hydrophobic interactions of SL-WPI could be provided by heating up to 80 °C.

Egg white derived peptides (EWDP) have been self-assembled by Du, Liu, Zhang, et al. (2019) using Cs and tripolyphosphate (TPP). The self-assembly of Cs-TPP with different Cs:TPP ratios (between 6:1 and 2:1) and several pHs was performed by ionic gelation in acetic solution (1% w/v) at room temperature. The surface charge of self-assembled Cs-TPP particles increased under acidic pH, due to the protonation of -NH$_2$ groups of Cs. The optimal ratio was found at 6:1 (Cs:TPP w/w), where self-assembled
particles had a particle size of 160 nm and a surface charge of +58 mV. The self-assembled Cs-TPP particle size increased to 425 nm as the pH increased to 6, this increase in particle size was associated with deprotonation of the -NH$_2$ groups. The self-assembled particles containing a high Cs ratio were also able to load EWDP more efficiently, due to the formation of H-bonds between Cs and EWDP. By last, these authors concluded that these particles can be used to manufacture functional food products where EWDP could be gradually delivered into the organism (Du, Liu, Zhang, et al., 2019). A similar study was developed by Wu, Li, Shen, Yuan, and Hu (2018) with the aim of obtaining self-assembled particles based on Cs and sodium alginate (NaAlg) loaded with lysozyme (protein), a natural compound and generally recognized as safe (GRAS) with antimicrobial properties against foodborne pathogens. Cs and NaAlg were crosslinked by using CaCl$_2$, thus promoting the formation of small Cs/NaAlg particles. The Ca$^{2+}$ from CaCl$_2$ stabilized the lysozymes within the self-assembled Cs-NaAlg particles. The authors speculated on the possibility that these systems can be applied as edible films, gels or particles for the supply of lysozyme in order to inhibit microbial growth in foods (Wu, Li, Shen, Yuan, & Hu, 2018). Another natural active compound with antimicrobial properties, which has been used to self-assemble proteins isolated from *Radix Pseudostellariae* (authorized Chinese medicinal plant) is curcumin. Curcumin and proteins isolated from *Radix Pseudostellariae* were self-assembled by Weng, Cai, Zhang, and Wang (2019) mixing the compounds in a 1:1 ration at pH 5.7, thus forming spherical shape particles and 70 nm of diameter, and as a result a marked improvement in the thermal and light stability of the active compound loaded into the self-assembled particles compared to the free active compound was observed.
4. Self-assembled proteins for food applications

The research studies analyzed in section 3 have suggested that proteins are very promising biopolymers for the development of different self-assembled structures for various food applications due to their important biological, chemical and physical properties. In this section, some of these applications will be reviewed.

4.1. Coatings

As described above, protein-based coatings are used as a barrier between the food and the environment, while maintaining the food safety and quality (Fritz, Fonseca, Trevisol, Fagundes, & Valencia, 2019; Valencia, Zare, et al., 2019). Some studies have reported the self-assembly of protein-based coatings with better surface and barrier properties or even with antimicrobial and antioxidant properties. Maity, Nir, Zada, and Reches (2014) developed a self-assembled coating based on peptides containing two adjacent fluorinated phenylalanine moieties. The self-assembled interactions were promoted by means of aromatic interactions between the dipeptide diphenylalanine and its fluorinated analogs. These dipeptides were adhered onto a third peptide (3,4-dihydroxy-L-phenylalanine), always using ethanol as solvent. These authors indicated that the spontaneous formation of a self-assembled structure with a hydrophobic surface could be used to reduce the formation of biofilms in the food industry (Maity et al., 2014).

Taking into account Murmu and Mishra (2017) self-assembled coatings derived from caseinate (protein), AG (polysaccharide) and Tulsi extract (TE) can also be used to extend the shelf life of coated guavas for 7 days at 28 ± 2 °C compared to control
guavas (uncoated), which had only 4 days of shelf life (Fig. 5). Murmu and Mishra (2017) suggested that self-assembly of proteins and polysaccharide in the presence of TE in order to obtain edible coatings is given though intermolecular H-bonds between the components. In this same line, Feng et al. (2018) used whey protein nanofibrils (WPNF) to manufacture self-assembled and plasticized coatings with trehalose (disaccharide) and glycerol, respectively. In general, self-assembled WPNF-based coatings were continuous, homogeneous, transparent and were shown to be less hydrophilic than non-self-assembled WPI coatings. It is worth noting that these self-assembled coatings achieved to protect the fresh-cut apple slices in terms of the best action to retain the total phenolic content and inhibit the browning and weight loss.

Nephomnysy, Rosen-kligvasser, and Davidovich-pinhas (2020) also studied the self-assembly of proteins. First, these authors dissolved the zein in ethanol at room temperature and then heated the zein dispersion at 90 ºC to induce ethanol evaporation which promoted the zein self-assembly and aggregation. As a result, the self-assembled material was stable by H-bonding interactions between the zein aggregates.

Active self-assembled protein-based coatings with antioxidant properties have also been developed by Yang et al. (2020) and Yi et al. (2020). In these research papers, lactoferrin/oat β-glucan/curcumin and pea protein isolate/methoxyl pectin/curcumin were self-assembled using spray-dried and emulsion stabilization approaches, respectively. In general, hydrophobic interactions and H-bonding were the main driving forces for the formation of ternary complexes. These self-assembly ternary systems could be used as natural antioxidant coatings to reduce oxidation of food lipids (Yang et al., 2020; Yi et al., 2020).
Emulsions are widely used in the food industry, and they consist of dispersions of two immiscible fluids, where one fluid is dispersed as discrete drops into the second fluid. Emulsions can be classified as water-in-oil (W/O) and oil-in-water (O/W) emulsions. Some examples of W/O and O/W emulsions are butter, margarine and spreads, and cream, mayonnaise and milk, respectively (Ghosh & Rousseau, 2010). Normally, emulsions are thermodynamically unstable and need stabilizers to ensure a shelf life during storage (Lorenzo, Zaritzky, & Califano, 2018). Proteins are natural polymers widely used as emulsion and foam stabilizers in the food industry due to their ability to form various structures, under different conditions (Feng, Li et al., 2019; Ghosh & Rousseau, 2010).

The development of self-assembled proteins for the development of new emulsions has been the objective of research in recent years (Table 1). WPI has been one of the most used materials to manufacture self-assembled emulsions. This is mainly due to its wide range of pIs (4.2-5.2), as well as its broad range of pH, pressure and temperature in which WPI can be used to make self-assembled structures. WPI can also be used to produce emulsions gels by one-step homogenization by means of simple stirring methods such as magnetic stirring, sonication and ultra-turrax (Sedaghat Doost et al., 2019).

WPNFs can, for example, be used to manufacture self-assembled rods on a nanometric scale (Feng, Li et al., 2019). In this context, Feng, Li et al. (2019) obtained WPNFs by dissolving WPI in an alkaline solution (pH 8) at room temperature, followed by enzymatic hydrolysis with endoproteinase GluC at 37 °C for 10 h, and acidification at pH 3, where the electrostatic interactions between β-sheet structures into WPNFs robs
were induced (Feng, Li et al., 2019). These authors achieved the production of O/W emulsions using Jiusan soybean oil, WPI and WPNFs by sonication; and concluded that WPNFs can reduce phase separation and prevent oxidation of Jiusan soybean oil compared to WPI-based control emulsions. In fact, possibly this happened due to the better hydrophobic interactions between WPNF and Jiusan soybean oil (Feng, Li et al., 2019).

Self-assembled emulsions of WPI with other proteins (e.g. gliadin, lactoferrin) or polysaccharides (e.g. almond gum, maltodextrin, NaAlg) have also been extensively investigated from the literature (see Table 1). Taking this into consideration, Sedaghat Doost et al. (2019) observed the formation of coacervate particles due to the electrostatic interaction between WPI and almond gum in the pH range between 4 to 5, being the best condition at pH 4.5, where the coacervates had a surface charge of -36.5 mV. These systems were applied to produce O/W emulsions using thymol (a natural active compound with antibacterial, anticancer, antifungal and antioxidant properties). As an outstanding result of this study, the self-assembly between WPI and almond gum achieved to guarantee the encapsulation of the thymol and the stability of the emulsion (Sedaghat Doost et al., 2019).

Self-assembled gels have also been used as emulsion systems (also known as emulgels). The strength of self-assembled WPI emulgels could be modulated by adjusting the pH and ionic strength, thus forming the strongest emulgels near the pI of WPI (pH between 4.2-5.2). In this way, the interactions between WPI and gliadin, lactoferrin and maltodextrin have been induced at acidic pH. These self-assembled systems have been applied as emulsifying agents for corn, linseed and palm olein oils and could have promissory applications as food matrices with bioactive properties (Fioramonti, Arzeni,
Another protein of increasing interest for the development of emulsions is zein, which has a special tertiary structure that can be self-assembled into micro- and nanoparticles through the evaporation of solvents or liquid-liquid approaches. Interestingly, zein shows high surface hydrophobia and can be self-assembled into particles which are not prone to adsorb onto the oil-water interface to form stable emulsions (Zou, Baalen, Yang, & Scholten, 2018). With this in mind, Zou, Guo, Yin, Wang, and Yang (2015) studied the effect of TA on the conformational structure of the protein in order to reduce the hydrophobicity and self-assemble of the zein particles to be used for manufacturing O/W emulsions with corn oil. The authors observed the formation of a novel self-assembled colloidal particle of zein-TA, which were stabilized by means of H-bond interactions between zein and TA. The authors also noted that protonation and ionization of TA was critical for understanding the colloidal behavior of zein. In this way, the gel strength could be efficiently modulated by changing the pH and ionic strength of the solution. In addition, the colloidal state of TA affected the nature of its interaction with Pro-rich proteins from zein. The intermediate concentration of TA in the neutral and partially protonated form at pH 5 facilitated the strong H-bonding interactions between the hydroxyl groups into TA and the carbonyl moieties onto the pyrrolidone rings of the Pro-rich domain in zein (Fig. 6). Another similar study done by this same research group, concluded that the strength of the multilayer O/W emulgels increased as the hydrophobicity of the self-assembled zein-TA particles decreased (Zou et al., 2018).

Other proteins such as flaxseed protein, gliadin, porcine bone protein hydrolysates and its derivatives, soy peptides and β-lactoglobulin can form self-assembled emulsions to...
stabilize food grade oils *(Table 1)*. In these studies, the self-assembly of the colloidal protein particles was induced by reducing the net charge density of the proteins or by increasing the ionic strength (Gonzalez-Jordan, Benyahia, & Nicolai, 2017; Li, He, et al., 2019; Liu, Han, Zhang, Liu, & Kong, 2019; Nikbakht Nasrabadi et al., 2019; Zhang et al., 2018).

4.3. Food additive delivery

Food additives can be defined as substances intentionally added to food products in order to alter positively their sensory attributes, such as taste and color, or to extend their shelf-life, such as active agents, among others. These compounds have no nutritive value and are not normally used as a typical ingredient of food (Hoadley, 2011; Pressman, Clemens, & Hayes, 2017). Active additives have been studied extensively to extend the shelf-life of foods. In addition, self-assembled proteins containing active compounds can be used to control and tune the release of active compounds as a function of time or a specific place to meet a target. For example, Belbekhouche et al. (2019) self-assembled cationic polycyclodextrin (PCD) and anionic alginate using the layer-by-layer method. These authors observed that self-assembled materials obtained had antimicrobial properties against *Staphylococcus aureus* (Gram positive) and *E. coli* (Gram negative), and this effect was more pronounced as the cationic PCD layers in the self-assembled material increased (Belbekhouche et al., 2019).

Chen et al. (2018) self-assembled zein and limonene (active food additive) through hydrophobic interactions using the anti-solvent method in order to encapsulate the active compound into core-shell microcapsules. The results obtained by Chen et al. (2018) showed that a reduction in the food additive:protein ratio allowed the
development of food additive-loaded NPs, thus suggesting that the core:shell ratio (w/w) significantly affected the capsule formation. Additionally, the self-assembled material shows a slow release of limonene and oxidation prevention, as well as could potentially be used as an additive to manufacture active food packaging with aroma delivery (Chen et al., 2018). Self-assembled zein with nisin (a peptide with antimicrobial properties) was also used by Feng, Ibarra-Sánchez, Luu, Miller, and Lee (2019) to reduce *Listeria monocytogenes* in fresh cheese by approx. 1 log CFU/g, thus extending the shelf life of fresh cheese in almost 7 days under refrigeration conditions.

4.4. Development of functional foods

Functional or nutraceuticals foods are foods that provide the nutrients for basic nutrition but can also contribute to reducing the risk of chronic diseases (Diarrassouba et al., 2015; Mohammad, Hosseini, Emam-djomeh, Sabatino, & Meeren, 2015). Recently, several research works have focused on the development of functional foods based on the self-assembly between proteins and active compounds. Self-assembly can reduce the instability of the active compounds against chemical, biological or physical degradation. In this way, active compounds with antioxidant properties such as curcumin, flavonoids and α-tocopherol have been self-assembled with several proteins by mixing the constituents in a pH range between 2 and 7 (see Table 2). These research studies have concluded that self-assembled proteins help to preserve the active properties of the aforementioned compounds. These active compounds are water insoluble (hydrophobic), but their solubility is also improved after self-assembly with proteins, thus opening a window of new applications of these self-assembled materials to develop
Other active compounds, such as egg protein lysozyme and β-carotene, have also been self-assembled with β-lactoglobulin (Table 2). According to Diarrassouba et al. (2015), self-assembled β-carotene-β-lactoglobulin capsules showed a particle size between 269 nm and 2.7 μm, and were highly water soluble and with good stability against aggregation, as well as stronger hydrophobic interactions between β-carotene and β-lactoglobulin were observed when the pH was increased to 4.2 (around pI of β-lactoglobulin ~4.7). Meanwhile, the self-assembly of egg protein lysozyme-β-lactoglobulin was due to the electrostatic interactions between the two proteins with opposite charge at pH 7.5 (Mohammad et al., 2015). These self-assembled β-carotene/β-lactoglobulin and egg protein lysozyme/β-lactoglobulin could be used to manufacture clear liquid food products of acidic pH and nutritional supplements, respectively (Mohammad et al., 2015). Mohammad et al. (2015) indicated that the previously indicated systems could be loaded with β-carotene, and used to manufacture clear liquid food products of acidic pH and nutritional supplements.

Essential fatty acids such as linoleic acid and its isomer could also be stabilized in self-assembled systems from OVA (see Table 2). Visentini et al. (2019) reported that electrostatic interactions between the systems indicated above are improved at pH 7.5, thus forming NPs between 25 and 92 nm, which could be applied in colloidal food products.
4.5. Other applications

Self-assembly of proteins has also been used to detect microorganisms in foods by using biosensors. For example, antibodies as receptors in biosensors have been self-assembled in proteins produced by *E. coli* and *S. aureus*, within the range of $10^2$-$10^6$ CFU/mL in the pure culture samples, thus allowing the detection of these pathogenic microorganisms. These systems also achieved to detect *E. coli* and *S. aureus* up to $2.05\times10^3$ CFU/g and $1.04\times10^3$ CFU/mL, respectively, in the chicken rinse water. These electrochemical immunosensors based on self-assembled microbial proteins with antibodies have great potential for rapid detection of pathogenic bacteria in the food industry (Li, Fu, Fang, & Li, 2015; Xu, Wang, & Li, 2016).

5. Conclusions and future aspects

The type of protein self-assembly required depends fundamentally on the expected properties for the designed food. It can, however, be summarized as conclusion: 1) high processing temperatures and pH values close to pI favor the self-assembly of proteins and apolar compounds *via* hydrophilic interactions and 2) values away from pI favor the self-assembly of proteins and polar compounds *via* ionic or hydrogen bonds. Finally, the perspectives in this field are barely beginning, and surely the development of new foods will be related to this macromolecular phenomenon.
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Author contributions

B. Tomadoni conducted the review of section 2 and corrected the sections 1, 3 and 4. C. Capello and G. A. Valencia carried out the review of sections 1, 3 and 4. T. J. Gutiérrez designed, revised, and corrected this manuscript, as well as made the abstract and section 5 of this review.
Conflicts of interest

The authors declare no conflict of interest.

References


Nikbakht Nasrabadi, M., Goli, S. A. H., Sedaghat Doost, A., Roman, B., Dewettinck,


https://doi.org/10.1021/acssynbio.6b00166

https://doi.org/10.1016/j.foodres.2018.10.027

https://doi.org/10.1016/j.ijbiomac.2018.04.074

https://doi.org/10.1533/9780857093639.150

https://doi.org/10.1016/j.foodres.2017.08.022

https://doi.org/10.1016/j.foodhyd.2018.04.007

https://doi.org/10.1016/j.foodhyd.2019.01.012


assembly of electrospun nano fibers into gradient honeycomb structures. 


Zou, Y., Baalen, C. Van, Yang, X., & Scholten, E. (2018). Tuning hydrophobicity of
zein nanoparticles to control rheological behavior of Pickering emulsions. *Food Hydrocolloids*, 80, 130–140. https://doi.org/10.1016/j.foodhyd.2018.02.014


Biography from the authors

Barbara Tomadoni received both degrees in Chemical Engineering (2013) and in Food Engineering (2014) from the National University of Mar del Plata (UNMdP, Argentina), before obtaining her Doctorate (2017) from the University of Buenos Aires (UBA, Argentina), studying green technologies for preservation of minimally processed fruits and juices. Today, Dr. Tomadoni is a teaching assistant at UNMdP, and a researcher at the Research Institute in Materials Science and Technology (INTEMA-CONICET, Argentina). Currently, Dr. Tomadoni is working on the development of hydrogels and seed coatings based on biopolymers for their use in agriculture to control moisture in soils and for sustained release of agrochemicals.
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Germán Ayala Valencia received a degree in Agroindustrial Engineering (2011) from the National University of Colombia, before obtaining his Master’s (2013) and Doctorate (2017) in Food Engineering from the University of São Paulo, Brazil, with a collaborative period spent in the Laboratoire de Physique Thermique at ESPCI Paris, France, between 2015 and 2016. Dr. Valencia is now a professor - researcher in the Department of Chemical and Food Engineering at the Federal University of Santa Catarina, Florianópolis, Brazil. Dr. Valencia mainly works in the Food Science and Technology area with emphasis on packaging, pigments, nanotechnology, use of agro-industrial waste and encapsulation of bioactive compounds.
Tomy J. Gutiérrez has a degree in chemistry (Geochemical option) from the Central University of Venezuela (UCV) (December, 2007), a degree in education (Chemical mention) from the same university (UCV, July, 2008), has a specialization in International Negotiation of Hydrocarbons from the National Polytechnic Experimental University of the National Armed Force (UNEFA) - Venezuela (July, 2011). He also has a Master’s and PhD degree in Food Science and Technology obtained in October, 2013 and April, 2015, respectively, both from the UCV. He has also PhD studies in Metallurgy and Materials Science from the UCV and postdoctoral studies at the Research Institute in Materials Science and Technology (INTEMA). Dr. Gutiérrez has been a professor - researcher at the UCV both at the Institute of Food Science and Technology (ICTA) and the School of Pharmacy at the same university. It is currently an adjunct researcher in the INTEMA - National Scientific and Technical Research Council (CONICET), Argentina. Dr. Gutiérrez has at least 20 book chapters, 40 publications in international journals of high impact factor and 5 published books. He has been a lead guest editor of several international journals such as Journal of Food Quality, Advances in Polymer Technology, Current Pharmaceutical Design and Frontiers in Pharmacology. He is also an editorial board member of several international journals such as Food and Bioprocess Technology (2018 Impact Factor 3.032) and Renewable Materials (2018 Impact Factor 1.429), from April and June 2019,
respectively, among others. Dr. Gutiérrez today is developing a line of research in nanostructured materials based on polymers (composite materials), which are obtained on a pilot scale to be transferred to the food, agricultural, pharmaceutical and polymer industries. It is also a collaborator of international projects between Argentina and Brazil, Colombia, France, Italy, Poland, Spain, Sweden and Venezuela.
Table 1. Emulsions made from self-assembled proteins.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Emulsion type</th>
<th>Optimal temperature (ºC)/pressure (MPa)/pH for self-assembly</th>
<th>Shape</th>
<th>Size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPI/Jiusan soybean oil</td>
<td>O/W</td>
<td>37/0.1/2</td>
<td>Rob</td>
<td>0.4-0.6 μm (length) 1-10 nm (diameter)</td>
<td>Feng et al. (2019)</td>
</tr>
<tr>
<td>WPI/almond gum/thymol</td>
<td>O/W</td>
<td>20/0.1/4-5</td>
<td>Spherical</td>
<td>5.4-6.5 μm</td>
<td>Doost et al. (2019)</td>
</tr>
<tr>
<td>WPI/NaAlg/maltodextrin/linsed oil</td>
<td>O/W</td>
<td>70/0.1/5</td>
<td>Spherical</td>
<td>0.4-1 μm</td>
<td>Fioramonti et al. (2015)</td>
</tr>
<tr>
<td>WPI/gliadin NPs/corn oil</td>
<td>O/W</td>
<td>25/0.1/5.0-5.8</td>
<td>Spherical</td>
<td>120.8 nm</td>
<td>Zhu et al. (2018)</td>
</tr>
<tr>
<td>WPI/NaAlg/palm olein oil</td>
<td>O/W</td>
<td>20/70/3</td>
<td>Spherical</td>
<td>0.8-55 μm</td>
<td>Ng et al. (2017)</td>
</tr>
<tr>
<td>WPI/lactoferrin/corn oil</td>
<td>O/W</td>
<td>20/82.7/6</td>
<td>Spherical</td>
<td>90.1-22,291 nm</td>
<td>Teo et al. (2016)</td>
</tr>
<tr>
<td>Zein NPs/tannic acid (TA)/corn oil</td>
<td>O/W</td>
<td>20/0.1/5</td>
<td>Spherical</td>
<td>99.1 nm</td>
<td>Zou et al. (2015)</td>
</tr>
<tr>
<td>Zein NPs/TA/sunflower oil</td>
<td>O/W</td>
<td>20/0.1/5</td>
<td>Spherical</td>
<td>25 μm</td>
<td>Zou et al. (2018)</td>
</tr>
<tr>
<td>Zein/TA/medium chain triglyceride oil</td>
<td>O/W</td>
<td>150/0.1/5</td>
<td>Spherical</td>
<td>N.I.</td>
<td>Zou et al. (2019)</td>
</tr>
<tr>
<td>Zein/TA/ sunflower oil</td>
<td>O/W</td>
<td>25/0.1/3</td>
<td>Spherical</td>
<td>125.1-5,000.7 nm</td>
<td>Li et al. (2019)</td>
</tr>
<tr>
<td>Zein/TA/porcine bone protein hydrolysates/porcine bone protein hydrolysate-rutin conjugates/soybean oil</td>
<td>O/W</td>
<td>22/0.1/5</td>
<td>Spherical</td>
<td>0.7 – 1.0 μm</td>
<td>Liu et al. (2019)</td>
</tr>
<tr>
<td>Flaxseed protein/mucilage/tricaprylin oil</td>
<td>O/W</td>
<td>4/0.1/3-7</td>
<td>Spherical</td>
<td>369.4 nm</td>
<td>Nasrabad et al. (2019)</td>
</tr>
<tr>
<td>Soy peptide NPs/Tween 80/corn oil</td>
<td>O/W</td>
<td>N.I./40/7</td>
<td>Spherical</td>
<td>104.1 nm</td>
<td>Zhang et al. (2018)</td>
</tr>
<tr>
<td>β-lactoglobulin/dextran/polyethylene oxide</td>
<td>W/W</td>
<td>80/0.1/7</td>
<td>Spherical</td>
<td>3.5-7.5 μm</td>
<td>Gonzalez-Jordan et al. (2017)</td>
</tr>
</tbody>
</table>

Table 2. Different types of functional foods obtained by self-assembly of proteins.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Functional compounds</th>
<th>Self-assembly pH</th>
<th>Food application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>α-tocopherol</td>
<td>7.4</td>
<td>Liquid foods</td>
<td>Ye et al. (2017)</td>
</tr>
<tr>
<td>Zein</td>
<td>Curcumin</td>
<td>4</td>
<td>Nutritional supplements</td>
<td>Dai, Wei et al. (2018)</td>
</tr>
<tr>
<td>β-casein</td>
<td>Flavonoids</td>
<td>2-7</td>
<td>Liquid foods</td>
<td>Li, Fokkink et al. (2019)</td>
</tr>
<tr>
<td>β-conglycinin</td>
<td>Curcumin</td>
<td>7</td>
<td>Food-grade protein</td>
<td>Liu, Li et al. (2019)</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>Egg protein lysozyme</td>
<td>7.5</td>
<td>Nutritional supplements</td>
<td>Diarrassouba et al. (2015)</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>β-carotene, folic acid, curcumin</td>
<td>4.2-7</td>
<td>Liquid foods</td>
<td>Mohammad et al. (2015)</td>
</tr>
<tr>
<td>OVA</td>
<td>Linoleic acid and its isomer</td>
<td>7.5</td>
<td>Colloids</td>
<td>Visentini et al. (2019)</td>
</tr>
<tr>
<td>OVA</td>
<td>Curcumin</td>
<td>7</td>
<td>Nutritional supplements</td>
<td>Liu et al. (2018)</td>
</tr>
</tbody>
</table>
Fig. 4. (a) Schematic illustration for self-assembly of pure β-casein (β-CNpure) and β-casein concentrate (β-CNconc) at different temperatures and CaCl$_2$ concentrations. (b) Transmission Electron Microscopy images of whey protein aggregates as a function of pH. Adapted with permission from Nicolai (2016) and Meng Li et al. (2019).
Fig. 6. Schematic illustration for the formation of particles based on self-assembly of zein and tannic acid at pH 5 and its particle stability at pH 3, 5 and 7. Adapted with permission from Murmu and Mishra (2017).
Fig. 1. Main sources of self-assembled proteins.
Fig. 2. Essential oil-loaded self-assembled casein.
Fig. 3. Forms and applications of self-assembled proteins in food industry.
Fig. 5. Schematic illustration for the formation of self-assembled film forming solutions based on sodium caseinate, arabic gum and Tulsi extract.
Highlights

✓ The self-assembled proteins (SPs) were reviewed and analyzed.
✓ Proteins from different sources (animal, vegetal and microbiological) can be self-assembled.
✓ SPs have been used as films, hydrogels, micelles/vesicles and particles.
✓ The multifaceted and tunable properties of SPs are promising.
✓ SPs can be used as coatings, emulsions, food additive delivery systems and functional foods.