

FOOD STRUCTURE AND FUNCTIONALITY

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Preface

Understanding the relationship between food structure and specific sensory attributes, as well as digestion and transport of food components within the gastrointestinal tract is necessary to design food products with specific functionalities, for example, healthy foods, foods with an increased feeling of satiety, food with increased protein, or reduced-fat content among others. However, with the recent advantages in food processing (e.g., nonthermal technologies and nanoencapsulation), new developments, data, and state-of-the-art come up in the field. Nowadays, modern food scientists and technologists often deal with new product development and functional foods, and thereby an essential guide connecting food structure and functionality is needed.

Food Waste Recovery Group provides insights into the food and environmental science and technology sectors, and this line is publishing in several books. The latter deals with food waste recovery technologies, biobased products, and industries, the valorization of food processing by-products (e.g., from olive, grape, cereals, coffee, and meat), sustainable food systems, saving food efforts, innovations strategies in the food and environmental science, innovations in traditional foods, nutraceuticals, and nonthermal processing. The group has also prepared textbooks for innovative food analysis, shelf-life, and food quality, personalized nutrition, nonalcoholic drinks, and customized guides for specific food components like carotenoids, dietary fiber, glucosinolates, lipids, polyphenols, and proteins.

Following the above considerations, the current book covers food structure and functionality, focusing on food products with tailored properties toward end-user preferences (e.g., improved texture, shelf stability, taste perception, and health). The ultimate goal is to support the scientific community, food engineers, food scientists, and food technologists who aspire to develop industrial and commercialized applications in the food industry. The book fills the gap of transfer knowledge between academia and industry, covering issues coming up to the table over the last decade with the development and applications of emerging and nonthermal technologies in the food industry.

The book consists of 12 Chapters. Chapter 4 provides an overview of the most important aspects that

govern the composition, structure, and mechanical properties of interfaces in food emulsions. The main theoretical concepts and experimental methods existing traditionally to study fluid interfaces are revised. Likewise, the characteristics of the pendant drop film balance are discussed before providing a detailed analysis of the interfacial properties of adsorbed layers.

Chapter 2 deals with the rheology, structure, and sensory perception of hydrocolloids. Hydrocolloids have long been used as food additives due to their influence on texture, rheology, structure, and mouthfeel of food products. Also, their use as functional ingredients in foods is currently on the rise due to their beneficial health effects. Starch and cellulose derivatives and gums are mostly used in food formulations. The different ways of modification of starch and cellulose are described as well as resulting changes in physicochemical and rheological properties more suitable for usage in food are also discussed.

In Chapter 3, the fundamental principles of microfluidics and their feasibility to process food materials and design food structures are thoroughly covered and discussed. Microfluidics has been studied as a rapid detection tool for food-safety related applications for a long time. Recently, more and more interests have been attracted to use microfluidics as microreactors to assemble biopolymers and to achieve desired functionalities. Attributed to the accurate flow control and unique bottom-up mechanism, the properties of the assembled biopolymer structures could be well-controlled.

High-pressure processing (HPP) has a decisive role in nonthermal food pasteurization being, among others, the most successful nonthermal processing technology, which has motivated both academia and industry to develop novel applications for HPP and new food products. HPP can lead to the development of new foods with improved texture aspects due to the “cold gelation,” a process that leads to the formation of proteins’ networks with a stable thermodynamic confirmation allowing a phase transition from liquid to solid. In Chapter 4, the effects of HPP on animal and plant-based proteins are presented and discussed. Important fundamental features of proteins, such as three-dimensional conformation, techno-functional properties, and other applications, are highlighted.

Chapter 5 addresses the way electric fields and electromagnetic waves exert an effect on protein's structure and functionality and, consequently, its impact on human health. It starts with background regarding electric fields and the electromagnetic spectrum, followed by the key technologies based on these principals, namely pulsed electric fields, ohmic heating, microwaves, and radiofrequency. Then, their use as an alternative process for protein extraction is reported regarding their mechanism and results are also found. Finally, the impact of the structural change of the protein on human health was addressed, covering the relationship between structure and protein digestibility and allergenicity.

Chapter 6 provides an overview of microscopy and spectroscopy techniques used to analyze the microstructure (structure) of food powders. With the knowledge of the powder structure, it is possible to propose the design conditions for quality products with better stability. The development of the structure of the powders will depend on the process used for their elaboration, among those of more significant industrial applications are the fragmentation (grinding), pulverization, lyophilization, drum drying, and spray drying, the latter being the most widely used.

Chapter 7 describes solid lipid nanoparticles and nanostructured lipid carriers, which are lipid-based nanocarriers used for encapsulating lipophilic compounds, such as carotenoids, polyphenols, essential oils, and some vitamins. First, the formulation, production process, and applications of solid lipid nanoparticles for encapsulating bioactive food components are discussed. Then, the discussion focuses on nanostructured lipid carriers. Finally, issues regarding safety are mentioned, and a summary of the current regulatory framework in various parts of the world is presented.

Chapter 8 summarizes the recent studies on the utilization of novel technologies in starch modification and their effect on the physicochemical, structural, rheological, and functionality of modified starch. The effect of these strategies on structure–function relationships of starch is also reviewed. The results provide useful information for the application of the novel method in starch-based industries. Moreover, these methods can be used for the designing of tailor-made products contained starch and their derivatives.

Chapter 9 presents current knowledge about the fabrication of different types of protein-based nanostructures, reviewing the leading strategies and

techniques to fabricate a variety of nanostructured proteins. Encapsulation, retention, protection, and release of bioactive compounds in protein-based nano-delivery systems are discussed. The effect of manufacturing variables on functional and physicochemical properties of the nanostructured proteins and bioavailability of encapsulated bioactives are also denoted, whereas the particular emphasis is given on the interactions between the bioactive ingredients and nanostructured proteins.

Chapter 10 revises the current knowledge of plant protein properties, their interactions with other polymers (proteins or carbohydrates) in food systems, the structure of complexes formed by these interactions at interfaces, and the applications of these complexes. The properties of polymers, environmental conditions, and processing technologies can affect interactions of plant proteins with other polymers and the applications of their complexes in the food industry.

Food-derived compounds have been explored as active components for the management of type 2 diabetes mellitus. One of the primary bioactivity mechanisms is through the inhibition of α -glucosidase, which is involved in the release of glucose from dietary carbohydrates during digestion in the gastrointestinal tract. Chapter 11 describes the regulation of carbohydrate digestion during normal and abnormal conditions, and the α -glucosidase inhibitory activity of food-derived compounds, including peptides, phenolic compounds, terpenoids, and nonstarch polysaccharides.

In Chapter 12, the role of various components of food in the pathobiology of metabolic syndrome is highlighted with particular attention to their role in the metabolism of essential fatty acids, an essential but often ignored component of food, and its impact on metabolic syndrome and cancer.

Conclusively, the current book is assisting food scientists, technologists, engineers, biochemists, and physical chemists working in the whole food science field as well as new product developers, researchers, academics, and professionals working in the food industry with food applications and food processing. University Libraries and Institutes could also use it all around the world as ancillary reading in undergraduate and post-graduate level multi-discipline courses dealing with food science and technology and food processing.

I would like to thank and acknowledge one by one all the authors for their fruitful collaboration and

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thousands of words that may contain gaps or errors. Suggestions, comments, and even criticism are always welcome. So please do not hesitate to contact me to discuss any relevant issues.

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9

Nanostructured proteins

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9.1 Fabrication of nanostructured proteins

There are many different techniques available for the fabrication of nanostructured proteins. The selection of the appropriate method depends on various factors such as the amino acid composition, physicochemical, and structural properties of the protein used as the carrier, characteristics of the bioactive ingredient, and the application area. A brief overview of the most commonly used methods for the preparation of protein-based nanoparticles is provided below.

9.1.1 Coacervation

Coacervation is one of the commonly used nanoencapsulation techniques. It generally occurs in a mixed polymer solution between oppositely charged groups on the polymers when the electrostatic attraction between them is high enough (Fathi et al., 2018). As a result of coacervation, a polymer-rich coacervate phase and a supernatant phase with low polymer content forms. In nanoencapsulation applications, the biopolymers used are generally proteins (gelatin and whey protein) and polysaccharides. Both negatively charged polysaccharides such as pectin and gum arabic and positively charged polysaccharides such as chitosan are commonly utilized for fabricating protein–polysaccharide nanoparticles with coacervation method (Tang, 2019). The coacervate phase can be treated with enzymatic or chemical cross-linking agents such as transglutaminase or glutaraldehyde to improve the stability of the particles formed. Furthermore, the surface of the particles can be coated with an additional biopolymer layer for different applications.

9.1.2 Heat-induced aggregation

When an aqueous solution of globular proteins is heated at a temperature, that is, higher than their thermal denaturation temperature, it results in the progressive unfolding of the structure, which in turn leads to exposure of some previously buried hydrophobic groups. More hydrophobic interactions occur between these unfolded proteins, resulting in aggregation. The formation of disulfide bonds may further stabilize protein aggregation. At high protein concentrations, gelation can occur. Heat-induced aggregation of globular proteins is reported to be affected by protein concentration, composition, heating conditions, pH and ionic strength, presence of other components in the solution (Tang, 2019).

9.1.3 Maillard conjugates

The stability of proteins and protein-based nanodelivery systems can be enhanced by forming protein–polysaccharide conjugates through the Maillard reaction. Dextrin, galactomannan, chitosan, inulin, pectin, carrageenan gum arabic, lactose, galactose, glucose, maltose have been used for forming conjugates with proteins

(de Oliveira et al., 2016; Feng et al., 2016). The Maillard reaction occurs between the carbonyl groups of reducing sugars and amine residues of proteins. Protein and carbohydrate solutions are mixed under controlled pH conditions for forming the Maillard conjugates. Heat treatment is also applied to accelerate the combination (Yi et al., 2016). Modification of proteins through Maillard reaction has been reported to improve not only the stability of the system but also their solubility, emulsifying properties, antioxidative properties and antimicrobial activity (Fathi et al., 2018). The increased stability of the protein–polysaccharide conjugates has been attributed to the strong steric repulsion provided by the polysaccharide part (Han et al., 2019).

9.1.4 Nanoemulsions

Nanoemulsions are considered as effective carrier systems for bioactive ingredients since they have various advantages in terms of physical stability, optical clarity, and enhanced bioavailability. Nanoemulsions have a mean droplet size smaller than 100 nm (Livney, 2015; Chen and Hu, 2020). However, more substantial droplet size distributions have been reported for protein-stabilized nanoemulsions (Tang, 2019). Their droplet size mainly depends on the shearing and mechanical forces applied during homogenization and formulation of the emulsion, which involves water, surfactant, and oil phases. Nanoemulsions are usually fabricated using high-pressure homogenizers, microfluidizers, and ultrasonic homogenizers. In some applications, a solvent evaporation technique can also be applied to improve the stability of the nanoemulsion. When nanosized colloidal particles are used as emulsifiers, the emulsion formed is regarded as a Pickering emulsion. Colloidal particles with partial wettability in aqueous and lipid phases and can accumulate at the interface can be used for Pickering stabilization (Sedaghat Doost et al., 2019). Various proteins have been used as Pickering stabilizers for nanoemulsions. Pickering emulsions are reported to have controlled size distribution, high stability, and surfactant-free formulation (Ding et al., 2019).

9.1.5 Electrohydrodynamic processes

Electrospraying and electrospinning are the electrohydrodynamic processes used for fabricating protein-based nanoparticles and nanofibers, where high-voltage electric fields are applied to protein solutions (Fathi et al., 2018). Proteins with relatively high surface charge are reported to be more suitable for fabricating nanoparticles and nanofibers with the electrohydrodynamic processes. In electrospinning, high-intensity electric fields are applied to protein solutions. Electrospraying works on the same principle as well. However, a lower voltage is required during the initial stage. The main advantage of the electrohydrodynamic processes is that they can be carried without the use of organic solvents and heat treatment, which makes these processes suitable for encapsulating sensitive bioactives (Tang, 2019).

9.1.6 Antisolvent precipitation

Antisolvent precipitation or nanoprecipitation is another commonly used method for producing protein nanoparticles. When an aqueous solution of proteins is dispersed into an antisolvent solution, the protein becomes gradually dehydrated, resulting in increased protein–protein interactions. As a consequence, the solubility of proteins decreases gradually, and precipitation occurs, forming nanoparticles (Fathi et al., 2018; Tang, 2019). Similar to coacervation, a cross-linking step can be included to improve the integrity and stability of the formed nanoparticles. This method is suitable for the fabrication of nanoparticles from hydrophobic proteins such as gliadin, kafirin, and zein (Tarhini et al., 2017).

9.2 Delivery systems with animal-based nanostructured proteins

9.2.1 Casein

Casein are the major proteins in milk and exist as colloidal aggregates dispersed in milk serum. Casein structure is organized in micelles to carry essential nutrients, such as protein, calcium, and phosphate. Caseins have been widely used as wall materials in microencapsulation. A summary of the recent studies focusing on fabrication, characterization, and applications of nanostructured caseins and other milk-derived proteins for delivery of bioactives is presented in Table 9.1.

TABLE 9.1 Formation, characterization, and applications of nanostructured milk proteins for delivery of bioactives.

Protein	Fabrication method	Bioactive ingredient	Application	References
Casein	Micellization	Vitamin D2	Improved photochemical stability	Semo et al. (2007)
Casein with pectin	Heat- and acid-induced aggregation	Rutin	Controlled release	Luo et al. (2015)
Casein with gum arabic	Complex coacervation	Eugenol	Stability against thermal degradation and acidic conditions	Jia et al. (2020)
Casein, β -lactoglobulin, kafirin	Antisolvent precipitation	Resveratrol	Improved radical scavenging capacity and stability	Khan et al. (2019)
Casein and zein with polysaccharides	Heat-induced aggregation	Curcumin	Controlled release, improved antioxidant activity, and better re-dispersibility	Chang et al. (2017a)
Casein and gliadin	Co-assembly	Proanthocyanidins	Improved antioxidant capacity, controlled release	Liu et al. (2017a)
Whey protein	Heat-induced aggregation	Vitamin D3	Improved storage stability	Abbasi et al. (2014)
β -Lactoglobulin	Heat-induced aggregation	Caffeine	Controlled release and improved stability	Guo et al. (2017)
Whey protein	Nanoemulsion formation	α -Tocopherol	Improved stability against processing conditions and storage	Relkin et al. (2014)
Whey protein with maltodextrin	Nanoemulsion formation	Folic acid	Improved stability	Assadpour et al. (2016)
Whey protein with almond gum	Self-assembly	Thymol	Improved storage stability	Sedaghat Doost et al. (2019)
Whey protein	Double emulsion formation	Olive leaf phenolics	Controlled release and improved stability	Mohammadi et al. (2016)
Whey protein	Electrospraying	Olive leaf extract	Protection of phenolics	Soleimanifar et al. (2020)
β -Lactoglobulin with alginate	Self-assembly	Curcumin, β -carotene, folic acid, and ergocalciferol	Improved stability	Hosseini et al. (2015)
α -Lactalbumin with dextran	Maillard conjugates	Curcumin	Improved dispersibility, stability against environmental stress, oxidative stability, and antioxidant activity	Yi et al. (2016)
Whey protein	Double emulsion formation	Gallic acid	Improved stability	Gharehbeglou et al. (2019)
Whey protein	Acid-induced aggregation	Lactoferrin	Improved stability against environmental conditions and processing	Raei et al. (2017)
Whey protein with pectin	Double emulsion formation	Folic acid	Controlled release	Assadpour et al. (2017)
Whey protein with pectin	Complexation	Orange oil	Improved protection against environmental conditions	Ghasemi et al. (2017)

[Semo et al. \(2007\)](#) developed a procedure for encapsulating hydrophobic nutraceutical compounds within casein micelles by the association to soluble caseins. Due to its hydrophobic nature, vitamin D2 was selected as a model nutraceutical in this study. It was incorporated within casein micelles by using the natural self-association characteristic of caseins. It was reported that concentration of vitamin D2 was approximately 5.5 times higher in the micelles compared to the serum. Cryogenic transmission electron microscopy images of the micelles revealed that both the particle size and morphology of the fabricated micelles were resembling naturally occurring casein micelles in skim milk. The samples were exposed to ultraviolet (UV) light for 3, 6, 12, and 24 h to investigate the effect of self-assembled casein micelles on photochemical stability of vitamin D2 incorporated in the micelles. It was reported that vitamin D2 was protected against degradation up to some extent inside the micelles. The

authors suggested that hydrophobic bioactives can be entrapped inside self-assembled casein micelles which are capable of protecting and carrying bioactives (Semo et al., 2007).

Caseins were studied in combination with various polysaccharides for fabrication of nanocomplexes. For example, Anal et al. (2008) studied the intermacromolecular complex formation between sodium caseinate and chitosan. The formed complexes between sodium caseinate and chitosan found to be soluble and stable at pH 4.8–6.0, where the biopolymers had opposite charges. When the chitosan concentration was 0.15%, it was observed that larger particles formed due to association of the complexes. The average particle size of chitosan–casein complexes at pH 4.8–6.0 range was 250–350 nm. When the pH was increased to >6.0, phase separation occurred as a result of the association of the nanoparticles. It was reported that the particle size further increased upon the addition of NaCl. Microscopy images of chitosan–caseinate mixtures revealed that the nanoparticles formed were intact, well-separated, and roughly spherical. It was reported that pH affected the electrical charge of the chitosan–caseinate complexes differently compared to the simple solutions of chitosan and sodium caseinate. It was suggested that the complexes between sodium caseinate and chitosan could be effectively used as carrier systems for bioactive ingredients (Anal et al., 2008).

In a later study, Luo et al. (2015) fabricated sodium caseinate–pectin nanoparticles for potential oral delivery applications. Sodium caseinate and pectin nanoparticles were produced by applying thermal treatment and glucono-D-lactone, which is hydrolyzed gradually and lowers the pH of the solution. Rutin, a plant-derived dietary flavonoid with significant pharmacological activities, was selected as a model compound for encapsulation. It was reported that a mixture of sodium caseinate and pectin at 1:1 ratio yielded the most uniform and the smallest nanocomplexes. Heating the mixture increased the yield of nanoparticles and improved its encapsulation efficiency of rutin. Transmission electron microscopy images revealed that sodium caseinate alone had 20 nm-sized structures, whereas pectin showed highly fibrous structures. The mixture of sodium caseinate and pectin had flocculated structures at pH 7, which were significantly different compared to the single biopolymers. Nanocomplexes formed at pH 3.7 were evident under a microscope, and heat treatment was reported to increase the density and sphericity of the particles. It was reported that hydrolysis of sodium caseinate in the simulated gastric medium was delayed by the formation of nanocomplexes. Controlled release of rutin was accomplished in such a way that the complexes released a limited amount of rutin in gastric medium and most of the rutin in an intestinal medium, which was verified by a release kinetics test in simulated gastrointestinal digestion (Luo et al., 2015).

In a more recent study, Jia et al. (2020) fabricated nanoparticles from sodium caseinate and gum arabic. A cross-linking step was applied with tannic acid. The fabricated nanoparticles were used for the encapsulation of eugenol, which is the key compound of clove oil with antimicrobial and antioxidant properties. The protein–polysaccharide nanoparticles were characterized by Fourier transform infrared (FTIR) spectroscopy and fluorescence spectrum analyses. It was observed that the secondary structure of the protein was significantly affected by cross-linking with tannic acid. Eugenol was encapsulated in the nanoparticles (~150 nm) with 70% efficiency. The stability of eugenol against thermal degradation was significantly improved by the encapsulation of eugenol in caseinate–gum Arabic nanoparticles. The cross-linked nanoparticles were also found to be stable at acidic conditions. Storage stability test conducted at different temperatures (4°C–40°C) confirmed the colloidal stability of the nanoparticles (Jia et al., 2020).

Casein was also studied in combination with various proteins of animal or plant origin for the fabrication of nanoparticles. For example, Khan et al. (2019) encapsulated resveratrol in protein nanoparticles. Nanoparticles were fabricated from kafirin, a prolamin-group protein obtained from sorghum, alone or in combination with beta-lactoglobulin or casein. The smallest particles were obtained with 2% beta-lactoglobulin or 0.2% casein. The highest encapsulation efficiency for resveratrol was observed in kafirin–casein particles. Different results were obtained in encapsulated resveratrol's antioxidant properties. It was reported that resveratrol's 2,2-Diphenyl-1-picrylhydrazyl (DPPH)-scavenging capacity was improved by encapsulation in kafirin/milk protein particles, whereas its 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) + scavenging capacity was decreased. The highest stability of encapsulated resveratrol was observed in kafirin/casein particles (Khan et al., 2019).

In another study, protein and polysaccharide-based nanocomplexes were fabricated using different biopolymers. Zein and sodium caseinate were the proteins used, whereas pectin, carboxymethyl cellulose, and gum arabic were the polysaccharides. A cross-linking step was applied to form covalent bridges between proteins and polysaccharides. Curcumin was selected as a model hydrophobic compound for encapsulation. It was reported that the cross-linking step was significantly affected by the polysaccharide type, which in turn allowed for higher stability in simulated gastrointestinal conditions. Morphology and dispersibility of powders in water were observed to change by spray drying. Pectin and carboxymethyl cellulose found to be more effective compared to

gum arabic in such a way that they formed smaller nanoparticles with spherical shape and high encapsulation efficiency. The nanocomplexes showed slower release under simulated gastrointestinal conditions, improved antioxidant activity, and better dispersibility after drying. It was concluded that, in order to fabricate efficient delivery vehicles from protein–polysaccharide complexes, application of a cross-linking step and selection of a proper polysaccharide could be essential (Chang et al., 2017a).

On the other hand, Liu et al. (2017a) followed a different approach and formed hybrid nanoparticles by co-assembling proteins with bioactive compounds. Proanthocyanidins, phenolic secondary metabolites of plants with antioxidant properties were encapsulated in order to improve their stability in various environmental conditions, including temperature, pH, and light. The authors proposed a strategy for fabricating protein–proanthocyanidin hybrid nanoparticles using casein/gliadin and proanthocyanidin. Transmission electron microscopy images revealed that nanocomplexes (~30 nm) fabricated under optimal conditions had regular shapes. It was suggested hydrophobic interactions and that hydrogen bonds were effective in interactions between proanthocyanidin and casein or gliadin. The antioxidant capacity of nanocomplexes was reported to improve compared to native proanthocyanidin with the application of salt, UV radiation, and heat treatment. Protein–proanthocyanidin hybrid nanoparticles were reported to be effective against hepatocellular liver carcinoma. Investigation of the release profile in simulated gastrointestinal fluids revealed that protein–proanthocyanidin nanoparticles had controlled release properties. It was concluded that proteins could be effective delivery vehicles for sensitive bioactives through fabricating hybrid nanoparticles by co-assembly (Liu et al., 2017a).

9.2.2 Whey proteins

Whey proteins are globular proteins obtained from whey, and are composed of α -lactalbumin, β -lactoglobulin, bovine serum albumin, immunoglobulins, and lactoferrin. Whey proteins received great attention as carrier materials in nanoparticle-based delivery systems due to their low cost, nutritional value, and various functional properties. Ha et al. (2019) recently discussed the effect of processing variables on functional and physicochemical properties of nanodelivery systems and bioavailability of encapsulated bioactives to produce efficient delivery systems for various bioactive ingredients.

Abbasi et al. (2014) prepared whey protein nanoparticles by aggregation method for encapsulating vitamin D3. The stability of the nanoparticles was monitored in the presence of air. Nanoparticles had higher amounts of extra vitamin concerning control. More compact structures were formed in the presence of calcium which inhibited oxygen diffusion into the particles. On the other hand, residual amount of vitamin was adversely affected as the vitamin concentration decreased. It was indicated that encapsulating vitamin D3 in whey protein nanoparticles delay its degradation during storage (Abbasi et al., 2014).

In another study, β -lactoglobulin nanoparticles were produced by simple heat-induced denaturation method at different pH and temperature values and their application for encapsulation of caffeine was investigated. Optimum conditions for fabricating β -lactoglobulin nanoparticles with high yield and stability were identified. Microscopy images indicated that β -lactoglobulin nanoparticles (200–300 nm) had spherical shapes. Stability test using different buffers indicated that the structure of the nanoparticles was mainly stabilized by hydrophobic interactions and hydrogen bonds. The highest encapsulation efficiency of caffeine was observed at 50:1 caffeine: β -lactoglobulin molar ratio. They indicated that caffeine's binding to β -lactoglobulin increased as a result of protein unfolding due to heating. During incubation in simulated stomach conditions, the nanoparticles were observed to degrade rapidly and total release of caffeine was achieved at intestinal conditions. Affinity of caffeine for partially denatured β -lactoglobulin was reported to be significantly higher compared to native β -lactoglobulin. It was suggested that the nanoparticles fabricated in the study could have potential applications in the binding of bioactive compounds for improved stability and bioavailability (Guo et al., 2017).

Relkin et al. (2014) fabricated nanoparticles from whey protein by high-pressure homogenization. For encapsulation, α -tocopherol was selected as a model hydrophobic bioactive with sensitivity to heat and oxygen. High-pressure homogenization accompanied by heat treatment was used to produce whey protein aggregates that were used alone as carrier materials or for stabilization of nanoemulsions. The processing treatment applied was reported to show smaller particle size, but higher degradation of the vitamin in whey protein aggregates alone compared to that of emulsions. Physical structure characterization with confocal laser scanning microscopy indicated that a high-pressure homogenization process with higher intensity resulted in a decrease in the stability of protein conformation and increased the interactions between the protein and vitamin. Emulsion droplet size was reported to decrease significantly. Higher intensity homogenization was observed to enhance the adsorption of proteins to oil droplets and decrease the crystalline fat content. It was suggested that the matrix structure should

be considered as an important factor in the encapsulation of α -tocopherol in whey protein aggregates or nanoemulsions stabilized with whey protein-based nanoparticles for improved stability against processing conditions and storage (Relkin et al., 2014).

In another emulsion-based study, Assadpour et al. (2016) optimized the production of whey protein and maltodextrin-based nanoemulsions using a low energy emulsion formation method for encapsulation of folic acid. Span 80 was used as a nonionic surfactant for producing nanoemulsions and the authors aimed to decrease the level of surfactant used in addition to shorten the duration of emulsification. The authors prepared a primary water-in-oil emulsion containing folic acid, and the secondary emulsion was formed using whey protein alone or a complex of whey protein and pectin. Then the double emulsions formed were spray-dried to obtain encapsulated folic acid in powder form. Emulsion formulation was optimized in terms of surfactant concentration, ratio of the dispersed phase, and folic acid concentration and the optimized formulation was encapsulated in whey protein and maltodextrin-based nanoemulsions. Mean droplet diameter of nanoemulsions found to be <100 nm. The authors reported that folic acid content did not affect droplet size. On the other hand, volume fraction of the dispersed phase and the concentration of the small-molecule emulsifier had a significant effect on droplet size. Emulsifier concentration was also found to affect the emulsion viscosity in such a way that higher concentrations resulted in higher emulsion viscosity which was attributed to the surfactant forming free micelles. Using the optimized emulsion formulation, the authors were able to encapsulate folic acid within whey protein and maltodextrin-based nanoemulsions with high encapsulation efficiency and emulsion stability using the low energy emulsion formation method (Assadpour et al., 2016).

Whey protein was also used in combination with almond gum for the fabrication of self-associated nanocomplexes through electrostatic interaction (Sedaghat Doost et al., 2019). Nuclear magnetic resonance technique was employed for characterization of almond gum soluble fraction. Nanocomplex formation was investigated with relation to pH, biopolymer concentration, and mixing ratios. Preparing the complexes at pH 3–4 resulted in the formation of insoluble complexes, whereas pH 4–5 resulted in insoluble complexes with a negative charge. Soluble whey protein–almond gum complexes formed at pH 4.5 were used for producing Pickering emulsions for stabilization of thymol. Complexation with almond gum decreased the contact angle of whey protein which has been stated as an important factor Pickering stabilization of emulsions. Whey protein–almond gum nanocomplexes were observed to adsorb on the surface of oil droplets. Pickering emulsions containing thymol found to be stable in terms of droplet size under 40 days of storage. At the same time, emulsions stabilized with whey protein alone at the same pH were instable due to increase in droplet size and creaming during storage. The authors suggested that thymol emulsion stabilized by whey protein–almond gum nanocomplexes can be used for antioxidant and antimicrobial applications (Sedaghat Doost et al., 2019).

Cabezas et al. (2019) fabricated nanocomplexes between whey protein and soluble soybean polysaccharides for potential application in improving the freeze–thaw stability of oil-in-water emulsions. The authors investigated the effect of heat treatment application for nanoparticle assembly and the order of polysaccharide addition on characteristics of the nanoparticles formed. Interactions between whey protein and soybean polysaccharides found to be pH-dependent, where whey protein/soybean polysaccharides dispersions were clear at pH 6–7 and became turbid as the pH decreased at pH 3.5–5.5 range. Therefore pH 3 was selected as the optimum pH for nanoparticle assembly. The polymers had opposite surface charges and showed clear appearance at this pH. Application of heat treatment at 90°C for 15 min resulted in a decrease in the nonsedimentable protein content of whey protein dispersions. The particle size of the nanocomplexes found to be larger compared to the single biopolymers. Heat treatment application resulted in the formation of significantly larger complexes. The authors suggested that heat treatment affected the organization of molecules during complexation. To support this hypothesis, the authors determined the aromatic surface hydrophobicity of the dispersions. It was reported that heating increased the surface hydrophobicity of whey protein dispersions, which was attributed to the exposure of previously buried nonpolar amino acids. Furthermore, the addition of soybean polysaccharides decreased the surface hydrophobicity of whey protein, which was suggested to be resulting from the formation of a shell-like layer of polysaccharides on the protein surface. Dispersions' turbidity monitored in the absence and presence of urea and dithiothreitol to investigate the internal structure of the complexes. As a result, it was suggested that the nanocomplexes formed mainly through hydrophobic interactions, hydrogen, and disulfide bonds. Interfacial behavior of the nanocomplexes was investigated to elucidate their potential application for improving the freeze–thaw stability of emulsions. The authors reported that denatured particles readily adsorbed at the oil–water interface and complexation with soybean polysaccharides further enhanced the absorption of proteins at the interface. Freeze–thaw stability of oil-in-water emulsions stabilized with whey protein–soybean polysaccharide nanocomplexes was reported to be significantly higher than that of emulsions stabilized with only whey protein. On the contrary, heated nanocomplexes were reported to result in lower freeze–thaw stability in the emulsion. It was concluded that; the fabrication

procedure of the nanocomplexes should be considered as an important factor when developing nanoparticle-stabilized emulsions for improved stability to environmental stress (Cabezas et al., 2019).

In another study, Mohammadi et al. (2016) fabricated double emulsions with whey protein for nanoencapsulation of olive leaf phenolics. Primary emulsion was a water-in-oil emulsion with olive leaf extract, whereas the external water phase contained whey protein–pectin complexes. The mean droplet diameter of the primary emulsion was measured as ~ 6 nm and increased to ~ 23 nm after 20 days of storage at 30°C. The mean droplet diameter of double emulsions stabilized with whey protein–pectin complexes (1443 nm) found to be significantly higher compared to double emulsions stabilized with whey protein alone (675 nm), which attributed to the increased thickness of the biopolymer layer around the oil droplets. The mean droplet diameter of double emulsions stabilized with whey protein–pectin complexes increased to 1992 nm after 20 days of storage at 30°C. On the contrary, the mean droplet diameter of double emulsions stabilized with whey protein alone decreased from 675 to ~ 348 nm during storage, which was explained by the breakdown of the liquid film between the droplets of the primary emulsion and resulting in the release of the droplets. The viscosity of double emulsions formed with whey protein–pectin complexes (128 mPa.s) found to be significantly higher compared to double emulsions formed with whey protein alone (~ 16 mPa.s) and only minor changes were observed in emulsion viscosity during storage. The encapsulation efficiency of olive leaf phenolics in whey protein-stabilized and whey protein–pectin-stabilized double emulsions was reported as 93.34% and 96.64%, respectively, which decreased to 72.73% and 88.81% after 20 days of storage. Double emulsions formed with whey protein–pectin complexes showed the lowest rate of phenolic release, whereas double emulsions formed with whey protein alone had the fastest release rate. Color analyses of the emulsions during storage showed that increased release in the double emulsions increased the “blueness/yellowness” value and decreased lightness. It was suggested that using whey protein–pectin complexes instead of protein alone for stabilization of double emulsions improves emulsion stability and allows a slower release of the entrapped bioactive due to different mechanisms (Mohammadi et al., 2016).

In another recent study, Soleimanifar et al. (2020) fabricated whey protein nanoparticles containing phenolic-rich olive leaf extract with electrospraying method and investigated the effect of formulation on the nanoparticles formed. The amount of whey protein and olive leaf extract had a significant effect on the size of the nanoparticles formed with electrospraying. Higher encapsulation efficiency values obtained at higher concentrations of whey protein and olive leaf extract. Net surface charge of the particles found to be negative. The chemical structure of nanoparticles was investigated with FTIR analysis, where the contribution of the ingredients (whey protein, olive phenolics, and emulsifier) was observed with specific bands. The average particle size of nanoparticles was reported to be between 232 and 660 nm with a heterogeneous nanoparticle population. Increasing whey protein concentration from 15% to 30% resulted in a decrease in particle size and an increase in encapsulation efficiency. On the other hand, increasing the olive leaf extract concentration from 500 to 1000 ppm increased in particle size. Oleuropein, tyrosol, caffeic acid, and hydroxytyrosol were identified as the key phenolics in the olive leaf extract. The optimum formulations in terms of retention of phenolic compounds were identified. It was reported that olive leaf extract was entrapped within the whey protein matrix, and the developed nanoparticle-based delivery system could be promising for encapsulation of sensitive bioactives (Soleimanifar et al., 2020).

Encapsulation of bioactive compounds with antioxidant properties in whey protein–polysaccharide nanocomplexes has been investigated extensively. For example, Hosseini et al. (2015) studied the encapsulation of four model compounds (curcumin, β -carotene, folic acid, and ergocalciferol) in β -lactoglobulin–alginate nanocomplexes. The authors reported that nutraceutical type and pH were the effective factors in complexation between β -lactoglobulin and nutraceuticals. Furthermore, binding sites on the protein and binding mechanism changed according to the nutraceutical used. It was reported that the highest binding affinity was observed in β -carotene, which was attributed to the more hydrophobic character of the compound. Binding constants were reported to change when the pH was lowered, depending on the characteristics of the compound. The nanocomplexes were observed to remain stable against aggregation. The authors suggested that β -lactoglobulin–alginate nanocomplexes formed could have the potential for encapsulating both hydrophilic and hydrophobic bioactives (Hosseini et al., 2015).

Yi et al. (2016) followed another approach and encapsulated curcumin in Maillard conjugates formed between α -lactalbumin and dextran. Characteristics of α -lactalbumin–dextran conjugates were studied. Antioxidant activity and physicochemical stability of curcumin encapsulated in the designed nanocomplexes were also investigated. Aqueous dispersibility of curcumin was reported to improve significantly with encapsulation within α -lactalbumin–dextran conjugates. The average particle size of α -lactalbumin and α -lactalbumin–dextran conjugates were measured as 60–70 nm, respectively. It was reported that the encapsulation efficiency of curcumin was no affected by glycosylation. However, glycosylation found to improve the stability of nanoparticles against environmental stress such as heat treatment, low pH, and high salt conditions. Stability of α -lactalbumin–dextran conjugates under environmental stresses such as pH,

heat, and salt was significantly higher compared to that of nanocomplexes prepared with α -lactalbumin only. Encapsulation within α -lactalbumin–dextran conjugates was reported to improve the oxidative stability and DPPH-scavenging activity of curcumin. An increase in antioxidant activity of nano encapsulated curcumin was attributed to the increased interaction between curcumin and free radicals due to increased surface area and dispersity in water. The authors suggested that α -lactalbumin-based nanoparticles can be efficiently used for encapsulation of hydrophobic bioactives for improved stability (Yi et al., 2016).

In another recent study with antioxidants, Gharehbeiglou et al. (2019) encapsulated gallic acid in double emulsions ($W_1/O/W_2$) stabilized with whey protein. The authors compared the performance of whey protein–pectin nanocomplexes with small-molecule surfactants in stabilizing nanoemulsions containing gallic acid. The W_1/O phase was stabilized with polyglycerol polyricinoleate. A wide range of droplet size (99.5–1520 nm) was observed in the study. The size of the nanoparticles found to increase with increasing whey protein concentration and the simultaneous use of whey protein and pectin. Increasing the concentration of whey protein and pectin was reported to increase the polydispersity index of double emulsions which is an indication of nonuniform oil droplet. The droplet size of emulsions formed with small-molecule emulsifiers (Tween 80) found to be smaller compared to that of emulsions formed with whey protein–pectin nanocomplexes. Net surface charge of whey protein–pectin-stabilized double emulsions were reported to be significantly higher than that of Tween 80-stabilized double emulsions. The resistance of double emulsions against sedimentation and creaming found to be similar for both whey protein–pectin and Tween 80-stabilized double emulsions. It was suggested that whey protein–pectin complexes can be utilized as a potential replacer for small-molecule emulsifiers in double emulsions carrying bioactives such as gallic acid (Gharehbeiglou et al., 2019).

Nanostructured whey proteins were used for encapsulating other bioactives with functional properties, too. For example, Raei et al. (2017) encapsulated lactoferrin, a glycoprotein responsible for carrying nutrients and also with other biological functions including antioxidant, antitumor, and antimicrobial properties. The authors aimed to design whey protein–pectin nanocomplexes for encapsulation of lactoferrin in order to improve its stability against environmental conditions and processing stresses. The authors investigated the effect of whey protein/pectin ratio, preparation pH, and acidification method (pre and postacidification) on physicochemical characteristics of nanocomplexes formed. Net surface charge of the nanoparticles found to decrease with decreasing pH in both acidification methods. Particle size increased at higher pectin/whey protein ratios. The postacidification method was reported to produce nanocomplexes with smaller mass compared to preacidification. The smallest particle size was observed in nanocomplexes fabricated with 2:1 whey protein/pectin at pH 3.5. For both pre and postacidification methods, the highest lactoferrin content and encapsulation efficiency were obtained at pH 3.5. Application of the preblending method and optimized biopolymer formulations can be effectively used for fabricating nanoparticles containing lactoferrin applicable in various food formulations (Raei et al., 2017).

Assadpour et al. (2017) studied the encapsulation of folic acid in double emulsions stabilized with whey protein alone or whey protein–pectin complexes. The double emulsions were spray-dried to produce encapsulated folic acid in powder form. Release characteristics of encapsulated folic acid were investigated at 37°C and pH 4, 7, and 11, respectively. Release rate of encapsulated folic acid found to be pH-dependent. The release rate at pH 11 found to be eight times higher than the release rate at pH 4 and four times higher than the release rate at pH 7. The authors suggested that high release at alkaline conditions and low release at acidic conditions could be useful for targeted release applications. Release profile of nanocapsules stabilized with whey protein alone and nanocapsules stabilized with whey protein–pectin complexes found to be similar. Surface of the nanocapsules stabilized with whey protein alone were observed to be smooth and had less surface dents and cracks. On the other hand, when pectin was incorporated, the surface of the particles appeared to have wrinkles and some shrinkage. FTIR spectroscopy analysis indicated the formation of whey protein–pectin complexes with the observation of the amide bond between the amino groups of protein and the carboxyl groups of pectin (Assadpour et al., 2017).

Flavor ingredients such as essential oils and aroma compounds were also encapsulated using protein-based nanodelivery systems. For example, Ghasemi et al. (2017) encapsulated orange oil, to provide some protection to the volatile compound against environmental conditions. Whey protein–pectin nanoparticles were fabricated with the complexation method. Effects of pH and concentration of biopolymers on the physicochemical properties of whey protein–pectin nanocomplexes carrying orange oil were investigated using an experimental design. The optimized formulation in terms of biopolymer concentration was prepared at different pH values (3, 6, and 9) freeze-dried to obtain encapsulated orange oil in powder form. The particle size of the nanocapsules was reported to be between 182 and 360 nm and affected by pH. The smallest nanocapsules were obtained at pH 6. The authors indicated that pH had a significant effect on the physicochemical characteristics of orange oil encapsulated in whey

protein–pectin nanocomplexes. As the pH was increased from 3 to 9, moisture content, water activity, the bulk, and absolute density of the powders were observed to decrease. On the other hand, increasing the pH about 9 had a tremendous increase in solubility (from 23% to 88%) of the encapsulated orange oil powder which was attributed to formation of weaker complexes at pH 9 which were easier to dissolve. The nanocomplexes prepared at pH 3 resulted in the highest encapsulation efficiency and the lowest score for yellowness, which indicated that orange oil was entrapped inside the complexes. Encapsulation efficiency found to be decrease from 88% to 70% as the pH was increased from 3 to 6. High encapsulation efficiency observed at acidic pH was attributed to formation of stronger complexes which were able to entrap higher amounts of orange oil. FTIR spectroscopy analysis was used to confirm the formation of complexes between whey protein and pectin. Scanning electron and atomic force microscopy analyses revealed that nanoparticles prepared at pH 3 were more spherical compared to pH 6 and 9. The authors concluded that whey protein–pectin nanocomplexes formed using the optimized formulation and pH can be employed for encapsulating orange oil ([Ghasemi et al., 2017](#)).

In a follow-up study, the same group encapsulated limonene, the major component of the citrus peel oils within whey protein–pectin nanocomplexes. Effects of biopolymer concentration and pH were investigated and the formulation was optimized. Nanoparticles formed were observed to be spherical. The particle size of the limonene–laden nanocomplexes was measured as 160 nm. Encapsulation efficiency was determined as 88% at pH 3. Using a ratio of 4:1 between whey protein and pectin resulted in the highest complex formation. It was suggested that whey protein–pectin nanocomplexes fabricated at the optimal conditions could be utilized for encapsulating appreciable amounts of limonene ([Ghasemi et al., 2018](#)).

9.2.3 Egg proteins

Egg protein-based nanoparticles were also among the recently investigated nanodelivery systems for bioactive ingredients. In a current study, Arzeni and Pilosof (2019) encapsulated folic acid in egg white-based nanoparticles and investigated the bioaccessibility of folic acid. The authors also aimed to evaluate the effect of binding folic acid on the profile of peptides post digestion in an in vitro system. Release of folic acid from the nanoparticles was monitored by using reversed phase-high performance liquid chromatography technique where specific wavelengths were used for detecting proteins, peptides, and folic acid. It was reported that the interaction formed between folic acid and egg white nanoparticles was not separated under the chromatographic conditions used in the study. On the other hand, following digestion, no significant difference was observed in the areas of the folic acid peak, which was attributed to the release of folic acid, which could contribute to the bioaccessibility of the vitamin. The authors reported that proteins aggregated slightly due to binding of folic acid; however, presence of folic acid did not affect the peptide profile after digestion. The authors suggested that nanostructured egg white proteins could be considered as effective carriers of folic acid ([Arzeni and Pilosof, 2019](#)).

Furthermore, ovalbumin, the main component of egg white protein was employed in recent studies for nanoencapsulation of bioactive ingredients with different functionalities. For example, [Visentini et al. \(2019\)](#) fabricated self-assembled ovalbumin nanoparticles with heat-induced aggregation method. Nanoparticles were formed at two different pH 11.35 and 7.5 and characterized. Inclusion complexes were formed between ovalbumin aggregates and linoleic acid (LA) and conjugated linoleic acid (CLA). It was reported that pH of the nanoparticle formation process had a significant effect on nanoparticles hydrodynamic diameter in such a way that nanoparticles formed at pH 7.5 had a much greater hydrodynamic diameter compared to that of nanoparticles formed at pH 11.35. On the other hand, no significant differences in net surface charge was observed. Size measurements indicated that fatty acid binding increased in nanoparticles size. Nanocomplexes were observed to have a round shape. Their size increased upon LA and CLA binding. For both pH values, ovalbumin nanoparticles were reported to bind higher amounts of LA compared to CLA which was attributed to the structural differences between the fatty acids. It was indicated that the physicochemical properties of protein nanoparticles and structural characteristics of fatty acids are important factors for designing inclusion nanocomplexes for polyunsaturated fatty acid (PUFA) delivery ([Visentini et al., 2019](#)).

Ovalbumin was also used in combination with various polysaccharides for producing nanoparticle-based delivery systems. For example, [Feng et al. \(2016\)](#) employed the Maillard reaction to fabricate conjugates of ovalbumin and dextran. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and far-UV circular dichroism techniques were used to confirm the covalent attachment of dextran to ovalbumin. It was reported that conjugation with dextran changed the secondary structure of ovalbumin significantly. Ovalbumin–dextran conjugates were then loaded with curcumin and heat treatment was applied near the isoelectric point of ovalbumin to form stable nanogels. The authors aimed to improve the bioavailability of curcumin in the ovalbumin–dextran

nanogels. Characteristic curcumin peaks were not observed in the FTIR spectra of the nanogels which indicated that curcumin was entrapped within the nanogels. It was reported that the physicochemical properties of the nanogels were not affected by encapsulation of curcumin within the nanogels. “transformation” of curcumin, which is an indication of the nondegraded curcumin fraction during the digestion was reported to be significantly higher in nanogels compared to the ovalbumin nanoparticles. On the other hand, bioaccessibility of curcumin in nanogels and nanoparticles found to be similar. The authors suggested that the ovalbumin–dextran nanogels fabricated can be utilized as curcumin nanocarriers for improved bioavailability (Feng et al., 2016).

In another study, Xie et al. (2019) fabricated ovalbumin/ κ -carrageenan nanocomplexes for the encapsulation of curcumin. The ovalbumin/ κ -carrageenan complex nanoparticles were reported to show good curcumin entrapping performance. Moreover, the inclusion of curcumin did not affect the emulsifying properties of the nanocomplexes significantly. The highest emulsifying activity of ovalbumin/ κ -carrageenan/curcumin complexes was observed at 48 h whereas the highest emulsion stability was observed at 36 h. Encapsulation of curcumin found to increase antioxidation activity, thermal, and light stability. The authors reported that the ovalbumin/ κ -carrageenan/curcumin nanocomplexes released only a small amount of curcumin in the simulated gastric juice during 3 h and most of the curcumin was still combined with the nanocomplexes. On the other hand, the release rate of curcumin from the nanocomplexes found to be fast ($\sim 70\%$) in the first hour in the simulated intestinal fluid. The authors indicated that the nanocomplexes formed can provide a protective effect for curcumin in the acidic conditions and controlled release in the intestine, which can be useful for enhancing the bioavailability of the compound (Xie et al., 2019).

9.2.4 Gelatin

In addition to milk and egg-sourced proteins, gelatin was also studied for fabricating nanoscale delivery systems. Jin et al. (2017) produced gelatin-based nanocomplexes for the Pickering stabilization of emulsions. The nanocomplexes were fabricated from gelatin, konjac glucomannan, and tannic acid through the self-assembly method using three levels of glucomannan content. It was observed that increasing glucomannan concentration resulted in the formation of more hydrophilic nanocomplexes with the larger size. Fluorescence microscopy images revealed that the nanocomplexes were positioned at the oil–water interface. The nanocomplexes found to be more effective in decreasing the interfacial tension between oil and water compared to gelatin and formed elastic films at the interface. The authors reported that, as the concentration of nanocomplexes increased, the equilibrium tension and droplet size decreased slightly, and the size distribution of the droplets became uniform. When the nanocomplex concentration was kept constant and the oil phase ratio was increased, an increase in droplet size was observed. The authors suggested that the gelatin–glucomannan–tannic acid nanocomplexes can be potentially used as an alternative to surfactants in food emulsions (Jin et al., 2017).

In a recent study by Ding et al. (2019), cross-linked gelatin nanoparticles were produced with an antisolvent precipitation method and used for encapsulating fish oil. The effects of preparation parameters and storage conditions on properties of fish oil-loaded cross-linked nanoparticles were investigated. It was reported that the droplet size of fish oil–laden Pickering emulsions was affected by the pH of the gelatin solution, homogenization time, and speed. Storage temperature had varying effects on the stability of the fish oil–laden Pickering emulsions. It was observed that emulsions stored at -18°C contained flocculated precipitates, whereas emulsions stored at 4°C were still in liquid form. The viscosity of the emulsions found to increase significantly during storage at 37°C and the emulsions stored at 60°C turned into gel form. Therefore storage at 4°C was determined to be the most suitable storage condition for the emulsions. The authors suggested that fish oil–laden cross-linked gelatin nanoparticles can find promising applications in beverages (Ding et al., 2019).

9.3 Delivery systems with plant-based nanostructured proteins

9.3.1 Soy protein

Soy protein contains mainly glycinin and β -conglycinin and has balanced proportions of charged, nonpolar, and polar amino acids. Soy protein has been investigated for its potential for the production of protein-based nanodelivery systems for various bioactive ingredients. Methods used for fabricating various nanostructured soy proteins and the feasibility of these structures as delivery systems for bioactive ingredients were reviewed recently (Tang, 2019). A summary of some of the recent studies on fabrication, characterization, and applications of nanostructured soy proteins for bioactive delivery is presented in Table 9.2.

TABLE 9.2 Formation, characterization, and applications of nanostructured soy proteins for delivery of bioactives.

Protein	Fabrication method	Bioactive ingredient	Application	References
Soy protein coated with sodium caseinate	Ultrasonication	Conjugated linoleic acid	Improved oxidative stability and controlled release	Gao et al. (2014)
Soy β -conglycinin	Antisolvent precipitation	Vitamin D3	Improved thermal and storage stability, controlled release	Levinson et al. (2014)
Soy protein	Cold-gelation	Vitamin B12	Improved bioavailability	Zhang et al. (2015)
Soy protein	Complexation with and without heat treatment	Curcumin	Improved solubility, stability, and bioaccessibility	Chen et al. (2015)
Soy β -conglycinin	Self-assembly	Curcumin	Improved stability and bioaccessibility	Liu et al. (2019a)
Soy protein	Complexation	Curcumin	Improved antioxidant and foaming properties	Xiang et al. (2018)
Soy protein	Pickering stabilized emulsions	Anthocyanins	Improved oxidative and physical stability	Ju et al. (2020)

[Liu et al. \(2017a\)](#) fabricated soy protein isolate nanoparticles as Pickering stabilizers. A nonthermal, Ca^{2+} -induced aggregation process was applied. The authors reported that turbidity and particle size increased significantly as the Ca^{2+} concentration increased, which indicated protein aggregation and nanoparticle formation. Aggregation due to increased Ca^{2+} concentration is reported to occur due to Ca^{2+} bridging between protein molecules and increased intermolecular interactions as a result of the screening of surface charges. Nanoparticles formed were cross-linked using varying concentrations of glutaraldehyde. Cross-linking treatment was reported to result in increased particle size and surface charge but decreased surface hydrophobicity. The authors indicated that nanoparticle formation by Ca^{2+} -induced aggregation occurred through hydrophobic interactions and hydrogen bonding, whereas cross-linking with glutaraldehyde established the integral integrity of the nanoparticles via the formation of amide bonds. Considering the emulsification properties of soy protein nanoparticles, the cross-linking with glutaraldehyde increased droplet size decreased emulsification performance and improved stability against coalescence. The fabricated protein-based nanoparticles suggested as potential Pickering stabilizers for various functional food formulations ([Liu et al., 2017b](#)).

[Gao et al. \(2014\)](#) fabricated soy protein-based lipophilic nanoparticles with ultrasonication technique for delivery of CLA. The CLA-loaded soy protein nanoparticles coated with sodium caseinate for improved colloidal stability. Encapsulation within protein-based nanoparticles reported to improve the oxidative stability of CLA compared to its free form. During release in the simulated gastrointestinal system, the release of CLA found to be constant through the whole digestion process, with only 20% being exposed in the gastric environment. Soy protein nanoparticles coated with sodium caseinate were proposed as effective delivery systems for hydrophobic bioactive ingredients that could provide oxidative stability and controlled release ([Gao et al., 2014](#)).

Micronutrients with different physicochemical properties were also encapsulated with nanostructured soy protein delivery systems. [Levinson et al. \(2014\)](#) fabricated soybean β -conglycinin nanoparticles for encapsulation of vitamin D. Since β -conglycinin is the relatively more amphiphilic component of soy protein. It was reported to have better emulsification properties compared to glycinin; it was selected as a potential delivery vehicle for vitamin D. Vitamin D- β -conglycinin nanocomplexes were fabricated with an antisolvent precipitation method. It was reported that the β -conglycinin solution remained transparent, with no significant increase in turbidity when Vitamin D was added. The average particle size of free Vitamin D was measured as 600–700 nm, whereas the average size of Vitamin D-loaded β -conglycinin nanoparticles was 50–400 nm, depending on the concentration of the vitamin. Encapsulation of vitamin D in β -conglycinin nanoparticles was reported to provide stability to the vitamin. When heat treatment was applied to the samples at 70°C for 30 s at pH 2.5; 75% of free vitamin D was degraded; whereas only 2.5% of encapsulated vitamin D was lost. A simulated shelf-life test was applied at 23°C and at two different pH values where only 30% and 10% loss was observed in encapsulated vitamin D at pH 6.8 and 2.5, respectively. On the other hand, 78%–87% degradation was reported for free vitamin. A release test in a simulated gastric environment indicated that more than 90% of vitamin D was protected in β -conglycinin nanoparticles at the end of 2 h digestion. The authors suggested that β -conglycinin can serve as an effective nanoencapsulation agent for hydrophobic nutraceuticals and can help provide stability to vitamins against environmental and processing conditions ([Levinson et al., 2014](#)).

On the contrary, [Zhang et al. \(2015\)](#) investigated the feasibility of soy protein nanoparticles for entrapment of a water-soluble nutraceutical; vitamin B₁₂ by studying its intestinal transport and absorption behavior. A cold-gelation method was applied to fabricate three different sized vitamin B₁₂-loaded soy protein-based nanoparticles by modulating preparation conditions. The effect of particle size of soy protein nanoparticles on intestinal uptake and transport mechanisms of vitamin B₁₂ was investigated. The nanoparticles did not affect the Caco-2 cells, which was attributed to the hydrophilic nature and biocompatibility of the protein. Nanoencapsulation of vitamin B₁₂ in soy protein was reported to increase the transport of the protein across the Caco-2 cell monolayers, depending on the particle size. The intestinal transport of vitamin B₁₂ was also reported to improve by nanoencapsulation. The authors suggested that the encapsulation of vitamin B₁₂ in soy protein nanoparticles can be an effective way for the oral delivery of the vitamin ([Zhang et al., 2015](#)).

In a recent study by [Ju et al. \(2020\)](#), soy protein–anthocyanin nanocomplexes were fabricated for Pickering stabilized emulsions. Covalently bonded nanocomplexes were formed between soy protein and anthocyanins purified from black rice extract. The authors reported that an increase in anthocyanin concentration increased the binding rate and the amount of soy protein conjugated with anthocyanins. Average particle size of soy protein–anthocyanin complexes found to be smaller compared to soy protein nanoparticles. Net surface charge of soy protein–anthocyanin complexes was reported to increase which was attributed to the lowering of isoelectric point of soy protein by the covalent conjugation with anthocyanins. On the other hand, surface hydrophobicity of the nanoparticles was observed to decrease due to complexation with anthocyanins and enhanced electrostatic repulsion with increased net surface charge. It was reported that radical scavenging activity of soy protein–anthocyanin nanocomplexes increased as the anthocyanin concentration increased. Pickering stabilized oil-in-water emulsions were prepared with soy protein–anthocyanin nanocomplexes. Lipid hydroperoxide and malondialdehyde content of the emulsions found to decrease significantly which indicated improved oxidative stability. A simulated intestinal digestion test indicated that the amount of free fatty acids released from Pickering emulsions was reduced when the anthocyanin concentration was increased, which was attributed to the formation of a strong interfacial structure by the soy protein–anthocyanin nanocomplexes which acted as a physical barrier limiting the lipolysis process. Creaming index of emulsions was reported to decrease, indicating improved emulsion stability, with the use of soy protein–anthocyanin nanocomplexes as Pickering stabilizers. The authors suggested that the fabricated Pickering emulsions could provide protection and extend the shelf life of sensitive lipophilic nutrients ([Ju et al., 2020](#)).

Curcumin was one of the most extensively studied bioactive compounds for encapsulation in protein-based nanodelivery systems. Different methodologies developed for entrapping this valuable compound for improved stability, water solubility, and bioavailability. [Chen et al. \(2015\)](#) formed nanocomplexes between curcumin and soy protein with and without heat treatment. The authors reported that complexation with curcumin did not affect the particle size and morphology of soy protein nanoparticles. On the other hand, a gradual increase was observed in protein denaturation and surface hydrophobicity as the temperature was increased from 75°C to 95°C. Encapsulation efficiency of curcumin was reported to decrease as the curcumin concentration increased. Nanocomplexation with soy protein was reported to enhance the solubility of curcumin by 98,000-fold compared to free curcumin. A stability test at 25°C for 4 h indicated that almost 90% of curcumin remained stable in soy protein nanocomplexes, whereas 80% of degradation was observed in free curcumin. Additionally, bioaccessibility of curcumin was reported to enhance upon complexation with soy protein verified by a simulated gastrointestinal digestion test. It was suggested that the bioavailability of lipophilic bioactive ingredients could be improved by complexation with soy protein nanoparticles ([Chen et al., 2015](#)).

In a recent study, [Liu et al. \(2019a\)](#) fabricated soy β -conglycinin nanoparticles with an ethanol-assisted assembly method for curcumin delivery. The authors reported that protein denaturation and aggregation occurred due to the unfolding of the structure of β -conglycinin as a result of ethanol treatment. When ethanol was removed from the medium by dialysis, nanostructured particles formed by reassembly of the denatured and aggregated protein. It was emphasized that ethanol concentration had a significant effect on nanoparticle size and morphology. Protein aggregation increased, and more compact particles formed when ethanol concentration was increased. Moreover, protein aggregation was reported to increase further, and larger particles formed when curcumin was present in the ethanol solution. The highest curcumin loading capacity observed in the reassembled β -conglycinin particles at 40% ethanol concentration. It was reported that curcumin encapsulated in reassembled particles showed significantly higher stability and bioaccessibility compared to that of free curcumin. It was suggested that the developed nanoscale delivery system could be efficiently used for improved stability, water solubility, and bioaccessibility of lipophilic ingredients ([Liu et al., 2019a](#)).

The same group ([Liu et al., 2019b](#)) developed another process for fabricating nanostructured soy β -conglycinin for curcumin delivery by the urea-assisted reassembly method. The structure of β -conglycinin was reported to

dissociate into its separate subunits gradually and even denature at high urea concentrations. When urea was removed from the medium with dialysis, nanostructured particles formed by reassembly of the previously denatured protein. It was suggested that, when curcumin was introduced into the medium, curcumin molecules interacted with the denatured subunits of β -conglycinin and were entrapped inside the reassembled nanoparticles. Higher amounts of curcumin were loaded in the urea-assisted nanoparticles compared to the previously reported loading amount for ethanol-assisted reassembled nanoparticles. Encapsulated curcumin was reported to show enhanced thermal stability, solubility in water, and bioaccessibility with a controlled release profile. Fabricating protein-based nanodelivery systems for poorly soluble nutraceuticals using an organic, solvent-free, and eco-friendly process was emphasized in the study (Liu et al., 2019b).

Xiang et al. (2018) followed a different approach and treated soy protein with glutaminase before its complexation with curcumin. Native and modified soy protein were compared in terms of physicochemical characteristics, effectiveness in complexing with curcumin, antioxidant properties, and storage stability. It was reported that glutaminase treatment resulted in changes in the secondary structure of the protein, increasing unfolding of the protein which also increased exposure of the previously buried hydrophobic groups. On the other hand, radical scavenging capacity and foaming properties of the protein were reported to improve by glutaminase treatment. The concentration of protein found to have a significant effect on the complex formation with curcumin. The highest antioxidant activity and entrapment efficiency for curcumin were achieved with 1% protein. The developed procedure for fabricating glutaminase-treated soy protein nanoparticles was proposed as a simple procedure for designing efficient delivery systems for curcumin (Xiang et al., 2018).

9.3.2 Corn protein

Zein is the major storage protein of corn, comprising 35%–60% of total protein. Zein belongs to the prolamine group with strong hydrophobicity and characteristic solubility profile. Due to its structural properties, zein has been shown to form micro- and nanoparticles through self-assembly and has been widely investigated as a carrier for nanodelivery of bioactives in food and pharmaceutical formulations. The tertiary structure of zein, self-assembly mechanisms, preparation, characterization, and application of zein-based micro- and nanoparticles have been reviewed (Luo and Wang, 2014).

Zou et al. (2016) fabricated curcumin-loaded zein nanoparticles by antisolvent precipitation improved stability and bioaccessibility. Nanoparticles were reported to be relatively small (~ 100 nm) with a uniform size distribution, and carry a positive surface charge ($\sim +20$ mV). A simulated gastrointestinal digestion test indicated that the bioaccessibility of curcumin increased as the nanoparticle concentration increased due to improved solubilization capacity. It was reported that the curcumin loading capacity of the zein nanoparticles was significantly higher compared to that of nanoemulsions and nanoliposomes containing curcumin. On the other hand, nanoemulsions showed the highest bioaccessibility for curcumin. The authors suggested that zein nanoparticles can be employed for improved bioaccessibility of curcumin (Zou et al., 2016).

Yao et al. (2018) also worked with curcumin-laden zein nanoparticles; however, they investigated the effect of electrostatic deposition of the gelatin-alginate coating on the characteristics of the system. Nanoparticles were fabricated with the antisolvent precipitation method and coated with fish gelatin–sodium alginate solutions. Stability of the nanoparticles against the changes in pH (3–7) was reported to improve significantly by biopolymer coatings. It was indicated that, no aggregation or sedimentation was observed around the isoelectric points of proteins, which suggested the protective effect of alginate in the coating against aggregation. Stability of the nanoparticles was also monitored against the changes in ionic strength. The authors observed an increase in particle size when the salt concentration increased from 0 to 400 mM, and particle aggregation and sedimentation at 500 mM which was attributed to the weak electrostatic interactions between nanoparticles due to high concentrations of salt. It was observed that the biopolymer-coated nanoparticles fabricated with the optimum formulation found to be stable against aggregation with no significant change in particle size during four weeks of storage at 4°C. It was reported that heat treatment at 80°C resulted in no significant change in particle size up to 40 min; however, some particle aggregation occurred at 60 min. High encapsulation efficiency ($\sim 88.5\%$) and curcumin loading capacity ($\sim 7.3\%$) were reported for the nanoparticles. Bioaccessibility of encapsulated curcumin (22.4%) was reported to improve significantly compared to its free form ($<8\%$) which was attributed to the improved solubility and stability against degradation in the gastrointestinal environment. Antioxidant activity of curcumin measured after digestion in terms of radical scavenging and ferric ion reducing capacity was reported to improve significantly with encapsulation within zein nanoparticles. The authors suggested that stability of curcumin-laden zein nanoparticles against environmental and processing conditions can be improved by application of biopolymer coatings (Yao et al., 2018).

Other lipophilic antioxidants were also encapsulated within zein-based nanoparticles in recent studies. For example, [Li et al. \(2019\)](#) fabricated zein colloidal nanoparticles with antisolvent precipitation method and used soluble soybean polysaccharide as a stabilizer. Complexation between soybean polysaccharides and zein was reported to occur through electrostatic interactions, hydrogen bonding, and hydrophobic effects. The authors indicated that zein nanoparticles were susceptible to aggregation at pH 5–7 in the absence of soybean polysaccharides where they observed increase in particle size followed by sedimentation. On the other hand, complexes formed between soybean polysaccharides and zein found to be stable against aggregation at pH 5–7 which was attributed to the structural features and high solubility of soybean polysaccharides. Zein–soybean polysaccharide nanocomplexes found to be relatively stable against aggregation at 0–75 mM salt concentration compared to individual zein nanoparticles. Increasing the salt concentration to 100 mM resulted in aggregation revealed by increased particle size and sedimentation due to the screening of the surface charges of protein by salt ions. Both individual zein nanoparticles and zein–soybean polysaccharide nanocomplexes found to be stable against aggregation during heat treatment at 80°C for 2 h. Surface hydrophobicity of zein nanoparticles was reported to decrease upon coating with soybean polysaccharides which was attributed to blocking of the hydrophobic parts on the surface of zein particles. The fabricated nanoparticles were evaluated to encapsulate quercetin, a natural flavonol with low solubility and stability. Encapsulation efficiency of quercetin was reported to increase from ~59% to ~83% after the soybean polysaccharide coating. Radical scavenging capacity and photochemical stability of quercetin were significantly improved with encapsulation within zein–soybean polysaccharide nanocomplexes. The authors indicated that the developed nanoparticles could be used as an efficient delivery system for hydrophobic bioactive molecules ([Li et al., 2019](#)).

In another recent study, [Ba et al. \(2020\)](#) developed a ternary system composed of zein–carboxymethyl chitosan–tea polyphenols and enriched it with β -carotene. When designing the ternary system, carboxymethyl chitosan was selected due to its controlled release and targeted delivery properties in encapsulation applications, whereas tea polyphenols were used for their antioxidant properties and ability to form strong covalent bonds with proteins. The ternary complexes were reported to have the highest stability against color fading compared to individual zein nanoparticles and zein–carboxymethyl chitosan complexes. High antioxidant activity and ability to inhibit β -carotene degradation were reported for the ternary complexes. The ternary nanoparticles fabricated in the study were proposed as an effective encapsulation system for lipophilic bioactives with enhanced storage stability ([Ba et al., 2020](#)).

Zein was also used in combination with sodium caseinate for developing nanostructured delivery systems. Due to zein's hydrophobic nature, individual zein-based nanoparticles are reported to be prone to aggregation during storage and when they are dried, their powders usually have low re-dispersibility in water. Sodium caseinate has been utilized as an emulsifier to improve the stability and water dispersibility of zein nanoparticles. In this context, [Chang et al. \(2017b\)](#) fabricated zein–sodium caseinate–pectin nanocomplexes with pH and heat-induced aggregation methods. The authors observed that the nanocomplexes lost their compact structure became heterogeneous at high zein concentrations. No significant change was observed in net surface charge of the nanoparticles due to heat treatment. It was reported that increasing zein concentration resulted in increased particle size and turbidity. At constant zein concentration, turbidity was reported to increase upon heating which was attributed to the partial denaturation of zein molecules and increased intermolecular interactions. The average particle size of zein–sodium caseinate–pectin nanocomplexes was reported to be approximately 200 nm with a uniform size distribution. Morphology of the nanoparticles was observed to be spherical with smooth surfaces. It was suggested that the main forces responsible for the formation and stabilization of the nanocomplexes were: electrostatic interactions, hydrogen bonds, and hydrophobic interactions. Preparation conditions found to be effective on the morphology of the nanoparticles. Pectin coating found to be effective in improving the stability of the nanoparticles against aggregation in a simulated gastrointestinal environment. The authors suggested that the zein–sodium caseinate–pectin nanocomplexes can be utilized as effective delivery vehicles for bioactives; however, their preparation conditions need to be optimized ([Chang et al., 2017b](#)).

[Xue et al. \(2018\)](#) also fabricated zein–caseinate composite nanoparticles for delivery of curcumin. Curcumin–laden zein–caseinate composite nanoparticles found to have a spherical shape, a narrow size distribution with an average diameter of approximately 187 nm, and a negative surface charge. The authors reported high encapsulation efficiency (~96%) for curcumin. It was suggested that curcumin and zein–caseinate composite nanoparticles were interacting through hydrogen bonding and hydrophobic attractions. When the freeze-dried curcumin–laden zein–caseinate nanoparticles were re-dispersed in water; the formed dispersion was observed to have a similar appearance, particle size distribution, and net surface charge as the original sample. The *in vitro* release profile found to be a controlled release of curcumin in simulated gastric and intestinal media without any burst effect. Stability of curcumin

against photodegradation and heat treatment was reported to be improved by encapsulation within zein–caseinate composite nanoparticles. It was also observed that bioavailability of curcumin encapsulated within nanoparticles was higher than that of free curcumin. It was suggested that the developed zein–caseinate composite nanoparticles could be employed as effective carriers for hydrophobic bioactives that can improve their stability, solubility, and bioavailability (Xue et al., 2018).

Han et al. (2019) followed a different approach and fabricated Maillard conjugates between corn protein hydrolysate and carboxymethyl chitosan for delivery of rutin, a flavonoid-class antioxidant compound with poor aqueous solubility. The idea behind using protein hydrolyzates instead of the native protein was explained as exploiting the benefits, including smaller particle size, low molecular weight, higher solubility, and absorption. The authors reported that heating at 60°C and 79% relative humidity (RH) for 48 h resulted in 53.3% degree of glycosylation and browning index of 0.6. High encapsulation efficiency (98.8%) for rutin was reported. It was suggested that the carbohydrate protrusions nanoparticles' surface resulted in an enhanced steric effect and inhibited particle aggregation due to the formation of salt bridges below the isoelectric point of corn protein hydrolyzate. The developed nanoparticles were proposed as effective delivery systems for hydrophobic bioactive compounds (Han et al., 2019).

9.3.3 Wheat protein

Wheat gliadins are highly polymorphic seed storage proteins that have been shown to possess controlled release and bioadhesive properties. Gliadin was recently investigated for its potential to form nanostructured delivery systems. Chen et al. (2019) fabricated nanoparticles from wheat gliadin and phospholipids using the antisolvent precipitation method. As the gliadin to phospholipid mass ratio increased, the average particle size of the nanoparticles was reported to increase, too. Nanocomplex formation between wheat gliadin and phospholipids was reported to improve the stability of the nanoparticles against pH, salt, and thermal treatment compared to individual gliadin nanoparticles. High phospholipid levels were shown to produce nanoparticles with better foaming properties compared to individual gliadin nanoparticles. The authors suggested that the nanostructured particles fabricated at the optimal conditions could be utilized as delivery vehicles for nutraceuticals and emulsifying and foaming agents (Chen et al., 2019).

Similarly, Yang et al. (2018) used the antisolvent precipitation method for fabricating gliadin–lecithin nanoparticles for curcumin delivery. The authors investigated the effect of the blending sequence of biopolymers on the characteristics of the nanoparticles and the delivery efficiency of curcumin. For this purpose, stepwise antisolvent precipitation (SASP) and antisolvent co-precipitation (ASCP) techniques were compared. Within the two techniques studied, ASCP found to be result in smaller particle size, improved delivery efficiency, lower turbidity, and higher encapsulation efficiency of curcumin. Furthermore, ASCP was reported to show the greater capability to allocate lecithin on the particle surface compared to SASP. Morphological observation with field emission scanning electron microscopy revealed that gliadin–lecithin–curcumin nanoparticles produced with ASCP had a smoother surface. Fluorescence spectrum analysis was used to confirm the entrapment of curcumin within the gliadin–lecithin nanoparticles. According to FTIR spectroscopy and circular dichroism analyses, it was suggested that electrostatic interactions, hydrogen bonding, and hydrophobic interactions were the main effective forces between curcumin, gliadin, and lecithin. ASCP was reported to result in higher antioxidant capacity and better stability for curcumin in the nanoparticles against photodegradation and thermal treatment. Overall, the blending sequence was shown to play a critical role in the fabrication of composite nanoparticle-based delivery systems (Yang et al., 2018).

On the other hand, Akman et al. (2019) used the electrospinning method for fabricating gliadin-based nanodelivery systems for curcumin. The electrospinning process was selected for its ability to produce nanofibers with greater specific surface area and potential to enhance the dispersibility and bioavailability of curcumin. It was reported that the optimization of the electrospinning parameters allowed for the fabrication of homogeneous and fine gliadin nanofibers with different amounts of curcumin. The authors observed that, when the gliadin concentration was increased, gliadin particles were gradually transformed into gliadin nanofibers, and thicker nanofibers were obtained with increasing concentration. Nanofibers were observed to have smooth surface characteristics and an average diameter of 258–375 nm. Increased curcumin loading was reported to result in increased encapsulation efficiency. X-ray diffraction patterns revealed that the majority of curcumin was encapsulated within the nanofibers. Entrapment of curcumin within gliadin nanofibers was reported to provide controlled release and protection of the radical scavenging capacity and antibacterial activity of curcumin. The authors suggested that gliadin nanofibers can be utilized as delivery vehicles for curcumin (Akman et al., 2019).

Wang et al. (2019) followed a different approach and investigated the effect of deamidation on gliadin-based nanoparticle formation. Deamidation was applied as it is known to be an effective technique for improving the solubility of wheat gluten by removing amino groups from glutamine and asparagine residues. It was indicated that stable nanoparticles were produced around pH 7–9 where native gliadin particles were unstable. Deamidation treatment was shown to change particle formation with more intraparticle interactions of N-terminal domain in deamidated gliadins. It was indicated that hydrophobic interactions and hydrogen bonding were the major driving forces for the nanoparticle formation. The optimum curcumin-to-protein mass ratio for encapsulating curcumin with high encapsulation efficiency (>91%) was identified as 1/20. Thermal stability of curcumin entrapped in deamidated gliadin nanoparticles found to be significantly higher than that of native gliadin nanoparticles (Wang et al., 2019).

Zeng et al. (2019) also encapsulated curcumin in gliadin-based nanoparticles. However, they used coacervation method for fabricating the nanoparticles. It was reported that soluble complexes were formed at pH 4–5, whereas coacervates were formed at pH 5–7 through electrostatic interactions. It was reported that the interaction between gliadin and chitosan was a spontaneous exothermic process at pH 3 and 5. The authors observed soluble and homogeneous gliadin–chitosan nanocomplexes and coacervates with a large amount of aggregation. The optimum pH for forming gliadin–chitosan nanoparticles was identified as 5.0 as soluble gliadin–chitosan soluble complexes were formed at this pH. The nanocomplexes fabricated at the optimized conditions were reported to have a smaller particle size (~570 nm) with more uniform distribution, higher net surface charge, and improved encapsulation efficiency (~85%) of curcumin. The gliadin–chitosan soluble complexes were reported to provide sustained release of curcumin in a simulated gastrointestinal environment (Zeng et al., 2019).

9.3.4 Pea protein

Pea protein is among the few commercially available plant proteins with increasing application in industrial use and academic research. Proteins in pea seeds are composed of main globulins (65%–80%), albumins, and glutelins. Globulins are composed of three different proteins: legumin, vicilin, and convicilin (Nesterenko et al., 2013). Either pea protein isolate or purified fractions of legumin and vicilin were explored for their potential to form protein-based nanoscale delivery systems.

Irache et al. (1995) formed legumin nanoparticles (~250 nm) with a coacervation method and applied a cross-linking step using glutaraldehyde. Nonstabilized nanoparticles or coacervates were also obtained by mixing an aqueous solution of legumin with a buffer. The authors investigated the effect of processing parameters on the size and yield of coacervates formed. Glutaraldehyde concentration was not found to have a significant effect on the size and yield of the legumin nanoparticles. While the nanoparticles formed found to be quite stable in pH conditions close to neutrality, nanoparticles stored under acidic conditions (pH 5.5; 37°C) showed a rapid degradation. This finding was attributed to the rapid increase in their particle size due to agglomeration of particles with change in their surface characteristics (Irache et al., 1995).

Ezpeleta et al. (1997) used the same method, coacervation followed by cross-linking with glutaraldehyde, for fabricating nanoparticles. However, they used a different fraction of the pea globulins, vicilin. The authors investigated the effects of processing parameters on characteristics of nano and microparticles obtained. Particle size was reported to increase with reaction time and glutaraldehyde concentration. The cross-linking process found to have a significant effect on particle stability when incubated in the presence of trypsin. It was reported that vicilin nanoparticles can be fabricated in aqueous conditions avoiding the use of organic solvents and stabilized by cross-linking. However, the stabilization step found effective in the size and degradation of nanoparticles (Ezpeleta et al., 1997).

Liang and Tang (2014) investigated the Pickering stabilization effect of pea protein at acidic pH. Most of the proteins in pea protein isolate found to be present in the nanoparticle form at pH 3.0 (~134–165 nm), depending on the concentration (0.25–3.0 g/100 mL). Increasing the protein concentration found to result in a significant reduction in the emulsion droplet size while their creaming stability also increased. Mainly at concentrations higher than 2 g/100 mL, no creaming occurred even after storage of 20 days. Increasing the concentration was reported to result in an increase in extent of droplet flocculation and formation of a network consisting of flocculated droplets. The emulsions formed at concentration values above 1.0 g/100 mL found to exhibit extraordinary stability against coalescence. The flocculated droplet network formation resulted in an increase in the amount of adsorbed proteins at the interface. The authors suggested that pea proteins could be potentially used as food-grade Pickering stabilizers for oil-in-water emulsions at acidic conditions (Liang and Tang, 2014).

In a follow-up study, Shao and Tang (2016) fabricated gel-like pea protein Pickering emulsions and investigated their potential as intestine-targeted and sustained-release delivery systems for β -carotene. The emulsions were produced by microfluidization at 6.0% (w/v) protein concentration and varying oil fractions (ϕ) of 0.2–0.6.

It was reported that increasing the oil fractions was favorable for the gel-like network strengthening of these emulsions. The gel structuring was reported to be mainly due to the droplet flocculation and attractive colloidal interactions. It was indicated by *in vitro* simulated digestion results that the release of β -carotene during the intestinal digestion of the emulsions could be modulated by altering the oil fraction. Although the gel-like emulsion at 0.6 oil fraction showed lower bioaccessibility of β -carotene (compared to that of 0.3), it was reported to exhibit a sustained-release behavior, which was indicated as an essential factor for achieving high bioavailability. Fabricated gel-like pea protein Pickering emulsions was proposed as a novel, plant-based delivery systems for lipophilic bioactive components (Shao and Tang, 2016).

9.3.5 Other plant-based proteins

In addition to commercially available plant proteins such as soy, corn, wheat, and pea, alternative plant protein sources were also investigated for their potential for the fabrication of protein-based nanoscale delivery systems. In this regard, proteins from amaranth and proso millet were investigated.

Aceituno-Medina et al. (2015) fabricated amaranth protein isolate: pullulan structures with electrospinning method for photoprotection of folic acid, which was selected as a model bioactive compound with high sensitivity to UV light exposure. The authors reported that thicker electrospun fibers were obtained by adding 100 mg of folic acid per g of the biopolymer to the biopolymeric solution used for electrospinning. High encapsulation efficiency (>95%) was reported for folic acid. Scanning electron microscopy observations revealed that smooth and homogenous fibers in terms of size distribution were obtained, indicating that the incorporation of folic acid did not negatively affect the electrospinning process. The proper distribution of folic acid along the fibers was observed with fluorescence microscopy. Thermal stability of folic acid was reported to increase after encapsulation within the amaranth protein: pullulan structures, which could be useful for food processing applications. UV–VIS spectra of the free and encapsulated folic acid were observed before and after 120 min exposure to a UV lamp. The authors observed no degradation of the encapsulated compound 2 h of UV exposure. On the other hand, the characteristic UV–VIS spectrum from the photodegradation compounds of folic acid was observed after UV irradiation of the unprotected vitamin. The amaranth protein: pullulan structures were suggested to have excellent potential for the encapsulation and protection of photosensitive bioactives for food-related applications (Aceituno-Medina et al., 2015).

Wang et al. (2018) designed the proso millet protein-based nanodelivery system for curcumin. Two authors extracted proso millet protein by either wet milling or 60% (v/v) aqueous ethanol and then used as the wall material to encapsulate curcumin. Prolamin-rich millet protein was reported to show significant differences in surface hydrophobicity depending on the extraction procedure. The millet protein–curcumin nanoparticles found to be spherical with a diameter of around 250–350 nm. Encapsulation efficiency was observed at a wide range in the study (varying from 11.2% to 78.9%), with millet protein extracted by ethanol showing better performance compared to that of the protein extracted by the wet milling process. Curcumin encapsulated in proso millet protein nanoparticles was reported to show a lower degradation rate compared to free curcumin at 60°C. DPPH and ABTS radical scavenging activity of encapsulated curcumin was not found to be affected by the encapsulation process. Compared to millet protein extracted with wet milling, ethanol-extracted millet protein exhibited a healthier interaction with curcumin during the encapsulation process due to its more unfolded structure. It was suggested that a higher binding affinity of ethanol-extracted millet protein with curcumin indicates that the hydrophobic regions in protein are more accessible for curcumin, favoring a stronger hydrophobic interaction. It was suggested that proso millet protein extracted with an appropriate method could be promisingly used in delivery systems for lipophilic components (Wang et al., 2018).

9.4 Conclusion

The potential of protein-based nanodelivery systems is being established rapidly, especially in encapsulation, protection, and delivery of bioactive ingredients. The nanostructured protein-based delivery systems have been shown to possess various advantages, including improved solubility, high bioavailability, and controlled release. However, they still have challenges such as cost issues, potential toxicity, and stability problems. Further research has to focus on interactions of nanostructured protein-based delivery systems with food components during the processing and shelf life of the product. More *in vitro* and *in vivo* studies are needed in the future in order to

understand how these systems behave within the human gastrointestinal tract after ingestion in order to develop safer and more efficient systems.

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