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Plant protein-based delivery systems for bioactive ingredients in foods

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The application of food-grade delivery systems for the encapsulation, protection and controlled release of bioactive food ingredients have recently gained increasing interest in the research fields of functional foods and pharmaceutics. Plant proteins (mainly soy proteins, zein and wheat gliadins), which are widely available and environmentally economic compared to animal derived proteins, can be made into various delivery platforms, such as micro- and nanoparticles, fibers, films and hydrogels. In this paper, we review the recent progress in the preparation of food-grade delivery systems based on plant proteins for bioactive ingredients, and highlight some of the challenges and directions that will be the focus of future research. The preparation and application of bifunctional particles, which were able to deliver the bioactives to oil/water interface and stabilize the interface, are also described, providing a novel perspective for the design of plant protein-based delivery system.

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1. Introduction

Driven by increasing demands for improving human health and wellness through diet, numerous attempts have been carried out to develop innovative functional foods that have been added value beyond their normal nutrition. The development of functional foods relies on the enrichment and fortification of food products by incorporation of bioactive ingredients, such as polyphenols, phytosterols, vitamins, minerals, functional lipids, bioactive peptides and even probiotic bacteria.[1–5] However, many of these bioactive ingredients could not be simply introduced into food stuff in pure form due to their limited physicochemical and biological properties. For instance, they may possess poor solubility in aqueous or lipid phase, and may be chemically or physically labile to food processing and storage conditions (temperature, light and oxygen), as well as digestive reactions in the human

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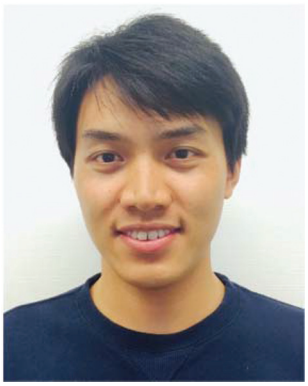
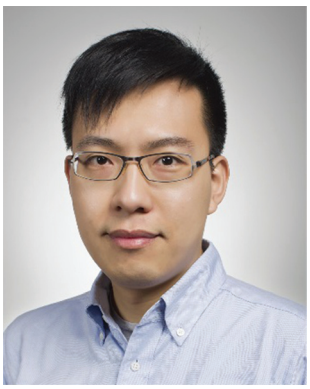


Zhi-Li Wan

Zhili Wan is a PhD candidate in the Department of Food Science and Technology at the South China University of Technology. Currently, he is completing his joint PhD study at the Laboratory of Physics and Physical Chemistry of Foods, in the Department of Agrotechnology and Food Sciences of Wageningen University from 2014 to 2015. His research focuses on the design of interface dominated food colloids (e.g. emulsions and foams) by utilizing the interactions of protein/protein fibrillar aggregates with biosurfactants, and on their application as delivery vehicles for functional ingredients in foods.



Jian Guo

Dr Jian Guo accomplished his PhD and postdoctoral research in Cereals, Oils and Vegetable Protein Engineering at South China University of Technology, Guangzhou, under the supervision of Prof. Xiao-Quan Yang. His current research interests include thermal aggregation behaviour of plant proteins and the preparation of edible functional particles for applications in food processing.

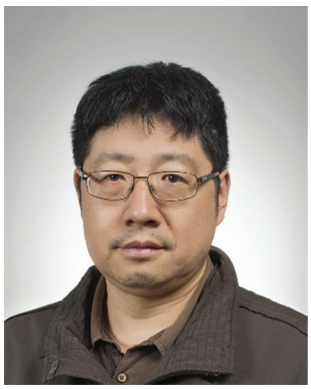
gastrointestinal tract (pH, presence of enzymes and other nutrients).[6–8] These would often compromise the overall functionality of food product, particularly hindering food sensory properties and decreasing the bioavailability of the bioactive ingredient.[1,2,9,10] For these reasons, the addition of micronutrients and nutraceuticals to foods has been a major scientific and technological challenge within the food industry.

An approach, which is receiving increasing attention as enabling the incorporation of bioactive ingredients in foods, is the use of food-grade delivery systems for their protection and controlled release behaviour.[4,5,9] Consequently, recent publications provide an overview linking structural properties of various food-grade delivery systems to their functionality, *e.g.*, stability, matrix compatibility, release characteristics and bioavailability, to gain scientific insights for their rational design and fabrication.[9,11–13] Numerous synthetic polymers have been used to formulate intelligent, modulated and selective drug delivery systems to protect and transport drug molecules to target functions in biomedical and pharmaceutical applications.[14–16] However, these materials are seldom used in food applications. The materials used for the manufacture of food-grade delivery systems have to be selected from a diverse range of natural biomaterials or compounds with granted GRAS (generally regarded as safe) status. Commonly used in the formulation of encapsulating bioactive ingredients are food biopolymers (proteins, carbohydrates), lipids, low molecular weight surfactants, co-polymers, or their mixed systems.[2–46,7,9]

Among these food-grade materials, food proteins are a versatile group of biopolymers that have high nutritional value along with considerable functional properties, including emulsification, gelation, foaming, and their applications as ingredients in food industry.[6,17–19] Their chemical and structural versatility makes them appropriate candidates for the delivery of bioactive ingredients in a wide range of platforms, such as particles, fibres, films and hydrogels, offering the possibility of delivering both hydrophobic and hydrophilic bioactive compounds.[6,20,21] Currently, proteins commonly used for food-grade delivery systems are mainly from animal origin including gelatin, casein, whey proteins and albumin (ovalbumin and serum albumin). For example, the self-assembly of some milk proteins has been reported to be successful in fabricating nanovehicles for the delivery of hydrophobic nutraceuticals, such as vitamin D and ω-3 polyunsaturated fatty acids in casein micelles, curcumin and resveratrol in β-lactoglobulin nanovehicles.[22–25] These versatile delivery vehicles also have the potential to become protective carriers for hydrophilic bioactive substances, such as tea polyphenols and riboflavin.[26,27]

Proteins extracted from crops such as soybeans, corn and wheat (generally called plant proteins or vegetable proteins) are commonly generated as by-products of edible oil, starch or other food processing products. Compared with animal-derived proteins, the production of plant protein with less consumption of natural resources is viewed as more "environmentally economic".[28] Furthermore, plant proteins are not only one of the macronutrients that provide building blocks for human body, but also offer some health benefits to humans which have not been found in the animal proteins.[29,30] Although foods made from plant proteins such as soy proteins (glycinin, β-conglycinin and lipophilic protein), corn and wheat proteins have been present in our diet for thousands of years, studies on the functionalities of plant proteins have been focused since the last century due to their potential as an alternative to animal-based sources of proteins.[31] In addition, recent studies have shown that they could also be developed into suitable carriers for bioactive compounds.[20,32] In contrast to delivery vehicles using hydrophilic animal proteins, hydrophobic plant proteins such as zein and gliadin have the capability of producing sustained-release particulate carriers, which might not require any further chemical treatment to harden them, thus not requiring the use of toxic chemical crosslinkers.[6,20,33,34] Development of plant protein-based delivery materials may provide opportunities to offer novel functional foods to consumers, particularly for vegan diets. Moreover, the use of plant protein-based materials as nutraceutical delivery systems also meets the present sustainable trends in the food production and pharmaceutical fields.[28] For these reasons, efforts have been made to explore the possibilities of utilizing plant proteins for the construction of natural vehicles for delivering various bioactive ingredients in foods over the past few years.[20,32]

Hence, in this review, we will provide an overview of the recent literatures available on various food-grade delivery systems based on plant proteins for the bioactive food ingredients in different platforms, such as particles, fibers, films and hydrogels. From nanoscale to macroscopic scale, these delivery vehicles with different dimensions and shapes are applied to various food systems. Some novel approaches used to fabricate and characterize these plant protein-based systems will be described in this review. As health issue has always been concerned, transport of the bioactive ingredients to the human gastrointestinal tract (GIT) is now the main job



Xiao-Quan Yang

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assigned to delivery systems, and the bioavailability of the systems has attracted great attention.[6,20,35,36] However, there are still considerable demands for developing delivery systems with the purpose of facilitating food processing and extending shelf life. The bioactive ingredients such as antioxidants and antimicrobials are difficult to transport to the required location, such as oil–water interface, without the help of delivery systems. Some plant protein-based particles were found to be able to deliver such substances to the oil–water interface and stabilize the interface simultaneously, which exhibited two distinct functions. The preparation and mechanism of these bi-functional particles are also discussed in this review. We intend to introduce this strategy for preparing novel functional plant protein-based ingredients to the food industry.

2. Micro- and nanoparticles

2.1 Delivery of bioactive ingredients

Due to the known characteristics of microencapsulation, easy surface modification and scale-up feasibilities, particulate systems in micron and nanometre scales provide better opportunities for targeted delivery of bioactive ingredients.[20,35,36] The design of particles with specific properties has recently been driven by the applications of nanotechnology in food and agricultural systems, especially by the development of bioactive food ingredients with improved aqueous solubility, physiochemical stability, oral bioavailability, and sensory attributes in functional foods.[37–39] Nanoparticles are generally preferred over micro-particles for nutrients and drug delivery because they can penetrate throughout the submucosal layers of tissue and have a relatively higher intracellular uptake due to their subcellular and submicron size,[40] thus leading to a higher nutrient or drug bioavailability.

Among various natural or synthetic polymer-based particulate systems potentially available to food applications, plant protein-based micro- and nanoparticles are preferably used for nutrient or drug delivery because they offer advantages over other materials in terms of biodegradability, abundant renewable sources, safety status *in vivo*, and many useful functional properties mentioned above.[6,20,34,41] Additionally, they also exhibit high loading capacity of various bioactives due to their amphiphilic structure, multiple binding sites, and a variety of possible binding mechanisms include electrostatic attractions, hydrophobic interactions, hydrogen and covalent bonding. Table 1 presents some of the studies available on developing micro- and nanoparticles from plant proteins as delivery systems.

2.1.1 Zein. Zein, which is usually manufactured from corn gluten meal, was found to be rich in α-helical conformation. As an amphiphilic molecule, zein possesses the capacity of self-assembly to form various mesostructures with different solvents, which makes it valuable in processed foods and pharmaceuticals.[42,43] Compared to other plant proteins, zein-based

Table 1 Overview of plant protein-based micro- and nanoparticles for bioactive ingredients delivery

Type of particles	Preparation	Encapsulated bioactives	Ref.
Zein microparticles	Spray drying or supercritical anti-solvent method	Food grade antimicrobials: lysozyme, thymol, nisin	56–58
Zein microparticles	Spray or freeze drying	Flax oil	59
Zein nanoparticles	Liquid–liquid dispersion method	Polyphenols: curcumin, quercetin, tangeretin, cranberry procyanidins	50–53
Zein nanoparticles	Phase separation or liquid–liquid dispersion method	Essential oils: oregano, red thyme, cassia and carvacrol oils	54, 55
Zein nanoparticles	Liquid–liquid dispersion method or electrospraying	Bioactive lipids: fish oil, DHA	49, 60
Zein nanoparticles	Supercritical anti-solvent	Lutein	63
Zein nanoparticles	Liquid–liquid dispersion method or electrospraying	Food coloring agents: curcumin, indigocarmine	50, 64, 65
Zein-chitosan complex nanoparticles	Low-energy phase separation method	Vitamin D_3	62
SPI-zein complex microparticles	Ca^{2+}-induced cold gelation method	Riboflavin	68, 69
SPI/FA-conjugated SPI nanoparticles	Ethanol desolvation method	Curcumin	70, 77
SPI nanoparticles	Ca^{2+}-induced cold gelation method	Vitamin B_{12}	77, 78
Soy protein nanocomplex	Ligand binding properties	Vitamin B_{12}, cranberry polyphenols, curcumin, RES and grape polyphenol	71–75, 81
SPI-CMCS complex nanoparticles	Ca^{2+} induced co-gelation method	Vitamin D_3	79
Soy protein-soy polysaccharide complex nanogels	High-pressure homogenization and heating procedures	Folic acid	80
Soy lipophilic protein nanoparticles	Ultrasonic treatment	Conjugated linoleic acid	83
Gliadin nanoparticles	Antisolvent precipitation method	All-*trans*-RA, vitamin E, mixture of linalool and of linalyl acetate, benzalkonium chloride	33, 84
Gliadin nanoparticles	Electrospray deposition	Cyclophosphamide	86
Barley protein microparticles	Pre-emulsifying process followed by microfluidizing	Fish oil, β-carotene	87, 88
Barley protein nanoparticles	High pressure homogenization	β-Carotene	89

particulate systems have been more widely studied as promising delivery vehicles specifically for hydrophobic active molecules (Table 1).[139,45] Recently, Wang *et al.* demonstrated the encapsulation of citral and lime flavour in self-assembled core–shell structures of zein, which are of interest for encapsulation purposes in food, pharmaceutical and cosmetics industries.[46] To further improve the loading capability of zein nanoparticles, Yang and co-workers reported a novel method to develop hollow zein nanoparticles by using sodium carbonate as sacrificial template for delivery of metformin.[47] Compared to conventional solid nanoparticles, hollow zein nanoparticles have smaller particle size, higher drug loading, a more sustained and controlled drug release manner, and could be used to directly deliver drugs to cells. Generally, the liquid–liquid dispersion process or anti-solvent precipitation method was used to fabricate zein nanoparticles with diameters between 100 to 400 nm, depending on the fabrication parameters, which has been covered in detail by Zhong *et al.*[48] During this process, non-polar bioactive ingredients can be easily encapsulated in zein nanoparticles if they can be co-dissolved in aqueous alcohol solutions together with zein.[48,49] To date, zein-based micro- and nanoparticles have been used for encapsulation, stabilization and controlled release of a variety of functional ingredients, such as polyphenols,[50–53] essential oils,[54,55] food grade antimicrobials,[56–58] bioactive lipids,[59,60] functional micronutrients,[61–63] and some food coloring agents.[64,65]

To produce zein particles on an industrial scale for encapsulation and delivery applications in food processing, several scalable approaches have been recently reported, such as spray drying,[57–59] supercritical anti-solvent,[56,63] and electrospraying technologies.[60,64] Zhong and co-workers successfully prepared spray-dried zein microcapsules to controlled release antimicrobials, such as lysozyme, thymol and nisin.[57,58] They also produced zein microparticles with encapsulated lysozyme using a supercritical anti-solvent process (SAS).[56] The microcapsules showed a sustained release of lysozyme over 36 days at room temperature, especially nearby neutral pH conditions and at the presence of salt. It has been reported that controlled release of lutein could also be achieved by preparing lutein-zein nanoparticles using solution enhanced dispersion by supercritical fluids (SEDS) technique.[63] The release of these nanoparticles displayed a near zero-order profile during the initial 40 min followed by about 90% release of the lutein within 120 min. Electrospraying technique was used by Torres-Giner *et al.* to stabilize docosahexaenoic acid (DHA) by encapsulation in zein ultrathin capsules (around 490 nm).[60] The encapsulated DHA showed a 2.5-fold reduction in the degradation rate constant, and the ultrathin zein-DHA capsules were more stable across relative humidity and temperature. In another study, curcumin loaded in zein nanoparticle produced by electrospray technique remained stable after three months of storage in dark conditions, without changes in the morphology or the curcumin content of nanoparticles.[64]

However, these above technologies have their own limitations for industrialization. For instance, spray drying technique is not suitable for encapsulating temperature-sensitive bioactives. Some organic solvents such as methanol, acetone and dimethyl sulfoxide (DMSO) were usually used in the SAS procedure.[56,63] For electrospray, it was recently reported that properties of zein particles were susceptible to the process parameters, such as zein concentration, flow rate and applied voltage.[60] Based on the advantages and problems of above-mentioned methods, there is no ideal method to produce zein particles. Recently, our group utilized a facile and continuous technique termed Flash NanoPrecipitation (FNP) to successfully generate zein particles with controlled particle sizes.[66] In the FNP process, an antisolvent stream and a solvent stream in a confined mixing chamber are rapidly mixed in a time shorter than the nucleation and growth time of polymer. Using this technology, the particle sizes are below 350 nm even at high zein concentrations (2.5–7.5% w/v) and can be well controlled by the flow rate of the zein solution and outlet configuration of confined impinging jet (CIJ) (Fig. 1). The properties of solvent systems have little influence on particle size in the FNP process, and scale-up is possible from laboratory apparatus to industrial continuous production. These features of FNP procedure are attractive for industrial applications and encapsulating bioactives with different solubility in ethanol–water binary solvent. Therefore, the FNP technique is a practical and applicable method to produce zein particles in large scale currently.

2.1.2 Soy proteins. Soy proteins, the by-product of soy oil processing, is now one of the most widely used protein ingredients in food processing. When different processing methods are conducted, soy protein aggregates with different structures and functionalities could be formed along different pathways.[67] In addition to zein, soy protein-based particles are also promising candidates as delivery systems for nutraceuticals or drugs (Table 1).

The microparticles made from soy protein isolate (SPI) were mainly fabricated by using spray-drying, coacervation, and cold gelation techniques.[32,68–70] Chen and Subirade reported the preparation of SPI/zein complex microspheres by cold gelation method (initiated by glacial acetic acid in the presence of calcium carbonate) for the delivery of hydrophilic nutraceuticals (riboflavin).[69] The obtained particles (about 15–25 μm) had spherical morphology with homogenous distribution throughout the matrix. Microspheres with SPI/zein ratios of 5 : 5 and 3 : 7 displayed near-zero-order release kinetics in simulated gastrointestinal fluids. Later on, the absorption rate and release profile of riboflavin in this delivery system were systematically evaluated with a dynamic artificial digestive system (TIM-1).[70] The release of riboflavin from pure SPI or zein microspheres in the stomach compartment is accomplished within 15 min, while the SPI/zein complex microspheres provided sustained release of riboflavin over 4 h and a near-zero-order nutrient availability for absorption profile in both fasting and prandial states. Incorporation of these microspheres into yogurt significantly delayed riboflavin release, which would increase the likelihood of gastric-sensitive nutrients reaching the intestine for absorption. Thus, these SPI-

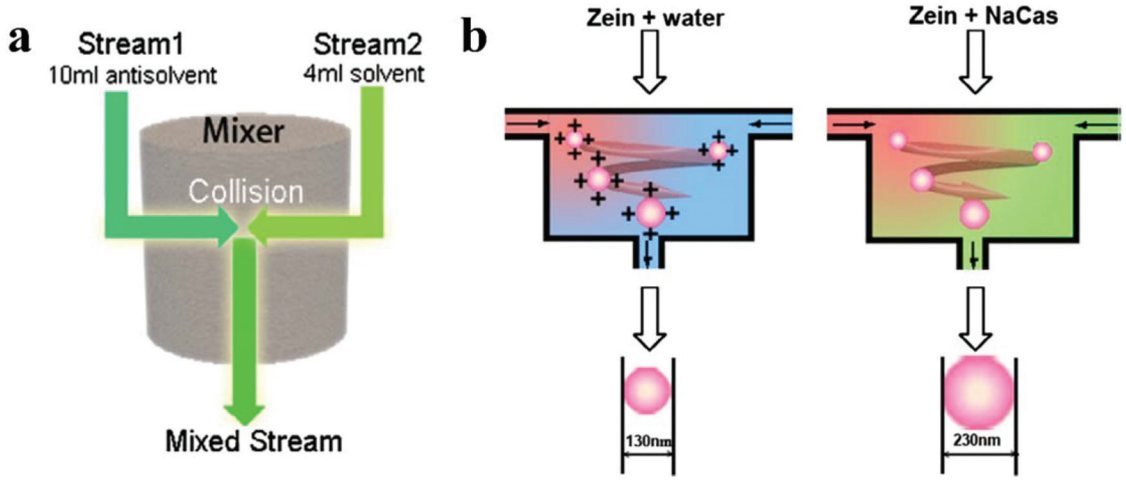

Fig. 1 Schematic representation of the Flash NanoPrecipitation (FNP) process (a) and a schematic illustration of plain and sodium caseinate (NaCas) zein particles (ZP) produced *via* FNP process (b). For Plain ZP, ethanol solution (red) and water (blue) were impinged into a confined impinge jet (CIJ) mixer. Then pH of mixed solution is below 4.0, so positive charges are shown around zein. For NaCas ZP, ethanol solution (red) and NaCas solution (green) were impinged into the CIJ mixer. The pH is neutral, so zein is uncharged. Particle sizes produced by 5% zein solution show at last.[66]

zein complex microspheres exhibited potential for the use as nutraceutical delivery vehicles in the creation of novel functional foods, such as yogurt enriched with vitamins.[70]

Due to the ligand binding properties, soy proteins can serve as an effective carrier for various bioactive molecules. They can bind these molecules to form complexes in nanoscale through physical interactions, mainly hydrophobic interactions, hydrogen bonds and van der Waals attraction. Recent studies suggest that soy proteins have the potential to be used as carriers for both hydrophobic and hydrophilic bioactive compounds, such as vitamin B_{12},[71] cranberry polyphenols,[72] curcumin,[73] resveratrol (RES),[74] and polyphenols from Concord grape pomace,[75] to improve their water solubility, stability and bioavailability.

Recently, SPI nanoparticles have been successfully prepared by two methods, ethanol desolvation as described by Teng *et al.*[76] and calcium-induced cold gelation as reported by Zhang *et al.*[77] SPI nanoparticles prepared by ethanol desolvation method exhibited desirable average size (150 nm), ζ-potential (−36 mV), and high encapsulation efficiency for curcumin (97.2%).[76] The release of curcumin in phosphate buffer saline (8 h) followed a biphasic pattern. Folic acid (FA) has been found to be an effective target-specific ligand for tumor cells. Therefore, the FA-conjugated SPI nanoparticles for target specific drug delivery were further prepared by this group.[78] Compared to SPI nanoparticles without FA, the FA-SPI nanoparticles showed a lower average size, a higher loading efficiency, and a faster and more complete release of curcumin in Tween 20-PBS buffer. Cellular uptake of the SPI nanoparticles was increased by at most 93% in Caco-2 cells upon the conjugation with FA.[78] The calcium-induced SPI nanoparticles (28–179 nm) exhibited uniform size distribution and spherical shape with an unique honeycomb-like core structure.[77] Nanoparticle characteristics could be modulated by changing pH and calcium concentration. *In vitro* study indicated that these nanoparticles were non-toxic and mainly distributed in cytoplasm when they were absorbed into Caco-2 cells. Zhang *et al.* further investigated the intestinal uptake and transport mechanisms of Ca^{2+}-induced SPI nanoparticles for vitamin B_{12} (VB_{12}) delivery.[79] SPI nanoparticles could potentially carry VB_{12} across the intestinal barriers *via* multiple endocytosis pathways including clathrin-mediated endocytosis and macropinocytosis pathways, which may enhance intestinal transport of VB_{12}. The intestinal transport and uptake of VB_{12}, studied in rat jejunum model with Ussing chambers technique, were improved up to 4-fold after being encapsulated into 30 nm SPI nanoparticles.

In addition, complex nanoparticles were also successfully developed from SPI and carboxymethyl chitosan (CMCS) by Ca^{2+} induced co-gelation method and employed as a delivery system for hydrophobic vitamin D_3 (VD).[80] These VD-loaded complex nanoparticles with an average size of 162–243 nm remained stable and suspended in aqueous phase after centrifugation and lyophilization. In comparison with pure SPI nanoparticles, the complex nanoparticles exhibited a reduced (42.3% compared to 86.1%) release of VD in simulated gastric fluid and an enhanced (36.0% compared to 8.2%) release under simulated intestinal condition. In another study, FA-loaded soy protein/soy polysaccharide complex nanogels were produced by using high-pressure homogenization and heating procedures.[81] The nanogels were dispersible and stable after 6 months of storage in acidic conditions (pH 3.0–5.0). Moreover, the nanogels could protect the loaded FA from decomposition in the presence of heat, light and oxygen at acidic conditions and release naturally structured FA at neutral pH, that is, in the intestine. These features suggest the complex nanogels are a suitable delivery system of FA in most food and beverages.

In a recent study, we found that soy lipophilic protein (LP, a group of protein fractions associated with lecithin) could transform into nanoparticles under an ultrasonic treatment in aqueous phase.[82] The lipophilic protein nanoparticles (LPP) had a core–shell structure, in which the hydrophobic proteins comprised the core with phospholipids covered over it, thus providing the potential as delivery vehicles for hydrophobic bioactives. Subsequently, we successfully incorporated conjugated linoleic acid (CLA) into LPP by ultrasonication.[83] The CLA-loaded LPP exhibited a mean particle diameter of 170 nm (Fig. 2) and a loading capacity of 26.3% (w/w). The encapsulation in LPP endowed the CLA with better oxidation stability and a sustained releasing profile in the simulated gastrointestinal fluids. These findings suggest that LPP could be used as a delivery system for hydrophobic bioactive ingredients in functional foods.

2.1.3 Wheat gliadins and barley proteins. Nanoparticles made from gliadin, a component of wheat gluten, have been prepared for nutrient/drug delivery and controlled release applications. For example, gliadin nanoparticles has been used as carriers for all-*trans*-retinoic acid (RA).[33] The obtained gliadin nanoparticles (500 nm) were stable in phosphate-buffered saline for up to 4 days, and the cross-linking induced by glutaraldehyde further increased their stability. A biphasic pattern of RA *in vitro* release was observed with an initial burst of approximately 20% RA followed by zero-order diffusion.[33] Gliadin nanoparticles (450–475 nm) were showed to be a suitable delivery and controlled release system for nutrients and drugs with different polarity (hydrophobic and amphiphilic).[84] It was found that the amounts of the entrapped drug increased with an increase in the drug hydrophobicity, confirming a strong interaction between gliadins and apolar compounds.[84] However, gliadin nanoparticles produced by antisolvent precipitation were only stable over a narrow range of pH, salt concentrations and temperatures, even after the strengthening with glutaraldehyde, which might limit their commercial applications in the food and beverage industry.[85] In another study, Gulfam *et al.* synthesized gliadin-based nanoparticles for delivery and controlled release of cyclophosphamide anticancer drug by using the electrospray deposition system.[86] Cyclophosphamide was gradually released from the gliadin nanoparticles for 48 h, and the breast cancer cells became apoptotic after being cultured with cyclophosphamide-loaded 7% gliadin nanoparticles for 24 h.[86]

Recently, Chen and co-workers developed barley protein-based microparticles by a pre-emulsifying process followed by microfluidizing without using organic solvents or cross-linking reagents.[87,88] The obtained microparticles (1–5 μm) have a spherical shape and porous inner structure with high encapsulation efficiency (92.9–100.2%) and oil loading efficiency (around 50%). These microparticles exhibited a strong capacity to protect fish oil against oxidation. In addition, *in vitro* study showed that barley protein microparticles have the ability to protect the encapsulated β-carotene in harsh simulated gastric conditions and steadily release it under simulated intestinal tract conditions.[88] In another study, barley protein nanoparticles with small sizes (90–150 nm) and narrow size distributions were prepared using high pressure homogenization without the use of any organic solvents or cross-linking reagents.[89] Interestingly, these nanoparticles were degraded by pepsin into smaller particles (20–50 nm), which could provide sufficient protection of the nutrient (β-carotene) in the simulated gastric fluid. Then, complete release occurred after 7 h of degradation by pancreatin in the simulated intestinal environments. *In vitro* studies showed that barley protein nanoparticles could be internalized by Caco-2 cells and accumulated in the cytoplasm.[89]

2.2 Stabilization of emulsion-based systems

As we know, many of functional ingredients are lipophilic, such as bioactive lipids, flavors and antioxidants. Emulsion, a dispersed system which consists of two or more immiscible liquid, is a feasible delivery system for these lipophilic components. Conventional oil-in-water (O/W) emulsions are currently the most widely used as vehicles for encapsulating and delivering lipophilic bioactives because of their relative ease of preparation. They can simply be produced by solubilizing the hydrophobic bioactives in an edible lipid phase and then

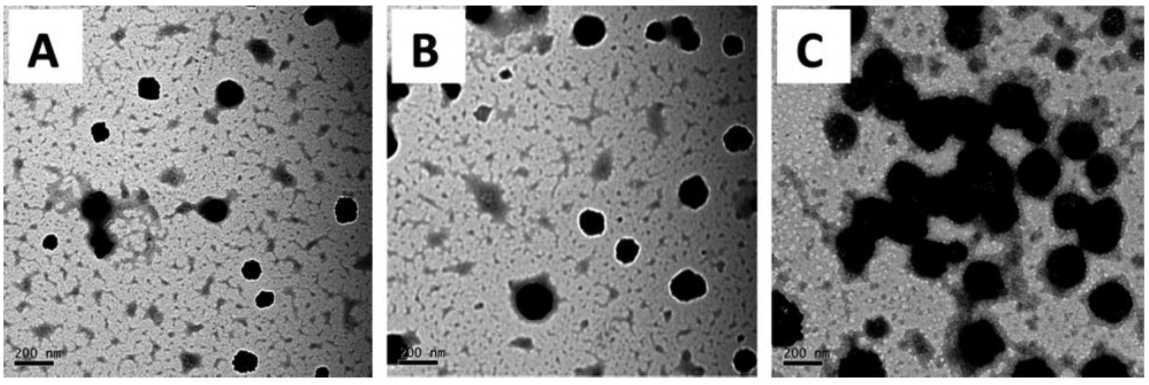

Fig. 2 Transmission electron microscope of the soy lipophilic protein nanoparticles LPP (A), LPP encapsulated with 2 mg mL^{-1} (B) and 5 mg mL^{-1} (C) of CLA.[83]

homogenising them with an aqueous phase containing food-grade emulsifiers.[4,11] Food proteins that derive from animals and plants are commonly used as effective emulsifiers and stabilizers to form, stabilize, and provide specific physicochemical properties to O/W emulsions systems within the food industry.[19,90] Their surface-active properties and capacity to build viscosity have been exploited in emulsion-based delivery systems.[4] Among plant proteins, soy protein has been extensively employed as a functional ingredient in food emulsions due to its higher emulsifying properties.[91] It was reported that SPI-stabilized O/W emulsions could be used to produce physically and oxidatively stable delivery systems for bioactive lipids, such as omega-3 fatty acids, and incorporate them into functional foods.[73,92,93]

In recent years, there has been a growing interest within the research fields of food and pharmaceutics in the emulsions stabilized by food-grade particles rather than conventional surfactants. This type of emulsion is called a Pickering emulsion, which could provide outstanding physical and chemical stability to the lipid phase and thus encapsulated bioactives.[94,95] The Pickering emulsions stabilized by inorganic particles (*e.g.* silica particles) or some biological origin particles (*e.g.* cellulose, chitin and starch) have exhibited more sustained release of some encapsulated lipophilic or hydrophilic ingredients/drugs,[96–98] are more stable against lipid oxidation,[99] and are slower in lipid digestion,[100] as compared to conventional surfactant-based emulsion systems. These findings indicate that Pickering emulsions can be developed into effective delivery vehicles for bioactive compounds with good functional performance. However, the application of particles derived from food proteins as the emulsifier is just at the initial stage. Several studies about emulsions stabilized by plant protein-based particles have been performed. Tang and Liu reported that soy protein aggregates (~100 nm) prepared by thermal treatment and the addition of sodium chloride could act as a kind of effective emulsion stabilizer.[101] The resulting emulsions showed extraordinary stability against coalescence and creaming. In another study, generation of Pickering emulsions using zein colloidal particles as interfacial stabilizer was reported.[102] Zein particles with an average particle size of 82 ± 16 nm were synthesized *via* an antisolvent precipitation procedure. These emulsions prepared by zein particles were found to be stable at pH above and below the isoelectric point of zein, and for low to moderate ionic strengths (1–10 mM).

2.3 Bifunctional nanoparticles

A great variety of delivery systems have been fabricated by various techniques. Researchers in food industry have been focusing on the delivery of functional ingredients in foods that might bring health benefits and pleasure to human beings. Meanwhile, these bioactive substances are also able to improve the processing efficiency and maintain the quality of the food products during processing, transportation and storage. And this effect also plays an important role in the food industry. One example is that of antioxidants which are used to enhance the oxidative stability and extend the shelf-life of emulsion-based products. However, the poor solubility of antioxidants in aqueous or lipid phases limits their application in this field. RES (*trans*-3,5,4′-trihydroxystilbene), a natural polyphenol compound mainly found in red grapes and peanuts, is one of this type of antioxidants. In our previous study, we used SPI as the vehicle for carrying RES by forming an SPI-RES complex, and then the RES was purposefully accumulated at the O/W interface by using this complex as an emulsifier.[74] The resulting O/W emulsion showed an increased oxidative stability with reduced amounts of lipid hydroperoxides and hexanal due to the high interfacial accumulation of RES. Zein/Chitosan Complex Particles (ZCPs), which were synthesized *via* antisolvent technique, were used in another study as carriers for curcumin, a hydrophobic polyphenol, so as to prepare an antioxidant emulsion.[103] The influence of the curcumin location on the antioxidant activity of the emulsions stabilized by ZCPs were investigated. Curcumin could be loaded in ZCPs when it was added during the preparation of ZCPs, and it was delivered to the O/W interface with the help of ZCPs, which was evidenced by observation using confocal laser scanning microscopy (CLSM) (Fig. 3B1 and B2).[103] The antioxidant only appeared on the O/W interface rather than the aqueous or lipid phase in this case. The amounts of primary and secondary oxidant products (including lipid hydroperoxides, malondialdehyde and hexanal) in this emulsion produced during storage were found to be significantly smaller than those in which curcumin was dispersed in the lipid phase (Fig. 3C1 and C2).[103] ZCPs made curcumin appear in the position which can maximize its antioxidant effect.

In addition to the physical and chemical stability, the damage caused by microorganism growth also greatly affects the shelf-life of emulsion-based products. In another example, we fabricated a mixed emulsion by using the soluble β-conglycinin (7S)-chitosan (CS, a positively charged polysaccharide) complex as emulsifier.[104] The obtained 7S/CS mixed emulsion exhibited good storage stability against microorganisms at acidic pHs. This was related to the functional positively charged groups of CS around oil droplet surfaces, which can interact with negatively charged cell membranes and provide electrostatic repulsive forces, thus resulting in the inhibition of microorganism growth. In other words, CS, as an antimicrobial agent, was loaded on 7S and then delivered to the oil droplet surface, thus endowing emulsions with good storage stability against microorganisms in acidic conditions.[104]

In general, particles only act as vehicles for encapsulating and protecting the functional ingredients against the external environment (section 2.1). Recently, the ability of the particles to stabilize interfaces (oil/water and air/water) has received a lot of attention (section 2.2) and such a single task are fulfilled by these particles. For the examples mentioned in this section, the bioactives were first entrapped in protein particles to form a functional complex. Due to the surface activity of proteins, these complexes would reach the O/W interface after the diffusion and absorption process. As the structural rearrangement of the adsorbed protein occurred, the encapsulated components would be released and accumulated at the interface,

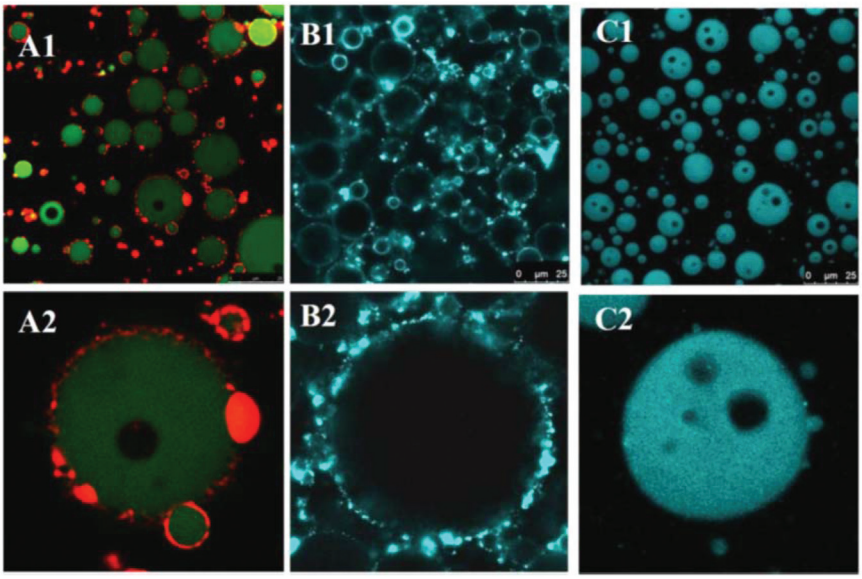

Fig. 3 Confocal laser scanning microscopy (CLSM) images of emulsions: ZCPs emulsion (A1 and A2, corn oil was stained with Nile Red (green) and zein was stained by Nile Blue A (red)), ZCPs-curcumin emulsion (B1 and B2, curcumin fluorescence was monitored by excitation by an argon laser at 488 nm (blue)) and ZCPs emulsion with curcumin (C1 and C2, curcumin fluorescence was monitored by excitation by an argon laser at 488 nm (blue)).[103]

and thus endow emulsions with good functional properties, such as improved oxidative stability and antimicrobial property.[74,103,104] Meanwhile, the functional complex and corresponding emulsion systems also served as vehicles to deliver the bioactives. Thus, these protein particles can not only act as a carrier for bioactive ingredients, but also act as a functional emulsifier to modify the microstructure of emulsion-based foods, which can serve multiple purposes simultaneously. The plant protein-based bifunctional particles provide a new perspective for designing novel functional delivery systems in food processing.

Furthermore, we also fabricated plant protein-based particles to deliver bioactives onto the O/W interface with the assistance of various surfactants. We introduced a novel biosurfactant stevioside (STE) to encapsulate RES by the formation of STE self-assembled micelles.[105] The physical and oxidative stability of SPI-based O/W emulsion were significantly improved by the incorporation of the STE-RES complex (Fig. 4).[105] In another work, STE was also found to be able to form complexes with soy proteins.[106] As the complex adsorbed at the O/W interface, soy proteins together with STE dominated the mixed interface. A synergistic effect in the interfacial tension decay and a plateau in the elasticity were observed due to the formation of complex. This endowed the corresponding emulsion with a long-term stability after 120 days, which did not take place in the emulsions stabilized solely by surfactant or protein.[106] Similar behaviour also was observed at the air–water interface, and the foams prepared by the complex exhibited good foaming capacity and considerable stability.[107] We applied this strategy to further deliver zein particles to the O/W interface.[108] Zein particles were modified using the ionic surfactant sodium stearate (SS) *via* ultrasonication. The resulting emulsions prepared by zein particle-SS complexes showed good stability against both coalescence and creaming, and oil gels without oil leakage could be obtained by a one-step freeze-drying of these emulsions (Fig. 5).[108]

3. Fibers

Fibers have diversified applications in food and biomedical fields, such as controlled nutrient/drug delivery and tissue engineering. Nanofibers and conventional microsized fibers can be produced by electrospinning, solution and melt-spinning, respectively.[20] Plant proteins, mainly zein and soy proteins, have been widely used to develop these two kinds of fibers for their delivery and controlled release applications. Electrospun fibers (200 nm to 2 μm) from SPI/poly(ethylene oxide) (PEO) blend and poly(lactic acid) were used for controlled release of an antimicrobial compound, allyl isothiocyanate (AITC).[109] The release of AITC could be controlled by varying the relative humidity, and exposure to air with elevated relative humidity triggered the release of AITC. The anthocyanin-rich red raspberry extract was also successfully incorporated into SPI-PEO composite electrospun fibers, which endowed the fibers with a high antibacterial activity against *Staphylococcus epidermidis*.[110] In addition, conventional protein fibers developed from soy protein (45 μm) have been reported to carry and controlled release three different drugs (metformin, 5-flouracil, diclofenac).[111] It was found that the affinity between the drugs and the fibers governed their release behavior, and drugs with higher affinity and lower

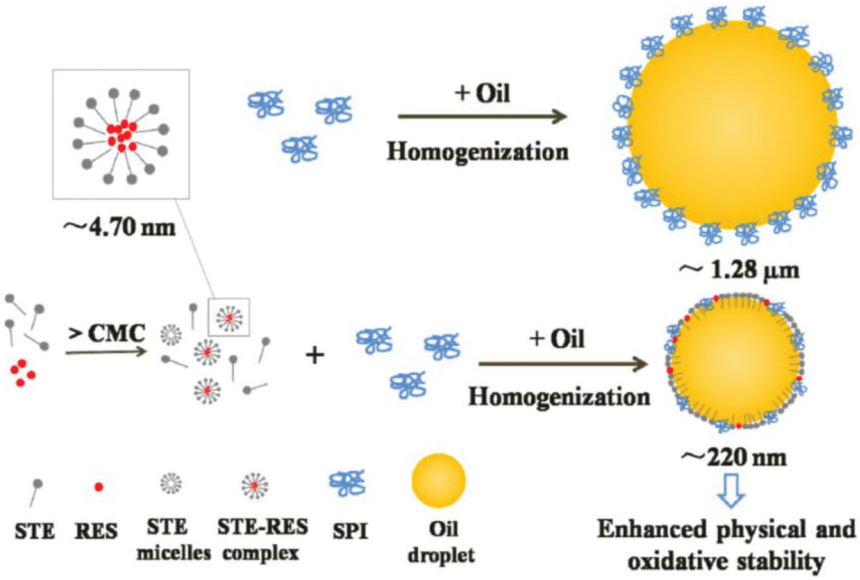

Fig. 4 Schematic illustration of the formation of oil-in-water (O/W) emulsion stabilized by soy protein isolate (SPI) and stevioside-resveratrol (STE-RES).[105]

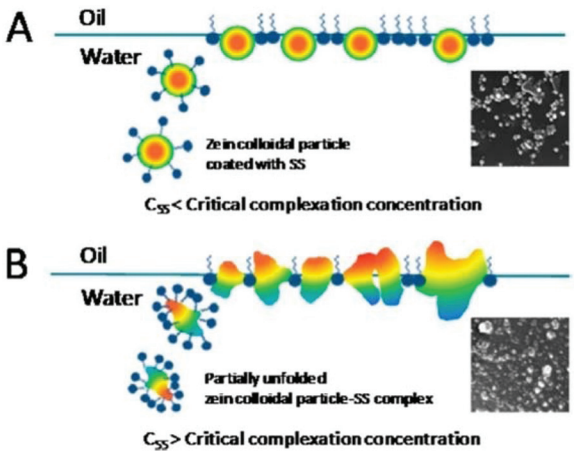

Fig. 5 Diagrammatic depiction of enhanced adsorption and targeted accumulation of zein particles at the oil/water interface with the synergism of sodium stearate (SS).[108]

diffusion coefficient had higher sorption loading capacity and a more sustained release rate.[111]

Compared to soy proteins, zein has been more widely studied for the development of nanofibers using electrospinning method due to its solubility in ethanol. To date, electrospun zein fibers have been used for encapsulation, stabilisation and controlled release of various bioactive ingredients, such as (−)-epigallocatechin-gallate (EGCG),[112] fish oil,[113,114] gallic acid,[115,116] and β-carotene.[117] Recently, Lim and co-workers have successfully prepared ultrafine zein fibers (150–600 nm) using electrospinning to encapsulate EGCG and fish oil, respectively, and they found that the stability of EGCG in water and the oxidative stability of fish oil during storage (14 days) were obviously enhanced.[112,113] The release kinetics of encapsulated fish oil were controlled by the extent of matrix swelling, erosion and diffusion of fish oil.[114] Gallic acid was also successfully incorporated into zein electrospun fibers at different loading ratios with average fiber diameters ranging from 327 to 387 nm.[115] The gallic acid was released rapidly from zein fibers primarily by a diffusion-controlled process.[116] The gallic acid loaded zein fibers were not cytotoxic and exhibited antimicrobial properties, which provide the potential as active packaging materials in food industry. The electrospun zein fibers also show an excellent outlook for their application in the encapsulation and stabilization of the light-sensitive bioactive antioxidant β-carotene.[117] The β-carotene was stable and well dispersed inside the zein fibers, and its light stability was significantly increased when exposed to UV-vis irradiation. To improve the mechanical and functional properties of zein fibers, composite electrospun fibers were made by blending with other biopolymers, such as chitosan.[118] The zein–chitosan composite fibers have also been demonstrated to have the potential as delivery vehicles for hydrophobic compounds such as alpha-tocopherol (α-TOC).[118] The release of α-TOC in simulated gastric fluid (SGF) without pepsin was triggered by swelling and driven by diffusion, however, α-TOC release in SGF was triggered by erosion in the presence of pepsin.

4. Films

Soy protein, zein and gliadin have been made into films for their potential use as carriers for delivery and controlled release of nutrients and drugs. SPI films have been used to study the release kinetics of drug delivery in simulated gastrointestinal conditions.[119] Two different drugs, hydrophilic

methylene blue and hydrophobic rifampicin, were applied, and the drug release profiles were controlled through the erosion of SPI films by zero-order kinetics.[119] In addition, SPI films have the potential as an effective delivery system for various active compounds, such as nisin, grape seed extract and organic acids.[120,121] These active SPI films could be applied for antimicrobial and antioxidant packaging for foods.

Recently, zein-based films have attracted increasing interest in active food packaging due to their ability to carry antimicrobials and antioxidants, such as lysozyme,[122–124] thymol,[125,126] and various phenolic compounds.[123,127] The release profiles from zein films could be changed by modifying film morphology and hydrophobicity using composite and blend film making technologies.[122–125] Arcan and Yemenicioğlu controlled the release of lysozyme from zein films by preparing composite films with different waxes and blend films with fatty acid (oleic acid).[124] The composites and blends showed 2.5- to 17-fold lower lysozyme release rates than control zein film. The zein-wax composite films also showed a sustained release rates of lysozyme, which caused a significant reduction in initial *L. monocytogenes* counts in fresh cheeses during cold-storage.[123] Mastromatteo *et al.* developed zein-based mono- and multilayer composite films loaded with spelt bran to control the release of thymol, and found that film thickness and spelt bran concentration could accelerate or delay the thymol release rate.[125] Recently, our group successfully fabricated a kind of novel thymol-loaded antimicrobial film based on zein colloidal nanoparticles coated with sodium caseinate as a stabilizer.[126] The zein self-assembly was changed by thymol migration during film formation, forming various particles or packed structure, which could affect the mechanical and barrier properties of the films (Fig. 6).[126] The release kinetic profile of thymol from zein nanoparticles-based films showed a two-step biphasic process, that is, an initial burst effect followed by a subsequent slower release, and the zein nanoparticles within the films matrices gave them the ability to sustain the release of thymol. Meanwhile, the films exhibited antimicrobial activity against *Escherichia coli* and *Salmonella*.[126] In addition, we further developed zein nanoparticle-stabilized emulsion films through microfluidic emulsification or in combination with solvent (ethyl acetate) evaporation techniques.[128] Both emulsion films exhibited excellent physical performance, such as water barrier capability, transparency and mechanical flexibility, and provide good potential as delivery and controlled release systems for lipophilic bioactive compounds. Gliadin films cross-linked by cinnamaldehyde were also used as carrier systems for the release of lysozyme.[129] The release rate was controlled by the reticulation of the protein matrix, and thus a greater degree of cross-linking led to slower release of lysozyme.

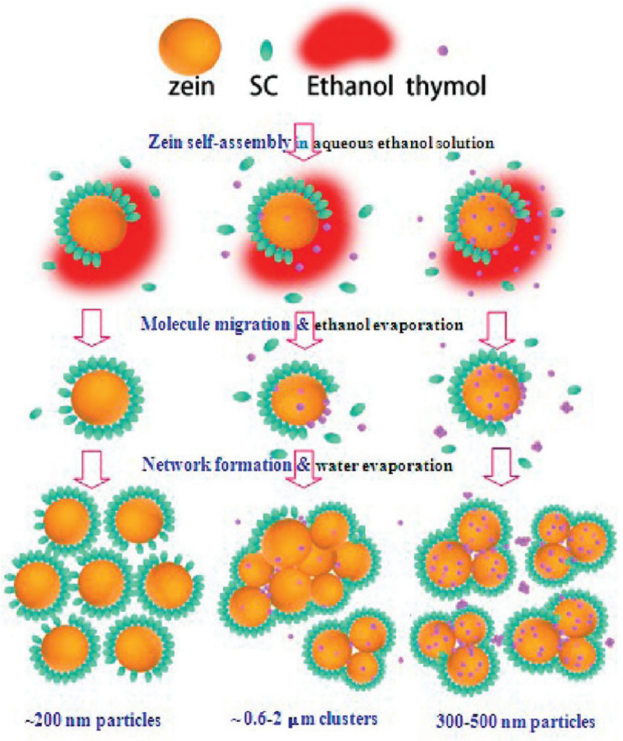

Fig. 6 Schematic illustration of formation of zein–sodium caseinate (SC) nanoparticle based films with or without thymol: (left) films without thymol (ZP_0); (middle) films at thymol-to-zein ratios of 10 and 20% (ZP_1 or ZP_2); (right) films at thymol-to-zein ratios of 30 and 40% (ZP_3 or ZP_4).[126]

5. Hydrogels

Hydrogels are three-dimensional networks constructed by physically or chemically cross-linked polymers, which are capable of holding a large quantity of water.[130,131] In this system, bioactive components can be entrapped, protected from hostile environments and delivered to the human GIT. The release behaviour of hydrogels can be manipulated by modifying their microstructure.[132] Compared to the hydrogels made from synthetic polymers, biocompatibility and biodegradability are the strength of the plant protein-based hydrogels. Since most of the bioactive ingredients are temperature-sensitive, cold-set gelation rather than that induced by heat treatment is preferable for the fabrication of protein-based hydrogel systems. On the other hand, the absorption of bioactives by the human body mainly occurs in the intestine tract. The protein-based gel networks also have to face the challenges in the gastric environment before they carry the compounds to the intestine tract. However, the hydrogels derived from plant proteins possess the capacity of pH response due to considerable amounts of acidic and basic groups in polypeptide chains of proteins,[132] and thus could achieve efficient delivery of bioactives along the human GIT.

Subirade and co-workers compared the controlled release behaviour of two types of cold-set soy protein hydrogels, filamentous or particulate, which were prepared by using different concentrations of calcium chloride.[133,134] They found that filamentous hydrogels exhibited a delayed release of ribo-

flavin due to their lower porosity when compared to the particulate hydrogels. In the presence of pepsin at pH 1.2, both the hydrogels provided good protection of riboflavin for at least 6 h and the release of riboflavin was independent of time or concentration (zero-order release), while the gels were digested in the presence of pancreatin at pH 7.5.[133,134] In other studies, soy protein hydrogels cross-linked by glutaraldehyde were studied *in vitro* for their potential as devices for the release of ionic compounds (amaranth and methylene blue).[135,136] Increasing the cross-linking extent and the concentration of salt in the gel generally led to the decrease of swelling/release rates without digestive enzyme. Amaranth, an anionic molecule, showed slower release in gastric conditions, whereas methylene blue, a cationic drug, showed the opposite trend.[136] These findings demonstrated the properties of the loaded compounds also affected their release behaviour in hydrogels.

Controlling the interaction between protein and polysaccharide, which depends on the environmental conditions (pH and ionic strength) and thus surface charge of these two biopolymers, is an effective strategy for fabricating pH-responsive hydrogel-based delivery systems. We have employed soy glycinin together with dextran sulfate (DS), a highly charged anionic polysaccharide, to prepare transparent hydrogels cross-linked by microbial transglutaminase (MTGase) using a two-step strategy.[137] With different combinations of DS amount and ionic strength, hydrogels with distinctive transparency and mechanical properties could be obtained.[137] With a decrease in the pH, more residues with positive charge appeared on the surface of soy glycinin, thus resulting in the enhanced interactions between glycinin and DS. It was also observed that this hydrogel deswelled in simulated gastric fluid and swelled in simulated intestinal fluid. The extent of deswelling and swelling increased when more DS was used in the hydrogel. Also, this hydrogel displayed a sustained drug release behaviour under simulated gastrointestinal conditions.

Compared to the conventional hydrogels, hydrogels with pore sizes in the micron range have open and interconnected pores, enhanced surface area, higher swelling capacity, faster swelling kinetics and response to the external stimuli, which make them become suitable nutraceutical delivery systems. However, the applied chemicals and complicated procedures in the preparation process are main limitations for fabricating edible porous hydrogels in large scale. Recently, we reported a fast and simple way to prepare soy protein porous hydrogels *via* high speed homogenizing in the presence of MTGase, that induced the protein cross-linking.[138] The foams produced during homogenization acted as the porogen template, and the porous architecture was set after homogenization by MTGase cross-linking.

Proteins from grains also have potential to develop hydrogels for delivering various bioactive ingredients. Recently, Scholten and co-workers described a novel method to prepare zein thermo-responsive gels by utilizing its specific assembly behaviour.[139] They found that zein, as amphiphilic triblocks, can assemble into a three-dimensional network in the presence of hydrophobic nucleation sites (oil droplets or hydrophobic silica) and good solvent quality (glycerol). The gel formation and collapse can be controlled by changing temperatures, nuclei hydrophobicity, or solvent polarity.[139] This thermo-responsive zein gels could find their use in the field of nutrient or drug delivery, especially for hydrophobic bioactives. Another study used a zein based *in situ* gelling system to carry a water-soluble glycopetide drug, pingyangmycin hydrochloride (PYM), and demonstrated that the release of PYM could be extended up to 7 days *in vitro* and 4 days *in vivo*.[140] The initial burst of PYM was significantly reduced from the zein–sucrose acetate isobutyrate (SAIB) *in situ* gels. It has been shown that complex hydrogels fabricated by blending zein with pectin did not swell in physiological environments, but were hydrolysed in the presence of pectinases.[141] The *in vitro* study showed the hydrogels have the capacity to endure protease attack and residence time variation.[141] Such pH- and enzyme-specific responses could be used to develop ideal hydrogel delivery systems for target delivery of bioactive food ingredients.

6. Conclusions and outlook

Plant proteins show great potential for developing promising delivery vehicles to incorporate and protect various bioactive ingredients, and control their release behaviour under the GIT conditions. As discussed in this article, plant proteins could be used to produce a wide range of delivery systems, such as micro- and nanoparticles, fibers, films and hydrogels, all of which can be tailored for the design of innovative functional foods. As the interest in functional foods is rapidly growing, the development of advanced plant protein-based delivery systems will expand the possible applications. Nevertheless, the delivery of functional ingredients in the complex food systems is rather challenging as it is essential to evaluate not only the impact of complex food matrix on the storage stability and bioavailability of the encapsulated ingredients, but also the effect of the delivery systems on the food product functionality, such as stability, texture, taste, appearance and bioavailability of the ingredients. To date, there are few studies on determining the compatibility of these delivery systems with the real food matrix and the processing pressure they have to withstand during the food manufacture, which will require more research in the future.

In addition, despite the success on the laboratory scale, many approaches applied to the preparation of these delivery systems, especially for soy protein- or zein-based particulate systems, still present limitations and difficulties for their large-scale production within the food industry. Hence, the development of novel technologies for the production of nano-sized delivery systems in large scale, such as the FNP technique, constitutes another issue for future research. Other than the dominating encapsulation and delivery applications, studies on the modifying the food microstructure using soy protein or zein colloidal particles, such as the formation and

stabilization of emulsions and foams, have also emerged recently. Future developments in this field are expected to design bi-functional plant protein (soy protein and zein) particles, which can serve multiple purposes simultaneously, such as functional ingredients delivery and the interfacial stabilization for food dispersions (*i.e.* emulsions and foams). Finally, more *in vivo* evaluations of these plant protein-based delivery systems are needed to address their biological fate in the human GIT as well as their efficacy and safety in physiological conditions.

Acknowledgements

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