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Interactions of vegetable proteins with other polymers: Structure-function relationships and applications in the food industry

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1	Interactions of Vegetable Proteins with Other Polymers:
2	Structure-Function Relationships and Applications in the Food
3	Industry
4	
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23 Abstract

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Background: In recent years, there has been increasing interest in vegetable proteins, due to their various health beneficial functions and wide applications in the food industry. Vegetable proteins combined with other edible polymers can be used to improve the quality and nutritional value of food products. In these complex food systems, interactions between different components are inevitable, and these interactions have a significant influence on the structure and functions of food products.

32 *Scope and approach :* This study reviews the current status of knowledge of 33 interactions between vegetable proteins and other polymers (proteins or 34 polysaccharides) in food systems and the structure of complexes formed by these 35 interactions. The study also provides a comprehensive review of the applications of 36 the complexes.

Key findings and conclusions: Vegetable proteins display different types of 37 interactions with other polymers (e.g., polysaccharides, or animal proteins) under 38 different conditions, thus forming a variety of complexes with different structures 39 (e.g., double networks, mosaic textures and cross-linked structures), which showed 40 different impact on properties of the final food products and their applications (e.g., 41 42 substitution for fat, or encapsulation for bioactive ingredients) in the food industry. However, previous studies mainly focused on leguminous proteins and vegetable 43 protein based mixtures of two polymers, further studies on other vegetable proteins 44

45	and more complex food systems containing vegetable proteins and other polymers are
46	required.
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48	Keywords: Vegetable protein; Polysaccharide; Interaction; Structure; Function;
49	Application
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67 1. Introduction

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69 Proteins are a very important component of the human diet, as they are essential 70 to the maintenance of muscle mass, immune responses, cell signaling and repair of 71 damaged cells (Henley, Taylor, & Obukosia, 2010). Animal and vegetable proteins are 72 two main sources of proteins in the diet. However, excessive consumption of animal proteins may lead to obesity (Bujnowski, et al., 2011), coronary heart disease (Clifton, 73 2011), high blood pressure (Elliott, et al., 2006) and increased serum and urine uric 74 75 acid (Tracy, et al., 2014). Many researches indicated that vegetable proteins had many health benefits, e.g., nutritional support to cirrhotic patients (Bianchi, et al., 1993), 76 improving obesity-induced metabolic dysfunction (Wanezaki, et al., 2015), 77 78 anti-cardiovascular disease (Lichtenstein, 1998) and anti-cancer activities (Lauerman, 1998). 79

As shown in Fig. 1, there are three main types of vegetable proteins: leguminous 80 proteins, oil seed proteins and cereal proteins (Zraly, et al., 2006). Based on various 81 82 health benefits of these vegetable proteins, many efforts have been made to develop vegetable proteins based food-grade films, hydrogels, emulsions, or foams for a 83 variety of applications in food, nutrition, biology and pharmaceutical industries 84 (Reddy & Yang, 2011). However, vegetable proteins are sensitive to processing and 85 environment. The denaturation of vegetable proteins may happen during extraction, 86 food processing or storage, which potentially can influence their performance in food 87 systems (e.g., in emulsions and foams). 88

89 In addition, the location of the proteins inside plan seeds can influence the extraction of proteins (Kasai & Ikehara, 2005). In order to improve protein 90 extractability, different extraction processes such as microwave heating (Choi, Choi, 91 Chun, & Moon, 2006) and ultrasound technology (Karki, et al., 2010) were 92 93 investigated, which may cause the protein denaturation (Fukase, Ohdaira, Masuzawa, 94 & Ide, 1994; Hafez, Mohamed, Hewedy, & Singh, 1985). During the extraction of proteins, many factors (e.g., the types of the solvent, the temperature and pH of the 95 reaction system, the agitation speed and extraction time) can be optimized to recover 96 proteins and prevent the loss of their solubility (Karaca, Low, & Nickerson, 2011; Wu, 97 Wang, Ma, & Ren, 2009). 98

Many strategies have been developed to prevent the denaturation of proteins 99 during food processing or storage, such as molecular modification of vegetable 100 proteins (Wang, Wang, & Sun, 2005) or mixing vegetable proteins with other 101 polymers (Liang, Wong, Pham, & Tan, 2016). In these multi-components food 102 systems, the interactions between vegetable proteins and other components will 103 inevitably take place in a variety of ways. These interactions can potentially have 104 great influences on the structures and properties of these food products (Zhao, Dong, 105 Li, Kong, & Liu, 2015). However, very limited information about an overall 106 summarization of the interaction between vegetable protein and other biopolymers 107 was known. Therefore, this study provides an overview of the current status of 108 knowledge about the interactions of vegetable proteins with food macromolecules, 109 structure-function relationships of vegetable-protein-based biopolymers and their 110

111 applications in the food industry.

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113 **2. Formation and structure of vegetable-protein-based complexes**

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When vegetable proteins are exposed to heating, ultrasonic, high pressure, 115 116 extreme pH or electrical force, they always denature and the hydrophobic groups buried in the native state are exposed to the surface (Jacoba, Harry Gruppen, & Ton 117 van Vliet, 2002; Nishinari, Fang, Guo, & Phillips, 2014). . Denatured vegetable 118 proteins can form films or gels, which can be used as package and encapsulation 119 120 materials for food products (Berghout, Boom, & van der Goot, 2015; Guerrero & de la Caba, 2010; Liu, Tellez-Garay, & Castell-Perez, 2004). Vegetable proteins can also 121 be used as emulsifiers in oil-in-water (O/W) emulsions or air-in-water dispersions, 122 due to their amphiphilic properties (Karaca, et al., 2011; Matemu, Kayahara, 123 Nakamura, 2011; Morales, 124 Murasawa, Katayama, Martinez, Pizones & Ruiz-Henestrosa, & Pilosof, 2015). 125

However, the structures of single protein formed gels or films are always fragile (Pan, Jiang, Chen, & Jin, 2014; Pan, et al., 2015) and the stabilities of single protein stabilized emulsions or forms are usually poor (Kasran, Cui, & Goff, 2013; Ventureira, Martínez, & Añón, 2012). The utilization of vegetable proteins combined with other biopolymers, e.g., polysaccharides or animal proteins, to form functional complexes is widely considered as one of the best methods for improving the functionalities of vegetable proteins (Table 1). 133

134 2.1. Protein-protein complexes

Protein-protein interactions have been well investigated with the objectives of clarifying structure-function relationships, improving food quality, and developing new products (Sarbon, Badii, & Howell, 2015). Interactions of proteins at oil-water or air-water interfaces can maintain the stability of emulsions or foams, respectively while the interactions between protein molecules in proteins solutions are essential to the formation of protein gels and films.

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142 2.1.1. Formation and structure of protein-protein complexes at interfaces

Single food protein stabilized emulsions are always sensitive to temperature, salt 143 144 and pH (Mcclements, 2004). Compounded utilization of two types of proteins with different structures as emulsifier is a simple and controllable way to improve the 145 stability of single protein stabilized emulsions (Liang, et al., 2016; Ventureira, et al., 146 147 2012). The study of Ji et al. (2015) can be used as a good example to clarify the structures of mixed proteins at oil-water interfaces. Sodium caseinate (SC) and soy 148 protein isolate (SPI) were shown to bind to oil-water interfaces to form negatively 149 charged compact interface structures at pH6.8 (Fig. 2), while pH and ionic strength 150 were shown to affect the surface charge and the particle size of a SC-SPI-stabilized 151 emulsion (Pizones Ruiz-Henestrosa, Martinez, Carrera Sánchez, Rodríguez Patino, & 152 Pilosof, 2014). Further investigations on the effect of concentration, mixture ratio, or 153 structure of proteins on the protein-protein interactions at oil-water interfaces are 154

155 needed.

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157 2.1.2. Formation and structure of protein-protein complexes in solutions

Protein-protein interactions in protein solutions follow three main pathways: 158 159 phase separation, synergistic interaction and aggregation (Firoozmand & Rousseau, 160 2015). In most cases, a mixture of two or more different proteins will lead to phase separation, e.g., coagulation and segregation. When phase separation occurs, two or 161 more proteins form independent phase-separated networks, and they may disturb the 162 assembly of a uniform network structure (Chronakis & Kasapis, 1993; Sarbon, et al., 163 2015). A mixture of two oppositely charged proteins can result in aggregation induced 164 by electrostatic attraction (Sarbon, et al., 2015). Synergistic interactions can lead to 165 better products with a uniform structure than those formed by each individual material 166 alone (Ngarize, Adams, & Howell, 2004). Denavi et al. (2009) found that the presence 167 of 25% (w/w) SPI led to conformational changes of gelatin, which produced a twofold 168 effect: self-aggregation of the gelatin polypeptide α -chains, and a certain degree of 169 intermolecular associations via C=O bonds between gelatin and SPI. 170

The type of protein has an enormous effect on protein-protein interactions in solutions. The primary sequence and secondary and tertiary structures of proteins influence the interactions between proteins. Taking SPI and myofibrillar protein isolate (MPI) as an example, these proteins have different denaturation temperatures due to differences in their subunit composition. Hence, it is difficult for them to interact with each other and form a uniform and compact structure under the same

heating condition, but an interwoven structure can be formed between SPI and MPI
by controlling reaction conditions (Bainy, Corredig, Poysa, Woodrow, & Tosh, 2010;
Denavi, et al., 2009).

The molecular weight of proteins is also one of the most important factors that 180 181 can significantly influence the protein-protein interactions in solutions (Ersch, et al., 2016). Proteins with low molecular weights can embed themselves in the matrix but 182 have different effects on the network structures formed by protein-protein interactions, 183 while proteins with high molecular weights may disturb the assembly of a network 184 structure or form an interwoven structure depending on their properties or reaction 185 conditions (Chen & Dickinson, 1999). Taking whey protein and blood plasma proteins 186 as an example of low molecular weight proteins, whey protein could occupy the 187 interaction sites of collagen molecules, weakening the ordered structure of collagen 188 networks (a crater-shaped form) (Walkenström & Hermansson, 1995); however, blood 189 plasma proteins could form a uniform network structure with collagen (Oechsle, 190 Häupler, Gibis, Kohlus, & Weiss, 2015). In terms of high molecular weight proteins, 191 e.g., gluten and SPI, phase separation occurred in mixture of collagen and gluten 192 while SPI could form an interwoven structure with collagen. By contrast, when the 193 concentrations of these co-gelling proteins were low, they could only fill in the pores 194 of collagen networks and had no significant effect on microstructure of collagen (Fig. 195 3) (Ahmad, Nirmal, Danish, Chuprom, & Jafarzedeh, 2016; Oechsle, et al., 2015). 196

197 Furthermore, protein-protein interactions and the resulting texturization (e.g.,198 gelation and film formation) depend greatly on the protein concentration. Low

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concentrations frustrate sufficient contact between protein molecules. High concentrations lead to a poor dispersity of proteins, and mixing or shearing forces may be then needed to favor a better dispersion of proteins, and form a favorable network structure (Grabowska, Tekidou, Boom, & van der Goot, 2014). Thus, in protein solutions, at least one of the proteins should be at an appropriate concentration to form a continuous network structure, while other proteins will fill in the gaps in the network in a continuous or dispersed manner depending on their properties.

Moreover, the pH can affect the surface charge and solubility of protein molecules and thus their interactions. Proteins molecules are nearly neutrally charged at pH values close to their isoelectric point (pI) and tend to aggregate, but can form a fine network structure at pH values far above or below their pI (Bengoechea, Romero, Aguilar, Cordobés, & Guerrero, 2010). For example, whey proteins can form aggregated particulate networks at pH values near their pI, but form fine-stranded networks at higher or lower pH values than pI (Alu'datt, Alli, & Nagadi, 2012).

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214 2.2. Protein-polysaccharide complexes

Proteins and polysaccharides can form fine complexes in two ways: covalent bond and/or non-covalent bond (Ji, et al., 2015). The covalent bond mainly refers to the Maillard reaction, which is a non-enzymatic glycosylation reaction between free amino groups of proteins and aldehyde group of reducing sugars (Liu, Ru, & Ding, 2012). This method usually involves thermal denaturing of a protein solution, and adding a polysaccharide solution as a Maillard-type cross-linking agent (Caillard,

221	Remondetto, & Subirade, 2009). The non-covalent bond includes hydro	ogen bond and
222	electrostatic attraction. Generally, uncharged polysaccharides can fo	rm complexes
223	with proteins mainly by hydrophobic interactions, whereas for ionic po	olysaccharides,
224	the complexes mainly are formed by electrostatic interactions (Chang,	Li, Wang, Bi,
225	& Adhikari, 2014; Wan, et al., 2014).	N Y

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227 2.2.1. Formation and structure of protein-polysaccharide complexes at interfaces

Protein-stabilized emulsions or foams are susceptible to environmental 228 conditions because proteins are easy to denature under exposure to some extreme 229 conditions (Martínez, Ganesan, Pilosof, & Harte, 2011). Adding polysaccharides to 230 emulsions can increase their stability by forming protein-polysaccharide complexes at 231 oil-water interface layers (Liu, Zhao, Zhao, Ren, & Yang, 2012; Martinez, 232 Carrerasanchez, Pizonesruizhenestrosa, Rodriguezpatino, & Pilosof, 2007; Yang, et 233 al., 2015). Surface activity, concentration and particle size of polysaccharides have 234 significant effects on the structures of protein-polysaccharide complexes (Baeza, 235 Sanchez, Pilosof, & Patino, 2004, 2005; Carp, Bartholomai, & Pilosof, 1999). For 236 instance, Wan et al. (2014) have shown that when stevioside at low concentration (0.1 237 wt%) was added to SPI-stabilized O/W emulsion, SPI still occupied the most part of 238 the droplet surface. Stevioside could only bind to the gaps between protein molecules. 239 When increasing the concentration to 0.25 wt%, stevioside showed stronger 240 interaction with SPI, thereby resulting in partial dissociation of the protein's rigid 241 structure. When the concentration of stevioside reached 2 wt%, a considerable number 242

of stevioside molecules bound to the droplet surface by replacing SPI-steviosidecomplexes due to their small particle size (Fig. 4).

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246 2.2.2. Formation and structure of protein-polysaccharide complexes in solutions

There are three different equilibrium situations in solutions containing mixed proteins/hydrocolloids, namely miscibility, thermodynamic incompatibility and complex coacervation (Giancone, Torrieri, Masi, & Michon, 2009). Formation of protein-polysaccharide complexes in solution follows two main pathways, phase separation and formation of synergistic networks.

Thermodynamic incompatibility between proteins and polysaccharides often 252 leads to separation (Li, et al., 2009), but two separate network structures formed by 253 segregation can still form a rigid structure by physically or chemically driven 254 intertwining (Zhao, et al., 2016). Hou et al. (2015) used a two-step enzymatic 255 sequential cross-linking method to form a protein-polysaccharide double network 256 structure. The first layer of network was formed by laccase-induced cross-linking of 257 sugar beet pectin (SBP). After adding and mixing an equal volume of soy glycinin 258 (SG) dispersion, the double network was formed under the action of microbial 259 transglutaminase (MTGase) in a water bath at 45 for 4 h (Fig. 5). Pires Vilela, 260 Cavallieri, and Lopes da Cunha (2011) mixed denatured SPI solution and heated 261 gellan gum solution together to form a homogeneous double-network structure by 262 using calcium chloride or potassium chloride as cross-linker. This double 263 protein-polysaccharide network structure was firmer than single network structure 264

formed by pure protein or polysaccharide. It has a wide range of promising applications in the food industry, such as use as controlled delivery systems for nutraceuticals (Nakayama, et al., 2004).

In most cases, mixing proteins with polysaccharides leads to phase separation 268 (Li, et al., 2009). The amount of branched chains and the molecular weight of 269 polysaccharide can affect their continuity and dispersity in this mixed systems (Min & 270 Yang, 2010). Polysaccharides with more branched chains and lower molecular weight 271 usually show better dispersity than those with few branched chains and higher 272 molecular weight, which are easy to agglutinate and form a continuous and 273 heterogeneous structure (Li, et al., 2008; Monteiro, Rebelo, da Cruz e Silva, & 274 Lopes-da-Silva, 2013). In addition, polysaccharides at low concentration can increase 275 the density of protein-polysaccharide aggregates, while polysaccharides at high 276 concentration may destroy the continuous network formed by proteins, because it is 277 hard to form a rigid structure by intertwining two independent networks (Chang, et 278 al., 2014; Li, Yeh, & Fan, 2007). 279

Miscibility and coacervation of proteins and polysaccharides are beneficial to the formation of an associative structure. Miscibility of protein and polysaccharide can form Maillard conjugates by covalent bonds while coacervation can form protein-polysaccharide complexes by electrostatic attraction (Giancone, et al., 2009; Yuan, Wan, Yang, & Yin, 2014). Polysaccharides can be used as a cross-linker to produce a protein network structure by linking denatured protein molecules (Fig. 6) (Caillard, Remondetto, & Subirade, 2010). Maillard reactions between SPI and

287 carboxymethyl konjac glucomannan (CMKGM) have been demonstrated by FTIR; meanwhile, FTIR results also suggested the coexistence of strong hydrogen bond 288 289 interaction between SPI and CMKGM (Wang, et al., 2014). Maillard reactions between vegetable proteins and carboxymethyl cellulose (CMC) (Su, Huang, Yuan, 290 Wang, & Li, 2010; Su, et al., 2012), glyceraldehyde (Caillard, et al., 2010), 291 292 glutaraldehyde (Caillard, et al., 2009), ribose and sucrose (Gan, Cheng, & Easa, 2008) in solutions have also been reported. However, for polysaccharides with a high degree 293 of polymerization, the Maillard reaction is slow. A novel method can be used to attach 294 functional groups to the polysaccharide surfaces using surface modification, followed 295 by using crosslinking agents to obtain protein-polysaccharide complexes (La Wang, 296 Li, Zhang, & Shi, 2016). For example, the chemical-crosslinking structure formed by 297 SPI, modified cellulose nanocrystal (MCNC), and ethylene glycol diglycidyl ether 298 (EGDE) could enhance mechanical properties and water resistance of the 299 SPI/EGDE/MCNC film, compared to the un-modified SPI/EGDE film (Fig. 7) 300 (Zhang, et al., 2016). 301

Properties of proteins and polysaccharides (e.g., charge density, molecular 302 weight and branched chain) and their concentrations or ratio have a big influence on 303 the protein-polysaccharide network structures (Ma, Dang, 304 & Xu, 2016). Polysaccharides can be classified as negatively-charged (e.g., xanthan gum (XG) and 305 pectin), naturally-charged and galactomannans), 306 (e.g., guar gum and positively-charged (e.g., chitin) polysaccharides. At high pH values (pH>pI), 307 negatively-charged proteins and negatively-charged polysaccharides can form a stable 308

309 dispersion due to electrostatic repulsion between protein and polysaccharide; at low pH (pH < pI), positively-charged proteins and negatively-charged polysaccharides can 310 311 form protein-polysaccharide complexes by electrostatic attraction (Chang, et al., 2014; Lam, Shen, Paulsen, & Corredig, 2007). In addition, different proteins are 312 differently charged at the same pH value, resulting in different strengths of 313 314 electrostatic attractions with polysaccharides. For example, glycinin can form a more stable complex structure than β -conglycinin with chitin at a wide pH range, because 315 glycinin carries greater positive charge than β -conglycinin at the same pH value 316 (Yuan, et al., 2014). 317

Therefore, the environmental pH must be properly controlled to ensure that the 318 proteins and polysaccharides are oppositely charged, which is essential for the 319 formation of a stable protein-polysaccharide complex by electrostatic attraction 320 (Spada, Marczak, Tessaro, & Cardozo, 2015; Yuan, et al., 2014). In addition, salts 321 (e.g., sodium, potassium, calcium and magnesium chloride) can influence the 322 structures of protein-polysaccharide complexes formed by electrostatic attractions, as 323 salts can shield charged-sites of both protein and polysaccharide molecules and 324 disrupt electrostatic attractions between them (Yuan, et al., 2014). Meanwhile, the 325 way of adding salts can affect the reaction rate and the final structures of 326 protein-polysaccharide complexes; slow diffusion of salts into protein and 327 polysaccharide solutions through a permeable membrane leads to a slower formation 328 of protein-polysaccharide complexes than the direct addition of the same amount of 329 salts. Slow diffusion of salts contributes to a sufficient interaction between proteins 330

331	and polysaccharides, which may be helpful in forming a homogeneous structure (Li,
332	et al., 2009; Pires Vilela, et al., 2011; Yuan, et al., 2014).

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334 **3. Structure-function relationships of vegetable-protein-based complexes**

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336 *3.1. Film formation*

Films are a kind of material with a unique function in selectively separating 337 compounds, which can be used in food packaging (Fabra, López-Rubio, & Lagaron, 338 2016). The most commonly used materials for film formation are polyvinyl chloride 339 (PVC), polyethylene (PE), polypropylene (PP) and polystyrene (PS) (Yabannavar & 340 Bartha, 1993). However, films formed by these synthesized polymers have serious 341 environmental concerns because they are not easy to degrade and remain intact in the 342 environment for long periods of time (Weng & Zheng, 2015). Thus, it is of interest to 343 develop renewable, biodegradable and nontoxic film-forming biopolymers, such as 344 natural biopolymers (e.g., starch, cellulose and proteins), bio-derived monomers (e.g., 345 polylactate) and polymers produced by microorganisms (e.g., polyhydroxybutyrate 346 and polyhydroxyvalerate) (Guerrero, Nur Hanani, Kerry, & de la Caba, 2011). 347

Solvent casting and extrusion are two technologies used to prepare polymer films
(Echeverría, Eisenberg, & Mauri, 2014; Guerrero, Beatty, Kerry, & de la Caba, 2012).
Polymer films must have good barrier properties for gas and water (e.g., low water
vapor permeability, WVP), mechanical properties (e.g., thickness, tensile strength,
elastic modulus, deformability and elongation) and physical properties (e.g., colour

and thermal stability). Based on these requirements, vegetable proteins are an ideal 353 source of film-forming materials. The properties of films formed by SPI, peanut 354 protein and zein have been well studied (Liu, et al., 2004; Song, Zhou, Fu, Chen, & 355 Wu, 2013; Wang, Marcone, Barbut, & Lim, 2012). Films made from vegetable 356 proteins show good mechanical and optical properties but high WVP (Otoni, 357 Avena-Bustillos, Olsen, Bilbao-Sainz, & McHugh, 2016). Mixing different proteins 358 together or mixing proteins with polysaccharides to form protein-protein or 359 protein-polysaccharide complexes is an effective way to improve barrier and 360 mechanical properties of protein-based films (Table 2) (Koshy, Mary, Thomas, & 361 Pothan, 2015; Wihodo & Moraru, 2013). 362

363

364 3.1.1. Film formation based on protein-protein interactions

Two or more types of vegetable proteins can be mixed together to form films 365 with improved barrier and mechanical properties compared with films formed by 366 single protein (Cho, Lee, & Rhee, 2010; Li, et al., 2015; Wang, et al., 2016). In 367 addition, vegetable proteins are often used to replace a portion of animal proteins, 368 which can reduce the cost and improve physical, mechanical or barrier properties of 369 films (Cao, Fu, & He, 2007; Denavi, et al., 2009; Gómez-Guillén, et al., 2009; 370 Oechsle, et al., 2016; Weng & Zheng, 2015). The addition of vegetable proteins can 371 improve the tensile strength, breaking forces or extent of elongation of films without 372 influencing their thickness (Denavi, et al., 2009; Oechsle, et al., 2016). Compared 373 with pure animal protein films, films formed by synergistic interactions of mixed 374

vegetable and animal proteins showed decreased WVP (Denavi, et al., 2009) while
films formed by phase separation of mixed vegetable and animal proteins showed
increased WVP (Weng & Zheng, 2015).

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379 3.1.2. Film formation based on protein-polysaccharide interactions

Many polysaccharides, e.g., cellulose, starch, gums and carboxymethyl konjac 380 glucomannan (CMKGM), can be used to prepare films in combination with vegetable 381 proteins due to their good film-forming ability, biocompatibility and biodegradability 382 (Fabra, et al., 2016; González & Alvarez Igarzabal, 2015; Pedro Guerrero, Garrido, 383 Leceta, & de la Caba, 2013; Jensen, Lim, Barbut, & Marcone, 2015; Li, Zhu, et al., 384 2015; Li, et al., 2015; Piazza, Dürr-Auster, Gigli, Windhab, & Fischer, 2009; Sun, 385 Sun, & Xiong, 2013; Wang, et al., 2014). Polysaccharides can improve the tensile 386 strength of films, but decrease the extent of elongation at breaking due to their 387 relatively dense and compact structures, unless they undergo complexation or 388 formation of network structure by Maillard reactions (González & Alvarez Igarzabal, 389 2015; Sun, et al., 2013). In protein-polysaccharide films, synergistic interactions 390 contribute to improved water vapor and oxygen barrier properties because of chemical 391 crosslinking or Maillard reactions between proteins and polysaccharides (Jensen, et 392 al., 2015; Li, Zhu, et al., 2015; Wang, et al., 2014). Meanwhile, phase separation is 393 also conducive to improving water vapor, in a different manner from that in 394 protein-protein films (Sun, et al., 2013). Possibly because interwoven compact 395

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- structures between proteins and polysaccharides have been formed, which inhibits the
 penetration of water into matrixes (González & Alvarez Igarzabal, 2015).
- 398

399 *3.2. Gelation*

400 Gels are a kind of special decentralized systems in which molecules are connected to each other and form a network structure under certain conditions. Gaps 401 in the networks may be filled with liquid or gas as a dispersed phase. Proteins and 402 polysaccharides are mainly responsible for gelation, and for this reason play important 403 roles in the food industry (Ersch, et al., 2016). Properties of gels formed by vegetable 404 proteins have been well studied (Berghout, et al., 2015; Dahesh, Banc, Duri, Morel, & 405 Ramos, 2016; Kim, Varankovich, & Nickerson, 2016; Rui, et al., 2016; Shand, Ya, 406 Pietrasik, & Wanasundara, 2007; Sun, et al., 2015); however, there are many good 407 reasons to mix different polymers to form favorable gels. Firstly, combined use of 408 different polymers (e.g., vegetable proteins and polysaccharides) could be an 409 attractive way to develop new food products with balanced nutritional value (Bainy, et 410 al., 2010; Chang, et al., 2014; Li, et al., 2007; Monteiro, et al., 2013; Sun & Arntfield, 411 2012). Secondly, gels formed by mixed polymers usually have better mechanical 412 properties than those formed by a single polymer due to the reactions between 413 different polymers and the formation of compact structures (Gan, Latiff, Cheng, & 414 415 Easa, 2009; Guo, et al., 2014; Hou, et al., 2015).

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417 *3.2.1. Gelation based on protein-protein interactions*

418 Mixing different vegetable proteins to form gels is a good way to improve the sensory and nutritional values of food (Alu'datt, et al., 2012; Bainy, et al., 2010). 419 420 However, inappropriate combinations or concentrations of proteins may lead to poor mechanical properties of gels (Sun & Arntfield, 2012). The concentration of one 421 422 protein in protein-protein mixtures should be high enough to act as filler to fill the gaps in the networks formed by the other protein. However, the concentration of this 423 filler protein should also not be so high that it will disturb network formation of the 424 other protein (Table 3) (Sun, Wu, Xu, & Li, 2012). In addition, some vegetable 425 426 proteins (e.g., black bean and mung bean protein isolate) can act as enzyme inhibitors rather than co-gelling agents or binders at low concentration, and they may prevent 427 the disintegration of the gel structures and improve the quality of food (e.g., surimi) 428 429 (Kudre, Benjakul, & Kishimura, 2013).

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431 3.2.2. Gelation based on protein-polysaccharide interactions

Understanding the structures and properties of protein-polysaccharide gels is 432 very important for designing products with desired properties and for developing new 433 products with novel textures (Chang, et al., 2014; Li, et al., 2007; Monteiro, et al., 434 2013). As shown in Table 3, the properties and concentration of polysaccharides had 435 great influences on the structures and properties of protein-polysaccharide gels. 436 Several strategies can be used to strengthen the mechanical properties of 437 protein-polysaccharide gels,. For example, MTGase-mediated ε -(γ -glutamyl)lysine 438 isopeptide bonding and Maillard reaction-induced cross-linking between proteins and 439

440	polysaccharides can improve the mechanical properties and microstructures of gels
441	(Gan, Latiff, et al., 2009; Guo, et al., 2014; Hou, et al., 2015).

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443 *3.3. Emulsification*

Vegetable proteins (e.g., SPI, pea protein and gluten) and dairy proteins (e.g., 444 casein and whey) are widely used as emulsifiers (Fernández-Ávila, Escriu, & Trujillo, 445 2015; Karaca, et al., 2011). There is a growing interest in mixing vegetable proteins 446 with animal proteins or utilizing vegetable proteins instead of animal proteins in 447 emulsification (Karaca, et al., 2011). The heat stability of mixed protein stabilized 448 emulsions can be increased due to protein-protein interactions (Liang, et al., 2016). 449 However, emulsions stabilized by mixed proteins are still sensitive to extreme 450 conditions. For example, after heating at 90°C for 15 min, casein/pea protein-stabilized 451 emulsions formed solid gels due to protein denaturation (Liang, et al., 2016). 452

Emulsions stabilized by proteins combined with polysaccharides usually show 453 better heat stability than those stabilized by only proteins (Zhao, et al., 2015). 454 Generally, polysaccharides cannot adsorb onto the surface of oil droplets and 455 accordingly cannot stabilize emulsions. However, they can improve the stability of 456 emulsions in association with proteins (Yin, Deng, Xu, Huang, & Yao, 2012). The 457 emulsification properties of protein-polysaccharide conjugates, e.g., peanut protein 458 isolate/dextran (Liu, et al., 2012), peanut protein isolate/maltodextrin (Chen, Chen, 459 Wu, & Yu, 2016), soy protein isolate/soy soluble polysaccharide (Yang, et al., 2015) 460 and soy protein isolate/fenugreek gum (Noshad, Mohebbi, Shahidi, & Koocheki, 461

462 2015) have been widely studied. Emulsions stabilized by these conjugates showed good stability in extreme environments (e.g., heating, ultrasonic, high pressure, 463 464 extreme pH or electrical force) (Fuguo Liu, Ma, Mcclements, & Gao, 2016). Formation of protein-polysaccharide conjugates by the Maillard reaction 465 generally requires a long reaction times at a suitable temperature and humidity (Liu, et 466 al., 2012). Compared with Maillard reaction, layer-by-layer deposition method and 467 electrostatic reaction are simpler, more effective and environment friendly strategies 468 to form protein-polysaccharide complex as emulsifiers (Yin, et al., 2012). The 469 layer-by-layer electrostatic deposition technique usually creates a multilayer coating 470 around oil droplets (Mcclements & Li, 2010). Noshad et al. (2015) found that the 471 emulsions with oil droplets coated by a three-component interfacial layers consisting 472 of SPI, octenyl-succinate starch (OSA starch) and chitosan, were more stable than 473 those coated with either a one (SPI) or two (SPI-OSA starch) component layer. 474 Another strategy to produce a protein-polysaccharide complex is that mixing proteins 475 and polysaccharides with opposite net charges by adjusting the pH value to form 476 dispersible complexes (Evans, Ratcliffe, & Williams, 2013). In this technology, 477 polysaccharide could interact with protein via electrostatic attractions and 478 hydrophobic interactions, meanwhile the neutral side chains of the polysaccharide 479 could stabilize the protein/polysaccharide complexes in aqueous solution (Wan, et al., 480 481 2014; Yin, et al., 2012).

482

483 *3.4. Foamability*

Among vegetable proteins, SPI is most frequently used protein as a foaming 484 stabilizer due to its favorable foaming ability and potential health benefits. Peanut 485 486 protein isolate (PPI) can also be employed as stabilizer of foam systems, but its foaming ability is not as good as that of SPI (Liu, et al., 2012). Mixing different 487 proteins together sometimes can improve their foam ability and surface activities 488 (Ventureira, et al., 2012). For instance, mixing soy globulin and β -lactoglobulin gave 489 better foaming ability than soy globulin or β-lactoglobulin alone (Pizones 490 Ruiz-Henestrosa, et al., 2014). Additionally, pH was shown to affect the surface 491 charge of proteins and electrostatic interaction between them, thus affecting the 492 structure and properties of foams (Pizones Ruiz-Henestrosa, et al., 2014). Interactions 493 between proteins and polysaccharides at interfaces can enhance of the foamability of 494 495 proteins adsorbed onto interfaces (Baeza, Sanchez, Patino, & Pilosof, 2005; Carp, Bartholomai, Relkin, & Pilosof, 2001). The molecular weight of polysaccharides has 496 a significant influence on the foam ability of proteins-polysaccharide complex. 497 Polysaccharides with low molecular weight have better foam stability, because they 498 have better dispersibility than those with high molecular weight (Martínez, et al., 499 500 2011).

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502 **4. Applications of vegetable proteins in the food industry**

503

504 *4.1. Use of vegetable proteins as fillers*

505 Vegetable proteins, used as substitutions for fat (Brewer, 2012; Guardeno,

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506	Hernando, Llorca, Hernandez-Carrion, & Quiles, 2012; Kumar, et al., 2011) or animal
507	proteins (Luo, Shen, Pan, & Bu, 2008), can make food healthier. For example, SPI
508	can be used to decrease the fat, lactose and calorie contents in food; however, adding
509	too much SPI may affect food flavor because of its beany flavor (Khiari, Pietrasik,
510	Gaudette, & Betti, 2014). Therefore, some other flavorful food ingredients (e.g., milk
511	powder and sugar) should be mixed with SPI to improve the sensory characteristics
512	(e.g., appearance, flavor and mouth feel) of final products (Sai Manohar, Urmila Devi,
513	Bhattacharya, & Venkateswara Rao, 2011).
514	In addition, vegetable proteins are commonly used as fillers or fat stabilizers to
515	improve the textures of meat products, such as surimi, pork meat gels and meat batters
516	(Luo, et al., 2008; Pietrasik, Jarmoluk, & Shand, 2007; Youssef & Barbut, 2011).
517	Meanwhile, in order to improve qualities of food products involving vegetable
518	proteins, it is becoming increasingly common to modify vegetable proteins by
519	different ways (e.g., by transglutaminase-catalyzed cross-linking, high pressure,
520	ultrasound, or microwave treatment) (Feng, et al., 2014; Guan, et al., 2011; He, et al.,
521	2014; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Pietrasik, et al., 2007).
522	However, the addition of vegetable proteins has a great influence on the texture and
523	sensory quality of food; inclusion of large amounts of vegetable proteins may destroy
524	the textures of meat products and introduce undesirable flavors (Luo, et al., 2008).

525

526 4.2. Use of vegetable proteins in extrusion

527 Extrusion cooking has been widely used in the food industry due to its high

nutrient retention rate. Food products prepared by extrusion showed porous structures 528 and high digestibility (Kręcisz, Wójtowicz, & Oniszczuk, 2015). However, extruded 529 530 food products always contain low levels of protein and fiber (Yu, Ramaswamy, & Boye, 2013). Vegetable proteins can be used to improve the protein content and thus 531 nutritive value of extruded food products (Kasprzak, et al., 2013; Konstance, et al., 532 1998; Yu, et al., 2013). Vegetable proteins also have a great influence on the flavor of 533 extruded foods. Variety of interactions between different ingredients in foods (e.g., the 534 Maillard reaction) during extrusion processing can lead to production of various food 535 536 flavors (Solina, Johnson, & Whitfield, 2007). The addition of vegetable proteins requires particular attention, because high level of vegetable proteins (>20% w/w) can 537 destroy the continuity, decrease the expansion ratio and increase the density of final 538 539 food products (Jin, Hsieh, & Huff, 1995; Zhu, et al., 2010).

540

541 4.3. Use of vegetable proteins in flour products

During bread making, sulfhydryl (SH) oxidation and SH/SS exchange reactions 542 occur between glutenins and gliadins to form a disulfide network (Deleu, Wilderjans, 543 Van Haesendonck, Brijs, & Delcour, 2016), but gluten in wheat flour can cause 544 allergic reactions and coeliac disease (Ziobro, Witczak, Juszczak, & Korus, 2013). 545 Thus, there has been an increasing interest in gluten-free breads, which incorporate 546 rice, corn, potato or cassava starch (Crockett, Ie, & Vodovotz, 2011; Ronda, Oliete, 547 Gómez, Caballero, & Pando, 2011). Gluten-free breads are usually characterized by 548 low nutritional value, so vegetable proteins (e.g., SPI, PPI and lupin isolate protein) 549

are often used to improve the nutritional as well as sensory properties of gluten-free
breads and traditional breads (Cadioli, Rodas, Garbelotti, Marciano, & Taipina, 2011;
Paraskevopoulou, Chrysanthou, & Koutidou, 2012; Villarino, et al., 2015; Ziobro, et
al., 2013).

In general, vegetable proteins can reduce the density, hardness, chewiness and 554 springiness of breads due to their high viscosity and water-holding capability (Ziobro, 555 et al., 2013). High level of vegetable proteins may increase the hardness of final 556 products (Crockett, et al., 2011; Ziobro, et al., 2013). The effect of vegetable proteins 557 on the volume of breads depends on the type of starch used in the formula (Ronda, et 558 al., 2011). Using modified vegetable proteins (e.g., by glycosylation or thermal 559 modification) is an effective method to reduce the adverse impact of vegetable 560 proteins (Campbell, Euston, & Ahmed, 2016). 561

Vegetable proteins can also be utilized to improve the quality of noodles or spaghetti. For example, soy globulins can cross-link semolina proteins during pasta making by disulphide linkages, and roasted soy flour is more effective in improving the quality of noodles or spaghetti than defatted soy flour, because the toasting process converts the free -SH groups into disulphide bonds (Lamacchia, et al., 2010). This reaction improves the tensile strength and elasticity of final products, but decreases the solubility of proteins (Gan, Ong, Wong, & Easa, 2009).

569

570 4.4. Vegetable-proteins-based encapsulation systems for bioactive ingredients

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Some food ingredients need to be encapsulated because of their instability,

unfavorable flavors, and the desire for their potential controlled release. Some gums and food proteins can be used as encapsulation materials. In recent years, there is an increasing interest in using vegetable proteins as encapsulation materials due to their renewability, biodegradability and health benefits (Tang & Li, 2013). Emulsions, spray-drying, films and cold-set hydrogels are the main technologies that involve the utilization of vegetable proteins as encapsulation materials.

Many lipophilic bioactive ingredients, e.g., omega-3 fatty acids, phytosterols and 578 carotenoids, can be encapsulated into vegetable proteins stabilized emulsions. For 579 example, SPI- and PPI-stabilized emulsions could effectively protect conjugated 580 linoleic acid from oxidation during storage and in vitro digestion (Fernandez-Avila, 581 Arranz, Guri, Trujillo, & Corredig, 2016). However, these conventional single 582 emulsions are not very stable under extreme conditions (e.g., after heating, ultrasonic, 583 high pressure, extreme pH or electrical force) (Cui, Chen, Kong, Zhang, & Hua, 2014; 584 Ji, et al., 2015). Thus, multilayer emulsions stabilized by vegetable proteins and other 585 polymers were developed. Xiang, Lyu, and Narsimhan (2016) found that, at pH 3.0, 586 positively-charged soy protein and negatively-charged pectin can form a double-layer 587 structure at oil-water interfaces by electrostatic attraction. An oil-in-water (O/W) 588 emulsion stabilized by a SPI-resveratrol complex showed better oxidative stability (of 589 encapsulated molecules or oil alone) than that stabilized only by SPI, due to the 590 antioxidant activity of resveratrol and the complexation of SPI with resveratrol (Wan, 591 Wang, Wang, Yuan, & Yang, 2014). 592



Spray-drying is another widely used encapsulation technology for a variety of

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food ingredients such as flavors, lipids and carotenoids. Many vegetable proteins such
as SPI (Chen, Li, & Tang, 2015), zein (Shukla & Cheryan, 2001), red bean isolate
proteins and mung bean isolate proteins (Fu Liu, Chen, & Tang, 2014) have been used
as encapsulation materials in spray-drying.

In order to develop multi-functional products and improve the functional 598 properties of vegetable proteins, some methods have been developed such as chemical 599 (e.g., glycosylation, acylation and cationization), enzymatic (e.g., hydrolysis and 600 cross-linking) or physico-chemical (e.g., preheating) modification. Emulsions 601 stabilized by these modified vegetable proteins showed reduced droplet size and 602 viscosity. Meanwhile, powders derived from these modified protein stabilized 603 emulsions also showed improved retention efficiency, dispersity and thermal stability 604 (Li, Wang, et al., 2015; Alla Nesterenko, Alric, Silvestre, & Durrieu, 2012; 605 Nesterenko, Alric, Silvestre, & Durrieu, 2014; Nesterenko, Alric, Violleau, Silvestre, 606 & Durrieu, 2014; Tang & Li, 2013; Zhang, et al., 2015). In addition, mixing several 607 different encapsulation materials together could also increase the encapsulation 608 efficiency. Mixing vegetable proteins with gelatin, gum arabic or stevioside has been 609 proved to produce stable dispersions and fine spray-dried powders from the stable 610 dispersions (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 611 2010; Porras-Saavedra, et al., 2015; Wan, Wang, Yang, Wang, & Wang, 2016). Wan et 612 al. (2016) found that SPI-stevioside complex could be rapidly absorbed onto the 613 surface of oil droplets, increase the nucleation rate and produce emulsions with small 614 droplet size. Furthermore, stevioside has a lower molecular weight than SPI, so it 615

could fill the gaps between SPI molecules in the interfacial layer and form a compact
interface layer, which could improve the stability of emulsion and thus the stability of
emulsion-encapsulated bioactive ingredients.

Compared to spray-drying, cold-set gel delivery systems are more suitable for 619 thermosensitive bioactive components (Lingyun Chen, Remondetto, & Subirade, 620 2006). This process consists of two distinct steps: first, preheating a protein solution 621 to obtain unfolded globular proteins with exposed reactive group, then adding 622 bioactive ingredients and cross-liners (e.g., calcium salts) (Maltais, Remondetto, 623 Gonzalez, & Subirade, 2005). Ca^{2+} can neutralize electrostatic repulsion and form salt 624 bridges between protein aggregates, allowing them to form a space-filling network. 625 Thus, this approach can achieve the encapsulation of nutrients at room temperature, 626 which is helpful in maintaining the chemical stability of encapsulated heat-sensitive 627 bioactive compounds (Hu, et al., 2015; Maltais, Remondetto, & Subirade, 2009, 628 2010). 629

630

631 5. Conclusions

Vegetable proteins can interact with other polymers in different ways, depending on their own molecular properties (e.g., molecular weight, particle size, or charge) and interaction conditions (e.g., initial concentration and ratio, pH, ionic strength or temperature). Accordingly, a variety of different structures (e.g., double networks, mosaic textures and cross-linked structures) can be formed to improve the mechanical, sensory, and functional properties of food products. Nowadays research about the

interaction of vegetable proteins with other biopolymers referred to very limited 638 source of vegetable proteins (e.g., leguminous proteins) and mainly focused on the 639 simple mixtures of two different types of vegetable proteins or mixtures of vegetable 640 protein with polysaccharides. Furthermore, along with the rapid growing of the 641 healthy and functional foods markets, there is an increasingly demand for the safe, 642 nutritional and health-beneficial food products. Therefore, new sources of vegetable 643 proteins and more complex food systems based on vegetable proteins for food 644 industry applications are highly worth to be further developed. 645

646

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Fig. 1. The classification of vegetable proteins commonly used in the food industry.

Fig. 2. Structure of a sodium caseinate and soy protein isolate (SC-SPI) co-stabilized emulsion droplet (pH 6.8, distilled water) loaded with vitamin A (VA) (Ji, et al., 2015).

Fig. 3. Influence of co-gelling proteins on the structures and storage modulus (G') of collagen matrices. Whey protein isolate and blood plasma proteins embedded in the surface of collagen, while whey protein isolate may interrupt the collagen interconnections and weaken the structure; blood plasma proteins could not increase G' value of collagen. Gluten led to phase separation in mixed systems. SPI formed a mixed interwoven structure with collagen (Oechsle, et al., 2015).

Fig. 4. Bulk and interfacial behaviors of soy protein isolate-stevioside (SPI-STE) mixtures with different stevioside concentrations at the oil/water interface. Stevioside at low concentration (0.1 wt%) can only bind to the gaps between protein molecules. Stevioside at intermediate concentration (0.25-1 wt%) can induce a partial dissociation of the protein's rigid structure with SPI. Stevioside at high concentration (2 wt%) can replace SPI-STE complexes due to their smaller particle size (Wan, et al., 2014).

Fig. 5. Possible mechanism of the formation of hierarchical microstructure in sugar beet pectin/soy glycinin (SBP/SG) double network gels (Hou, et al., 2015).

Fig. 6. Schematic of Maillard reaction induced formation of soy protein gels (Caillard, et al., 2010). **Fig. 7.** Reaction among SPI, modified cellulose nanocrystal (MCNC), and ethylene glycol diglycidyl ether (EGDE). (a) SPI; (b) EGDE; (c) MCNC; (d) crosslinking networks in SPI -based films (Zhang, et al., 2016).

Table 1

Summarize of interactions between vegetable proteins and other polymers.

Table 2

Selected examples of structures and properties of films formed by vegetable proteins and other

polymers.

Table 3

Selected examples of structures and properties of gels formed by vegetable proteins and other

polymers.

Table 1

Summarize of interactions between vegetable proteins and other polymers.

Group	Interactions	Main Influence Factors	References
Protein-Protein	Phase separation Synergistic interaction	Protein sources (the structure and molecular weight of proteins) determine the denaturation	(Bainy, Corredig, Poysa, Woodrow, & Tosh, 2010: Denavi, et al., 2009: Oechsle,
	Aggregation	temperature, dispersibility and functionality of proteins.	Häupler, Gibis, Kohlus, & Weiss, 2015)
		Protein concentration affects the dispersibility and texturization of proteins.	(Grabowska, Tekidou, Boom, & van der Goot, 2014)
		The pH value and ionic strength of reaction system	(Alu'datt, Alli, & Nagadi, 2012;
		affect the surface charge and solubility of proteins	Bengoechea, Romero, Aguilar, Cordobés, &
		and thus protein-protein interactions.	Guerrero, 2010; Pizones Ruiz-Henestrosa,
			Martinez, Carrera Sánchez, Rodríguez
			Patino, & Pilosof, 2014)
Protein-Polysaccharide	Miscibility	Properties of polysaccharides (e.g., charge density,	(Chang, Li, Wang, Bi, & Adhikari, 2014;
	Thermodynamic	molecular weight and branched chain) and proteins	Lam, Shen, Paulsen, & Corredig, 2007; Ma,
	incompatibility	(e.g., charge density) affect the continuity, dispersity	Dang, & Xu, 2016; Yuan, et al., 2014)
	Complex coacervation	Concentration or mixture ratio affects the	(Chang at al. 2014; Li Vah. & Ean. 2007;
		concentration of mixture fatio affects the	(Chang, et al., 2014; LI, Ten, & Fan, 2007; Were et al. 2014)
		interfaces and the dispersity of polymore in	wall, et al., 2014)
		solutions	
		The nH value of reaction system affects the surface	(Spada Marczak Tessaro & Cardozo
		charge of proteins and thus protein-polysaccharide	2015: Yuan et al. 2014)
		interactions.	2010, 1uiii, et ui., 2011)
		Salts can shield charged-sites of both protein and	(Li, et al., 2009: Pires Vilela, et al., 2011:
		polysaccharide molecules, and the means of adding	Yuan, et al., 2014)
		salts can affect the reaction rate.	
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 Table 2

 Selected examples of structures and properties of films formed by vegetable proteins and other polymers.

Compositions	Conditions	Observations	Contrast	Structure	References
Protein-Protein		0	7		
SPI-Corn zein(CZ)	Pouring the heated CZ solution onto a dried SPI film; casting.	Mechanical properties: Tensile strength (TS) increased but percentage elongation at break (EBA) decreased dramatically. Barrier properties: Lower water vapor permeability (WVP) but higher oxygen permeability (OP). Physical properties: Yellowness increased	SPI film	CZ layer laminated on SPI film	(Cho et al., 2010)
SPI/Zein + microwave	Different ratios of SPI to zein (3:1, 2:1, 1:1, 1:2, 1:3 and 0:1); pH 12.0; casting.	Mechanical properties: TS and breaking distance increased; microwave treatment could increase mechanical properties. Barrier and physical properties: None.	Zein film	Phase separation	(Wang et al., 2016)
SPI/Gelatin	Different ratios of SPI to gelatin (0:100, 25:75, 50:50, 75:25 and 100:0); pH 10.5; casting	Mechanical properties: Higher breaking forces at ration of 50S:50G and 25S:75G; similar thickness. Barrier properties: Lower WVP. Physical properties: Yellowish colour increased.	Gelatin film	Synergistic networks	(Denavi et al., 2009)
SPI/Gelatin + transglutaminase	MTGase was added to the gelatin solution with or without SPI; casting	Mechanical properties: Similar thickness; TS decreased, while EAB increased markedly in the absence of MTGase. Barrier properties: WVP increased slightly ($P < 0.05$). Physical properties: No significant changes ($P > 0.05$) in the colour.	Gelatin film	Phase separation	(Weng & Zheng, 2015)
SPI/Collagen or Gluten/Collagen	Collagen (2.75%) with SPI or gluten (1.25%); extrusion.	Mechanical properties: Thickness decreased slightly and TS increased. Barrier and physical properties: None.	Collagen film (2.75%)	Phase separation	(Oechsle et al., 2016)
Protein-Polysaccharide					
SPI/CMKGM	Mixing CMKGM and SPI solutions; pH 8.0; casting.	Mechanical properties: TS and EAB increased. Barrier properties: OP decreased; the water adsorption reduced and the surface wettability improved with the increase of CMKGM. Physical properties: The roughness decreased with the increase of CMKGM.	SPI or CMKGM film	Synergistic networks (Maillard reaction and hydrogen bonding)	(Wang et al., 2014)

SPI/Cellulose	5 g of fiber: 95 g of	Mechanical properties: TS and Young's modulus (YM) increased but	SPI film	Synergistic	(Jensen et al
Si i/ Centelose	SPI: nH 12:	FAB decreased	51111111	networks	2015)
	casting	Barrier properties: Lower OP	<u>_</u>	(chemical	2013)
	casting.	Darrier properties. Lower Or.		(chefinical	
		Physical properties: None.		reaction)	
SPI/Starch	SPI with 0, 2, 5,	Mechanical properties: TS and EAB increased but YM decreased.	SPI film	Phase	(González &
nanocrystals	10, 20 and 40% of	Barrier properties: MVP increased.		separation	Alvarez
	SNC; casting.	Physical properties: None.			Igarzabal, 2015)
PPI/Peanut starch	PS and PPI were	Mechanical properties: Thickness and TS decreased; EBA increased.	PS film	Phase	(Sun et al., 2013)
	mixed at different	Barrier properties: WVP and water-vapor transmission rate (WVTR)		separation	
	ratios (10:0, 8:2,	dropped markedly.		•	
	6:4, 5:5 and 0:10);	Physical properties: The opacity slightly elevated and colour			
	casting.	intensified.			
PPI/Gum Arabic	PPI: Gum Arabic	Mechanical properties: TS increased but EBA decreased.	PPI film	Synergistic	(Li, W. Zhu et
	1:1; pH 8.0;	Barrier properties: MVP decreased.		network	al., 2015)
	casting.	Physical properties: None.		(disulfide	
	6			bonds)	

perties: None.

 Table 3

 Selected examples of structures and properties of gels formed by vegetable proteins and other polymers.

Compositions	Conditions	Observations	Structure	References
Protein-Protein				
Pea protein/Myofibrillar protein isolate (MPI)	4% total protein level with or without MTG; 0.6 M NaCl; pH 6.0.	Storage modulus (G') decreased as pea protein level increased. MTG increased G' and peak force values.	Phase Separation	(Sun & Arntfield, 2012)
PPI/Chicken salt-soluble proteins (SSP)	Mixing SSP and PPI (0%, 2%, 2.5%, 3%, 3.5%); 0.6 M NaCl; pH 6.8.	Water-holding capacity (WHC) increased as PPI level increased. Breast and thigh SSP showed the highest strength and springiness on addition of 2.5% and 3.5% PPI, respectively. PPI also could increase G' value of gels.	Phase Separation	(Sun et al., 2012)
Protein-Polysaccharide				
SPC/Corn starch (CS)	CS and SPC mixed at ratios of 0, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.	G' value decreased and the continuous phase changed from SPC to CS with increasing CS level.	Phase Separation	(Li et al., 2007)
SPI/Galactomannans	Mixing SPI (6-10%) and galactomannans (0.2%-0.5%); pH 7.0.	Galactomannans with less branching could decrease the gelling temperature and increase G' value more significantly.	Phase Separation	(Monteiro et al., 2013)
SPI/Gellan Gum	Mixtures contained 8.0 wt.% SPI and 0.3 wt.% gellan gum; 200 mM KCl; 30 U/g SPI MTGase.	Fracture strain and stress of the mixed gels were higher than that of gellan gum gels but lower than that of SPI gels; trend for Young's modulus was the opposite. The mixed gels were firmer with increasing gellan gum level (0-0.4%).	Phase Separation	(Guo et al., 2014)
SPI/Xanthan gum or Guar gum	Mixing SPI (4%, 6% and 8%) with XG (0- 0.2%) or GG (0-0.3%).	The apparent viscosity, and G' and G'' values of the mixed gels increased with the increase in the gum (XG, GG) concentration.	Phase Separation	(Chang et al., 2014)
SPI/Ribose or Sucrose	Mixing MTGase-incubated or non- MTGase-incubated SPI (0.1 g/mL) with 2% ribose or 2% sucrose.	Mixed gels produced by pre-cross-linked SPI showed higher G' values than those produced by non-pre-cross-linked SPI. SPI-ribose gels showed lower G' values than SPI-sucrose gels.	Synergistic networks (Maillard reaction)	(Gan, Latiff, et al., 2009)
Sugar beet pectin (SBP)/Soy glycinin (SG)	Mixing SG with SBP with or without laccase (4 U/g SBP); 20 U/g SG MTGase; pH 7.0.	The double network gel formed by SG-SBP with laccase had higher G' value and mechanical toughness (fracture strain and stress) than the single network gel formed by SG-SBP without laccase.	Phase Separation	(Hou et al., 2015)



Fig. 1. The classification of vegetable proteins commonly used in the food industry.



Fig. 2. Structure of a sodium caseinate and soy protein isolate (SC-SPI) co-stabilized emulsion droplet (pH 6.8, distilled water) loaded with vitamin A (VA) (Ji et al., 2015).



Fig. 3. Influence of co-gelling proteins on the structures and storage modulus (G') of collagen matrices. Whey protein isolate and blood plasma proteins embedded in the surface of collagen, while whey protein isolate may interrupt the collagen interconnections and weaken the structure; blood plasma proteins could not increase G' value of collagen. Gluten led to phase separation in mixed systems. SPI formed a mixed interwoven structure with collagen (Oechsle et al., 2015).

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Fig. 4. Bulk and interfacial behaviors of soy protein isolate-stevioside (SPI-STE) mixtures with different stevioside concentrations at the oil/water interface. Stevioside at low concentration (0.1 wt%) can only bind to the gaps between protein molecules. Stevioside at intermediate concentration (0.25-1 wt%) can induce a partial dissociation of the protein's rigid structure with SPI. Stevioside at high concentration (2 wt%) can replace SPI-STE complexes due to their smaller particle size (Wan et al., 2014).

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Fig. 5. Possible mechanism of the formation of hierarchical microstructure in sugar beet pectin/soy glycinin (SBP/SG) double network gels (Hou et al., 2015).

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Fig. 6. Schematic of Maillard reaction induced formation of soy protein gels (Caillard et al., 2010).



Fig. 7. Reaction among SPI, modified cellulose nanocrystal (MCNC), and ethylene glycol diglycidyl ether (EGDE). (a) SPI; (b) EGDE; (c) MCNC; (d) crosslinking networks in SPI -based films (Zhang et al., 2016).

Highlights

• Many factors can affect the interaction of vegetable proteins with food macromolecules.

• The structure-function relationship of vegetable proteins based biopolymers or materials is discussed.

• Understanding structures of complex food systems containing vegetable proteins has an important implication for applications of vegetable proteins.