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

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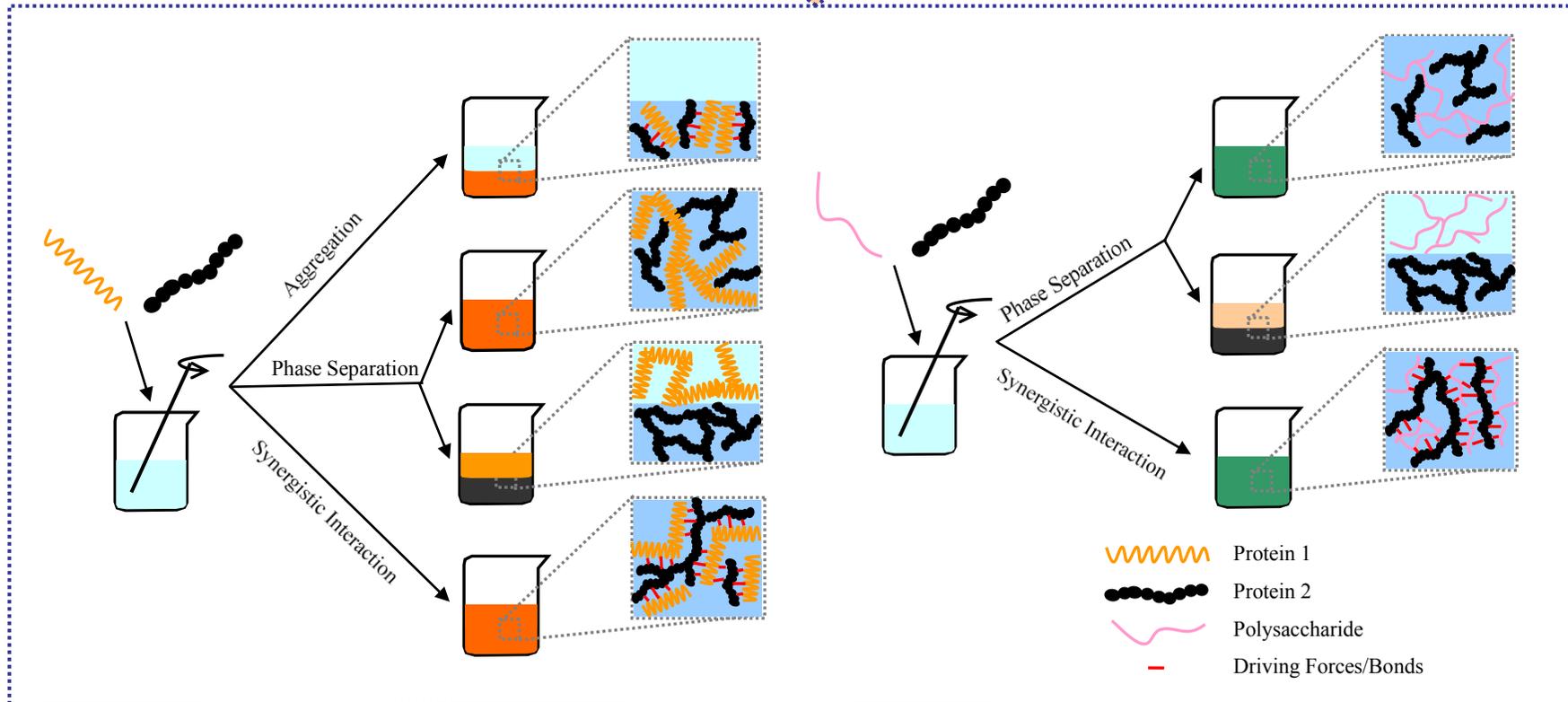
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Interactions of vegetable proteins with other polymers (proteins or polysaccharides) lead to different structures.



Different structures have different impact on food properties and influence applications of vegetable proteins.



23 **Abstract**

24

25 *Background:* In recent years, there has been increasing interest in vegetable proteins,  
26 due to their various health beneficial functions and wide applications in the food  
27 industry. Vegetable proteins combined with other edible polymers can be used to  
28 improve the quality and nutritional value of food products. In these complex food  
29 systems, interactions between different components are inevitable, and these  
30 interactions have a significant influence on the structure and functions of food  
31 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.

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

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  
73 proteins may lead to obesity (Bujnowski, et al., 2011), coronary heart disease (Clifton,  
74 2011), high blood pressure (Elliott, et al., 2006) and increased serum and urine uric  
75 acid (Tracy, et al., 2014). Many researches indicated that vegetable proteins had many  
76 health benefits, e.g., nutritional support to cirrhotic patients (Bianchi, et al., 1993),  
77 improving obesity-induced metabolic dysfunction (Wanezaki, et al., 2015),  
78 anti-cardiovascular disease (Lichtenstein, 1998) and anti-cancer activities (Lauerman,  
79 1998).

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

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

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

111 applications in the food industry.

112

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

114

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

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

133

134 *2.1. Protein-protein complexes*

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

141

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

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

155 needed.

156

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

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

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

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

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

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

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

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

213

## 214 *2.2. Protein-polysaccharide complexes*

215 Proteins and polysaccharides can form fine complexes in two ways: covalent  
216 bond and/or non-covalent bond (Ji, et al., 2015). The covalent bond mainly refers to  
217 the Maillard reaction, which is a non-enzymatic glycosylation reaction between free  
218 amino groups of proteins and aldehyde group of reducing sugars (Liu, Ru, & Ding,  
219 2012). This method usually involves thermal denaturing of a protein solution, and  
220 adding a polysaccharide solution as a Maillard-type cross-linking agent (Caillard,

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

226

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

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

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

245

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

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

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

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

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

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

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

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

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

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

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).

333

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

335

#### 336 *3.1. Film formation*

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

348 Solvent casting and extrusion are two technologies used to prepare polymer films  
349 (Echeverría, Eisenberg, & Mauri, 2014; Guerrero, Beatty, Kerry, & de la Caba, 2012).

350 Polymer films must have good barrier properties for gas and water (e.g., low water  
351 vapor permeability, WVP), mechanical properties (e.g., thickness, tensile strength,  
352 elastic modulus, deformability and elongation) and physical properties (e.g., colour

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

363

#### 364 *3.1.1. Film formation based on protein-protein interactions*

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

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

378

### 379 *3.1.2. Film formation based on protein-polysaccharide interactions*

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

396 structures between proteins and polysaccharides have been formed, which inhibits the  
397 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  
401 connected to each other and form a network structure under certain conditions. Gaps  
402 in the networks may be filled with liquid or gas as a dispersed phase. Proteins and  
403 polysaccharides are mainly responsible for gelation, and for this reason play important  
404 roles in the food industry (Ersch, et al., 2016). Properties of gels formed by vegetable  
405 proteins have been well studied (Berghout, et al., 2015; Dahesh, Banc, Duri, Morel, &  
406 Ramos, 2016; Kim, Varankovich, & Nickerson, 2016; Rui, et al., 2016; Shand, Ya,  
407 Pietrasik, & Wanasundara, 2007; Sun, et al., 2015); however, there are many good  
408 reasons to mix different polymers to form favorable gels. Firstly, combined use of  
409 different polymers (e.g., vegetable proteins and polysaccharides) could be an  
410 attractive way to develop new food products with balanced nutritional value (Bainy, et  
411 al., 2010; Chang, et al., 2014; Li, et al., 2007; Monteiro, et al., 2013; Sun & Arntfield,  
412 2012). Secondly, gels formed by mixed polymers usually have better mechanical  
413 properties than those formed by a single polymer due to the reactions between  
414 different polymers and the formation of compact structures (Gan, Latiff, Cheng, &  
415 Easa, 2009; Guo, et al., 2014; Hou, et al., 2015).

416

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

430

### 431 *3.2.2. Gelation based on protein-polysaccharide interactions*

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

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).

442

### 443 3.3. Emulsification

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

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

462 2015) have been widely studied. Emulsions stabilized by these conjugates showed  
463 good stability in extreme environments (e.g., heating, ultrasonic, high pressure,  
464 extreme pH or electrical force) (Fuguo Liu, Ma, McClements, & Gao, 2016).

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

482

483 *3.4. Foamability*

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

501

#### 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,

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

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

540

#### 541 *4.3. Use of vegetable proteins in flour products*

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

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

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

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

569

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

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

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

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

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

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

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

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

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

630

## 631 **5. Conclusions**

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

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

646

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**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**

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

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

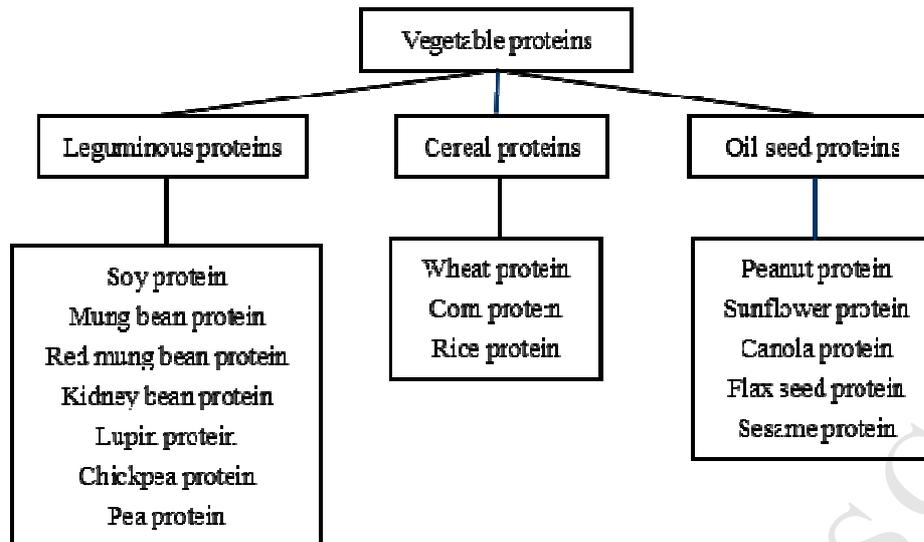
Compositions	Conditions	Observations	Contrast	Structure	References
<b>Protein-Protein</b>					
SPI-Corn zein(CZ)	Pouring the heated CZ solution onto a dried SPI film; casting.	Mechanical properties: Tensile strength (TS) increased but percentage elongation at break (EAB) decreased dramatically. Barrier 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)
<b>Protein-Polysaccharide</b>					
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 SPI; pH 12; casting.	Mechanical properties: TS and Young's modulus (YM) increased but EAB decreased. Barrier properties: Lower OP. Physical properties: None.	SPI film	Synergistic networks (chemical reaction)	(Jensen et al., 2015)
SPI/Starch nanocrystals	SPI with 0, 2, 5, 10, 20 and 40% of SNC; casting.	Mechanical properties: TS and EAB increased but YM decreased. Barrier properties: MVP increased. Physical properties: None.	SPI film	Phase separation	(González & Alvarez Igarzabal, 2015)
PPI/Peanut starch	PS and PPI were mixed at different ratios (10:0, 8:2, 6:4, 5:5 and 0:10); casting.	Mechanical properties: Thickness and TS decreased; EBA increased. Barrier properties: WVP and water-vapor transmission rate (WVTR) dropped markedly. Physical properties: The opacity slightly elevated and colour intensified.	PS film	Phase separation	(Sun et al., 2013)
PPI/Gum Arabic	PPI: Gum Arabic 1:1; pH 8.0; casting.	Mechanical properties: TS increased but EBA decreased. Barrier properties: MVP decreased. Physical properties: None.	PPI film	Synergistic network (disulfide bonds)	(Li, W. Zhu et al., 2015)

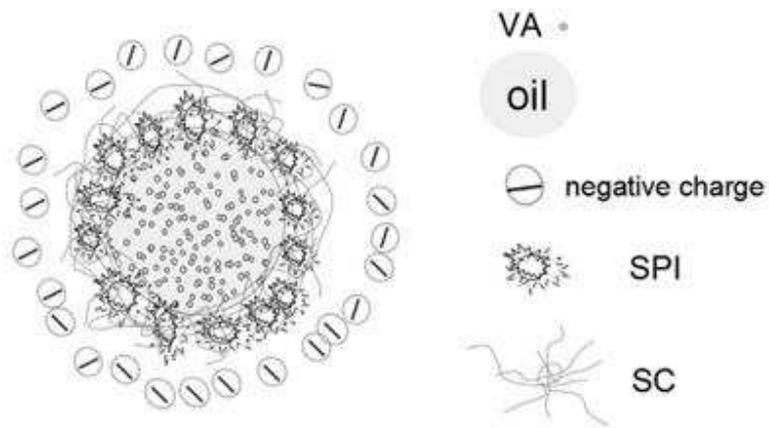
**Table 3**

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

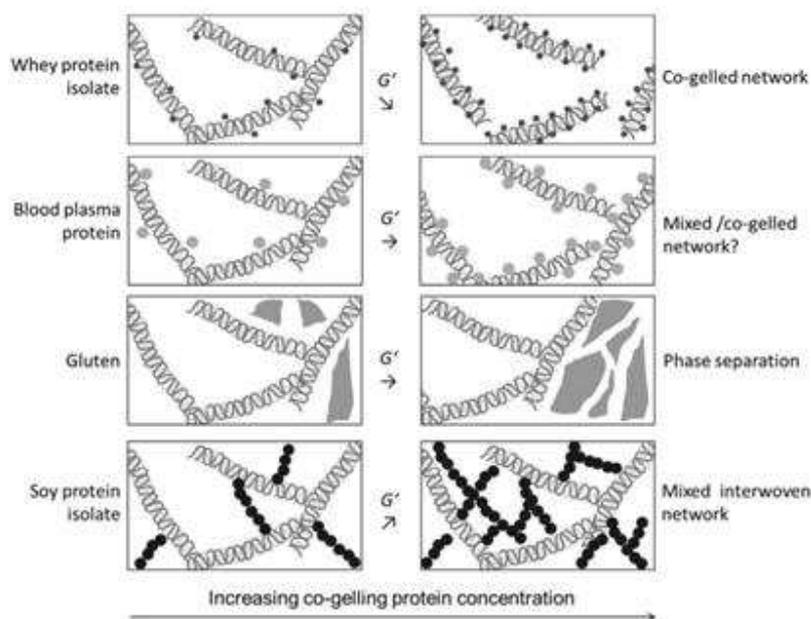
Compositions	Conditions	Observations	Structure	References
<b>Protein-Protein</b>				
Pea protein/Myofibrillar protein isolate (MPI)	4% total protein level with or without MTG; 0.6 M NaCl; pH 6.0.	Storage modulus ( $G'$ ) decreased as pea protein level increased. MTG increased $G'$ and peak force values.	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)
<b>Protein-Polysaccharide</b>				
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)



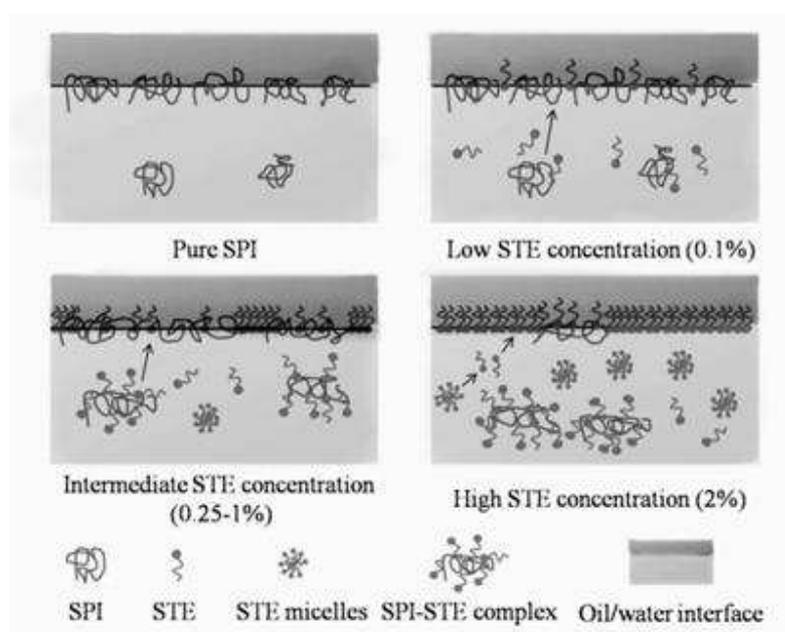
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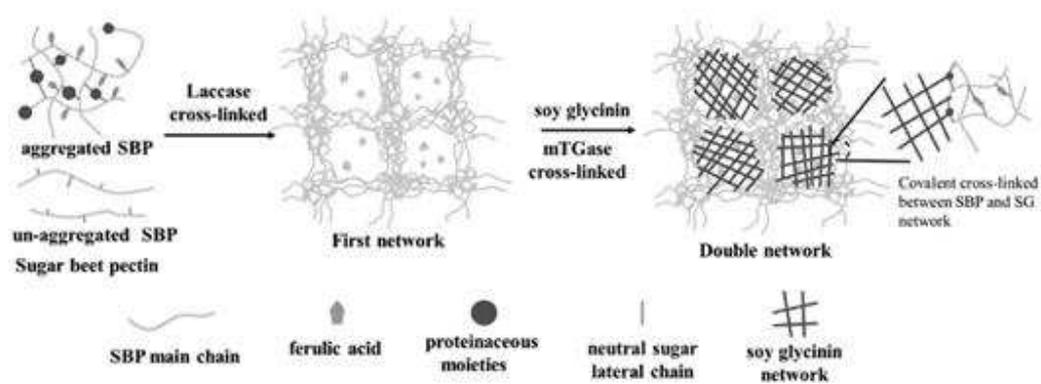
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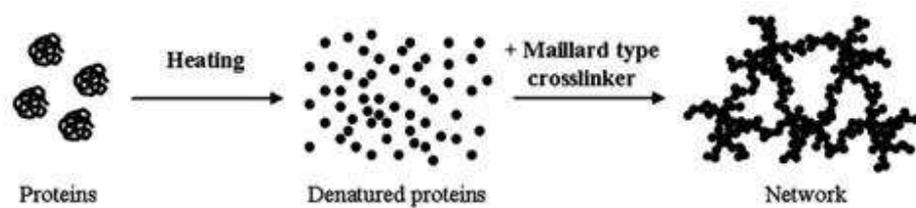
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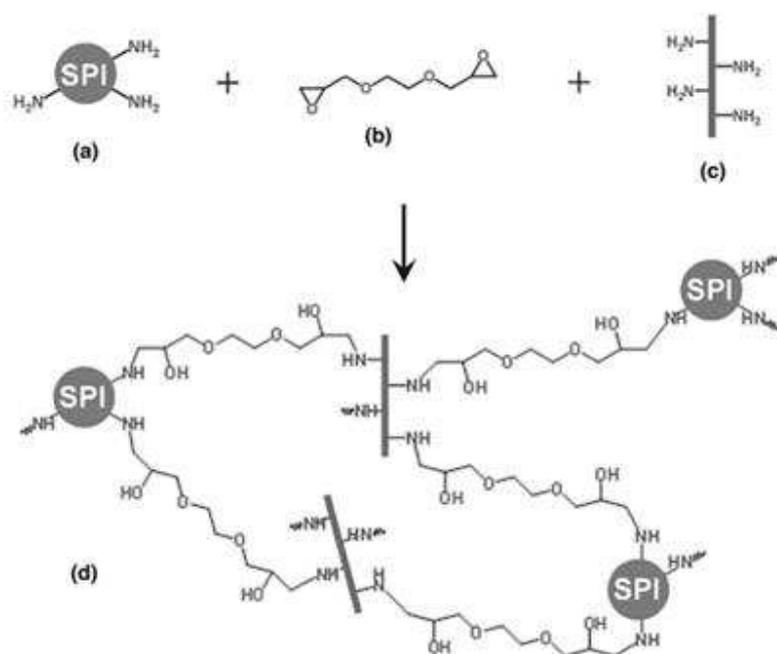
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**Highlights**

- 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.