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Review

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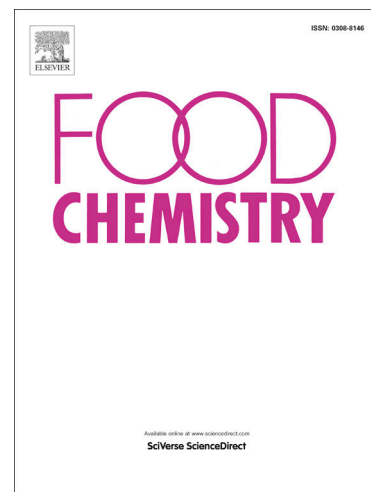
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1 **Faba bean protein: a promising plant-based emulsifier for improving**
2 **physical and oxidative stabilities of oil-in-water emulsions**

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17 **Abstract**

18 Faba bean is a protein-rich, sustainable, but understudied legume. Faba bean protein isolates
19 (FBPIs) can serve as promising emulsifiers. This review aims to summarize the research on
20 FBPIs as emulsifiers and various modification methods to improve the emulsifying
21 functionalities. The emulsifying activities of FBPIs depend on several physiochemical
22 characteristics (e.g. solubility, surface hydrophobicity, surface charge, interfacial activity).
23 Physical modifications, especially via linking FBPIs electrostatically to polysaccharides can
24 effectively increase the interfacial layer thickness/compactness and maintain the interfacial
25 protein adsorption. Chemical modifications of FBPIs (e.g. acetylation and Maillard reaction)
26 could improve the interfacial activity and affect the droplet-size distribution. Enzymatic
27 modifications, usually either via hydrolysis or cross-linking, help to optimize the molecular
28 size, solubility, and surface hydrophobicity of FBPIs. It is critical to consider the
29 lipid/protein oxidative stability and physical stability when optimizing the emulsifying
30 functionality of FBPIs. With suitable modifications, FBPI can serve as a promising
31 emulsifier in food production.

32 **Keywords:**

33 Faba bean protein; emulsion; physical stability; oxidative stability; protein modifications

34 **I Introduction**

35 The oil-in-water (O/W) emulsion commonly exists in food products. The formation of
36 emulsions is associated with increased positive free energy in the interface, which makes the
37 emulsions thermodynamically unstable (i.e. natural tendency to separate into oil and water
38 phases over time) (Sharma & Shah, 1985). In addition, emulsions have greater interfacial
39 area; this could increase the contacts between unsaturated lipids in the oil phase and
40 prooxidant compounds (e.g. metal ions) and oxygen dissolved in the aqueous phase, making
41 emulsions highly susceptible to oxidation (Berton-Carabin, Ropers, & Genot, 2014).
42 Instability of food emulsions causes undesirable sensory outcomes (e.g. appearance, texture,
43 mouthfeel, and flavor) and nutrition loss; consequently, various emulsifiers are routinely
44 added to food emulsions (D. J. McClements, Bai, & Chung, 2017).

45 With increasing awareness of food safety and growing demands for natural ingredients,
46 proteins are receiving more attention. Proteins can serve as natural emulsifiers, because the
47 amphiphilic properties of proteins allow them to self-aggregate, and to form continuous and
48 homogeneous layer around oil droplets (Dickinson, 1986). Considering the economic cost
49 and sustainability issues, more research interests are focused on plant proteins as substitutes
50 for animal proteins (Multari, Stewart, & Russell, 2015). The application of different plant
51 proteins as emulsifiers has been studied and reviewed. For example, Burger and Zhang (2019)
52 discussed the impacts of origin, processing, and environmental conditions (e.g. temperature,
53 pH, and ionic strength) on emulsifying properties of pea proteins. Based on the stability
54 behavior of model emulsions prepared by soy protein isolate and sodium caseinate,
55 Dickinson (2019) summarized the critical factors to control and inhibit the flocculation of
56 O/W emulsions; these factors included protein/oil ratio, emulsification conditions, protein
57 solubility, protein denaturation, pH conditions, ionic strength, and calcium ion content; the
58 author further emphasized covalent protein-polysaccharide conjugate as a consistently

59 successful strategy. Kim et al. (2020) compared the emulsifying properties of several plant
60 proteins (soy, peas, cereal, and oilseed) with dairy proteins and highlighted that emulsifying
61 properties were highly dependent on protein sources, structure, molecular weight, and
62 adsorption behavior. Compared to the plant proteins mentioned above, faba bean protein is
63 considered economical but underutilized.

64 Faba bean contains high level of proteins (ranging from 27% to 34% of dry weight) (Samaei
65 et al., 2020). It not only has excellent adaptability in a wide range of climates and
66 environmental conditions, but also is considered as a sustainable crop in that it offers
67 ecosystem services via biological nitrogen fixation and diversifications of cropping systems
68 (Lizarazo, Lampi, Liu, Sontag-Strohm, Piironen, & Stoddard, 2015). Like many plant
69 proteins mentioned above, faba bean proteins can serve as emulsifiers. However, the
70 unmodified plant proteins usually exhibit unfavorable emulsifying activities, which could be
71 attributed to the suboptimal intrinsic physiochemical properties (e.g. surface charge,
72 hydrophobicity, and molecular weight) that are decisive factors for emulsifying properties
73 (Schwenke, 2001). Various efforts have been devoted to improving the emulsifying
74 properties by physical, chemical, and enzymatic modifications. The majority of these efforts
75 are focused on modifying the protein configuration, changing the surface charge or
76 hydrophobicity, increasing the interfacial layer thickness/compactness, as well as obtaining
77 desirable molecular weight and solubility (Chen, Chen, Ren, & Zhao, 2011; Luisa, Gaspar,
78 & de Goes-Favoni, 2015). Different modifications on FBPIs for improving the emulsifying
79 property and the stability of corresponding emulsions have been reported, making FBPI a
80 promising plant-based emulsifier in O/W emulsions. The focus and objective of this review
81 is to provide an update on applications of unmodified and modified FBPIs as emulsifiers in
82 O/W emulsions, and to give insights into the possibilities to enhance faba beans utilization.

83 **2 O/W emulsions and emulsion stability**

84 Emulsions are colloidal systems that consist of two immiscible liquids, where one of the
85 liquids is dispersed as spherical droplets in the other liquid (Lam & Nickerson, 2013). In
86 most emulsion-type foods, these two liquids usually refer to oil droplets dispersed in a water
87 phase, hence classified as O/W emulsions (Bakry et al., 2016).

88 **2.1 Physical stability**

89 Because the contact between oil and water is thermodynamically unstable, O/W emulsions
90 suffer from various physical instabilities evidenced by changes in the spatial distribution or
91 structural organization (Elwell, Roberts, & Coupland, 2004). Creaming, flocculation, and
92 coalescence are the major examples of physical instabilities (**Figure 1**). Creaming in O/W
93 emulsions occurs when oil droplets move upward and accumulate at the top of the aqueous
94 phase due to density difference; over time, a creamed layer of oil droplets is floating on the
95 top, leaving an aqueous phase at the bottom (Robins, 2000). Flocculation is generally defined
96 as the aggregation of droplets when two or more oil droplets come in close proximity but
97 still maintain individual integrity (David Julian McClements, 2015). Coalescence happens
98 when two or more oil droplets nearby merge into a single larger oil droplet with the
99 partitioning layer disrupted; as a result of the merging, the average droplet size increases
100 over time while the interfacial area is reduced (Tcholakova, Denkov, & Lips, 2008).

101 **2.2 Oxidative stability of protein-emulsified O/W (P-O/W) emulsions**

102 P-O/W emulsions are not only physically unstable, but also are prone to various adverse
103 oxidative deterioration due to the lipid and protein contents. Lipid oxidation can generate off
104 flavor, undesirable color, and potentially toxic by-products such as malondialdehyde and 4-
105 hydroxynonenal, while protein oxidation might induce loss in sensory quality and nutrition
106 (Esterbauer, 1993; Lund, Heinonen, Baron, & Estevez, 2011). Moreover, the oxidative

107 stability has a strong interplay with physical stability. Some lipid oxidation products such as
108 lipid hydroperoxides are surface active and can interact with protein interfacial layer,
109 consequently inducing coalescence of oil droplets and protein oxidation; surface active
110 antioxidants such as *p*-hydroxyphenylacetic acid and its conjugates with a butyl or dodecyl
111 group were able to effectively adsorb at the interface and scavenge free radicals (Yuji, Weiss,
112 Villeneuve, Lopez Giraldo, Figueroa-Espinoza, & Decker, 2007). Thus, it is critical to
113 improve both oxidative stability and physical stability.

114 Lipid oxidation is usually elucidated as pathways of autoxidation, enzymatic oxidation, and
115 photo-oxidation (Bartosz, 2013; Dey & Neogi, 2019; Frankel, 2014). In food products,
116 oxygen scavengers are commonly used to remove or decrease the level of oxygen in package;
117 the light that can accelerate lipid oxidation is usually blocked by glass or plastic containers
118 while lipoxygenases and other enzymes that catalyze the oxidation of free polyunsaturated
119 fatty acids are mostly deactivated during thermal processing (Bartosz, 2013; Dey et al., 2019;
120 Frankel, 2014). Autoxidation via free radical chain reactions are particularly important; the
121 chain reactions generally consists of three well-established steps: initiation, propagation, and
122 termination (**Figure 1**) (Bartosz, 2013; Frankel, 2014). In emulsions, hydroperoxides can act
123 as surface-active compounds, in that they often accumulate at the interfacial layer around oil
124 droplets. The decomposition of lipid hydroperoxides at the oil droplet surface into highly
125 reactive radicals is proposed to be the most likely mechanism for initiating and accelerating
126 lipid oxidation in O/W emulsions (Decker & McClements, 2001).

127 In P-O/W emulsions, the physical instability and lipid oxidation can be perceived readily
128 due to visible structural alterations, rancidity, or color changes; on the other hand, protein
129 oxidation is usually underestimated. In fact, protein oxidation not only leads to depletion of
130 essential amino acids, but also causes undesirable changes in protein functionalities such as
131 emulsifying properties (Lund et al., 2011). More importantly, protein oxidation *in vivo*
132 contributes to a variety of diseases such as Alzheimer's disease, Parkinson's syndrome,

133 rheumatoid arthritis, muscular dystrophy, cataractogenesis, inflammatory bowel's disease
134 (IBD), and cataractogenesis (Estevez & Luna, 2017). Similar to lipid autoxidation, protein
135 oxidation is usually initiated by reactive oxygen species that originate from irradiation,
136 oxygen, metal-catalyzed systems (e.g. Fe(II), Mn (II)), peroxides, as well as non-protein
137 radicals and free radicals including hydroxyl radicals ($\text{HO}\cdot$), peroxy radicals ($\text{HO}_2\cdot$), and
138 superoxide anion radicals ($\text{O}_2\cdot^-$); these radicals then effectively initiate formations of protein
139 radicals; oxidative modifications of protein not only lead to changes in amino acid side chain
140 such as thioloxylation, aromatic hydroxylation, and formation of carbonyl groups, but also
141 produce peptide backbone cleavage and cross-linking (Stadtman & Levine, 2003).

142 **2.3 Interplay between lipid oxidation and protein oxidation in P-O/W** 143 **emulsions**

144 In P-O/W emulsions, lipid oxidation and protein oxidation are closely interrelated. Proteins
145 can either be freely distributed in the aqueous phase as unadsorbed, or adsorbed at the
146 interfacial layer. The unadsorbed proteins can affect lipid oxidation by several mechanisms.
147 Firstly, aqueous proteins can chelate or bind metals by forming metal complexes, altering
148 the physical or redox state of metal ions, or changing the physical location of metal ions
149 (Berton-Carabin et al., 2014). Secondly, proteins in the aqueous phase can reduce
150 hydroperoxides; for example, sulfur-containing amino acids can reduce hydroperoxides into
151 the inactive hydroxylic derivatives (Pokorny, Yanishlieva, & Gordon, 2001). Thirdly,
152 unadsorbed proteins can trap or quench free radicals (Kong & Xiong, 2006; Wang & Xiong,
153 2005). Tryptophan and cysteine (rich in electrons and therefore nucleophilic) are
154 preferentially oxidized prior to lipid oxidation in an O/W emulsion system, so that the radical
155 transfer reactions for lipid oxidation could be partially inhibited (Elias, McClements, &
156 Decker, 2005).

157 Apart from the aqueous phase in a P-O/W emulsion, proteins are the major components for
158 constructing the interfacial layer where lipids and prooxidants such oxygen, transition metals,
159 and free radicals contact. Thus, the interfacial properties of proteins could strongly influence
160 lipid oxidations (Berton-Carabin et al., 2014). Firstly, interfacial proteins determine the
161 surface charges of oil droplets. As transition metals are positively charged, a positively
162 charged protein-interfacial layer of a P-O/W emulsion could repel the prooxidant metals,
163 thus slowing down the lipid oxidation (Hu, McClements, & Decker, 2003). Secondly,
164 increased thickness and packing density of the protein-interfacial layer also decrease lipid
165 oxidation by preventing free radicals, oxygen, and other prooxidants in the aqueous phase
166 from reaching the fatty acids in the oil droplets (Gürbüz, Kauntola, Diaz, Jouppila, &
167 Heinonen, 2018). Lastly, proteins at the interface may protect lipid from oxidation by acting
168 as antioxidants as they do in the aqueous phase (Berton, Ropers, Guibert, Sole, & Genot,
169 2012). It needs to be pointed out that neither of the above mentioned factors is the single
170 determining factor, as lipid oxidation is the collective result of various internal and external
171 factors.

172 Lipid oxidation also plays an important role in protein oxidation. Under oxidative conditions,
173 the onset of lipid oxidation normally takes place faster than the oxidation of protein (Lund,
174 Hviid, & Skibsted, 2007). It is likely that protein oxidation is initiated and promoted by lipid
175 oxidation products (Lund et al., 2011). Various protein oxidative products are formed and
176 accumulated after reactions of amino acids/peptides/proteins with lipid-derived oxidative
177 products such as hydroperoxides and aldehydes. Pre-oxidized reactive lipid species serve as
178 sources of radicals and create protein radicals; for example, protein oxidation is initiated via
179 abstracting hydrogen atoms from protein molecules by peroxy radicals formed during lipid
180 oxidation (W. Wu, Zhang, Kong, & Hua, 2009).

181 **2.4 Emulsifiers**

182 In food industry, emulsifiers are widely used to facilitate the homogenization process and
183 maintain emulsion stability (Padial-Dominguez, Espejo-Carpio, Garcia-Moreno, Jacobsen,
184 & Guadix, 2020). Emulsifiers can reduce the interfacial tension between oil and water phase,
185 and consequently decrease the energy needed during homogenization; in addition,
186 emulsifiers can readily adsorb to the surface of freshly formed oil droplets due to the
187 amphiphilic nature, and consequently form a protective layer that prevents oil droplets from
188 aggregation (Kim et al., 2020). Considering the role of protein in lipid oxidation in emulsion,
189 as well as the consumer needs for more sustainable and environmentally friendly food
190 products, increasing research are directed to investigating plant proteins as emulsifiers
191 (Burger et al., 2019). Recent years, the potentials of faba bean proteins as emulsifiers have
192 been explored and reported.

193 **3 Faba bean proteins**

194 **3.1 Protein composition**

195 Faba bean storage proteins mainly consist of legumin-like globulins (11S) and vicilin-like
196 globulins (7S), as well as less amount of albumins (2S), glutelins, and polyamines (El Fiel,
197 El Tinay, & Elsheikh, 2002). Legumin-like globulins (11S) are hexameric proteins
198 containing six intermediary subunits with a molecular weight (MW) of 300-400 kDa (Bailey
199 & Boulter, 1970). Each intermediary subunit comprises of an acidic α subunit (~ 36 kDa)
200 and a basic β subunit (~ 22 kDa) (Wright & Boulter, 1974). The α subunits are hydrophilic
201 and situated at the surface, whereas β subunits contain more hydrophobic amino acids (e.g.
202 leucine, valine, and phenylalanine) hidden in the core. The α and β subunits are mainly
203 connected by a single disulfide bond and closely packed via electrostatic and hydrophobic
204 interactions to form a stabilized rigid structure (Wright et al., 1974). Vicilin-like globulin

205 (7S) is a trimeric protein containing three subunits, with an average MW of 150 ± 2.5 kDa;
206 vicilin fraction accounts for ~ 20% of the total globulins (Wright et al., 1974).

207 The thermal denaturation midpoint temperatures of 11S and 7S globulins in faba bean are
208 higher than those in soybean (95.4 °C vs. 93.5 °C for 11S, and 83.8 °C vs. 78.5 °C for 7S) at
209 ionic strength of 0.5; this indicates faba bean protein has comparable or better thermal
210 stability than soybean protein and is suitable for the production of foods requiring high
211 thermal stabilities (Kimura, Fukuda, Zhang, Motoyama, Maruyama, & Utsumi, 2008).
212 Despite the rich protein content in faba bean, its use is relatively limited, not only because
213 of the poor understanding of its functional properties, but also due to the presence of anti-
214 nutritional and negative factors (Rahate, Madhumita, & Prabhakar, 2021).

215 **3.2 Emulsifying property of FBPIs**

216 Our recent research interests have been devoted to exploring the use of FBPIs in food
217 production, with a focus on its emulsifying functionalities (Liu, Bhattarai, Mikkonen, &
218 Heinonen, 2019; Liu, Damodaran, & Heinonen, 2019; Liu, Pei, Peltonen, & Heinonen, 2020);
219 meanwhile, the potentials of FBPIs as emulsifiers have been studied and reported by other
220 groups (**Table 1**). The emulsifying property of FBPIs mainly depends on several
221 physiochemical characteristics.

222 **3.2.1 Solubility**

223 Solubility is essential for the emulsification by facilitating the migration of proteins to and
224 spreading at the oil-water interface (W. U. Wu, Hettiarachchy, & Qi, 1998). With an
225 isoelectric point (pI) of ~ 4.5, the solubility of FBPIs markedly decreases at near pI (~ 4 to
226 6) and mostly solubilizes at pH of 8-9 (Otegui, Fernandez-Quintela, De Diego, Cid,
227 Macarulla, & Partearroyo, 1997). pH is a decisive factor for solubility, as it affects
228 hydrophobicity and surface charge of proteins which in turn influence the equilibrium
229 between the protein-solvent (hydrophilic) and the protein-protein (hydrophobic) interaction,

230 and electrostatic repulsion, respectively (Karaca, Low, & Nickerson, 2015). For example,
231 heating at pH of 11.0 significantly increased the solubility of FBPIs (from 43% at pH 3.0,
232 14% at pH 6.0, and 35% at 7.0 to more than 90%); correspondingly, the FBPIs with greater
233 solubility produced more stable emulsions with smaller particle size (Farhad Alavi, Chen,
234 Wang, & Emam-Djomeh, 2021). Similarly, Felix et al. (2019) reported that FBPIs were 19%
235 and 71% more soluble at pH 3.0 and 8.0 than at pH 5.0, respectively; meanwhile, emulsions
236 prepared at pH 3.0 and 8.0 demonstrated smaller droplet sizes and better rheological
237 properties than that at pH 5.0. MWs influence protein solubility as well. FBPI hydrolysates
238 with smaller MWs exhibited higher solubility because smaller peptides produced by
239 hydrolysis can form stronger hydrogen bonds with water and become more soluble.
240 Hydrolysis of FBPIs by alcalase improved the solubility by 6-10% at pH 8; accordingly,
241 moderately hydrolyzed FBPIs (degree of hydrolysis at 4%) showed increased surface charge
242 and surface hydrophobicity which favored the emulsifying activity (Liu, Bhattarai, et al.,
243 2019). Similarly, Xu et al. (2016) reported that hydrolysis of rice glutelin by trypsin changed
244 the MW, increased flexibility, altered surface hydrophobicity, and greatly increased the
245 solubility (from < 10% to > 60%), which in turn increased the stability of emulsions.

246 **3.2.2 Surface hydrophobicity and surface charge**

247 Surface hydrophobicity and surface charge of proteins remarkably influence the
248 emulsification (Schwenke, 2001). The amphiphilic nature of proteins allows them to remain
249 in the aqueous phase while simultaneously adsorb at the surface of oil droplets and generate
250 stabilizing electrostatic forces and steric hindrance (Claesson, Blomberg, & Poptoshev,
251 2001). The hydrophobic patches of proteins, which relate to the hydrophobic amino acid
252 residues exposed at the surface, are the precondition for protein adsorption at the oil-water
253 interface (Jiang, Cheng, & Sun, 2020). Johnston et al. (2015) reported the hydrophobicity in
254 decreasing order with: chickpea (~137 arbitrary units, AU) > lentil = soya (~70 AU) > FBPI
255 (~24 AU), which might partly explain the relatively poorer emulsifying capacity of

256 unmodified FBPI. Compared to the unmodified FBPI, moderate hydrolysis of FBPIs by
257 alcalase increased the surface hydrophobicity by 18%, which could be attributed to exposing
258 more buried hydrophobic groups; correspondingly, the emulsions prepared by hydrolyzed
259 FBPI demonstrated greater physical and oxidative stability (Liu, Bhattarai, et al., 2019). Heat
260 treatment increased the surface hydrophobicity from 181 to 504 relative fluorescence units,
261 which could partly explain the increase in emulsion activity index (EAI) from 25.1 to 27.4
262 m^2/g (Nivala, Nordlund, Kruus, & Ercili-Cura, 2020). However, it needs to be stressed that
263 the balance between hydrophobicity and hydrophilicity is the key factor that determines
264 accessible surface area, unfolding, and re-orientation at the interface (T. Xu et al., 2020).

265 Surface net charge is another critical factor contributing to the surface characteristic. A high
266 protein surface charge not only promotes greater hydration of proteins, but also induces high
267 repulsive interfacial charges (Schwenke, 2001). In FBPIs, the net negative charge, for
268 example at pH 7.0, is mainly due to the negatively charged side chains of aspartic acid
269 ($\text{pK}_a = 3.65$) and glutamic acid ($\text{pK}_a = 4.25$) residues spatially located on the protein surface
270 (Lide, 2004). Thus, the net charge of FBPIs in emulsions is primarily dependent on pH and
271 other components at the interface. For example, the surface charges were changed drastically
272 when adjusting the pH of FBPI solutions, with $\sim 30, 10, -10, -20,$ and -30 mV at pH of 3.0,
273 4.0, 5.0, 6.0, and 7.0, respectively; the emulsions prepared under different pH displayed
274 diverse physical stability as determined by changes in particle size, which was proposed to
275 be partly explained by the surface charge effects (Farhad Alavi et al., 2021).

276 3.2.3 Interfacial tension

277 Last but not least, interfacial tension is another important factor for emulsifications. In the
278 preparation of P-O/W emulsions, proteins migrate to the oil-water interfaces where they re-
279 align themselves to position the surface hydrophobic amino acids toward the oil phase and
280 hydrophilic moieties within the aqueous phase. These migrated proteins form an interfacial

281 film surrounding oil droplets that maintains stability via electrostatic repulsive forces, steric
282 stabilization, and decreased interfacial tension (Schwenke, 2001; Singhal, Stone,
283 Vandenberg, Tyler, & Nickerson, 2016). It has been demonstrated that FBPIs, as well as
284 protein isolates from chickpeas, lentils, and soybeans, can efficiently decrease the interfacial
285 tension by ~50% in canola oil-water mixtures (Johnston et al., 2015).

286 **4 Modifications to enhance the FBPIs' emulsifying properties**

287 Most plant proteins, including FBPIs, do not exhibit desired emulsifying properties for food
288 industries, due to suboptimal physiochemical attributes (Gumus, Decker, & McClements,
289 2017a, 2017b, 2017c; Karaca, Low, & Nickerson, 2011). Based on the key physiochemical
290 characteristics for emulsification discussed above, various modification methods are
291 reported to improve the emulsifying activity of FBPIs, as well as to increase the stability of
292 FBPI-stabilized emulsions (**Figure 2**). Generally, the modifications of proteins are
293 categorized into physical, chemical, and enzymatic methods (**Table 1**).

294 **4.1 Physical modifications**

295 Physical association with polysaccharides via electrostatic interaction can effectively
296 improve the emulsifying activity of proteins. In general, a protein-polysaccharide complex
297 could be formed first via electrostatic interaction and then introduced as emulsifiers (i.e.
298 complex model); or the polysaccharides could be added to existing protein-emulsified O/W
299 emulsions to form an extra outer layer around the droplets (i.e. layer-by-layer/LBL model)
300 (Berton-Carabin et al., 2014). The two different preparation methods (complex vs. LBL
301 model) are expected to have a major impact on the interfacial properties, especially the
302 thickness and compactness (Berton-Carabin et al., 2014). We recently investigated how
303 different combinations of FBPIs and chitosan (complex vs. LBL model) affected O/W
304 emulsion stability (**Table 1**) (Liu et al., 2020). Compared to the complex model, the

305 interfacial structure in the LBL model appeared to be more compact and denser; one likely
306 explanation was that the FBPI formed a denser inner layer around the oil droplet which
307 facilitated a more uniformed secondary layer by chitosan (Liu et al., 2020). As a result, the
308 LBL model produced both better physical stability and greater oxidative stability (Liu et al.,
309 2020).

310 The combination of other polysaccharides and plant-based proteins, as well as different
311 preparation methods in the O/W emulsion systems have been reported. For example, adding
312 xanthan gum to wheat protein-emulsified emulsions improved emulsion stability to high
313 ionic strengths (Qiu, Zhao, & McClements, 2015). Emulsions emulsified with soluble
314 glycinin-chitosan complex displayed improved stability at certain ratio due to synergistic
315 effect of the two molecules (Yuan, Wan, Yang, & Yin, 2014). Extra pectin coating enhanced
316 spray-dry stability of pea protein-stabilized O/W emulsions, which was proposed to be
317 attributed to increased steric effects (Gharsallaoui, Saurel, Chambin, Cases, Voilley, &
318 Cayot, 2010). Conversely, some studies showed that additions of cationic polyelectrolytes
319 to protein-emulsified emulsions promoted droplet aggregation, creaming instability, and
320 viscosity enhancement (Nylander, Arnebrant, Cárdenas, Bos, & Wilde, 2019). The
321 inconsistent conclusions might be mainly attributed to the different experiment conditions
322 such as the type and the ratio of polysaccharides and proteins, environmental pH and ionic
323 strength, and preparing methods, etc. The potential application of combing FBPIs and these
324 polysaccharides and different preparation procedures need further investigation.

325 Another means of physical modification is by modulating the pH. Felix, et al. (2019)
326 investigated the effects of pH on the interfacial viscoelastic properties of FBPI layer in O/W
327 emulsions; FBPI dispersions were subjected to pH of 3.0, 5.0, and 8.0 and the interfacial
328 properties were determined by dilatational measurement and interfacial small amplitude
329 oscillatory shear measurements. They found that the strength of interfacial FBPI film at O/W
330 interface depended on the pH values; the measurement of adsorption kinetics for penetration

331 and unfolding of the protein at the interface showed that protein adsorption took place
332 significantly faster at pH 3.0 and 5.0 than at pH 8.0 (Felix, Romero, et al., 2019). This group
333 further examined the microstructural characteristics of emulsion stabilized by FBPIs at
334 different pH, as well as the *in vitro* antioxidant activities; the emulsions were prepared with
335 250 mL of 30 mg/mL FBPIs adjusted to different pH values (3.0, 5.0, and 8.0) and 250 mL
336 of sunflower oil; smaller droplet sizes and better rheological properties were obtained at pH
337 3.0 and 8.0; in addition, the emulsions at pH 3.0 displayed the lowest extent of lipid oxidation
338 (Felix, Cermeño, et al., 2019). Both studies demonstrated that interfacial properties are
339 important targets of modification for improving the emulsifying functionalities of FBPIs.

340 Treatments by heating and high-pressure are other conventional modification methods of
341 proteins (Tcholakova, Denkov, Ivanov, & Campbell, 2006). Our group showed that heating
342 faba beans with an air oven at 170°C for 30 min prior to emulsification could modified the
343 surface hydrophobicity of proteins via exposing or burying more hydrophobic amino acid
344 groups (Gürbüz, Liu, et al., 2018). High pressure homogenization could dramatically
345 improve FBPI solubility by dissociating large insoluble protein aggregates; high pressure
346 homogenization also increased surface hydrophobicity by promoting certain level of protein
347 unfolding (Yang, Liu, Zeng, & Chen, 2018). However, the improved solubility and surface
348 hydrophobicity did not translate into better emulsifying property. The authors proposed that
349 the negative effects of high pressure homogenization on emulsifying capacity could be
350 attributed to the reduced viscoelasticity of the interfacial film and the flocculation effect
351 caused by the soluble supramolecular aggregates; the unadsorbed supramolecular aggregates
352 might also be responsible for the formation of flocs and destabilization of the emulsion
353 (Yang et al., 2018).

354 **4.2 Chemical modifications**

355 Many studies have reported that chemical modifications could improve the functionality of
356 proteins (e.g. solubility, foaming, and emulsifying) and the rheological behavior and stability
357 of P-O/W emulsions (Oliver, Melton, & Stanley, 2006).

358 In the early 1990s, chemical modification focused on acetylation of FBPIs. Schmandke et
359 al. (1990) compared the emulsifying properties of unmodified and acetylated FBPI (degree
360 ~97%); they found that acetylation improved the flow, gelifying, surface and emulsifying
361 properties, as well as the physical stability of emulsions. This group later examined the
362 association of low methoxylated pectin and acetylated FBPI as emulsifiers in O/W emulsions.
363 They found that the combination could increase the protein content in the protein film around
364 the oil droplets; as a result, the apparent viscosity and flow behavior index of the emulsions
365 were increased and creaming was inhibited accordingly (Schultz, Schmidt, Krause, Seifert,
366 & Schmandke, 1991). Another group compared the emulsifying activities of unmodified and
367 acetylated FBPIs (~96%) with a focus on the ultrastructure of formed emulsions. They
368 reported that acetylation could improve the emulsifying activity, which was associated with
369 tangible difference in different droplet-size distribution and ultrastructure (Krause &
370 Buchheim, 1994). This group also proposed that the increased emulsifying activity by
371 acetylation was due to increased interfacial activity indicated by changes in Gibbs'
372 adsorption isotherms and the inflection point of the adsorption isotherms (Krause &
373 Schwenke, 1996).

374 Maillard reaction is another common chemical modification for improving emulsifying
375 properties; it starts between the non-protonated amine group from proteins and the
376 electrophilic carbonyl carbon of a reducing sugar in polysaccharides (Martins, Jongen, &
377 van Boekel, 2000). One group investigated the effects of heating under alkaline pH alone or
378 in the presence of maltodextrin on emulsifying properties of FBPIs (Farhad Alavi et al.,

379 2021). The FBPI-maltodextrin conjugates were obtained by heating the FBPIs and
380 maltodextrin (w/w ratio of 2:1) at 90 °C for 30 min at pH of 7.0 or 11.0; then, emulsions
381 were prepared with 10% (v/v) sunflower oil and 10 mg/mL unmodified and modified FBPIs;
382 they found that FBPI-maltodextrin samples heated at pH 11.0 produced emulsions that were
383 stable against flocculation and phase separation for 15-day storage at pH 6-7 or in the
384 presence of salt; the improved emulsifying functionality was partly attributed to increased
385 surface hydrophobicity, increased solubility, and steric hindrance (Farhad Alavi et al., 2021).
386 Similarly, covalent attachment of polysaccharides to β -lactoglobulin can increase steric layer
387 thickness, and consequently improve stability of emulsions against calcium induced
388 flocculation (Wooster & Augustin, 2006). The Maillard type complex of wheat protein and
389 dextrans could form a thicker interfacial layer and provide enhanced steric stabilization of
390 the O/W emulsions (Wong, Day, & Augustin, 2011). Conjugation of soy protein isolates
391 (SPI) with citrus pectin and apple pectin under controlled dry-heating conditions was shown
392 to improve emulsifying properties of SPI by increasing the solubility and modifying the
393 surface hydrophobicity (Ma et al., 2020). Other chemical modification involves attaching
394 specific groups. For example, covalent binding between rice protein hydrolysates and
395 chlorogenic acid was shown to improve the emulsifying activity of rice protein hydrolysates
396 and resulted in more physically and chemically stable emulsions; the authors proposed that
397 the complex could form a thicker interfacial layer around the oil droplets (Pan et al., 2019).
398 By optimizing the different aspects of physiochemical characteristics, these chemical
399 modifications of plant proteins can improve the emulsifying activities. However, in the light
400 of consumer awareness of food safety and demands for eco-friendly foods, chemical
401 modification might not be favored. To address these concerns, enzymatic modifications on
402 proteins have been pursued.

403 **4.3 Enzymatic modifications**

404 Enzymatic modifications of proteins are favored by commercial manufacturers because of
405 food safety, lower costs, easier control of the reactions, and acceptability by consumers and
406 regulatory agencies. In order to improve the emulsifying properties, enzymatic modifications
407 of proteins applied in P-O/W emulsions are generally either via decreasing the molar mass
408 of proteins by proteolytic enzymes, or increasing the molar mass by cross-linking enzymes
409 (Chen et al., 2011; Luisa et al., 2015).

410 We recently investigated the effects of different degrees of hydrolysis (DH) by alcalase on
411 the emulsifying activity of FBPIs (**Table 1**) (Liu, Bhattarai, et al., 2019). DH of 4% produced
412 suitable molecular weight and surface net charge that in turn promoted better interfacial layer
413 stability, as well as increased repulsive electrostatic force; DH of 4% also increased the
414 surface hydrophobicity and expose more buried hydrophobic groups, thus improving the
415 adsorption of proteins at the interface and emulsifying capability (Liu, Bhattarai, et al., 2019).
416 Another advantage of hydrolysis treatment is that protein hydrolysates exhibit higher
417 capacity to inhibit lipid oxidation, indicated by higher metal-chelating activities and radical-
418 scavenging activity; in particular, alcalase is a commonly used protease which could produce
419 hydrolysates which have higher antioxidant activities and are more resistant to digestive
420 enzymes (Bucko, Katona, Popovic, Petrovic, & Milinkovic, 2016; Shen, Zhou, Zhang, Yuan,
421 Zhao, & Zhao, 2020; Thaiphanit, Schleining, & Anprung, 2016). Similarly, hydrolysis of
422 FBPIs by alcalase could produce emulsions with significantly less oxidation (i.e., less
423 conjugated dienes and hexanal) while maintaining protein oxidative stability compared to
424 the unmodified FBPI (Liu, Bhattarai, et al., 2019).

425 It has been underlined that enzyme specificity, protein confirmation, enzyme dose, and
426 environmental conditions (e.g. pH, ionic strength, temperature, other components such as
427 inhibitory substances, etc.) all together influence the emulsifying activities of hydrolysates

428 (Tavano, 2013). Our work showed that the hydrolysates with higher DHs (9 and 15%) had
429 decreased emulsifying activity which might be caused by unduly decreased surface
430 hydrophobicity and increased surface load (Liu, Bhattarai, et al., 2019). Similarly, another
431 hydrolysis study reported that excessive hydrolysis of whey proteins may accelerate the
432 desorption of proteins/peptides at interfacial layer, and thereby decrease stability of
433 emulsions (Schroder, Berton-Carabin, Venema, & Cornacchia, 2017). Therefore, it is critical
434 to determine the optimum DH for the best stable P-O/W emulsion. Choosing optimal
435 reaction conditions such as pH and temperature based on the type of enzyme is always the
436 prerequisite (Tavano, 2013).

437 Another major enzymatic modification is through inter- or intramolecular cross-linking
438 reactions of proteins. One commonly used enzyme is microbial transglutaminase (MTG).
439 MTG catalyzes acyl transfer reactions between γ -carboxamide of peptide or protein-bound
440 glutamyl residue (acyl donor) and primary amino group (acyl acceptor). When lysine
441 residues act as acyl acceptor, ϵ -(γ -glutamyl)- lysine “isopeptide” covalent bonds are formed,
442 resulting in polymerization or amine incorporation (Luisa et al., 2015). We have investigated
443 the effects of MTG treatment on the emulsifying activity of FBPIs in O/W emulsion (**Table**
444 **1**). Apparent cross-linking in FBPIs was induced by MTG treatments, which led to 5%-8%
445 increases in protein net surface charges and 19%-135% increases in emulsion particle size;
446 moreover, MTG-treated FBPIs showed inhibiting effects against lipid oxidation (e.g. less
447 conjugated dienes and hexanal production) (Liu, Damodaran, et al., 2019). This may be
448 attributed to thicker interfacial layer around oil droplets, larger emulsion droplet size, and
449 protective effect of proteins (Azuma, Kimura, Hosokawa, & Miyashita, 2009; Berton-
450 Carabin et al., 2014). However, MTG-FBPIs moderately promoted protein oxidation
451 (120 min > 240 min \approx 0 min). Moreover, MTG treatment for 120 and 240 min but not 60 min
452 showed decreased emulsifying activity and physical stability of emulsion, which could be

453 explained by excessive surface hydrophobicity by cross-linking (Liu, Damodaran, et al.,
454 2019). Another group examined the combined effect of heating and MTG on emulsifying
455 properties of FBPI; FBPIs were treated with heat (90 °C, 5 or 30 min) and MTG (0.6, 6 or
456 60 U); then, emulsions were made with 20% sunflower oil and 8.0 mg/mL unmodified or
457 modified FBPIs (Nivala et al., 2020). They found that cross-linking by MTG was facilitated
458 by heat treatment; heat/MTG treatment caused an increase in EAI from 25.1 m²/g to 28.9
459 m²/g (Nivala et al., 2020).

460 On the other hand, cross-linking of proteins may induce undesirable results. For example,
461 one study showed that emulsions with more cohesive MTG cross-linked casein at the
462 interface of oil-water did not show better lipid oxidative stability compared to untreated
463 emulsions (Kellerby, Gu, McClements, & Decker, 2006). This might be attributed to the
464 ability of prooxidants in the aqueous phase to diffuse through the interfacial layer where they
465 might have longer time to contact with lipid hydroperoxides and promote the decomposition
466 into free radicals; consequently, more free radicals would induce lipid oxidation in the
467 droplet core (Kellerby et al., 2006). Similar to hydrolysis, it should be emphasized that
468 choosing an optimum level of enzymatic treatment for proteins is important for achieving
469 desired functionalities. Apart from the major two types of enzymes discussed above, a few
470 other enzymes have been reported. For example, enzymatic deamidation by protein-
471 glutaminase was shown to improve the emulsifying activities of evening primrose seed cake
472 protein by increasing the solubility of protein and enhancing protein's ability to form a layer
473 around fat globules (Hadidi, Ibarz, & Pouramin, 2021). The effects of deamidation of FBPIs
474 needs further investigation.

475 Besides the modifications of FBPIs discussed above, there are other factors that need be
476 considered. FBPIs were commonly extracted via the wet methods (alkaline extraction and
477 isoelectric precipitation) followed by lyophilization or spray drying, which might change the
478 native protein structure and influence the functional properties. Recently, Keivaninahr et al.

479 (2021) compared the emulsifying properties of faba bean protein extracted by the
480 conventional wet method and by air classification. They found that the latter method
481 produced proteins with lower protein content but higher solubility; the proteins obtained by
482 air classification also produced emulsions with better or comparable characters in terms of
483 particle size, zeta potential, and microstructure. Another factor to consider was the role of
484 other ingredients in plants. Karefyllakis et al. (2019) suggested milder and simpler extraction
485 processes which could not only yield proteinaceous fractions, but also preserve other native
486 multicomponent (e.g. lipid, phenols, and carbohydrates). In particular, the multicomponent
487 from sunflower seeds exhibited comparable or even better emulsifying properties than the
488 pure proteins. Moreover, this approach is more sustainable and efficient in that it reduces the
489 energy and chemicals that are required for isolating pure proteins. The combined effects of
490 different modifications and other extraction approaches (e.g. air classification, mild methods
491 for preserving other native multicomponent) on emulsifying properties of FBPIs warrant
492 future investigation.

493 **5 Conclusions**

494 To meet the increasing consumer demands for natural, healthy, and sustainable food
495 ingredients, the plant protein can serve as an alternative emulsifier in O/W emulsions to
496 replace synthetic emulsifiers. Faba bean is a promising source of plant protein due to its high
497 protein content, easy cultivation, and high yields. However, the emulsifying functionality of
498 unmodified FBPIs is relatively inadequate due to undesirable physiochemical characteristics
499 (e.g. low solubility, suboptimal surface hydrophobicity and surface charge). Various
500 physical, chemical, and enzymatic modifications have been examined to produce desired
501 physiochemical characteristics for emulsifying activities via different mechanisms (**Figure**
502 **2**). After suitable modifications, the emulsifying activity of FBPIs and emulsion stability
503 could be remarkably improved, making modified FBPIs as promising plant-based

504 emulsifiers in food production. It is important to optimize the modification conditions to
505 achieve favorable outcomes. However, more research is needed on the effects of other types
506 of safe-for-consumption modifications on FBPIs and the underlying working mechanisms
507 need to be elucidated.

Journal Pre-proofs

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756 **Table 1.** Applications of unmodified and modified FBPIs as emulsifiers in O/W emulsions since 1990.

	Methods	Key findings	Reference
	Protein solutions were obtained by acid precipitations, alkaline dissolutions, centrifugation, and reconstitution from commercial protein concentrates. Emulsions were prepared with 10% fish oil and 20 mg/mL of pea, lentil, or faba bean protein solutions with whey protein as control.	Whey protein produced smaller initial particle size and better stability; initial oxidative stability: pea < lentil < faba bean < whey in whole emulsions; whey < PI = FBPI in washed emulsions.	(Gumus et al., 2017c)
	Protein isolates were obtained by acid precipitations, alkaline dissolutions, and lyophilization from commercial protein concentrates. Emulsions were prepared with 10% (w/w) algae oil and 5% (w/w) protein isolates from pea (PPI), lentil (LPI), faba bean protein (FBPI).	The LPI stabilized emulsions showed better stability to environmental stresses (pH, salt, and temperature) than FBPI and PPI.	(Gumus et al., 2017a)
Unmodified	Protein isolates were obtained by acid precipitations, alkaline dissolutions, and lyophilization from defatted legume flours. The physicochemical and emulsifying properties of protein isolates from chickpea (CPI), faba bean (FBPI), lentil (LPI) and soy (SPI) were compared.	Solubility: CPI (94 %) > LPI (90 %) > FBPI (85 %) > SPI (50 %); surface charge: CPI ≈ LPI ≈ FPI (-47 mV) < SPI (-44 mV); all could effectively decrease interfacial tension (from 16.73 to 8.42 mN/m).	(Johnston et al., 2015)
	Protein isolates were obtained by either isoelectric precipitation or salt extraction, and lyophilization from defatted legume flours. The physicochemical and emulsifying properties of protein isolates from chickpea (CPI), faba bean (FBPI), lentil (LPI), and pea (PPI) were compared.	Surface hydrophobicity (isoelectric precipitation): FBPI ≈ SPI < LPI < CPI ≈ PPI; surface charge (salt-extracted): FBPI (-18.3 mV) > CPI ≈ LPI ≈ PPI (-20.4 mV) > SPI (-21.7 mV); emulsion capacity: PPI ≈ CPI < FBPI < SPI ≈ LPI; emulsion stability (ESI): PPI < FBPI < CPI ≈ SPI ≈ LPI.	(Karaca et al., 2011)
Physical	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 0.5% (w/v) FBPIs electrostatically associated with chitosan via either a complex model (by a soluble FBPI-chitosan complex) or LBL model (by FBPI first and thereafter a secondary layer of chitosan added via electrostatic attraction).	Both LPL and complex model increased interfacial layer thickness; but only with the LBL model produced increased compactness and protein adsorption, resulting better physical and oxidative stability.	(Liu et al., 2020)

<p>FBPIs were prepared from a commercial protein concentrate produced via direct milling of faba beans followed by dry densification. FBPI dispersions were subjected to three pH values (3.0, 5.0, and 8.0); interfacial properties were determined by dilatational measurement and interfacial small amplitude oscillatory shear measurements.</p>	<p>The strength of interfacial FBPI film at O/W interface depended on the pH values; protein adsorption took place significantly faster at pH 3.0 and 5.0 than at pH 8.0.</p>	<p>(Felix, Romero, et al., 2019)</p>
<p>FBPIs were prepared from a commercial protein concentrate produced via direct milling of faba beans followed by dry densification. Emulsions were prepared with 250 mL of 30 mg/mL FBPIs adjusted to different pH values (3.0, 5.0, and 8.0) and 250 mL of sunflower oil.</p>	<p>Smaller droplet sizes and better rheological properties were obtained at pH 3.0 and 8.0; the emulsions at pH 3.0 displayed the lowest extent of lipid oxidation during storage.</p>	<p>(Felix, Cermeño, et al., 2019)</p>
<p>FBPIs were obtained by filtration of aqueous extraction from raw or heat-treated faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 3% (w/v) FBPIs that were heated with either oven at 170 °C for 30 min or microwave at 950 watts for 1.5 min.</p>	<p>Oven heating showed better ability to increase surface hydrophobicity while decreasing native lipoxygenase and peroxidase, resulting decreased lipid oxidation.</p>	<p>(Gürbüz, Liu, et al., 2018)</p>
<p>Faba bean proteins were extracted by alkaline solution, isoelectric precipitation, and lyophilization from dehulled faba bean flours. FBPIs were treated with high pressure (15 kpsi) homogenization.</p>	<p>Homogenization increased protein solubility, surface hydrophobicity by inducing certain level of protein unfolding; but did not improve emulsifying property due to competition of supramolecular aggregates with protein molecules at the interface.</p>	<p>(Yang et al., 2018)</p>
<p>FBPIs were obtained by alkaline extraction, isoelectric precipitation, and spray drying from dehulled faba bean flours. FBPIs were subjected to alkaline shifting or ultrasound treatment alone, or their combination. Emulsions were prepared with 10% (w/w) canola oil and 10 mg/mL modified FBPIs.</p>	<p>The combined treatment increased protein surface hydrophobicity and decreased free sulfhydryl groups; FBPI solubility was significantly increased (>95% at pH 3 and 7; >80% at pH 6.0); meanwhile, FBPIs modified by the combined treatment produced emulsions with smaller particle size and improved stability at pH values 3 and 7.</p>	<p>(F. Alavi, Chen, & Emam-Djomeh, 2021)</p>
<p>FBPIs were obtained by alkaline extraction, isoelectric precipitation, and spray drying from dehulled faba bean flours. FBPIs were heated under alkaline pH alone or in the presence of maltodextrin; emulsions were prepared with 10% (v/v) sunflower oil and 10 mg/mL unmodified and modified FBPIs.</p>	<p>The heating under alkaline pH improved the protein solubility (from 43% at pH 3.0, 14% at pH 6.0, and 35% at 7.0 to more than 90%). FBPI-maltodextrin samples heated at pH 11.0 produced emulsions that were stable against flocculation and phase separation.</p>	<p>(Farhad Alavi et al., 2021)</p>

	FBPIs were obtained by neutral aqueous extraction, acidic precipitation, and spray drying from dehulled faba bean flours. Various degree of acetylation was induced by treating FBPI with acetic anhydride.	Acetylation increased the interfacial activity, Gibbs' adsorption isotherms, and the inflection point of the adsorption isotherms.	(Krause et al., 1996)
Chemical	FBPIs were obtained by neutral aqueous extraction, acidic precipitation, and spray drying from dehulled faba bean flours. The ultrastructure of unmodified and acetylated FBPI (~96%) were compared.	Acetylation improve emulsifying activity, which was associated with tangible difference in different droplet-size distribution and ultrastructure.	(Krause et al., 1994)
	FBPIs were obtained by alkaline extraction from faba bean flours. Acetylation (~97%) was induced by treating FBPI with acetic anhydride.	Acetylation increased the flow, gelifying, surface and emulsifying properties, as well as the physical stability.	(Schmandke et al., 1990)
	FBPIs were obtained by aqueous extraction at pH 7.5 followed by isoelectric precipitation from faba bean flours. Acetylation was in combination with methoxy pectin.	The combination increased the protein adsorption at interfacial phase and viscosity but decreased the creaming capacity.	(Schultz et al., 1991)
	FBPIs were obtained by alkaline extraction, acid precipitations, and lyophilization from defatted faba bean flours. FBPIs were treated with heat (90 °C, 5 or 30 min) and MTG (0.6, 6 or 60 U); emulsions were made with 20% sunflower oil and 8.0 mg/mL unmodified or modified FBPI.	Heat treatment increased surface hydrophobicity; heat treatment facilitated cross-linking by MTG; heat/MTG treatment caused a minor increase in EAI.	(Nivala et al., 2020)
Enzymatic	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 1% (w/v) FBPIs that were hydrolyzed by alcalase to different degree of hydrolysis (DH of 4%, 9%, and 15%).	DH of 4% produce increased physical and oxidative stability due to suitable molecular weight, surface net charge and hydrophobicity; higher DHs (9% and 15%) induced decreased surface hydrophobicity and decreased emulsifying activity.	(Liu, Bhattarai, et al., 2019)
	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 3% (w/v) FBPIs that were cross-linked by microbial transglutaminase (MTG) for 60, 120, or 240 mins.	MTG induced significant cross-linking, increased protein surface charge and particle size, and decreased lipid oxidation; longer MTG treatment (120 and 240 min) decreased emulsifying activity and physical stability of emulsion.	(Liu, Damodaran, et al., 2019)

758 **Figure 1.** Emulsion stability. Emulsion is thermodynamically unstable and highly
759 susceptible to oxidation. Physically, emulsion instability usually includes creaming,
760 flocculation, and coalescence. Lipid oxidation in emulsion mainly involves autoxidation via
761 free radical chain reactions. In protein-containing emulsions, protein oxidation is also
762 initiated by metal ions and oxygen radical and is closely related to lipid oxidation, producing
763 peptide backbone cleavage, side-chain modification, and cross-linking. Protein oxidation can
764 also be initiated and prompted by lipid oxidation products.

765 **Figures 2.** The mechanisms by which physical, chemical, and enzymatic modifications of
766 FBPIs affect the emulsifying functionalities. The emulsifying activities depend on several
767 physiochemical characteristics such as solubility, surface hydrophobicity, surface charge,
768 interfacial activity, etc. These characteristics of FBPIs can be optimized by various
769 modifications via: increasing the interfacial layer thickness and compactness (1); increasing
770 steric hindrance by either electrostatically or covalently linked to other polysaccharides or
771 proteins (3, 4, 7); improving surface hydrophobicity by inducing protein unfolding and
772 changing configuration (2), acetylation (3), Maillard reaction (4), hydrolysis (6), or cross-
773 linking to other proteins (7); inducing greater electrostatic repulsion by increasing surface
774 charge (5); increasing protein solubility by reducing molecular size and increasing surface
775 charge (5, 6).